

**IJUP'**<sup>12</sup>



# IJUP'12

5<sup>th</sup> MEETING OF YOUNG RESEARCHERS  
OF UNIVERSITY OF PORTO

# CREDITS

Livro de Resumos IJUP'12

5º Encontro  
de Investigação  
Jovem da U.Porto

© Universidade do Porto  
AA ID+i  
T. 22 040 81 46  
secidi@reit.up.pt

## **Design**

Tiago Campeã  
Rui Mendonça

## **Impressão e acabamentos**

Invulgar – artes gráficas

## **Tiragem**

1300 exemplares

## **Depósito Legal**

340336/12

## **ISBN**

978-989-8265-82-1

## **SCIENTIFIC COMMITTEE**

Ádrian Silva  
Aurora Teixeira  
Elisa Keating  
Filipe Magalhães  
Georgina Correia da Silva  
Gonçalo Furtado  
Graciela Machado  
Hélder Bastos  
Helena Madureira  
Jorge Gonçalves  
Jorge Teixeira  
José Albino Lima  
Laura Oliveira  
Manuela Aguiar  
Marcela Segundo  
Maria José Oliveira  
Miguel Madeira  
Patrícia Antunes  
Paula Santos  
Pedro Sousa Gomes  
Rita Faria  
Rute Pedro  
Sofia Rodrigues  
Vitor Vasconcelos

### **Secretariat**

Paula Coelho

WEDNESDAY, 22 <sup>nd</sup>		THURSDAY, 23 <sup>rd</sup>		FRIDAY, 24 <sup>th</sup>	
08:40	09:00	REGISTRATION	REGISTRATION	REGISTRATION	REGISTRATION
09:00		<b>PARALLEL ORAL SESSIONS I</b>	<b>PARALLEL ORAL SESSIONS IV</b>	<b>PARALLEL ORAL SESSIONS VIII</b>	
	09:30	A0- Stress & Heart Physiology I	A1- Endocrinology & Metabolism	A1- Molecular Medicine II	
	09:30	A1- Arts	A2- Environment I	A2- Chemistry	
	10:00	A2- UNICER I	A3- Geography & Sociology	A3- Sport Sciences	
	10:00	A3- Psychology & Education Sciences I	A4- Biological Sciences I	A4- Biological Sciences IV	
	10:30	A4- Economics & Management	A5- Engineering III	A5- Engineering IV	
	10:30	A5- Communication Sciences			
	11:00	<b>WELCOME SESSION</b>	<b>COFFEE BREAK &amp; POSTER VIEWING</b>	<b>COFFEE BREAK &amp; POSTER VIEWING</b>	
	11:00				
	11:30	<b>WORKSHOP</b>	<b>PARALLEL ORAL SESSIONS V</b>		
	11:30	Estratégias para sobreviver ao vale da morte: Aproximar a I&D do mercado	A1- Innovation in Health		
	12:00		A2- Environment II		
	12:00		A3- Virtualities		
	12:30	<b>POSTER VIEWING</b>	A4- Biological Sciences II		
	12:30		A5- Astronomy		
	12:30				
	13:00	Exhibition Opening <i>MNEMOSINE</i> , at Galeria dos Leões	<b>LUNCH BREAK</b>		
	13:00	<b>LUNCH BREAK</b>			
	13:30				
	13:30				

# PROGRAM

- ORAL SESSIONS
- POSTER VIEWING

13:30 ..... 13:30	
14:00 ..... 14:00	
14:30 ..... 14:30	<p><b>PARALLEL ORAL SESSIONS II</b></p> <p>A1- Pharmaceutical &amp; Med. Chemistry A2- UNICER II A3- Psychology &amp; Education Sciences II A4- AGR0 F000 I A5- Engineering I</p>
15:00 ..... 15:00	<p><b>PARALLEL ORAL SESSIONS VI</b></p> <p>A1- Molecular Medicine I A2- Legal Studies &amp; Criminology I A3- History &amp; Cultural Studies I A4- Biological Sciences III A5- Architecture I</p>
15:30 ..... 15:30	
16:00 ..... 16:00	<p><b>COFFEE BREAK &amp; POSTER VIEWING</b></p>
16:30 ..... 16:30	<p><b>COFFEE BREAK &amp; POSTER VIEWING</b></p>
17:00 ..... 17:00	<p><b>PARALLEL ORAL SESSIONS III</b></p> <p>A1- Clinical Care A2- Psychology &amp; Education Sciences III A3- Literature &amp; Language Studies A4- AGR0 F000 II A5- Engineering II</p>
17:30 ..... 17:30	<p><b>PARALLEL ORAL SESSIONS VII</b></p> <p>A1- Stress &amp; Heart Physiology II A2- Legal Studies &amp; Criminology II A3- History &amp; Cultural Studies II A4- Public Health &amp; Epidemiology A5- Architecture II</p>
18:00 ..... 18:00	
18:30 ..... 18:30	



# TABLE OF CONTENTS

Foreword .....	13
<b>Oral Sessions I .....</b>	<b>17</b>
A0- Stress & Heart Physiology I .....	19
A1- Arts .....	25
A2- UNICER I .....	31
A3- Psychology & Education Sciences I .....	39
A4- Economics & Management .....	47
A5- Communication Sciences .....	55
<b>Oral Sessions II .....</b>	<b>65</b>
A1- Pharmaceutical & Medicinal Chemistry .....	67
A2- UNICER II .....	75
A3- Psychology & Education Sciences II .....	83
A4- AGRO FOOD I .....	91
A5- Engineering I .....	99
<b>Oral Sessions III .....</b>	<b>109</b>
A1- Clinical Care .....	111
A2- Psychology & Education Sciences III .....	119
A3- Literature & Language Studies .....	127
A4- AGRO FOOD II .....	137
A5- Engineering II .....	145
<b>Oral Sessions IV .....</b>	<b>155</b>
A1- Endocrinology & Metabolism .....	157
A2- Environment I .....	165
A3- Geography & Sociology .....	173
A4- Biological Sciences I .....	183
A5- Engineering III .....	193
<b>Oral Sessions V .....</b>	<b>203</b>
A1- Innovation in Health .....	205
A2- Environment II .....	213
A3- Virtualities .....	221
A4- Biological Sciences II .....	227
A5- Astronomy .....	237

<b>Oral Sessions VI</b> .....	<b>249</b>
A1- Molecular Medicine I .....	251
A2- Legal Studies & Criminology I .....	259
A3- History & Cultural Studies I .....	267
A4- Biological Sciences III .....	277
A5- Architecture I .....	287
<b>Oral Sessions VII</b> .....	<b>295</b>
A1- Stress & Heart Physiology II .....	297
A2- Legal Studies & Criminology II .....	305
A3- History & Cultural Studies II .....	311
A4- Public Health & Epidemiology .....	321
A5- Architecture II .....	329
<b>Oral Sessions VIII</b> .....	<b>339</b>
A1- Molecular Medicine II .....	341
A2- Chemistry .....	349
A3- Sport Sciences .....	357
A4- Biological Sciences IV .....	367
A5- Engineering IV .....	375
<b>Posters</b> .....	<b>385</b>
Posters from Wednesday, 22 <sup>nd</sup> .....	387
Posters from Thursday, 23 <sup>rd</sup> .....	475
Posters from Friday, 24 <sup>th</sup> .....	561
<b>Index</b> .....	<b>652</b>





## FOREWORD

O programa de iniciação à I&D da U.Porto (IJUP) tem, no seu encontro anual o seu momento de maior visibilidade.

O encontro deste ano - já a sua 5ª edição, reúne mais de 1100 participantes, maioritariamente estudantes dos primeiros ciclos e de mestrado integrado. Os resultados dos trabalhos em que participam vão ser apresentados nas mais de 500 comunicações orais e na forma de poster. Um acontecimento único num local simbólico - o edifício da Reitoria, representando-se bem o espírito de cooperação que pretendemos criar dentro da nossa universidade.

Mas o IJUP é mais do que este encontro anual. Ele revela o dinamismo e a abertura da nossa comunidade científica para acolher estes estudantes, permitindo-lhes uma experiência de aprendizagem única. Nele se revelam novas vocações e paixões para a investigação científica e para a prática do método científico, num processo que aproxima a criação do conhecimento dos seus destinatários. E isso é ainda mais relevante na medida em que criará canais facilitadores da ligação do sistema científico e tecnológico às entidades onde, no futuro, esses estudantes irão a exercer a sua atividade profissional.

O IJUP deve ser motivo de orgulho de toda a comunidade U.Porto. Sem incentivos de políticas públicas, tem sido possível colocar de pé um programa único no ensino superior Português e até mesmo a nível internacional. Com este programa, encontraram-se pretextos para maior cooperação entre centros de investigação e unidades orgânicas. Com este programa a U.Porto mostra-se e passa a conhecer-se melhor... E este maior conhecimento do nosso potencial só nos pode dar razões para enfrentar o futuro com confiança!

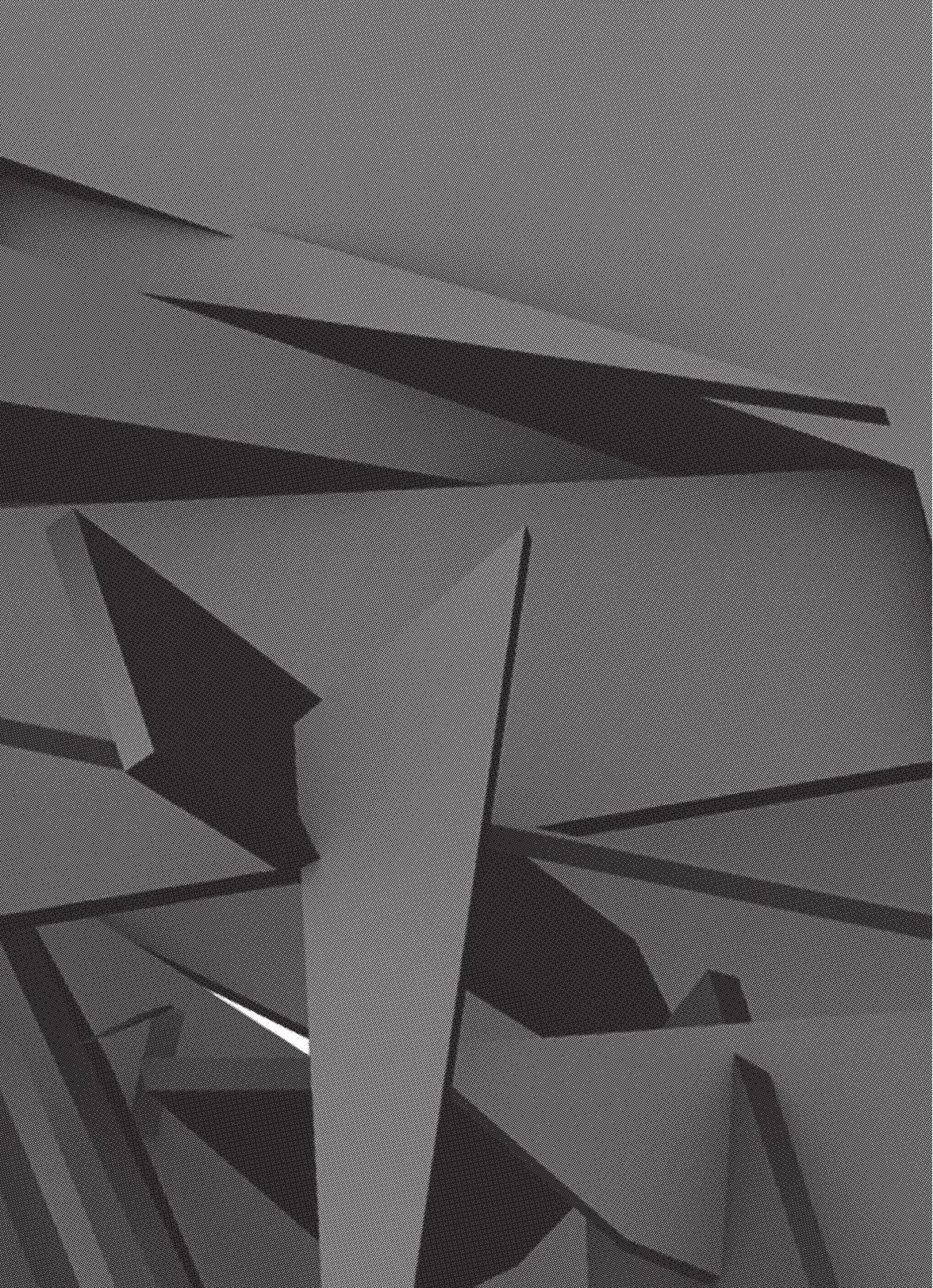
O nosso obrigado a todos os que tornam este programa possível e que deste modo também materializam a nossa ambição de nos diferenciarmos enquanto universidade e afirmarmos a U.Porto como uma universidade de investigação.

Bem hajam!

Marques dos Santos  
Reitor da Universidade do Porto







# Oral Sessions I



Oral Sessions I > A0

# Stress & Heart Physiology I



# Characterization of heart rate variability in critical situations

**P. F. Silva<sup>1</sup>, A. Carvalho<sup>1</sup>, A. P. Rocha<sup>1,2</sup>**

<sup>1</sup> Departamento de Matemática, Faculdade de Ciências, Universidade do Porto, Porto, Portugal.

<sup>2</sup> CMUP, Centro de Matemática da Universidade do Porto, Porto, Portugal.

Time and frequency analysis of Heart Rate Variability (HRV) can provide a quantitative and non-invasive tool to access the integrity of the cardiovascular system and has been applied in various clinical situations [1]. It allows to follow the clinical evolution of patients, namely those being monitored at intensive care units [2,3].

In this work we characterize HRV signals both in time and frequency domains, using standard techniques from time series analysis and statistical signal processing. The main objective consisted in studying the behaviour of typical measures in HRV description [1] and in using them with automatic classification techniques, available through R Project, to study group behaviour, according to three clinical status (brain death, coma and control group), with data retrieved from [2].

The main results are summarized in Fig. 1. Standard measures although correlated are highly discriminant. In spite of still unexplained dynamics, preliminary results indicate that automatic classification is feasible, but further investigation is needed.

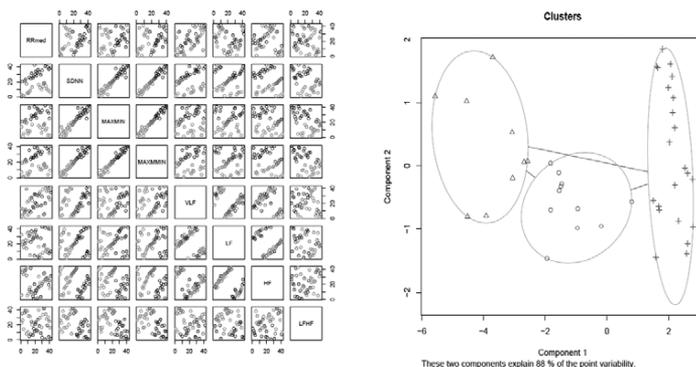


Figure 1: Left - scatter-plot of the collected measures. Right - clustering of the studied cases

## References:

- [1] Task Force of ESC & NASPE (1996). *Heart rate variability, standards of measurement, physiological interpretation, and clinical use*. Eur Heart J 17, 354-381.
- [2] Freitas, J., Puig, J., Rocha, A.P., Lago, P., Teixeira, J., Carvalho, M.J., Costa, O., Falcão de Freitas, A. *Heart Rate variability in brain death*. Clinical Autonomic Research, 6, 141-146, 1996.
- [3] Rocha, A. P. et al, *Cardiovascular Variability Monitoring in Pediatric Coma*, IJUP 09, 2<sup>nd</sup> Meeting of Young Researchers of U. Porto, Porto, February 25- February 27, 2009.

# Effects of exercise in the hepatic regeneration

**M. Ferreira**<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Sports, University of Porto, Portugal.

The acknowledged benefits of exercise gave it an important role in people's lives. Due to those benefits, exercise influence in various structures and metabolic pathways are deeply studied and described by the scientific research.

Located in the right upper quadrant of the abdominal cavity, liver intervenes in a lot of processes as bile production, immunologic response, enzymatic secretion and storage of many substances.

Diverse pathologies, drugs and toxins cause liver cells destruction changing hepatic efficiency but the rapid cell repopulation shows its high and effective regenerative capacity.

The main objective of this study is to determinate the possible interaction of physical exercise in the liver regeneration capacity.

The sample was composed by forty rodents (Wistar 8 week age) that were divided randomly in two groups. The first one was a control group. The second group was exercised in treadmill 60min/day, 20m/min during eight weeks. Two/three animals of each group were sacrificed every seven days. To identify the cell proliferation we used bromodeoxyridine (BrdU), it was given to all rodents, in water, seven day before sacrifice. Samples of the livers were collected and processed in paraffin. Afterward, tissues were cut in sections of 5µm with a microtome, selected and organized in slides.

Eosin/hematoxylin staining protocol was used to microscopic observation and photographed tissues.

Using the antibody/antigen reaction, the BrdU imunohistochemical protocol allowed the identification of new nuclei formed during BrdU administration period. By the end of this process, marked nuclei will be identified and quantified by optic microscopy and image processing software.

At this point, the results are only preliminary but the differences on the number of new marked nuclei in rodents livers that exercise and control's livers may be indicators of the effects caused by physical exercise in hepatic regeneration. Other expected result is that the number of marked nucleus will vary according to the exercise time, demonstrating difference between acute and chronic practice.

## Effect of intermittent hypobaric hypoxia and endurance training in cardiac mitochondrial function

**M. Balça<sup>1</sup>, IO. Gonçalves<sup>1</sup>, J. Lumini-Oliveira<sup>1,2</sup>, I. Marques-Aleixo<sup>1</sup>, E. Passos<sup>3</sup>, S. Rocha-Rodrigues<sup>1</sup>, A. Ascensão<sup>1</sup> and J. Magalhães<sup>1</sup>**

<sup>1</sup>Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal

<sup>2</sup>Faculty of Health Sciences, University of Fernando Pessoa, Portugal

<sup>3</sup>Department of Biochemistry, Faculty of Medicine, University of Porto, Portugal

**Background:** Endurance training (ET) and intermittent hypobaric hypoxia (IHH) exposure are recognized cardioprotective strategies against several deleterious stimuli being mitochondria involved in the process. The present study aimed to analyze whether a combination of these two non-pharmacological approaches may provide additive effects increasing heart mitochondrial function.

**Methodology:** Twenty-eight adult male rats were divided into normoxic-sedentary (NS), normoxic-exercised (NE), hypoxic-sedentary (HS) and hypoxic-exercised (HE). IHH consisted of 5h/d at an atmospheric pressure equivalent to 6000m and ET of 1h/d of treadmill running for 5 weeks. Mitochondrial oxygen consumption, transmembrane potential ( $\Delta\Psi$ ) and calcium-induced permeability transition pore (MPTP) were evaluated in vitro in isolated cardiac mitochondria. OXPHOS subunits, cyclophilin D (Cyp D), adenine nucleotide translocator (ANT) as well as Bax and Bcl2 were semi-quantified by Western Blotting.

**Results:** Respiratory control ratio increased in all groups compared to NS whereas no significant alterations in  $\Delta\Psi$  endpoints. Moreover, mitochondrial susceptibility to calcium-induced MPTP decreased in NE, HS and, even further, in HE compared to NS. HS significantly decreased the levels of complex I and II OXPHOS subunits compared to NS; however HE completely reverted the levels of complex II OXPHOS subunits. Cyp D expression was not altered in any group, but ANT increased significantly when IHH and ET were combined. The Bcl-2/Bax ratio increased in NE and HS; however only a trend to increase was observed in HE.

**Conclusions:** Data confirm that both IHH and ET modulate cardiac mitochondria to a more resistant phenotype. Moreover, data suggest, for the first time, that the combination of both strategies may have an additive effect on some particular features of mitochondrial function.

**Acknowledgements:** supported by IJUP to JM, FCT grants (SFRH/BPD/4225/2007 to AA, SFRH/BPD/66935/2009 to JM, SFRH/BD/61889/2009 to I-MA, SFRH/BD/62352/2009 to IO-G, SFRH/BD/71149/2010 to EP

# **Endurance training and chronic intermittent hypobaric hypoxia modulate in vitro salicylate-induced hepatic mitochondrial dysfunction**

**S. Rocha-Rodrigues<sup>1</sup>, IO. Gonçalves<sup>1</sup>, J. Lumini-Oliveira<sup>1,2</sup>, I. Marques-Aleixo<sup>1</sup>, E. Passos<sup>3</sup>, A. Ascensão<sup>1</sup> and J. Magalhães<sup>1</sup>**

<sup>1</sup>Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal

<sup>2</sup>Faculty of Health Sciences, University of Fernando Pessoa, Portugal

<sup>3</sup>Department of Biochemistry, Faculty of Medicine, University of Porto, Portugal

**Introduction:** Mitochondrial function is modulated by multiple approaches including physical activity, which can afford cross-tolerance against a variety of insults. To analyze the effects of endurance training (ET) and chronic intermittent hypobaric hypoxia (CIHH) on liver mitochondrial bioenergetics and whether these effects translate into benefits against in vitro salicylate mitochondrial toxicity.

**Methodology:** Twenty-eight young-adult male rats were divided into normoxic-sedentary (NS), normoxic-exercised (NE), hypoxic-sedentary (HS) and hypoxic-exercised (HE). ET consisted of 1h/d of treadmill running and CIHH of simulated atmospheric pressure of 49.3kPa 5h/d during 5wks. Mitochondria were isolated from livers and oxygen consumption, transmembrane electric potential and calcium-induced mitochondrial permeability transition pore induction (MPTP) were evaluated in the presence and absence of salicylate. Aconitase, MnSOD, caspase 3 and 8 activities, -SH, MDA, sirtuin 3 (SIRT3), cyclophilin D (Cyp D), heat shock proteins 70kDa (HSP70), OXPHOS subunit contents were assessed.

**Results:** ET and CIHH decreased basal hepatic mitochondrial state 3 and state 4 respiration, although no alterations were observed in transmembrane electric potential endpoints evaluated in control mitochondria. In the presence of salicylate, ET and CIHH decreased state 4 and lag phase of ADP phosphorylation. Moreover, ADP lag phase in hypoxic was further lower than in normoxic groups. Neither ET nor CIHH altered the susceptibility to calcium-induced MPTP. CIHH lowered MnSOD and increased aconitase activities. ET per se decreased aconitase activity. ET and CIHH decreased caspase 8 activity whereas no effect was observed on caspase 3. The levels of SIRT3 increased with ET and CIHH and Cyp D decreased with CIHH. No differences between groups were observed in the levels of HSP70 and OXPHOS subunits.

**Conclusion:** Data suggest that ET and CIHH do not alter general basal liver mitochondrial function, but may attenuate some adverse effects of salicylate.

**Acknowledgements:** supported by IJUP to AA, FCT grants (SFRH/BPD/4225/2007 to AA, SFRH/BPD/66935/2009 to JM, SFRH/BD/61889/2009 to I-MA, SFRH/BD/62352/2009 to IO-G, SFRH/BD/71149/2010 to EP

Oral Sessions I > A1

# Arts



## Painting and Printing Techniques: intersections and contaminations

A. M. Rocha<sup>1</sup>

<sup>1</sup> Department of Fine Arts - Painting, Faculty of Fine Arts, University of Porto, Portugal

The present research project intends to explore relations that can be established between painting and printing techniques, based on testing of technical procedures and different ways of graphic registration and plastic expression.

Starting from a working path in painting, printing techniques arise as a reference to be explored. Thus, began a process of producing a body of work from the production of monotypes, a process almost obsessive, trying to discover all the possibilities of this technique. The results are a nature-based abstraction, images or scenarios of organic forms, fluid, irregular, changing, based on structures and systems of the microcosm and the macrocosm. This process results in tonal surfaces graphically rich, but light and faint at the same time. I am interested in exploring a large variety of visual effects, including transparencies, opacity, density, tonal variation and chromatic effects.

Nature appears as a source of encouragement. Details of the composition of a rock, phenomena such as sedimentation of rivers and irrigation, images of maps and cartography and the satellite and aerial views of Google Maps, serve as a basis for further development and production of images. The work resulted from a symbiosis between attentive observations of the natural world and a creative visual expression, through plastic materials.

It is also important for this work the experiences and process of producing images that will define, restructuring, removing, replacing, and discovering new relationships that will strengthen the experimental practice of painting. I am interested to understand and explore the natural qualities of the materials, resulting in a greater concern with the process of creation, as important as the finished work.

At the same time, has been developed a collaborative multidisciplinary research project, about glass modulation and printing. I am developing the technique of *colography* with *carborundum* [1], by constructing the images, in relief, on a matrix. This technique has plastic results similar to painting with heavy, rich, tonal areas, loaded with color.

References:

[1] Goetz, H. (1974), *Gravure au carborundum: nouvelle technique de l'estampe en taille douce*, Maeght Editeur, Paris.

## **Glass and Print: Creation of alternative substrates and matrix for printmaking**

**Célia Esteves<sup>1</sup>, Joana Paulino<sup>2</sup>, Graciela Machado<sup>3</sup>, Teresa Almeida<sup>4</sup>**

<sup>1</sup> Department of Drawing, Faculty of Fine Arts, University of Porto, Portugal.

<sup>2</sup> Department of Drawing, Faculty of Fine Arts, University of Porto, Portugal.

<sup>3</sup> i2ADS - Institute of Research in Art, Design and Society, Department of Drawing,  
Faculty of Fine Arts, University of Porto

<sup>4</sup> Research Unit VICARTE “Glass and Ceramic for the Arts”, i2ADS - Institute of Research in Art,  
Design and Society, Department of Fine Arts, Faculty of Fine Arts, University of Porto

The present project, in the field of glass applied to the artistic creation, intends to define several production methods compatible with printmaking printing technologies. Despite its increasing presence in contemporary art forms, and numerous applications to architecture and design, the combination of glass and print are not fully explored in Portugal. Through the co-operation of specialists in materials, glass techniques and printmaking, the project aims to allow new processes to the artists as much as to independent publishing and construction industry [1]. Hence, this project rethinks and gives meaning to tools and technologies, helping people to connect, understand, share and create.

The technical revision, innovative at the national context, will be accompanied by the development of alternative production methods that will encounter to the artistic vocabulary specific to the printmaking context. The investigation foresees an original comparative study with the traditional support – paper – as well as an assessment of digital technology potential integration, confirming it. Printing techniques, such as screenprinting, lithography, etching or engraving, and glass techniques, such as kiln-casting, sand-casting, sand-blasting, handblasting, slumping and lamination, are being reviewed to strengthen its combination on the contemporary art context in addition to introduce auxiliary experimental methods. Therefore, and in the concerns of science materials, it is essential to study solutions and technology designed to maximize compatibility and assist the transfer and creation of images into glass – both as decal or direct processes –, the durability and resistance of the matrix, the possibility of layers forming in glass and the manipulation of hot glass matrix production [2].

The collaborative nature of the project, as well as the mixtures of creativity and production areas, will determine how the results emerge. Thus, it is crucial to understand this model as an informer of the emergence of novel artist practice scenarios, used here as an investigation methodology that challenges the traditional art research as much as contributes to new products and services.

References:

[1] Marques, C, “A Expressividade do Betão – Aplicação de Técnicas de Impressão”, 2011.

[2] Petrie, K, “Glass and Print”, 2006.

# OS PAINÉIS DE AZULEJO DA ESTAÇÃO DE S. BENTO HISTÓRIA, CONTEXTO E ICONOGRAFIA

**Ednilson Leandro Pina Fernandes and Luís Alberto Esteves dos Santos Casimiro**

Department of Ciências e Técnicas do Património, Faculty of Letras, University of Porto, Portugal.

Under the scope of the Internship in the Cultural Office of CP (Portuguese public railway company), we prepared this report called “Os Painéis de Azulejos da Estação de S. Bento: história, contexto e iconografia” (“The Tile Panels of S. Bento Train Station: history, context and iconography”) which is a proposal concerning subjects to be included in a catalogue about S. Bento Train Station vestibule's tile panels.

Since we find relevant to analyse the panels taking in account its space and context, we present the history of the place where they belong (from the former monastery which occupied that place to the architect who planned the train station) and of the artist responsible for its craft.

Accordingly, we approach our subject in four chapters,

The first two are reserved to the history of the monastery [1] (Mosteiro de S. Bento de Ave-Maria) [2], which inspired the name of the train station, from its erection to its controversial dissolution in the end of the XIX century to make way for the train station; and to the erection of the S. Bento Train Station, namely, its controversial localization, the hesitations in choosing the project, with reference to the education and works by Marques da Silva [3], who was responsible for the architecture of the building.

Under the scope of the analysis of the tile panels, which are the object of our study, the third chapter is devoted to the presentation of its author, the painter Jorge Colaço [4] with reference to his artistic career and major works. To conclude our study, we present the iconography [5] of the panels previously identified as relevant for us to achieve our purpose.

## References:

- [1] DIAS, Marina Tavares; MARQUES, Mário Morais. (2002), *Porto desaparecido*. Lisboa: Quimera,
- [2] ALVES, Joaquim Jaime B. Ferreira. (1988), *O Porto na época dos Almadás: arquitectura, obras públicas*. Porto: Câmara Municipal.
- [3] CARDOSO, António Cardoso Pinheiro de. (2007), *Estação de S. Bento Marques da Silva*. Porto: Instituto Arquitecto José Marques da Silva.
- [4] Pamplona, Fernando de. (2.<sup>a</sup> Ed.) (1987), *Dicionário de Pintores e Escultores Portugueses ou que trabalharam em Portugal II*. Porto: Livraria Civilização Editora, Vol. II, pp. 109-110.
- [5] MARTINS, Fausto Sanches. (2001), *Azulejaria portuense: história e iconografia*. Lisboa: Inapa.

# TRANSMUTATION OF BEING: Investigation into Image Construction as a time-based process within an installation-based context

Mariana Moranduzzo<sup>1</sup>

<sup>1</sup> Department of Painting, Faculty of Fine Arts, University of Porto, Portugal.

This project approaches the thin line between the image and the object and is mainly concerned with how the pictorial can survive without a bi-dimensional support so as to surpass the rigidity of the rectangular format and its intrinsic flatness, emerging from the plan to acquire a physical presence.

I explored a time-based artistic strategy by experimenting the possibility of gradual change as a way of re-thinking the art object during the artistic process of image or object making. Transition and transformation are key-concepts for this project proposal. Therefore, I approach the multiplicity through repetition and transmutation of forms. I'm interested in the hybrid, the potential and the remains or trails of existence.

In the context of printmaking, the possibility of changing gradually by means of acid bites, layering or re-printing is also connected to this idea of time as awareness of mutation. (Fig. 1) Transformation is present in the image construction, both in a cumulative and subtractive approach. Some of my work developed within this project is characterized by an extensive use of overlapping. Some of the three-dimensional objects are built by layering diverse thin pieces of paper that never fully cover those underneath, so that the object holds the capacity to tell the story of its own process (Fig. 2).

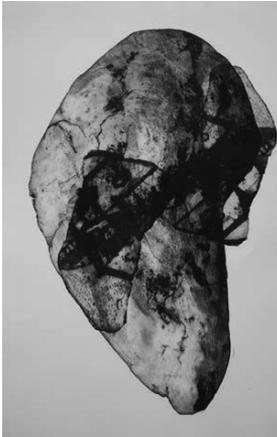


Fig. 1



Fig. 2

Oral Sessions I > A2

# UNICER I



## Proteolytic activity of surplus yeast extracts

**Tiago Almeida<sup>1</sup>, Cristina Moura<sup>1</sup>, Elsa Vieira<sup>1</sup>, Olívia Pinho<sup>2</sup>, Isabel Ferreira<sup>1</sup>, Beatriz Carvalho<sup>3</sup>**

<sup>1</sup> REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Portugal

<sup>2</sup> Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Portugal.

<sup>3</sup> UNICER – Bebidas de Portugal SGPS, SA, Leça do Balio, 4466-955, S. Mamede de Infesta, Portugal

The surplus yeast is the second largest brewing by-product and presents rich chemical composition. The most abundant classes of macromolecules in yeast cells are proteins and carbohydrates. It is also rich in vitamins and minerals. The total mineral content of yeast is approximately 5–10% of the cell dry weight. This fraction comprises a multitude of elements, specially potassium and phosphorus. Several compounds of industrial interest can be isolated from brewer's yeast biomass, such as  $\beta$ -glucan, enzymes, proteins, vitamins, amino acids, cytochromes, the purine components of DNA and RNA [1].

The goal of this work was to evaluate the proteolytic activity of surplus yeast extracts using standard casein assay and by RP-HPLC using a protein substrate very resistant to hydrolysis, such as elastin. Comparison with activity of commercial enzymes was also performed.

Six yeast extracts (a mixture of enzymes, peptides, nucleotides and other soluble components of yeast cells) were produced by the breaking down of yeast cells using a mechanic process.  $\beta$ -glucan from cell walls was separated after centrifugation. Two yeast extracts were obtained from first use of yeast (Gn0 and Hn0) and the other four were obtained from reused yeast surplus (Gn+1, Gn+2, Hn+1, Hn+2). Protein content of extracts was evaluated by Bradford method and its proteolytic activity was confirmed by casein assay (Fig. 1). Good correlation was obtained between protein content and enzymatic activity of extracts.

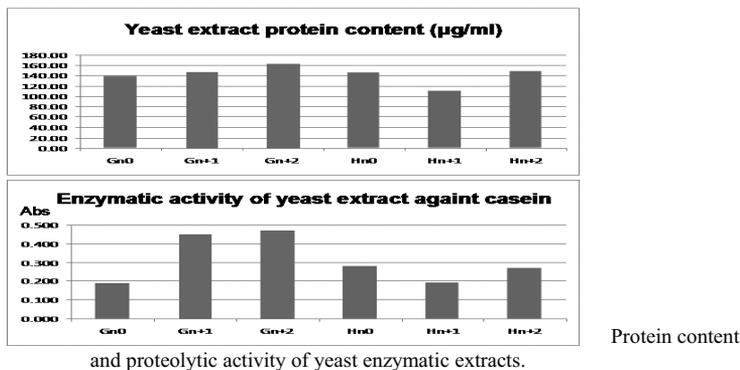


Fig. 1 -

and proteolytic activity of yeast enzymatic extracts.

Hydrolysis of elastin and peptide formation was monitored by RP-HPLC/UV. Extensive peptide formation was observed after 24h incubation. Similar proteolysis was observed for commercial enzymes, namely alcalase.

[1] Ferreira, I.M.P.L.V.O., Pinho, O., Vieira, E., Távora J.G. (2010). *Brewer's Saccharomyces yeast biomass: characteristics and potential applications*. Trends Food Sci & Technol, 21, 77-84.

# ***Saccharomyces cerevisiae* brewing biomass as a promising source of nucleotides for *flavour* enhancers production**

**Elsa Vieira<sup>1</sup>, Olívia Pinho<sup>2</sup>, Isabel Ferreira<sup>1</sup>, Tiago Brandão<sup>3</sup>**

<sup>1</sup>REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Portugal; <sup>2</sup> Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Portugal; <sup>3</sup> UNICER – Bebidas de Portugal SGPS, SA, Leça do Balio, 4466-955, S. Mamede de Infesta, Portugal

*Saccharomyces cerevisiae* biomass surplus is the second by-product from brewing industry. Due to the global pressure towards sustainable environmental technology, an alternative use to this by-product is paramount. Yeast is often reused for four to six times in wort fermentation. Yeasts contain between 6-12% RNA and DNA [1-3]. For this reason, several research groups have been dedicated to the selection of specific strains rich in nucleic acids and optimized methods of production of *flavor* enhancers, namely, 5'IMP, 5' GMP and 5'AMP. On the other hand, since the yeast contains a wide variety of nucleases, the optimization of the conditions of RNA autolysis provides a high yield of production of different nucleotides isomers, as well, as nucleosides and nucleobases [4].

The goal of this work was to evaluate the composition in nucleotides of brewer's yeast biomass presenting different reuses in order to estimate potential application of yeast extracts as food ingredients with *flavor* enhancer properties.

Experimental planning included the analyses of 48 yeast samples, ranging from zero to seven reuses. After yeast disruption using 0.6 mm glasses beads, followed by RNA hydrolysis under the experimental conditions optimized (24 hours, 60°C, pH 5), 5 nucleosides and 9 nucleotides were quantified, using a HILIC methodology, previously validated and optimized. Total RNA content was quantified by spectrophotometry.

The RNA levels quantified in the yeast biomass varied between 4-8% (dry weight). 2'AMP and 2'GMP were the major compounds in yeast biomass hydrolysates, representing 47.4%±3.6 (RSD=7.7%) of total nucleotides quantified. These results are consistent with those obtained by other authors [4-5]. Nucleotides 5'AMP, 5'GMP and 5'IMP represented 28.95%±2.6% (RSD=9.1%) of total nucleotides. Concerning to nucleosides, the predominant compounds were guanosine (0.092±0.024g/100g dry weight (RSD=25.72%) and adenosine (0.035 ± 0.002g/100g dry weight (RSD= 5.626%).

In conclusion, 5'AMP, 5'IMP e 5'GMP that present *flavor* enhancer properties represent less than 30% of the total nucleotides, thus, it is important to improve the yield of formation of these compounds by external addition of 3'RNAases.

## References:

- [1] Hellborg, L. and Piškur, J. (2009), *Yeast diversity in the brewing industry*, in Preedy, VR Press, “*Beer in health and disease prevention*”. New York, Elsevier, pp. 1068-1073.
- [2] Aleksander, P., Piotr, A., Tadeusz, T., Makarewicz, M. (2009). Accumulation and release of metal ions by brewer's yeast during successive fermentations. *J. Inst. Brew.* 115(1), 78-83
- [3] Ferreira, I.M.P.L.V.O., Pinho, O., Vieira, E., Távora J.G. (2010). Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. *Trends Food Sci & Technol*, 21, 77-84.
- [4] Zhao, J. and Fleet, G.H. Degradation of RNA during autolysis of *Saccharomyces cerevisiae* produces predominantly ribonucleotides. *J Ind Microbial Biotechnol* 2005 Aug; 32: 415-423.
- [5] Charpentier, C., Aussenac, J., Charpentier, M., Prome, J.C., Duteurtre, B., Feuillat, M. (2005). Release of nucleotides and nucleosides during yeast autolysis: kinetics and potential impact on flavor. *J Agric Food Chem*, 53(8): 3000-3007.

## **Immunofluorescent study of VEGF, angiopoietins, VEGFR1, VEGFR2 and Tie2 in the corpus cavernosum of diabetic rat after epigallocatechin gallate treatment**

**C. Lombo<sup>1</sup>, D. Fernandes<sup>1</sup>, I. Tomada<sup>1</sup>, C. Morgado<sup>1</sup>, I. Tavares<sup>1</sup>, D. Neves<sup>1</sup>**

<sup>1</sup> Department of Experimental Biology, Faculty of Medicine and IBMC of Universidade do Porto, Portugal.

**Diabetes type 1 is a chronic disease that often initiates in youth and increase the risk of developing complications such as cardiovascular disease or erectile dysfunction.** This is partially due to oxidative stress in affected cells, that in addition to macromolecular damage, inactivates nitric oxide (NO). This condition strongly affects blood vessels of corpus cavernosum (CC), decreasing erectile capability of the penis [1]. Thus, we hypothesise that treatment with antioxidants present in the green tea in the initial phase of diabetes type 1 may protect CC from structural and molecular changes induced by oxidative damage. In support of this hypothesis a previous study demonstrated that green tea long-term ingestion decrease lipid perivascular deposition and vascular endothelial growth factor (VEGF) and its receptor VEGFR 2 expression in CC of aged rats [2]. VEGF is the main angiogenic factor in tissues that induces proliferation and survival of endothelial cells after binding to VEGFR2. In vivo, VEGF crosstalks with other angiogenic growth factors, such as angiopoietins (Ang) 1 and 2, competing for binding to receptor Tie2.

Wistar rats were divided into three groups (n=8): non-diabetic control rats (C), rats injected intraperitoneally with a streptozotocin solution (STZ, 60 mg/kg) and rats injected with STZ and treated with the green tea antioxidant (EGCG-epigallocatechin gallate) solution (2g/L) (STZ/EGCG). Rats were sacrificed 10 weeks after the STZ injection. The CC was removed and immediately fixed and processed for dual-labelling immunofluorescence detection of the endothelial protein PECAM (platelet/endothelial cell adhesion molecule) and  $\alpha$ -actin, VEGF and receptors VEGFR1 or VEGFR2, and Ang1 or Ang2 and Tie2. Morphometrical analysis of smooth muscle content in CC after immunohistochemical detection of  $\alpha$ -actin was performed by ImageJ<sup>®</sup>.

The expression of PECAM, VEGFR2, Ang2 and Tie2 was restricted to the endothelium and of  $\alpha$ -actin and Ang1 to smooth muscle layer in all experimental groups. On the other hand, VEGF was observed both in the endothelium and on smooth muscle, often co-localizing with VEGFR1. Untreated STZ rats presented reduced perivascular smooth muscle layer comparatively to other groups.

The data here reported suggest that EGCG consumption since an early phase of diabetes prevents structural diabetes-induced modifications in blood vessels of CC. The role of VEGF and angiopoietins need to be further evaluated in the perspective of preventing erectile dysfunction during diabetes. Financial Support of IJUP/UNICER Project

### References:

- [1] Agarwal, A., et al. *Role of oxidative stress in the pathophysiological mechanism of erectile dysfunction*, J Androl 2006 (27):335-347.
- [2] Neves, D., et al. *Does regular consumption of green tea influence expression of vascular endothelial growth factor and its receptors in aged rat erectile tissue?*, AGE 2008 (30): 217-228.

# **Epigallocatechin Gallate prevents spinal oxidative stress damage and neuropathic pain in STZ-diabetic rats**

**D. Raposo, P. Pereira-Terra, C. Morgado, I. Tavares**

Department of Experimental Biology, Faculty of Medicine and IBMC  
University of Porto

Diabetic neuropathy is a devastating complication of diabetes, which is frequently associated with neuropathic pain. For this pain condition it seems to contribute the hyperactivity detected in the spinal nociceptive neurons of diabetic rats. However, the mechanisms underlying the spinal hyperactivation remain unclear. Considering recent data showing that the antioxidant treatment with alpha-lipoic acid reversed spinal hyperactivity and ameliorated pain responses while decreasing the spinal oxidative damage in STZ-diabetic rats suggest that oxidative stress may concur for the painful neuropathic condition induced by diabetes.

This study aimed to evaluate the effects of the treatment with Epigallocatechin Gallate (EGCG), a potent antioxidant present in green tea, in the behavioral pain responses and oxidative stress damage at the spinal cord of STZ-diabetic rats.

Diabetes was induced by intraperitoneal injection of STZ (60 mg/kg body weight) in male Wistar rats. Control animals (CTR) received the vehicle solution. Three days post-injection one set of STZ-diabetic rats started to receive EGCG (2g/L; STZ+EGCG) in the drinking water while CTR and the other STZ-diabetic (STZ+H<sub>2</sub>O) animals maintained the normal water consumption. Mechanical hyperalgesia and tactile allodynia were behaviourally evaluated by Randall-Sellito and dynamic plantar aesthesiometer, respectively, before diabetes induction, at 4 weeks post-injection and at the end of treatment (10 weeks post-injection). The animals were, then, sacrificed and spinal cords were removed. Oxidative stress damage was evaluated in spinal sections by immunodetection of 8-hydroxy-2'-deoxyguanosine (8-OH-dG), a marker DNA/RNA oxidation. The expression of 8-OH-dG was quantified by densitometry in the spinal dorsal horns of 10 randomly taken spinal sections from the L4-L5 segment. Means were compared by ANOVA followed by the Tukey *post hoc* test for multiple comparisons.

STZ rats developed hyperglycemia, which was maintained until the end of the experiments and was not affected by treatment with EGCG. The STZ+H<sub>2</sub>O rats developed mechanical hyperalgesia and tactile allodynia, which were less severe and not detected, respectively, in the STZ+EGCG rats. The expression of 8-OH-dG was significantly higher in STZ+H<sub>2</sub>O than in CTR and STZ+EGCG rats.

The treatment with EGCG prevented the diabetes-induced oxidative stress damage at the spinal dorsal horn, ameliorated the mechanical hyperalgesia and prevented the development of tactile allodynia. The antioxidant treatment with EGCG should, then, be considered for the prevention of diabetic neuropathy.

Supported by IJUP/ UNICER project.

## **The effects of EGCG, a potent antioxidant present in green tea, on the serotonergic neuronal population of pain control centres of the brain in STZ-diabetic rats**

**A. Miranda<sup>1,2</sup>, J. Silva<sup>1,2</sup>, P. Pereira-Terra<sup>2,3</sup>, D. Raposo<sup>2</sup>, C. Morgado<sup>2,3</sup>, I. Tavares<sup>2,3</sup>**

<sup>1</sup> School of Health Sciences, University of Minho, Portugal

<sup>2</sup> Department of Experimental Biology, Faculty of Medicine of Porto, University of Porto, Portugal; <sup>3</sup> IBMC, University of Porto, Portugal

Painful diabetic neuropathy is a common and debilitating complication of diabetes. This condition affects more than 25% of diabetic patients with neuropathy and is characterized by spontaneous pain, mechanical hyperalgesia and tactile allodynia. Using the streptozotocin (STZ)- diabetic rat as animal model, previous findings showed that during diabetes there is a decrease in the numbers of serotonergic neurons in the rostroventromedial medulla (RVM), a key pain control areas in the brain. Whether such decrease is due to oxidative stress and neuronal death remains unknown. Since serotonergic RVM neurons are involved in descending nociceptive modulation, it is also important to evaluate if changes in the numbers of serotonergic neurons in the RVM account for painful diabetic neuropathy.

This study aimed to evaluate the effects of the antioxidant treatment with Epigallocatechin gallate (EGCG) on serotonergic neuronal population of the RVM and on behavioral pain responses in STZ-diabetic rats.

Diabetes was induced in male Wistar rats (250-300g) by an intraperitoneal injection of STZ (60 mg/Kg). Control rats (CTR) received only the vehicle solution. Three days later, one set of STZ-diabetic rats started to receive EGCG (2g/l; STZ+EGCG) in the drinking water while normal tap water was given to the remaining experimental groups (STZ+water and CTR). Behavior responses to noxious and innocuous mechanical stimulation were evaluated before and 10 weeks after treatment onset using the paw pressure test and the dynamic plantar aesthesiometer, respectively. The animals were then sacrificed and the brainstem sections containing the RVM were immunoreacted against tryptophan hydroxylase (TpH), the rate-limiting enzyme in serotonin synthesis, to identify the serotonergic neurons. The number of immunoreactive neurons for TpH (TpH-IR) was counted in the RVM. Means numbers of TH-IR neurons in the RVM were compared between the 3 groups by One-Way Analysis of Variance (ANOVA) followed by Tukey post hoc test for multiple comparisons.

STZ rats developed hyperglycaemia, which was not affected by the EGCG treatment. The EGCG prevented tactile allodynia and mechanical hyperalgesia detected in untreated STZ rats, along with the decrease in the number of TpH-IR neurons at the RVM.

By preventing the decrease in the numbers of RVM serotonergic neurons involved in descending pain modulation, EGCG may present beneficial effects on painful diabetic neuropathy. Further studies will evaluate the neurobiological mechanisms underlying the antioxidant effects of EGCG in serotonergic pain control neurons. New perspectives on the development of therapeutic strategies using antioxidants to prevent diabetic neuropathic pain should be considered.

Supported by IJUP/ UNICER project.



Oral Sessions I > A3

# **Psychology & Education Sciences I**



# Bologna Process and teaching and learning in UPorto: an analysis to institutional reports

**B. Mendes<sup>1</sup>, R. Trindade<sup>1</sup>**

<sup>1</sup> Faculty of Psychology and Education Sciences University of Porto, Portugal.

Having as problematic the teaching and learning processes and the implementation of Bologna Process in Portuguese Higher Education system, it was sought to collect indicators of transformations within Higher Education Institutions, as well as to illustrate if it is possible to affirm the emergence of a new educational paradigm and of new forms to conceive teaching and learning challenges in Higher Education.

It was performed the documental analysis of thirty institutional reports on the implementation of Bologna Process, from eleven Component Units of UPorto University, of the year 2007/2008. It was aimed to perceive the ways how the roles of teachers, students and the cultural and technological patrimony are enounced, to identify constraints, reflections, proposals and solutions found within the transition to Bologna, and the way how these allowed to reconfigure or to answer to pedagogical challenges.

The model proposed in Bologna Process seems to be delineated under presupposes of Pedagogies of Learning (Altet, 1997), where it is privileged the relationship between learners and knowledge, and where teachers occupy a non-relevant place. According to this perspective, the development of cognitive and relational competencies assumes primacy over the confront with cultural patrimony, which is not object of explicit valorisation and interpellation (Houssaye, 1996, *in* Trindade, 2009).

In this way, the dilemma of teaching through competencies in Higher Education is analyzed according to Perrenoud's (1999:7) idea that the misunderstanding is in believing that while developing competencies we are quitting to transmit knowledge. We discuss the vision of the university teacher as a mediator between students and knowledge, a conception that meets what Cosme (2009) presents as the *action of teaching as an action of qualified interlocution*.

In the reports analyzed, the discussion of the management of teaching and learning process are not dissociated from the organizational, structural and administrative constraints that shape the vision about the pedagogical challenges. The analysis performed shows these pedagogical challenges: to control and plan in better ways the work developed inside and outside the classroom contact time; to redefine the times of accomplishment and the learning outcomes; to increase the components of experimentation; to valorise assessment processes capable to express the acquisitions and the learning of educational processes centred on the construction of knowledge, on the development of specific or transversal abilities, skill or attitudes.

## References:

- [1] Altet, M. (1997). *As pedagogias da aprendizagem*. Lisboa: Instituto Piaget.
- [2] Trindade, R. (2009). *O Ensino Superior como espaço de formação: do paradigma pedagógico da aprendizagem ao paradigma da comunicação*. Documento policopiado, pp.1-29.
- [3] Perrenoud, P. (1997). *Construir as competências desde a escola*. Porto Alegre: Artmed.
- [4] Cosme, A. (2009). *A ação docente como uma ação de interlocução qualificada*. Porto: Livpsic.

## **Students' autonomy and responsibility in the context of Physical Education classes: reflections of a Pre-service teacher**

**B. Gonçalves<sup>1</sup>; T. Leandro<sup>2</sup>; P. Batista<sup>3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal

<sup>2</sup> Secondary School Dr. Manuel Gomes de Almeida, Portugal

<sup>3</sup> CIF12D, Faculty of Sport, University of Porto, Portugal

Student's autonomy and responsibility throughout classes is essential for achieving success in learning. Taking this into account, this study is a result from an analysis of the class written reflections focused on students' responsibility and autonomy in Physical Education classes. This analysis was taken at the Program of Master degree in Teaching Physical Education in Primary and Secondary level from Faculty of Sport, University of Porto, in academic year 2011/2012. The main purpose of this work was to understand how students behave in Physical Education classes and to suggest strategies to increase their level of commitment and participation. The students that take part of this study were at the 10<sup>th</sup> degree from Dr. Manuel Gomes de Almeida secondary school, at Espinho. The class written reflections were submitted to a thematic analysis based on three predefined categories: i) initial level of autonomy and responsibility; ii) strategies and iii) results. From the analysis it was made clear that: i) the class had a lack of autonomy and responsibility; ii) the chosen strategies came real through the analysis of the teacher in search new ways of acting with students, when managing different learning activities, putting a clear focus on the students involvement and on demanding more autonomy and responsibility from the students; iii) the results were translated in an increasing level of student autonomy and responsibility, having a direct impact in an enhancement of the classes' quality and students' learning process.

**Key-words:** Practicum; Physical Education; Reflection; Autonomy and Responsibility.

# **Educational Intervention Modes in Higher Education as a Response to Current Challenges: A Case Study**

**R. Encarnação<sup>1</sup> and R. Trindade<sup>1</sup>**

<sup>1</sup> Department of Educational Sciences, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Current changes in Higher Education, from a paradigm that's centered around information transfer to one that focuses on a meaningful teaching and learning process, made way for a set of newfound challenges for both Higher Education Institutions and their Faculty, demanding their educational context to be looked upon with different objectives in mind.

Assuming that this paradigm shift can be eased with the introduction of educational support and intervention modes based on the addition of a specialized element to the pre-existing relationship between teachers and the teaching-learning process, we will discuss four methods of this kind that can help Faculty improve: Mediation, Consultancy, Supervision and Educational Advisory.

After presenting the origins and main aspects of each of these methods, we will try to ascertain whether the activity of educational support and development units can be understood within the intervention types discussed above, focusing on a single, exploratory and qualitative case study: the Department of Medical Education of the Nova University of Lisbon's Faculty of Medical Sciences.

In order to understand if it's activity model can be framed within the aforementioned intervention modes and, if so, if these can be seen as valid answers to the challenges currently faced by Higher Education Institutions and Faculty, we'll cross-reference available data from recent studies with data previously collected during a curricular internship at the Department with exploratory interviews done for this particular study.

# Revisiting strategies for class control through written reflection: the "look" of a pre-service teacher of Physical Education

**B. Pinheiro<sup>1</sup>, T. Sousa<sup>2</sup> and O. Teixeira<sup>3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal

<sup>2</sup> CIFED, Faculty of Sport, University of Porto, Portugal

<sup>3</sup> Basic School Júlio Dinis, Portugal

This work represents a moment to share experiences and problems experienced by pre-service teacher, in the context of practicum in teaching physical education at elementary and secondary level. The aim of this work is to analyze the behavior of pupils in the 7<sup>th</sup> year in a school of Vila Nova de Gaia, using different control strategies in order to promote a better learning environment. The class has 22 students between 12 to 15 years old, corresponding to the period of puberty. This age is characterized by the start of puberty, i.e. in feminine gender the menarche and genitals development in men's. As such, this development causes changes in the body, as well as the behaviors and attitudes of students.

The development of control strategies and its implementation began after detected disruptive behaviors, systematic in students. The presentation of the strategies is conditioned first instance for practice, by the behavior of students in the classroom. In this sense the written reflections of all lessons about the behavior of students, the events of the classroom and the action of the pre-service teacher, should assist to acquiring consistency of those strategies and taking new decisions. It is intended that the reflection is, beyond a point of interpretation of the practice, an opportunity to modify future practice. The renovation of the action of teacher and students. [2]

The analysis performed on written reflections already prepared presents the following strategies associated with some evidences. (i) The initial strategy adopted for the control of the class focused on punishment with the realization of physical exercises, which reflected negatively to provoke greater disorder and even fun for students. (ii) The implementation of an agenda for the registration of behaviors in a board related to psychosocial was another strategy used. In this way, if the frame had less than five fouls, students could choose the warm-up exercise for the next lesson. This control strategy is not adapted to the class because the disruptive behaviors persisted. (iii) The individual dialog and the proposal of being teacher assistant in lessons has promoted greater dynamic and maintains a stable behavior. (iv) Preventive interventions promoting an improvement in the attitudes of the students. So, do specific routines during the class, different by sport. Plan my classes to be more dynamic, in that the waiting time is very small, as a way to prevent disruptive behaviors.

The actions produced have been contributing to a more constant of good behavior of the class, unlike that were used previously. The teacher's intervention has been more solid and consistent. The written reflections have made an important contribute to the consistence of the pre-service teacher action.

## References

[1] Weineck, J. (1991), *Biologia do Esporte*, Manole, São Paulo.

[2] Schön, D. A. (1983). *The reflective practitioner: How professionals think in action*. New York: Basic Books.

# The Relationship between the Teacher and the Student: The Importance of Affectiveness in the Teaching-Learning Process

**J. Almeida<sup>1</sup>, T. Leandro<sup>2</sup>, e P. Batista<sup>3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal.

<sup>2</sup> Escola Secundária Dr. Manuel Gomes de Almeida, Portugal.

<sup>3</sup> CIFID, Faculty of Sport, University of Porto, Portugal.

The process of the Professional Teaching Practice is an intense phase in a student teacher's life. In fact, there are many dilemmas and challenges that they have to face during their entry into the real context of teaching. Garcia [1] states that in their first years of professional activity the young teacher usually faces the "reality shock", which is characterized by a transition period between being a student and a teacher. In this context, it acts as a phase of uncertainty and tension, but also of intense learning because it consists of tasks that pertain the building of professional knowledge and preservation of emotional balance. Therefore, this study results in an analysis in first person of the log produced during the first phase of Professional Teaching Practice (1<sup>st</sup> term), centered on the teaching element. The aim was to identify the dilemmas and difficulties that I struggled with during these first months of experience as a teacher. Given the fact that in the first revisit to my written reflections clearly identified that one of the main dilemmas I faced reported to the typology of the pedagogical (affective) relationship established with the students, I have focused this presentation on this dilemma only. The Professional Teaching Practice occurred in Escola Secundária Dr. Manuel Gomes de Almeida, in the Master's Degree of Teaching Physical Education in Basic and Secondary Schools, in the school year 2011/2012.

The analysis made it become evident that: i) the establishment of a positive pedagogical relationship promotes by far the conduct of the teaching-learning process, ii) the affective relationship between teacher and students is fundamental, not only for the growth of the student but also for the development and growth of the teacher; the establishment of a good teacher / student relationship is dependent on knowing and understanding of each student individually, including their background and their (personality and physical) characteristics, which are divergent from individual to individual, and this only happens if the teacher is able to establish an empathic relationship and closeness with their students. In conclusion, in the educational act the options are taken in the benefit of the student. That is the reason why the teacher has to take into account the student's uniqueness, that is, their needs and motivations. Indeed, the engine of educational process is the student; they are the center of all pedagogical action. Thus, everything that the teacher does should have as a central focus the development and training of their students.

Keywords: Student teacher; Professional Teaching Practice; Pedagogical Relationship; Physical Education.

Referencies:

[1] Garcia, M. (1999), *Formação de Professores: para uma mudança educativa*. Porto – Portugal: Porto Editora.

## **Aesthetics training for teachers**

**M. M. Barroso**<sup>1</sup>

<sup>1</sup> Faculty of Physical Education, University of Brasília, Brazil.

The university education has always been closely connected with the transmission of content, Maffesoli (1998) says that the more stuck we are at the pure consciousness the more distant we are from the world around us, in that sense, discusses the need on issues that go beyond the specific content and to treat the context sensitive.

The Licenciatura (Teacher credential) program seek to graduate teachers, but is the Teacher Credential or teach at the school enough to make a student becomes a teacher? In the opinion of Pereira (2003), the teacher is always a process of continuous training that depends on the mediation of others.

In science the knowledge related to the reason are privileged at the expense of sensitivity to knowledge, we must redeem the sense of reason. Rios (2003) clarifies that the sensitivity and creativity are not restricted to the arts, is interconnected with human life.

An aesthetic experience would enable a more comprehensive reading of the world, since the subject would see the ordinary in several ways. There are several ways to have an aesthetic experience, for example, when watching a movie we identify with a character and feel emotions in our everyday situations that would not live, so do not feel. As proposed by Amorim and Castanho (2008) schools should provide aesthetic experiences into their curriculum.

Teaching is an art, so it is necessary to awaken the aesthetic sense of teachers with the aim of being able to deal with the human formation of their students, thus enabling the understanding from their students that they are authors of their own lives.

### References:

- [1] Maffesoli, M. (1998), *Elogio da razão sensível*, Vozes, Petrópolis.
- [2] Pereira, M. V. (2003), *Traços de fundamentalismo pedagógico na formação de professores*, (no prelo).
- [3] Rios, T. A. (2003), *Comprender e ensinar: por uma docência de melhor qualidade*, Cortez, São Paulo.
- [4] Amorim V. M. and Castanho M. E. (2008), *Por uma educação estética na formação universitária de docentes*, *Educ. Soc.*, 29 (105), 1167-1184.

Oral Sessions I > A4

# **Economics & Management**



# The antecedents of burnout syndrome in a retail bank and its effects on the affective commitment.

**A. Fernandes<sup>1</sup>**

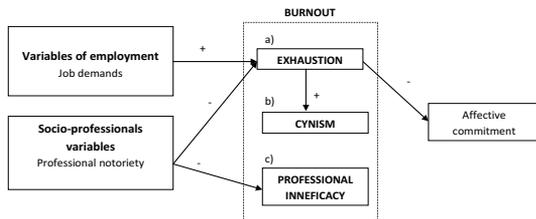
<sup>1</sup> Faculty of Economy, University of Porto, Portugal.

In the recent decades we have assisted the globalization, privatization and modernization of the labour market. This phenomenon led to rapid changes in the work life. The professionals, in general, quickly needed develop and improve their abilities to adopt new types of work and adjust the pressure to increase productivity and work quality. This context may lead to burnout: feelings of emotional exhaustion, alienation from others (colleagues, superiors and/or customers) and feelings of lack of personal accomplishment [1]. The globalization phenomenon was also witnessed in the Portuguese retail bank and had employment implications. There was an intensification of work, and were required flexibility, versatility and ability to perform different tasks. Jacques and Amazarrray consider that retail banking dynamics and client exposure may lead to burnout [2].

This study explores the levels of burnout in the retail banking business, and their influence on levels of affective commitment. In order to accomplish this goal, quantitative data was collected through the use of a questionnaire. The sample was composed by 166 Portuguese banking employees, working to a private bank. Their working places were located in the districts of Viana do Castelo, Braga, Vila Real, Bragança and Viseu.

The hypotheses were tested using correlation analysis, variance analysis (ANOVA) and regressions analysis. The results indicate an average value for each of the components of burnout: exhaustion, cynicism and professional inefficacy. At same time, the results suggest that these banking employees are affectively committed to the organization.

No demographic variables were identified with predictive power. From the employment variables, job demands emerge as a predictor of exhaustion. And from the socio-professional variables is extracted professional notoriety as a predictor of exhaustion and professional inefficacy. In addition, results from the regression analysis showed that exhaustion is the only burnout component that could predict affective commitment. The data revealed that the process development happens according to the model of Leiter (1993) [3].



**Figure 1- Model Found**

## References

- [1]Schaufeli, W. B., Leiter, M. P. e Maslach, C. (2008), *Burnout: 35 years of research andpractise*, Careers Developente International, 14 (3), pp.194-220.
- [2]Jacques, M. G. C. e Amazarrray, M. R. (2006), *Trabalho bancário e saúde mental no paradigma da excelência*, Boletim de Saúde, 20 (1), pp 93-105.
- [3]Leiter, M. P. (1993), *Burnout as a developmental process: consideration of models*, in Schaufeli, W. B, Maslach, C. e Marek, T. (Eds.). *Professional Burnout: Recent Developments in Theory and Research*, Washington DC, pp. 237-250.

# Macroeconomic Fundamentals of Poverty and Deprivation: an empirical study for developed countries

**Duarte Cabral Moncada Guimarães<sup>1</sup>, Sandra T. Silva<sup>2</sup> and Ana Paula Ribeiro<sup>3</sup>**

<sup>1</sup> Master Programme in Economics, Faculty of Economics, University of Porto, Portugal.

<sup>2</sup> CEF.UP and Faculty of Economics, University of Porto, Portugal.

<sup>3</sup> CEF.UP and Faculty of Economics, University of Porto, Portugal.

Fighting poverty is now at the top of the political agenda of the most relevant international institutions, like the World Bank (WB) and the International Monetary Fund (IMF). Poverty is defined nowadays as a multidimensional phenomenon, but some caveats still persist when it comes to the measurement of the different dimensions of poverty and also on how to encompass them. Such caveats have to be solved towards a comprehensive, and properly assessable, definition to enable the implementation of adequate policies.

The literature points, on the one hand, to the evolution of poverty concept from a pure material deprivation to a multidimensional phenomenon, encompassing both physiological and social deprivations. In this regard, most of the applications are targeted to the measurement of poverty in the less developed countries. On the other hand, in order to better understand poverty and contribute to its reduction, microeconomic decisions must be encompassed with macro outcomes. However, the research on the role of Macroeconomics in explaining poverty is rather scarce. So, it is crucial to study, in detail, the macroeconomic transmission mechanisms using a broader matrix that covers not only economic growth and poverty, but also the role of macroeconomic stabilization and institutions.

In this context, this work goes deep on reviewing the macroeconomic fundamentals, as well as the corresponding mechanisms, of poverty. Moreover, we review several studies focused on assessing poverty. This review is our departing point to propose our own measure, the Index of Multiple Deprivation for Developed Countries (IMD\_D), to assess deprivation in 18 countries of the European Union (EU), from 2005 to 2008. This index aims at encompassing and measuring different deprivation dimensions, specifically for developed countries. Finally, we use a panel data econometric model to study the relation between macroeconomic variables and the IMD\_D, in order to check the robustness of the most relevant theoretical explanations.

Taking the 4-year (2005-2008) average, we ranked the 18 countries using the IMD\_D indicator; Portugal is the sixth most deprived country. From our study became clear that in developed countries monetary poverty is likely to overestimate the extent of deprivation while, from the literature, the opposite is likely to occur in developing and underdeveloped countries. Applied to developed countries, our results confirm most of the theoretical arguments in terms of the expectable effects of relevant macroeconomic mechanisms. Other things equal, public investment, GDP growth rate, and governance quality, if increasing, impose downturn pressures on the IMD\_D. Conversely, other things equal, income dispersion and government budget imbalances, if increasing, impose upturn pressures on the IMD\_D.

# Fiscal Sustainability in an Overlapping Generations Model with Endogenous Growth and Public Debt

J. Silva<sup>1</sup>, L. Fonseca<sup>2</sup>, P.B. Vasconcelos<sup>3</sup>, and O. Afonso<sup>4</sup>

<sup>1</sup> Faculty of Economics, University of Porto, Portugal.

<sup>2</sup> Faculty of Economics, University of Porto, Portugal.

<sup>3</sup> CMUP and Faculty of Economics, University of Porto, Portugal.

<sup>4</sup> CEFUP and Faculty of Economics, University of Porto, Portugal.

The question of whether a given level of government debt is “sustainable” has grown significantly in recent years. Noteworthy, the discussion induced by the recent economic international crisis is an example. The usual point of view has been that large and persistent deficits generate an explosive path for the debt stock. As a result, a default on the debt and/or bad consequences for the rest of the economy emerges.

Our work presents fiscal sustainability in a three-period overlapping generations (OLG) economy with endogenous growth coming from human-capital formation through educational spending. The OLG model is widely used in most contemporary theoretical studies concerning Public Financing of government debt, since future generations are expected to pay at least a part of the debt undertaken by the present generation of the nation.

The analytical framework used in this study permits to analyze the interaction between public debt dynamics and the outlook for economic growth, thereby providing a rationale for fiscal rules ensuring sustainability [1-3]. The economy is assumed to be populated by three types of agents: Households, that maximize their utility function and accumulate human capital; Firms, with a constant elasticity of substitution production technology; and Government, that is allowed to run budgetary imbalances.

Using computational requirements, the model is implemented, calibrated and used to simulate the effects of a demographic shock under different fiscal policy scenarios. Results point out that the existence of steady-state (long-run equilibrium) is not sufficient to ensure fiscal sustainability. The stability properties of the economy depend on the set of fiscal rules, which propagate for a timely reaction to temporary shocks, thereby avoiding possibly disruptive fiscal adjustment in the future. It is also clear that the more the adjustment is delayed, the larger must be the scale of the set of fiscal rules.

## References:

- [1] Diamond, P.A. (1965), *National Debt in a Neoclassical Growth Model*, American Economic Review Vol. 55, n°5 (December 1965), pp. 1126-1150.
- [2] De la Croix, D. and Michel, P. (2002), *A Theory of Economic Growth – Dynamics and Policy in Overlapping Generations*, Cambridge: Cambridge University Press.
- [3] Moraga, J. and Vidal, J. (2010), *Fiscal sustainability and public debt in an endogenous growth model*, in Journal of pension economics and finance, Cambridge, Vol. 9, n° 2 (April 2010), pp. 277-302.

# Organising Capacity for Sustainable Urban Planning

**M. Inês Santos<sup>1</sup>, Luís Carvalho<sup>2</sup>**

<sup>1</sup> Master student in Urban Economics and Urban Management, Faculty of Economics, University of Porto, Portugal

<sup>2</sup> European Institute for Comparative Urban Research, Erasmus University Rotterdam & Faculty of Economics, University of Porto

For almost 25 years now *sustainability* has been a trendy concept, whose real meaning and implications are still under discussion. Namely in cities, it is widely accepted that it refers to dealing simultaneously with economic, social and environmental issues [1], and that it requires strong organisational and integrative skills from urban planners [2]. In the realm of urban studies, van den Berg et al. (1997) developed a framework with a combination of elements “designed to respond to fundamental developments and create conditions for sustainable development”: strategic networks, leadership, vision, political support, societal support and communication [3]. However, sustainability can be placed on a *weak-strong continuum*: “the degree to which individuals are required to change their lifestyle and behaviours” [4]. If sustainability notions among urban players differ along this continuum, different organisational needs and outcomes may be expected. Moreover, sustainability notions may vary over the duration of a project. Thus, for planning theory and practice, it is important to assess if and how the framework of organising capacity is appropriate enough to deal with different sustainability notions.

This study analysed the relationship between weak/strong sustainability notions and organising capacity. Based on an intensive, longitudinal case study on the Riverside intervention in Coimbra (analysed through a number of rounds of problems-solutions and involved actors) [5], the study suggests that (1) stronger sustainability notions are associated with higher levels of organising capacity; (2) societal participation (allied to communication strategies) are key to implement stronger planning practices and, therefore, stronger sustainability conceptualisations; (3) over time, project actors have increasing difficulties in sharing common notions of sustainability; (4) the organising capacity framework does not assess the ability of project actors to share common notions of sustainability, neither ensures their interaction produces a pro-sustainability outcome.

References:

- [1] Camagni, R., Capello, R. and Nijkamp, P. (1998), *Towards sustainable city policy: an economy-environment technology nexus*, Ecological Economics, vol. 24, 1998, pp. 103-118.
- [2] Campbell, S. (1996), *Green Cities, Growing Cities, Just Cities? Urban Planning and the Contradictions of Sustainable Development*, Journal of the American Planning Association, vol. 62 no. 3, Summer 1996.
- [3] Berg, L. van den et al. (1997), *The Organising Capacity of Metropolitan Regions*, Environment and Planning C: Government and Policy, 15, 1997, pp. 252-257.
- [4] Lombardi, Rachel D., Porter, L., Barber, A. and Rogers, C.D.F. (2010), *Conceptualising Sustainability in UK Urban Regeneration: a Discursive Formation*, Urban Studies 1-24, 2010.
- [5] Teisman, Geer R. (2000), *Models for Research into Decision-Making Processes: on Phases, Streams and Decision-Making Rounds*, Public Administration, vol. 78 no. 4, 2000, pp. 937-956.

# The impact of international technological-knowledge diffusion on economic growth

O. Decoud<sup>1</sup>, O. Afonso<sup>2</sup>, and P.B. Vasconcelos<sup>3</sup>

<sup>1</sup> Faculty of Economics, University of Porto, Portugal.

<sup>2</sup> CEFUP and Faculty of Economics, University of Porto, Portugal.

<sup>3</sup> CMUP and Faculty of Economics, University of Porto, Portugal.

It is widely discussed that in opened economies, the international diffusion of technological knowledge affects economic growth.

Initially, the research discusses the theoretical concepts and empirical importance of the diffusion of technological knowledge, bearing in mind the existing literature. In particular, it highlights the discussion of the diffusion of technological knowledge focuses on the importance of specific channels of diffusion (in particular, international trade), the spatial distribution of technological knowledge, the technological “gap”, and the technological “catch-up”.

In line with Connolly (2003) [1], international patent data – from recognized international institutions – for 50 countries between 1980 and 2009 is used to create a proxy of imitation and innovation. Some of the considered variables are: Imports of High Technology, Infrastructure Transport and Communications and Foreign Direct Investment. By using econometric techniques, the goal is to determine which one(s) of these channels has(have) a significant statistically weight in the diffusion of technological knowledge and, consequently, on economic growth. For this purpose, countries will be classified into two groups called leading (developed) countries and imitators (developing or followers) countries.

This work intends to better understand the impact of international technological-knowledge diffusion on economic growth, bearing in mind the three above mentioned diffusion channel. Indeed, the existing literature usually considers just one channel.

**Keywords:** Technological-knowledge diffusion, economic growth, innovation, imitation, regression analysis.

**JEL classification:** C30, O30, O31, O34, O40

## REFERENCES

[1] Connolly, M. (2003), *The dual nature of trade: measuring its impact on imitation and growth*, Journal of Development Economics, 72(1), October, pp. 31–55

## The internationalization profiles of Portuguese SMEs

**P. Oliveira<sup>1</sup>, A. Teixeira<sup>2</sup>**

<sup>1</sup> Department of Industrial Engineering and Management, Faculty of Engineering of University of Porto, Portugal.

<sup>2</sup> CEF.UP, Faculty of Economy of University of Porto, INESC-Porto, OBEGEF, Portugal.

Given the (increasing) view point that firms' internationalization strategy is the unique path to overcome the Portuguese dismissal economic growth, the present paper offers a comprehensive picture of the internationalization behavior of Portuguese SME, constituting therefore an important tool for political action.

On the basis of the literature review and the factorial and cluster analyses performed, we propose three main segmentation criteria, one ('Whole encompassing segmentation': Experienced Medium Low-Tech firms; Low skill, Low-Tech firms; Young High-Tech firms) based on language skills, SME business experience, foreign market dependency, introduction of organizational innovation, exporting to 'High income countries' and education level of executive teams. The second segmentation proposal ('Intermediate segmentation': Young small-sized firms; Young micro-sized firms; Mature small-sized firms; Young medium-sized firms; Mature medium-sized firms; Foreign equity firms; Highly productive firms) has as criteria the SME size, business experience, foreign capital presence, and average productivity. The last segmentation proposal ('Parsimonious segmentation': Medium-sized firms; Small-sized manufacturing firms; Micro-sized firms; Non-manufacturing small-sized firms; Export active small-sized firms; Potential exporters; Promising exporters firms) is based on firm size, the SME export intensity and industry.

Given the need for a parsimonious segmentation criterion, we convey that the most adequate segmentation criterion is the one combining SME size, export intensity and industry. This restricted number of criteria does not, however, affect the quality of the proposed SME segmentation, and has the advantage of being statically adequate and user/cost friendly.

Oral Sessions I > A5

# Communication Sciences



# United Nation's Intervention in Libya: media coverage

**J. Dias, M. Cruz and M. Macedo<sup>1</sup>**

<sup>1</sup> Department of Communication Sciences, Faculty of Letters, University of Porto, Portugal.

Many authors allow us to take and constantly reiterate the power of the media in social and political landscape. Media are the primary communication vehicle through which the political power structure communicates with society. When media presents the reality that we not know, they propose us some interpretations for the same reality, shaping our knowledge (Silva, 2010: 6).

Civil war in Libya became increasingly larger and with more supporters, deserving attention of the global community. In fact, due the violence, there was a need for international intervention in particular by the UN. The United Nations approved the intervention in Libya on 17 March with the 1973 Resolution. According to Hallin (1986: 116-117), at the beginning of the conflict, media tend to convey what is in the sphere of consensus among government agencies, promoting and defending the official version of the national interest. In fact, even Pedelty (1995:222) (cit. Novais, 2007:555) ensures that: "much of the world's news coverage is still arranged According to national boundaries and constructed in terms of national interest".

In our article, we attempt to understand if the media coverage of United Nation's intervention in Libya was completely neutral, with no governmental influences behind. We examined the articles and news published in the online version of The New York Times (USA) and Le Monde (France) and the printed version of Público (Portugal). Our analysis was based on the methodology Meta Performance Analysis (Novais, 2007), with four dimensions of analysis: selection and use of sources of information, terminology, criticism / attacks on government officials, present and absent subjects. "(...) An original combination of quantitative, qualitative, as well as the content of the performance assessment reporting. Designated in this work the 'meta-performance' analysis" (Ibid.:554).

Our research question relates to: UN action was presented in a totally neutral, with no forces of government, political ideology? We have formulated several hypotheses that allowed us to get to the bottom line. In general, we based our investigation in: identification of sources, characterization of Qaddafi, identification of the news' focus, tones, and also the identification of missing issues during media coverage.

The missing issues contributes substantially to the research question as they are the mirror of manipulation / or not by governments on the media. Indeed, this has been mentioned as a fact fundamental to this investigation, since, trying to analyze the bias and neutrality, it's therefore necessary to highlight what has been published. Civilian deaths caused by the intervention aren't a frequent topic and is rarely mentioned, for example, in Le Monde. In parallel, the violation of the no-fly zone wasn't published in this newspaper.

We conclude that the coverage of United Nation's intervention in Libya was partial and influenced by governmental sources. We also conclude that there were certain themes that failed to be covered.

## References:

- [1] SILVA, Mafalda (2010) Cobertura mediática de África na imprensa europeia, no contexto da II Cimeira EU - África, disponível na Internet via <http://www.bocc.uff.br/pag/silva-mafalda-cobertura-mediatica-de-africa-na-imprensa-europeia.pdf> [6] (consultado a 25 de Novembro de 2011)
- [2] HALLIN, Daniel (1986) The Uncensored War, Berkeley: University of California Press [116-117]
- [3] NOVAIS, Rui (2007) National Influences in Foreign News: British and Portuguese Press National Influences in Foreign News: British and Portuguese Press [555-570]

## **The military intervention in the Libyan conflict: a comparative analysis of six international newspapers news content**

**H. Figueiredo<sup>1</sup>, J. Gonçalves<sup>1</sup>, L. Santos<sup>1</sup> and M. Ramalhosa<sup>1</sup>**

<sup>1</sup> Department of Communication Sciences, Faculty of Arts, University of Porto, Portugal.

Libya is going through a civil war sparked by protests with social and political demands. To restore order in the country, the UN Security Council adopted the Resolution 1973. Although this decision was not consensus within the UN, military intervention began two days later.

In order to determine if there is or not different treatment of the news content by the media according to the position of the country in relation to conflict, we analyzed the production of six newspapers representing each side.

The results show that there is a differential treatment of news content from newspapers, reflecting the different positions taken by their countries. We also conclude that military subjects and intra-media influences prevail, as well the critical capacity of the media falls short compared to previous conflicts.

# **The assault on the "Liberty Fleet" Comparative study of traditional and online press coverage of the Israeli attack on the boat pro-Palestinian in 2010**

**Marisa Ferreira**

Department of Communication Sciences, Faculty of Letters, University of Porto, Portugal.

The present investigation aims to analyze the existence of major differences between the coverage by traditional media and online media, focusing on the particular case of assault on pro-Palestinian fleet of humanitarian aid to May 31, 2010. For the implementation of this study were chosen four media, two mainstream media (public and Morning Post) and two online media (Diary Digital Diary and Portugal). To support all the research we resorted to the use of the following methodologies: critical discourse analysis, content analysis and framing (framing theory). With the development of the study showed the existence of differences in coverage of the traditional press and online media as well as differences in the frame made of the information. The level of editorial position it was only found in the traditional press.

**Key-words:** Israel, Palestine, Middle East, Conflict, The Freedom Flotilla, Attack

# **Politics 2.0: the use of the social networks facebook and twitter in electoral campaigns and the 2011 Portuguese presidential election case study**

T. Moreira

Department of Journalism, Faculty of Letters, University of Porto, Portugal

The incredible growth of social networks had increased the academic research in this area and also on the studies of the possibilities that marketing can collect from these tools. The political and electoral marketing arises in this context as a fertile field for the development of new conclusions and useful recommendations in strategies development at the online world.

This research reinforces the online social networks importance in Portugal political campaigns, identifying aspects of their application, their potential and how they are used as political tools in web political marketing.

Facebook and twitter are taken as the largest and the most prominent online social networks and are, therefore, the analysis subject in the empirical study that explores the official campaign period of the 2011 Portuguese presidential elections.

This study is performed through an analysis of the 2011 Portuguese Presidential campaign. The analysis is based on three parts: questionnaire, campaign advisors interviews and social networks profiles analysis in which the candidates were involved.

Analysis reveals the unprecedented relationship between politicians and voters and the connection of the campaign budget and the online politicians activity and recognition in the social networks. It was found that social networks are used by politicians to catch the attention of young voters. Another conclusion of this study is that twitter was a marginal social network in the 2011 Portuguese Presidential campaign.

# New challenges in cyberjournalism Is there any business model?

I. Leite<sup>1</sup>

<sup>1</sup> Faculty of Engineering, University of Porto, Portugal.

The ancient Chinese curse or saying — “May you live in interesting times.” — is upon us. We are in the midst of a new revolution fueled by advancements in the Internet and technology. Currently, there is an abundance of information and the size of social interaction has reached a colossal scale. Within a span of just one generation, the availability of information and our access to them has changed dramatically from scarcity to surplus”[1].

Multimedia nowadays is one of the keypoints (the main one?) to a new way of journalism, close to readers. Know how to work with different components (images, audio, video, graphics, animations) is be able to inform in an enriched and direct way. Social networks, including Facebook and Twitter, turn out to be strong allies in communication and engagement with the audience. There is talk even of the term community host: **The job of engaging with those formerly known as “the audience” is in some ways becoming a new online “beat”** [2] It is important to reflect on this changing environment, which brings questions about the new business models to digital media, the public, field agents and news gathering. New model?

**Key words:** Entrepreneurial journalism, Social Media, Cyberjournalism

References:

[1]Forecast 2020: web 3.0 and collective intelligence,  
<http://glennremoras.com/2010/07/28/forecast2020/>, accessed at November 12<sup>th</sup>

[2] Community Host: An Emerging Newsroom ‘Beat’ Without a Guid  
<http://www.nieman.harvard.edu/reports/article/102521/Community-Host-An-Emerging-Newsroom-Beat-Without-a-Guide.aspx>, accessed at November 12<sup>th</sup>

# The Standardization of news on Twitter: Political Journalism

J.Pereira

Department of Ciências da Comunicação, Faculty of Letras, University of Porto, Portugal.

With this work it's intend to verify how the press works communicates on Twitter, specifically with political news, checking if there is (or there isn't) convergence on themes, actors and language approached by the different news organizations.

The proposal is to verify if there is standardization on political news on Twitter.

This work is relevant because it tests, if not the quality, at least the range and difference on news coverage on Twitter helping readers, users, and newsrooms to interact in a better or different way as so they see to fit better their interests.

There were 5 news Twitters (I, JN, Público, Correio Manhã, Agência Lusa) followed by two weeks, from 5 to 16 December 2011 and retrieved all political tweets from them to achieve a total of 797.

Then a grid was formed in which the tweets were broken back to its 7 main components: tweet (fully copied), source, date, hour, title of linked article, actors and a short summary of the tweet. This selection was made attending to two ("What" and "Who") of the 5 W's of journalism [1] that always appear on tweets.

Following this methodology it was discovered that there is standardization on political news on Twitter. The strongest one is verified when comparing every news organization with Agência Lusa, as expected in the early stages of the study.

This results show that newsrooms are losing their individuality when on Twitter and that there isn't much of difference from news organization to news organization at this microblog.

## References:

[1] Hart, J. (2002), *The Five W's of online help*, <http://www.techwhirl.com/techwhirl/magazine/technical/5wsonlinehelp.html>, 2002, accessed at June 2011.

## Touching the image: the Fine Arts edition

**Joel Maia, Graciela Machado (internship supervisor and editor/publication coordinator),  
Eduardo Aires (project supervisor)**

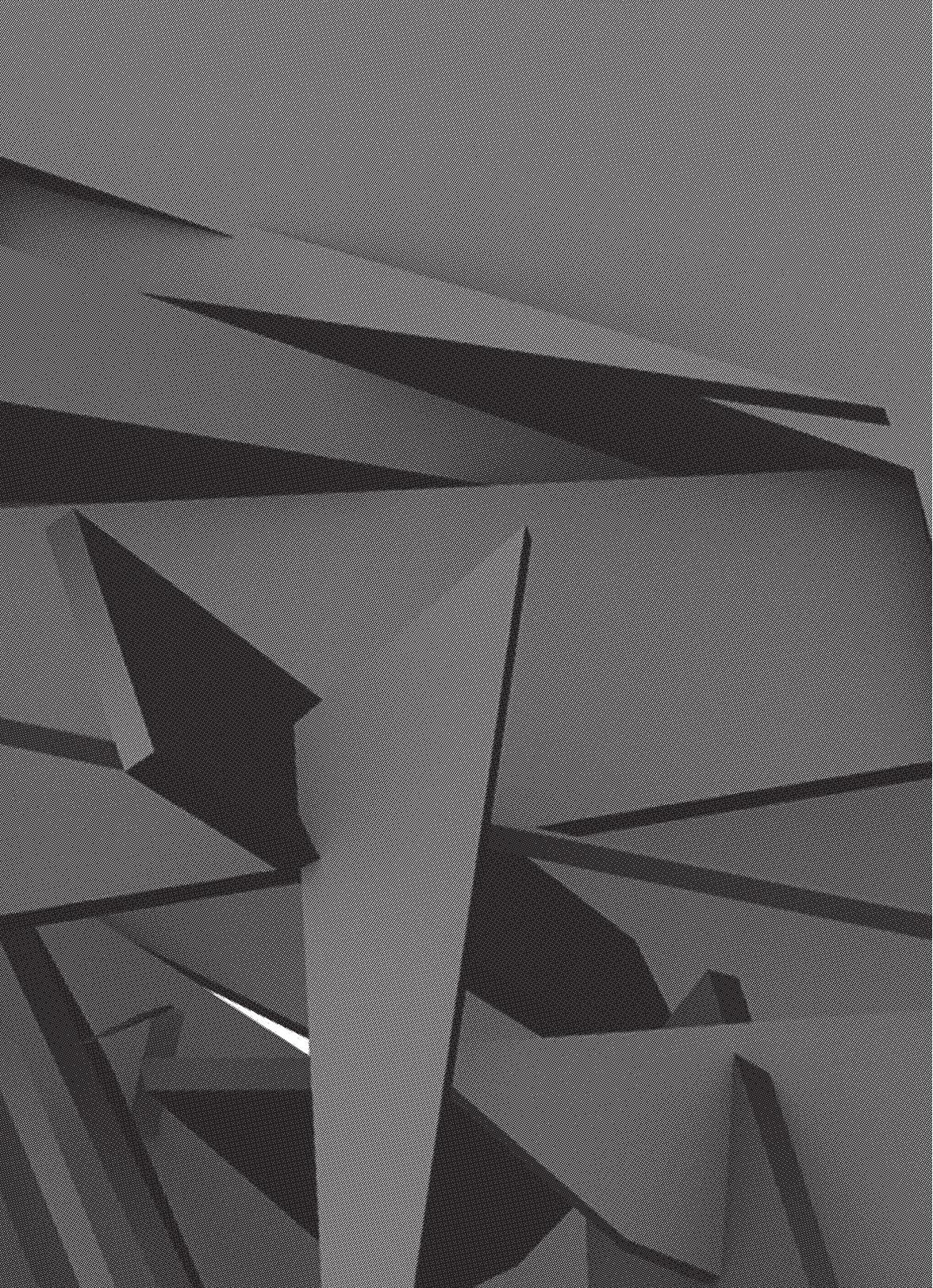
Fine Arts College of Porto University

"Touching the image" is the final result of a project started and later developed by me, within the course of my curricular internship in the communications office of Porto University's College of Fine Arts (FBAUP). More than a simple graphic publication (a portfolio/archive of posters by authors from FBAUP), this editorial project permeates the unique relationships established not only between different subjects, but also between the direct and indirect interventions *from* and *on* the means itself: a clear and enticing overflow of the boundaries of the curricular. There is a deliberate emphasis on the relationship set by this project between design – in a clearly scientific perspective – and the arts – which breathe, exude and embody the emotional and human role of the artistic creation. I dare state that this intertwining itself questions the subordination each area is submitted to regarding pre-established concept, along with the duplicity of the act of "experiencing" they bring about.

Since the 1950's the College of Fine Arts (still named Higher School of Fine Arts) has instigated, consolidated and simultaneously developed a "tradition" of poster production through the means, techniques and analog printing processes available through the college's workshops; this same tradition shaped the very way in which – albeit the availability of a distinctly superior level of technology – the production of posters is perceived and thought of by the creators and designers who train or work at FBAUP, as by the very students who, in a way, see their own academic, artistic and creative path influenced. One can equally observe an inspiring overlapping of knowledges, technologies, tools and experiences which, combined with the different time frames that envelop them, lead to an expressive exploration and transposition of the past to the present, in the creation of the acclaimed "printing tradition". "Touching the image" is not just about a simple publication, an editorial project that gathers a set of historical and contemporary posters: more than that, "Touching the image" explores a story, a tradition: conveys in itself the processes, studies, knowledges, obstacles and boundaries, barriers and solutions, successes reached by a distinct set of both current and former students and teachers. "Touching the image" is in itself a long and solid process of research, that uncovered and rediscovered, met and acknowledged, redesigned and rekindled the simmering creativity of the printing processes and techniques present at the "living museum" that are the college's workshops; and that most of all influenced the way design is thought of in its relationship with the arts, the way it is explored and transformed into an effective vessel to them.

Consequently, it is inherent to this publication a role of "outwards dissemination" of the college's unique editorial and creative productivity, of its own academic functioning and its way of conceiving the relationship between design and the arts. All of this is supported and grounded by the timely intervention of the various teachers at the college, along with the different areas of expertise

(Francisco Laranjo, Graciela Machado, Miguel Carvalhais, Susana Barreto and Dario Alves), who pour into "Touching the image" a consistent knowledge that travels back and forth between the arts, design, digital and analog, past and present in a true celebration of the image.



# Oral Sessions II



Oral Sessions II > A1

# Pharmaceutical & Medicinal Chemistry



## Resolution of chiral xanthenes by HPLC and docking studies

**A. Santos**<sup>1,2</sup>, **C. Fernandes**<sup>1,2</sup>, **A. Palmeira**<sup>1,2</sup>, **M.E. Tiritan**<sup>1,3</sup>, **C. Afonso**<sup>1,2</sup> and **M. Pinto**<sup>1,2</sup>

<sup>1</sup> Centro de Química Medicinal da Universidade do Porto, (CEQUIMED-UP), Portugal.

<sup>2</sup> Departamento de Química, Laboratório de Química Orgânica e Farmacêutica, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal.

<sup>3</sup> Centro de Investigação em Ciências da Saúde, Instituto Superior de Ciências da Saúde-Norte, (CICS-ISCN), Gandra, Portugal.

The marketing of single enantiomeric drugs by the pharmaceutical industry has been steadily increasing in last decades as a means to improve drug potency and selectivity while decreasing toxic and side effects [1]. The CEQUIMED-UP research group has been active in synthesizing molecules based on the xanthone scaffold for pharmacological activity evaluation, including chiral xanthone derivatives (CXDs) [2].

This work describes the HPLC-UV resolution of seven synthetic CXDs racemates using the commercial Pirkle-type chiral stationary phase (CSP) (*S,S*)-Whelk-O 1 (5  $\mu\text{m}$ , 100  $\text{\AA}$ , 25 x 0.46 cm i.d.), in normal-phase, polar organic and reversed-phase elution conditions. The majority of the racemates was resolved and excellent enantioselectivity and resolution were achieved for CXDs possessing an aromatic ring next to the stereogenic center (for example,  $\alpha = 7.55$  and  $R_s = 17.58$ ).

The chromatographic results were used as a starting-point to elucidate the mechanisms underlying chiral recognition. Docking studies were subsequently performed for three CXD racemates using the PyRx/AutoDock Vina software [3,4]. Data regarding the CSP-enantiomer molecular conformations and interactions were retrieved. The results attained were in accordance with the experimental HPLC data, regarding enantioseparation and the elution order of the enantiomers.

In conclusion, docking studies were successfully used to elucidate the chiral recognition mechanisms between the (*S,S*)-Whelk-O 1 CSP and the CXDs. The use of docking for chiral separation purposes is a promising method which might be used to aid in the choice of the most adequate CSP for a given separation or in the design of CSP able to resolve specific analytes.

Acknowledgments: CEQUIMED-UP (PEst-OE/SAU/UI4040/2011), FERDER, POCl, for financial support.

### References:

- [1] Shaikh, S., Muneera, M.S. and Thusleem, O.A. (2009), *Chiral chromatography and its application to the pharmaceutical industry*, Pharmaceutical Reviews, 7 (1).
- [2] Pinto, M.M.M., Tiritan, M.E., Fernandes, C.S.G. and Cass, Q.B. (2009), *Fases estacionárias quirais baseadas em derivados xantônicos*, Patent 20091000070209, Code 0199.
- [3] Trott, O. and Olson, A.J. (2010), *Software news and update AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading*, Journal of Computational Chemistry, 31 (2), 455-461.
- [4] Zhao, C.F. and Cann, N.M. (2008), *Molecular dynamics study of chiral recognition for the Whelk-O 1 chiral stationary phase*, Analytical Chemistry, 80 (7), 2426-2438.

## **Sodium quantification in meals: from methodology to diagnosis**

**C. Resende<sup>1</sup>, S. Rodrigues<sup>1</sup>, O. Pinho<sup>1</sup> and P. Graça<sup>1</sup>**

<sup>1</sup> Faculty of Nutrition, University of Porto, Portugal.

Hypertension is associated with high salt intake and is related to the development of cardiovascular diseases, which represent a large percentage of chronic diseases in the world. Western countries have a high salt intake and therefore, there are need to establish policies for diagnosis and intervention around the topic.

This study aimed to estimate the amount of salt present in meals served in a Restaurant, in the Oporto city, and to verify the applicability of a technique of flame photometry in the analisys of complete meals. Based on the quantification of sodium in foods, a comparison was made with the recommendations proposed by the World Health Organization (5 g salt/day) for this mineral; perception of the users about the amount of salt in the foods analyzed was also investigated through a questionnaire.

The results of the quantification showed high levels of salt in meals, when compared to the recomendations. The data relating to salt perception showed a discrepancy between the perception of users about the salt and the quantified values. The technique used was effective and thus may be a facilitator for running diagnostics on meals, favoring future interventions in the reduction of salt intake.

# Flavonoids profiles of *Bauhinia forficata* Link: authenticity and relation with biological potential

S. T. Silva, J. Vinholes, P. Valentão and P. B. Andrade

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

*Bauhinia* genus (Fabaceae) comprises more than 500 species of flowering plants, which are commonly known as cow's foot due to their bilobed leaves. In Portugal, cow's foot is sold in some herbal shops under the denomination of *Bauhinia forficata* Link. This species is used as diuretic, tonic, cleanser, in the control of hypoglycaemia and to reduce glycosuria [1]. In order to ascertain *B. forficata* Link authenticity MeOH:H<sub>2</sub>O (1:1) extracts were prepared for two certified *B. forficata* Link (BCERT1) and *B. forficata* Link subsp. *pruinosa* (Vogel) Fortunato & Wunderlin (BCERT2) and two commercial (C1 and C2) samples and their phenolic profiles were determined by HPLC-DAD-ESI/MS<sup>n</sup>. In addition, different enzymatic ( $\alpha$ -glucosidase and cholinesterases inhibition) and chemical (DPPH<sup>•</sup>, O<sub>2</sub><sup>-•</sup> and <sup>•</sup>NO scavenging) microassays were performed to assess samples biological potential.

Thirty-nine flavonoids were found among the analyzed samples, but just kaempferol-3-*O*-(2-rhamnosyl)rutinoside was common to all of them. BCERT1 and BCERT2 shared 5 compounds, kaempferol derivatives being the most representative ones. In contrast, C1 and C2 showed higher contents of quercetin derivatives, and myricetin derivatives and flavonoids-(galloyl)glycosides were also found. Our results indicate that C1 and C2 probably correspond to other *Bauhinia* species, since their phenolic profiles are completely distinct from the certified samples. In addition, the previously proposed chemical marker for *B. forficata* species [2], kaempferitrin (Fig. 1), was found only in BCERT1, confirming that the commercial samples may not correspond to *B. forficata* Link.

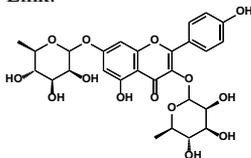


Fig. 1. Chemical structure of kaempferitrin (kaempferol-3,7-di-*O*-rhamnoside).

In a general way, commercial samples were more effective than certified ones in all microassays, which might be explained by their high contents in quercetin derivatives. However, health issues like toxicity are not known for these samples, which may render their use unsafe. Therefore, metabolomics studies should be used to assure the quality and safety of plant material commercially available.

## References

- [1] Martins, R. E., Castro, D. M., Castellani, D. C., & Dias, J. E. (1998). *Plantas Medicinais*, Ed. Universidade Federal de Viçosa, p. 155.
- [2] Engel, I. C., Ferreira, R. A., Cechinel-Filho, V., & Meyre-Silva, C. (2007). Controle de qualidade de drogas vegetais a base de *Bauhinia forficata* Link (Fabaceae). *Brazilian Journal of Pharmacognosy*, 18, 258-264.

# Macroalgae chemical profile *versus* cholinesterases activities

**R. Matos, M. Barbosa, J. Vinholes, P. B. Andrade and P. Valentão**

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Biodiversity within macroalgae offers the possibility of finding a wide variety of natural compounds with interesting properties. Our search for new bioactive compounds from this source has led to the chemical characterization of 18 species of red (Rhodophyta), green (Chlorophyta) and brown (Phaeophyta) seaweeds. Samples were submitted to ethanol extraction and were further analyzed by gas chromatography–mass spectrometry (GC–MS). All extracts were evaluated for their potential to inhibit cholinesterases, namely acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), which are associated with the etiology of Alzheimer’s disease (AD).

GC–MS analysis revealed the presence of amino acids, phloroglucinol, sugars, fatty acids and sterols. Brown macroalgae extracts showed a distinct and dose-dependent behavior in the AChE and BuChE inhibitory microassays (Fig. 1), which seems to be related with their chemical composition.

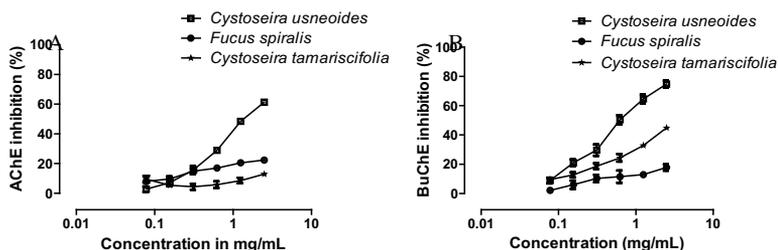


Fig. 1. AChE (A) and BuChE (B) inhibitory activities of three brown algae ethanol extracts. Results show mean  $\pm$  standard error of three assays, performed in triplicate.

Our results, indicates that we are dealing with very promising extracts, since they were able to inhibit both AChE and BuChE, which consequently may result in a higher efficacy in AD treatment. Besides the enhancement of the acetylcholine content in the synaptic cleft by inhibition of both AChE and BuChE, extracts able to inhibit the last can also prevent the formation of new  $\beta$ -amyloid plaques [1].

## References

[1] Çokuğraş, A. N. (2003). Butyrylcholinesterase: Structure and physiological importance. Turkish Journal of Biochemistry, 28, 54-61.

## Chiral xanthone derivatives: Synthesis, physicochemical properties and anti-inflammatory activity determination

**I. Ramos<sup>1,2</sup>, C. Fernandes<sup>1,2</sup>, M.E. Tiritan<sup>1,3</sup>, C. Afonso<sup>1,2</sup>, M. Pinto<sup>1,2</sup>, M. Lúcio<sup>4</sup>, Paula C.A.G. Pinto<sup>4</sup>, M. Lúcia M.F.S. Saraiva<sup>4</sup>, S. Reis<sup>4</sup>**

<sup>1</sup> Centro de Química Medicinal da Universidade do Porto, (CEQUIMED-UP), Portugal

<sup>2</sup> Departamento de Química, Laboratório de Química Orgânica e Farmacêutica, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal.

<sup>3</sup> Centro de Investigação em Ciências da Saúde, Instituto Superior de Ciências da Saúde-Norte, (CICS-ISCN), Gandra, Portugal.

<sup>4</sup> REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Portugal.

Xanthenes or *9H*-xanthen-*9*-ones comprise an important class of *O*-heterocycles which are well known for their important biological activities [1]. Chiral xanthone derivatives (CXDs) are of great interest since they can be associated with enantioselectivity when they interact with biological targets [2].

The present work describes the synthesis of a small library of CXD with potential anti-inflammatory activity. The methodology for the synthetic pathways involves the reaction of carboxyxanthone building blocks with both enantiomers of commercially available chiral compounds, using a coupling reagent [3]. All the synthesized compounds were structurally elucidated by spectroscopic methods (IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR). Lipophilicity (log P) and protein binding affinity for CXD were evaluated. Log P was determined by a micelle/buffer model and by derivative spectrophotometry. Drug-albumin affinity was studied by spectrofluorimetry. The results showed that the evaluated compounds present interaction and penetration into membrane phospholipids which may be relevant to understand their rate of body distribution. High partition coefficients are also important to confer to the compounds the ability to permeate biological membranes.

In order to study anti-inflammatory activity for the new CXDs, the inhibition of enzymes involved in the inflammatory process, namely phospholipase A2 (PLA2) and cicloxygenases (COX-1 and COX-2) was evaluated. The effect on the hydrolytic efficiency of PLA2 was investigated by fluorimetry using the AcryloDated Intestinal fatty acid binding protein (ADIFAB) as a fluorescent indicator for measurement of free fatty acid released from phospholipids. The evaluation of the inhibition of COX-1 and COX-2 activities was performed by using the COX Inhibitor Screening Assay Kit

The studied CXD showed to be active but less than indomethacin.

References:

[1] M.M.M. Pinto *et al*, *Curr. Med. Chem.* (2005), 12(21) 2517-2538.

[2] H. Marona *et al*, *Bioorg. Med. Chem.* 17 (2009), 1345-1352.

[3] Balalaie, S. *et al*. *J. Iran. Chem. Soc.*, (2007), 4(3), p. 364-369.

Acknowledgments: CEQUIMED-UP (PEst-OE/SAU/UI4040/2011), FERDER, POCI, U.Porto/Santander Totta, for financial support.

## Sunscreens: Photodegradation and stabilization

**S. Afonso, J.P. Sousa e Silva, I. F. Almeida, M. H. Amaral, P.A.L. Lobão, P.C. Costa, J.M. Sousa Lobo**

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Nowadays, there is a need to develop more effective sunscreens to ensure optimal photoprotection. Chemical UV-filters are compounds incorporated on sunscreen formulations to absorb specific wavelengths of ultraviolet radiation, UVA (320-400 nm), UVB (290-320 nm) or both. Some UV-filters are photochemically unstable, which impair their absorbance after UV exposure [1]. The aim of this study was to evaluate the photodegradation of the UVA filter 4-*tert*-butyl-4'-methoxydibenzoilmethane (avobenzone) after exposure to UV radiation and also to improve its stability by using antioxidants such as vitamin C (Vit. C), vitamin E (Vit. E) and ubiquinone (UBQ).

Avobenzone (AVB) solutions (5 µg/mL) mixed with antioxidants at different concentrations were prepared in dimethyl sulfoxide. A control solution of avobenzone was used. Samples were kept in sealed quartz cells and irradiated at 750 W/m<sup>2</sup> in an Atlas Suntest CPS+ with a xenon lamp (~45°C) equipped with a special UV filter used to simulate UV radiation that reaches the earth. The irradiation time was 1 hour and the incident dose of UV was 2700 kJ/m<sup>2</sup>. To measure the decrease in absorbance of AVB, UV measurements were carried on a Jasco V-650 spectrophotometer and the spectra were recorded from 250 nm to 400 nm (~ 2 hours after irradiation). The area under the curve (AUC) was calculated for UVA range, before and after irradiation. The AUC ratio defined as  $R_{AUC} = \frac{AUC_{after}}{AUC_{before}}$  was used to compare photostabilities. If  $R_{AUC} > 0.80$ , the sunscreen was considered photostable [2].

The results are presented in Table 1. The stabilization effect is dependent on the antioxidant concentration. Higher concentrations of antioxidants did not stabilize the filter, except in the case of vitamin E. AVB + vitamin C at 2.5µg/mL, AVB + vitamin E at 10µg/mL and AVB + ubiquinone at 2.5µg/mL were the only combinations with an AUC ratio higher than the control solution of AVB. The present study shows that vitamin C (2.5µg/mL), vitamin E (10µg/mL) and ubiquinone (2.5µg/mL) increase the photostability of AVB.

Table 1- AUC ratios of avobenzone control and in combination with Vit. C, Vit. E and UBQ at different concentrations.

	AVB (5 µg/mL)	AVB + Vit. C (2.5 µg/mL)	AVB + Vit. C (5 µg/mL)	AVB + Vit. C (10 µg/mL)	AVB + Vit. E (5 µg/mL)	AVB + Vit. E (10 µg/mL)	AVB + UBQ (2.5 µg/mL)	AVB + UBQ (5 µg/mL)
$R_{AUC}$	0.18	<b>0.33</b>	0.15	0.15	0.18	<b>0.21</b>	<b>0.21</b>	0.12

References:

- [1] Damiani, E., Astolfi, P., Giesinger, J., Ehliis, T., Herzog, B., Greci, L., Baschong, W. (2010), *Assessment of the photo-degradation of UV-filters and radical-induced peroxidation in cosmetic sunscreen formulation*, Free Radical Research, 44 (3), 304-12.
- [2] Gonzalez, H., Tarras-Wahlberg, N., Stromdahl, B., Juzeniene, A., Moan, J., Larko, O., Rosén, A., Wennberg, A. (2007), *Photostability of commercial sunscreens upon sun exposure and irradiation by ultraviolet lamps*, BMC Dermatology, 7 (1), 1-9.

Oral Sessions II > A2

# UNICER II



## The antioxidant treatment with EGCG prevents the diabetes-induced decrease of brainstem pain-related noradrenergic neurons

**J. Silva<sup>1,2</sup>, A. Miranda<sup>1,2</sup>, P. Pereira-Terra<sup>2,3</sup>, D. Raposo<sup>2</sup>, C. Morgado<sup>2,3</sup>, I. Tavares<sup>2,3</sup>**

<sup>1</sup> School of Health Sciences, University of Minho, Portugal

<sup>2</sup> Department of Experimental Biology, Faculty of Medicine, University of Porto, Portugal

<sup>3</sup> IBMC, University of Porto, Portugal

Painful diabetic neuropathy (PDN) is a common complication of diabetes, which is accompanied by changes at the central and peripheral nervous system. Streptozotocin (STZ)-diabetic rats with PDN present spinal nociceptive neuronal hyperactivity along with decrease in the numbers of noradrenergic neurons of the A5 and A7 cell groups. The A5 and A7 noradrenergic neurons project to the spinal dorsal horn and modulate nociceptive transmission by releasing noradrenaline. Accordingly, diabetic rats present lower levels of noradrenaline at the spinal cord along with higher nociceptive behavioral responses, which are an indication of the existence of painful diabetic neuropathy. Diabetes was shown to induce oxidative damage in neurons from those areas suggesting that oxidative stress may be underlying the changes detected in the noradrenergic descending pain modulation pathway during diabetes.

This study aimed to evaluate the effects of the antioxidant treatment with Epigallocatechin Gallate (EGCG), a potent antioxidant present in green tea, in the noradrenergic neurons of the A5 and A7 cell groups, spinal noradrenaline level and behavioral pain responses in STZ-diabetic rats.

Diabetes was induced in male Wistar rats by intraperitoneal injection of STZ (60 mg/Kg). Controls received citrate buffer. At 3 days post-injection, one group of STZ rats started to receive EGCG (2g/l) in drinking water while the other experimental groups received only water. Mechanical hyperalgesia and tactile allodynia were evaluated before the injections and at 10 weeks after treatment onset, using the paw pressure and the dynamic plantar aesthesiometer tests, respectively. The noradrenergic neurons were identified by immunodetection of tyrosine hydroxylase (TH), an enzyme involved in noradrenaline synthesis. Countings of TH-immunoreactive neurons in the A5 and/or A7 cell groups was performed according to a blind procedure. Spinal lumbar enlargement was used to evaluate the levels of noradrenaline by ELISA. All data were compared by ANOVA followed by Tukey's post hoc test for multiple comparisons.

STZ rats developed hyperglycemia, which was not affected by EGCG. The treatment with EGCG prevented the mechanical hyperalgesia and tactile allodynia developed by untreated STZ rats and the reduction in the numbers of TH-IR neurons detected in the A5 and A7 of untreated STZ rats. It also normalized the levels of noradrenaline in the spinal cord of STZ-diabetic rats.

EGCG prevents the decrease of noradrenergic neurons in the A5 and A7 and of the spinal levels of noradrenaline during diabetes, which may justify its beneficial effects on pain behavioral responses. Treatment with EGCG could be a promising strategy to prevent the neurodegenerative events affecting the noradrenergic neurons of the A5 and A7 during diabetes and, consequently, the diabetic neuropathic pain.

Supported by IJUP/ UNICER project.

## Monitoring a Draught Beverage Machine

**Ricardo Reis<sup>1</sup>, Clito Afonso<sup>1</sup>, João Faria<sup>1</sup>, Karla Gonçalves<sup>1</sup>, José Jacinto<sup>1</sup>, Joaquim Gabriel<sup>1,2</sup>, Armando Araújo<sup>1</sup>, Armando Oliveira<sup>1</sup>, Jorge Pires<sup>3</sup>, Cristina Silva<sup>3</sup>**

<sup>1</sup> Faculty of Engineering, University of Porto, Portugal

<sup>2</sup> IDMEC – Pólo FEUP

<sup>3</sup> Unicer Bebidas, SA, Leça do Balio, 4466-955, Matosinhos, Portugal

This work aims to improve the performance of the refrigeration systems for cooling beverage pressure existing in restaurants and bars. After reviewing several systems, we realized that improvements of the actual solution may be possible. This is the main motivation for this collaborative work.

The operation of such refrigerating systems is based on the classic vapor compression cycle. The evaporator is housed in a tank containing cold water where the drink from the barrel is cool down when passing through a heat exchanger. The drink, once cooled, follows to a column sum of two tubes, to the tap, ready to be consumed. The system is composed by two circuits, the circuit for the cooling fluid and the circuit of the beverage. Thus, both circuits have to be monitored at several critical sites using pressure, temperature and flow transducers. Also, the beverage consumption, ambient temperature, electrical energy should also be monitored. There are three main electrical consumers: the compressor, the fan and the ventilator.

The data of all these transducers is going to be collected through a data acquisition board to a computer and subsequently analyzed using homemade software.



**Figure 1** View of the experimental setup

As referred above, in these systems the cold water is used to cool down the beverage. Thus an interesting topic for further developments would be to develop a new solution able to cool directly the beverage in order to improve the efficiency of whole system.

The authors would like to thanks the support of the UNICER Bebidas and University of Porto, through the Projectos Pluridisciplinares, Concurso 2010.

## Can brewers yeast, a by-product of brewing industry, be used in fish diets?

**C. Castro<sup>1,2</sup>, A. Perez-Jiménez<sup>2</sup>, F. Coutinho<sup>1</sup>, H. Peres<sup>2</sup>, B. Costas<sup>2</sup>, P. Pousão-Ferreira<sup>3</sup>, T.M. Brandão<sup>4</sup> and A. Oliva-Teles<sup>1,2</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal. <sup>2</sup> CIMAR, Centro de Investigação Marinha e Ambiental, Porto, Portugal. <sup>3</sup> IPIMAR/CIMSul, Olhão, Portugal. <sup>4</sup>Unicer Bebidas, SA - Via Norte - Leça do Balio - Portugal

Nowadays, due to social, economic and environmental reasons industry has to base its business strategy on sustainability. In the case of brewing industry its sustainable development depends, among other factors, in the valorization of its by-products such as surplus yeast. Yeasts have been used in aquafeeds both as a dietary protein source or nutraceutical (probiotic) due to its nutritional value and immunostimulatory properties [1]. Thus, the present study aimed to evaluate the feasibility of using yeast by-product from brewing industry (UNICER) as a probiotic by accessing its effects on growth performance, hematology and serum biochemical parameters of two marine fish species of interest for aquaculture: meagre (*Argyrosomus regius*) and white sea bream (*Diplodus sargus*). For this purpose triplicate groups of fish (each replicate: 6 meagres, initial body weight (IBW) of  $122.3 \pm 0.1$ g or 10 white sea bream, IBW of  $85.5 \pm 0.2$ g) were randomly distributed to 100L tanks and kept at  $24.6 \pm 0.2$  °C. During 4 weeks, fish were hand fed to satiation (2 meal/day, 6 days a week) with one of 3 isonitrogenous (52% crude protein) diets: unsupplemented (control) or supplemented with live brewer's yeast *Saccharomyces cerevisiae* at 1 or 2%. At the end of the trial, growth parameters (final weight, FBW and daily growth index, DGI) and feed efficiency (FE), hematological parameters (hemoglobin concentration, hematocrit, red blood cells, white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) and serum metabolites (total protein and amino acids, total lipids, cholesterol, triglycerides and glucose) were measured. Dietary supplementation with yeast did not improved growth, however, FE significantly improved in meagre fed the yeast supplemented diets. No significant alterations were observed on serum biochemical parameters of both species. Only in white sea bream, total plasma amino acids were significantly elevated in fish fed 1% dietary yeast diet compared to the control diet. Regarding hematological parameters, while in meager only WBC significantly increased in fish fed the yeast-supplemented diets, in white sea bream a decrease in hematocrit was observed in the yeast fed groups along with lower levels of MCV in fish fed the 1% yeast-supplemented diet, compared to the control diet. The present results demonstrate the potential of *S. cerevisiae* to improve feed utilization, particularly in meager, without affecting growth performance. Furthermore, considering the different effects of dietary yeast-supplementation on hematological parameters of the two species, additional studies are required to assess the immunomodulatory potential of brewer's yeast on meagre and white sea bream.

### References:

[1] Oliva-Teles, A. and Gonçalves, P. (2001), *Partial replacement of fishmeal by brewers yeast (Saccharomyces cerevisiae) in diets for sea bass (Dicentrarchus labrax) juveniles*, Aquaculture, 202, 269–278.

# Monitorization of wort and beer proteolysis by size exclusion and reverse phase liquid chromatography

**Cristina Moura<sup>1</sup>, Tiago Almeida<sup>1</sup>, Elsa Vieira<sup>1</sup>, Olívia Pinho<sup>2</sup>, Isabel Ferreira<sup>1</sup>, Beatriz Carvalho<sup>3</sup>, Tiago Brandão<sup>3</sup>**

<sup>1</sup> REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Portugal

<sup>2</sup> Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Portugal

<sup>3</sup> UNICER – Bebidas de Portugal SGPS, SA, Leça do Balio, 4466-955, S. Mamede de Infesta, Portugal

The majority of beer protein lies in the 10–40 kDa size range. Mostly, the origin of this protein is malted barley [1]. Beer proteins and polypeptides contribute to mouthfeel, flavour, texture, body, colour, and nutritional value [1]. Protein Z, LTP1, and other proteins and polypeptides present in beer have been associated to foam formation and/or stabilization [1-3]. Protein Z has also been related to beer haze [2].

The goal of this work was to study the evolution of proteolysis during wort fermentation/stabilization and the effect of yeast biomass reuse in the protein and polypeptide degradation. For this purpose wort and beer samples from two different tanks (1000 hl and 3000 hl) were analysed. Three cycles of beer production were followed in each tank, one produced with new yeast and the other two produced with consecutive reused yeast biomass. Protein hydrolyses was evaluated by size-exclusion liquid chromatography (SE-HPLC) and by reversed-phase high performance liquid chromatography (RP-HPLC) both coupled to UV detection.

SE-HPLC analyses separated five different fractions, one with 40 kDa (Peak 1), three other fractions corresponding to proteins and polypeptides with molecular weight between 17 and 1 kDa (Peaks 2, 3 and 4), and the fifth fraction corresponding to amino acids (Peak 5). Hydrolysis presented as percentage of protein degradation, was evaluated by the difference of peak area at time zero and peak area at each point. Around 40% protein and polypeptide degradation was observed after 3 days fermentation (except for protein fraction with 40 kDa that at the beginning suffered low degradation but was 75% hydrolysed in the final product). Protein and polypeptide degradation increased to 65-70% at the end of fermentation. Worts and beers produced with reused yeast presented lower content of proteins and polypeptides. Results from RP-HPLC separation were analysed as three fractions, namely, peptides, polypeptides and proteins, similar degradation pattern was observed. No significant differences were observed in qualitative and quantitative profiles of protein/polypeptides/peptides of beers obtained with reused yeast biomass.

[1] Ferreira, I. M. P. L. V. O., Jorge, K., Nogueira, L. C., Silva, F., & Trugo, L. C. (2005). *Effects of the combination of hydrophobic polypeptides, iso-a-acids, and malto-oligosaccharides on beer foam stability*. Journal of Agriculture and Food Chemistry, 53, 4976–4981.

[2] Perrocheau, L., Rogniaux, H., Boivin, P., & Marion, D. (2005). *Probing heat-stable water-soluble proteins from barley to malt and beer*. Proteomics, 5, 2849–2858.

[3] F. Silva, L.C. Nogueira, C. Gonçalves, A.A. Ferreira, I.M.P.L.V.O. Ferreira, N. Teixeira (2008) *Electrophoretic and HPLC Methods for Comparative Study of the Protein Fractions of Malts, Worts and Beers Produced From Scarlett and Prestige Barley (Hordeum vulgare L.) Varieties*. Food Chem. 106, 820-829.

## Enzyme Selection for Bioethanol from Brewery Spent Grains

N. S. Caetano<sup>1a</sup>, A.C. Melo<sup>2a</sup>, V.F.M. Silva<sup>1a</sup>, **R.F. Moura**<sup>2a</sup>, T.M. Brandão<sup>3</sup>, C.A. Silva<sup>3</sup>,  
T.M. Mata<sup>2a</sup>

<sup>a</sup>LEPAE – Laboratory for Process, Environmental and Energy Engineering, FEUP.

<sup>1</sup>Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto (IPP), R. Dr. António Bernardino de Almeida, s/n, 4200-072 Porto, Portugal.

<sup>2</sup>Department of Chemical Engineering, Faculty of Engineering, University of Porto (FEUP), R. Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal.

<sup>3</sup>UNICER, Bebidas S.A., Via Norte - Leça do Balio, Apartado 1044, 4466-955 S. Mamede de Infesta, Portugal

The needs for transportation fuels and fossil fuel prices rise led to the use of renewable liquid fuels, with the advantage of reducing CO<sub>2</sub> and other pollutant emissions to the atmosphere. Bioethanol, together with biodiesel, is one of the most important biofuels in the current European Union (EU) strategy of renewable energy, that can be used instead or as a blend with gasoline (or diesel) with a target of at least 20% of the EU energy consumption from renewable sources by the end of 2020.

Bioethanol technology is well developed for conventional crops such as sugarcane or corn (in Brazil or USA) but its usage for fuel has raised social and economic questions as these are mainly used for feeding purposes. Researchers have driven their attention to other inexpensive and more sustainable sugar sources, such as lignocellulosic residual materials that still contain significant amount of sugars [1]. Brewery spent grains (BSG) is a lignocellulosic material rich in cellulose and hemicelluloses that can provide a source of sugars for bioethanol production via fermentation [2]. Xiros and coworkers [3] concluded that a bioconversion yield of 65 g<sub>ethanol</sub>/kg<sub>dry matter</sub> could be achieved but some additional research is needed to make the process commercially interesting.

This study aims to evaluate the possibility of producing bioethanol from BSG, as a higher added value solution for this brewery's organic waste management. The enzymatic hydrolysis is one of the most important steps in this process that will allow for sugar release from the cellulose and hemicellulose polymer chains, aiming to attain the highest conversion of biowaste to fermentable sugars. Thus, several commercial enzymes were tested in this study: Viscozyme L, Ultraflo L, Glucanex 100g and Ban 480L, from Novozymes. The results obtained in this study show that Viscozyme L is the enzyme that can lead to the larger sugars release (32.3 g<sub>sugar</sub>/100 g<sub>BSG</sub>, of which xylose and glucose production, respectively 16.7 and 6.2 g<sub>sugar</sub>/100 g<sub>BSG</sub>, is about twice the one achieved with any of the other enzymes). This means that there is still work to do to increase the ethanol production from BSG.

**Agradecimento:** Financiamento do Projecto Pluridisciplinar, Concurso 2010, n° 103 “Valorização da Drêche do Processo Cervejeiro para a Produção de Bioetanol”, pela UNICER.

[1] Mata, T.M.; Martins, A.A.; Sikdar, S.K.; Costa, C.A.V.; Caetano, N.S. (2011) *Biofuels sustainability evaluation through the supply chain*, SDEWES2011, September 25 – 29.

[2] White, J.S.; Yohannan, B.K.; Walker, G.M. (2008), *Bioconversion of brewer's spent grains to bioethanol*, FEMS Yeast Research, 8(7), 1175-1184.

[3] Xiros, C.; Topakas, E.; Katapodis, P.; Christakopoulos, P. (2008), *Evaluation of Fusarium oxysporum as an enzyme factory for the hydrolysis of brewer's spent grain with improved biodegradability for ethanol production*, Industrial Crops and Products, 28(2), 213-224



Oral Sessions II > A3

# **Psychology & Education Sciences II**



## Students school performance and attention: presentation of a research project

**S. Henriques<sup>1</sup>, T. Silva<sup>2</sup>, P. Batista<sup>3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal.

<sup>2</sup> Secondary School Doutor Manuel Gomes de Almeida, Espinho, Portugal.

<sup>3</sup> CIFI2D, Faculty of Sport, University of Porto, Portugal.

This work is a presentation of a research project to be developed in the practicum context, integrated into the master's degree in Teaching Physical Education in Basic and Secondary Education, at the Faculty of Sport, University of Porto, in the 2011/2012 year. The design came after a meeting of the Class's council, that I teach, at the end of the first period. It found that the student's had low classifications levels, and the analysis done by the generality of the teachers, pointed to the students low attention's levels as a cross characteristic to all the students. These evidences led me to question if there was any relation between the teachers' perception relatively to the students' weak attention' levels and the low grades obtained. Thus, I proposed me to realize a study to inspect this possible relation between these two variables. The sample will be twenty-two students from the 10th year of Secondary School Dr. Manuel Gomes de Almeida, at the school's year 2011/2012. The ages are between 14 and 15 years ( $14.9 \pm 0.4$ ). The students will be divided into three distinct categories taking into account the score obtained in the first period and the Special Educational Needs. The first category will include all students with positive ratings, the second category will include students with one or more negative ratings, and, finally, the third category will contain the students identified with Special Educational Needs. For the attention's evaluation will be used the Toulouse-Piéron's test [1] which assesses the attention on attentional speed, accuracy and attentional fatigue resistance components. The analysis procedures will comprehend the basic descriptive statistics (mean and standard deviation) and inferential statistics (Student t test and Mann-Whitney test). The significance level is set at  $p \leq 0,5$ . It was intended that the results' study be evidence, or not, that the teachers' perception about the relation between these two variables in order to define strategies that allow students to improve their levels scoring.

### References:

[1] Toulouse, E., Piéron, H. (1982), *Perception and Attention's test*, Applied Psychology's Publications, 20, 3-12.

## Strategies to optimize good behaviours in Physical Education classes: learning through reflections

C. Santos<sup>1</sup>, T. Sousa<sup>2</sup>, A. Azevedo<sup>3</sup>

<sup>1</sup> Faculty of Sport, University of Porto, Portugal

<sup>2</sup> CIFI<sup>2</sup>D, Faculty of Sport, University of Porto, Portugal

<sup>3</sup> Secondary School of Oliveira do Douro, Porto, Portugal

This study is based on personal reflexions referred to the experience of a pre-service teacher in Physical Education teaching practicum context, of Master degree on Physical Education, Faculty of Sport, of the academic year 2011/2012. The practicum was made at a Secondary school of Vila Nova de Gaia with a 8<sup>th</sup> degree class.

The purpose of this study was to analyze the students' behaviour along the lessons taught during the first semester, in Handball and Athletics. The study was supported in written reflections developed at the end of each lesson, in which the experienced events were described, interpreted and this was the basis for applying further strategies in the practice context. From the analysis of the reflexions the following evidences emerged: (i) - After the adoption and consolidation of certain rules and routines the students' behaviour has considerably improved; (ii) - The strategy of using cards (yellow and red like a referee) allowed us to evaluate the students' behaviour at different lesson moments. The first yellow card was used as a warning and showing a second one corresponded to a red card, precluding, on a temporary basis, the participation in the classroom practice. Throughout that time the student had the possibility to reflect on his attitude and understand why that situation happened; (iii) - The assigning of the captain's armband in each lesson also allowed to give responsibility to students because they understand that the "captain" refers not only to the one who shows the best performance in motor skills, but rather the one that has a proper behavior to the different situations, the one that complies with the rules, respects, cooperates and engages in the requested tasks; (iv) - During the lessons, appealing to the team work during the exercises enabled the development of psychosocial concepts such as cooperation, unity, mutual aid, reducing disruptive behaviours; (v) - The use of the instruction strategy explanation with demonstration as well as the questioning led to an increase of students' awareness on the tasks, preventing the occurrence of disruptive behaviours; (vi) - A good classroom organization and management, with reduced waiting times and higher periods of motor engagement, prevent disruptive behaviours.

In that experience the different strategies used were fundamental to improve the students' general behaviour. In this way, a good learning atmosphere, win the students' trust and the control of the class was achieved. However, it is important that each class is regarded as unique, and all the aspects worked on so far should be constantly reminded and consolidated.

**Key-words:** PHYSICAL EDUCATION; PRE-SERVICE TEACHERS; INDISCIPLINE; PRACTICUM; REFLECTION

## **A narrative of a Physical Education pre-service teacher about the class control**

**A. Cerca<sup>1</sup>, T. Silva<sup>2</sup> e P. Batista<sup>3</sup>**

<sup>1</sup>Faculty of Sport, University of Porto, Portugal.

<sup>2</sup>Secondary School Doutor Manuel Gomes de Almeida, Espinho, Portugal.

<sup>3</sup>CIFI2D, Faculty of Sport, University of Porto, Portugal.

This study was the result of a self-assessment done by a pre-service teacher that teaches a large class, where the majority was male. The practicum took place in the context of the Physical Education master degree teaching at the primary and secondary levels, FADEUP, during the 2011/2012 academic year at School Doctor Manuel Gomes de Almeida, in Espinho. The group which was being studied was at 10 degree of social economic sciences course. The body of this study was mostly taken from the lessons board diary written during the period of September 27<sup>th</sup> until December 13<sup>th</sup> of the same school year, with a total of 22 lessons. Thus, this study is composed of an analysis in the first person, which alone enabled the advancement of the reflection process explained in writing. The purpose of this study was to make a self-assessment of the teaching practice, centered on class control. The focus on the issue of class control is recurrent in the reflection stage and the first step to establish itself as a structuring element of pedagogical skills. The analysis included two distinct but equivalent steps. The first stage proceeded to the exploitation of data to detect the prevalence of situations that make up the conceptual field of the term control group, and then it was made a content analysis centered on aspects related to class control, with reference to the following points: i) dilemmas; ii) strategies and iii) results. The analysis was made clear that i) the dilemmas that occurred showed on the attitudes of the less positive students; ii) the strategies outlined were implemented by: management of learning tasks, creation of working groups, the teacher's attitude, promotion of the responsibility and students independence, positive reinforcement and motivation, iii) The results obtained were expressed in increase levels of controlling the group, translating into more students getting involved, being responsible, obeying the rules, being motivated and involving themselves more with the class and the teacher.

## Contribution of the Sport Education Model for the students involvement in learning of Acrobatic Gymnastics

**D. Ferreira<sup>1</sup>; P. Mota<sup>2</sup>; P. Batista<sup>3</sup>**

<sup>1</sup>Faculty of Sport, University of Porto, Portugal

<sup>2</sup>High School of Almeida Garrett, Vila Nova de Gaia, Portugal

<sup>3</sup>CIFID2, Faculty of Sport, University of Porto, Portugal

This work represents part of a personal report referred to the experience of a Physical Education pre-service teacher in the professional training context related to the implementation of the Sport Education Model. The Sport Education Model aims to training students' sportingly competent, cults and enthusiasts (Siedentop, 1987; Hastie, 2004). Graça e Mesquita (2009, p.59) said that is a model that "meets the need to give a slant to the affective and social learning". In this context, the purpose of this study was to examine how students of a class of secondary school became involved in Acrobatic Gymnastics lessons organized under the aegis of Sport Education model. The application of the Gymnastic unit occurred in the first period of the academic year of 2011/2012, in a school in the city of Vila Nova de Gaia. The teaching process was conducted by a pre-service teacher of the course of Masters in Physical Education Teaching in Primary and Secondary Schools, Faculty of Sport, University of Porto. The *corpus* of study was the board diary developed during the period in which the model was applied and the blog built by students during the unit. In the board diary were described events of seven lessons of sixty minutes each, among the 7<sup>th</sup> October and 18<sup>th</sup> November. The blog was built by students and worked as a space for sharing and dissemination among students of this class, teacher and some parents. This included a digital newspaper, interviews with the captains/coaches and teachers, a gallery of all classes and training, as well as the events classification. The analysis of the report and the blog information resulted in the following evidences: (i) students were greatly involved in the process, reflecting the high degree of motivation in the classroom and outside the classroom; (ii) it was visible that the students learned not only in motor skills but also in the psychosocial domain; (iii) like the students, the teacher (the author) was also heavily involved, and this investment has found reflection in student involvement; (iv) the enthusiasm and festivity levels were present throughout the unit, focusing more on the final event preparing where the commitment of the students in the various tasks exceeded the expected; (v) the blog has been a crucial factor in prolonging the students involvement outside the classroom context, revealing it was also an aggregator element for your interest and curiosity that attracted not only students but also parents. From a personal standpoint it should be stressed that the adoption of this model was extremely gratifying. I can even say that I cannot imagine the teaching of this mode in a different configuration, which was so enthusiastic! Throughout the course I have always tried to involve students in this process that it had viewed so complex but which in the end became extremely rewarding. Note also that the application of this model enabled the student-teacher relationship development not only in the class context but also outside the classroom.

### References:

- [1] Siedentop, D., Hastie, P., Mars, H. (2004). *Complete Guide to Sport Education*. Champaign: Human Kinetics.
- [2] Rosado, A., Mesquita, I. (2009). *Pedagogia do Desporto*. Lisboa: FMH Edições.

## **The (re)construction of Professional Identity in the practicum context. Main results of a research project.**

**C. Ferreira<sup>1</sup>, P. Gomes <sup>1</sup>; A. L. Pereira <sup>1,2</sup>; A. Graça <sup>1,3</sup>; P. Batista<sup>1,3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal.

<sup>2</sup> IS-FLUP, Institute of Sociology, Faculty of Arts and Humanities, University of Porto, Portugal.

<sup>3</sup> CIFI<sup>2</sup>D, University of Porto, Portugal.

The initial training of Physical Education (PE) teachers is changing, mainly, since the implementation of the Bologna Process. Therefore, a professional identity (PI) emerges with a new twist due to the fact that the PI is historical, situational and relational construct [1]. Thus, the research about the (re)construction of the PI as an outcome of an interface between the personal experiences of teachers and the social, cultural, and institutional context in which they function on a daily basis [2] assumes great importance. The main goal of this paper is to present some main conclusions about a research project guide by the following questions: i) how pre-service teachers experience the impact of real teaching experience in school context? how they (re)construct their PI?; ii) how cooperating teachers, while supervisors of the professional practicum process, (re)construct their PI?; and iii) how was the functioning of a group of practicum in the school context? In order to answer these questions three studies of interpretative nature were developed, accomplished with the use of a set of methods to collect data such as: semi-structured individual interviews, focus groups, pre-service teachers' portfolios, participant and non-participant observation, field notes and video records. The participants of the study were 12 PE pre-service teachers and 15 cooperating teacher from the Faculty of Sport, University of Porto, through the academic year of 2010/2011. Data were submitted to thematic analysis using Nvivo9. The findings show that: (i) the pre-service teachers revealed difficulties in idealizing their professional identity as teachers, being that the experiences gained while students/athletes and the negative experiences during the initial contact of practicum influenced their work as teachers; ii) to be a cooperating teacher allows to improve the teaching practice; the sharing and conducting learning the pre-service teacher for updates knowledge are the main reasons for the initiation and maintenance of the supervising activity; iii) the group of practicum teaching in community practice in school context, being that to be the pre-service teacher is (a) taking responsibility for the students' learning process and their integral formation as human beings; (b) to respect the school rules and the disciplinary group and, simultaneously, cooperating on the development of extra-curricular activities; (c) to reflect on their own actions as teachers, the individual and collective way, in order to improve the teaching process and improve and acquiring new competences; (d) to share new knowledge with the students, teachers and the members of the community of practice.

Acknowledgments: authors acknowledge financial support given by University of Porto and Santander Totta through "Projetos Pluridisciplinares 2010\_código 83"

### References

- [1] Luehmann, A. (2007), Identity Development as a Lens to Science Teacher Preparation, *Science Education*, 91(5), 822-839.
- [2] Day, C., Flores, M. and Viana, I. (2007), Effects of national policies on teachers' sense of professionalism: findings from an empirical study in Portugal and in England, *European Journal of Teacher Education*, 30(3), 249-265.

## Identifying features of the teaching profession

Ana Raquel Simões<sup>1</sup>

<sup>1</sup> Department of Sociology, Faculty of Arts, University of Porto, Portugal.

Our research had as its subject the professional identities of primary and secondary education teachers of the public school. The main goal was to analyze the identifying features that arise from speeches that are based on representations and opinions about the current socio-professional status of the teacher. In this last one we considered some of the educational policies that were being implemented at the time of the research and generating dissatisfaction among teachers, such as changes to the Teaching Career Statute and to the new model of Performance Evaluation.

We carried out a qualitative research strategy, considering this work as a case study of exploratory and descriptive nature. There were made twenty-five interviews to teachers of a secondary school in the District of Porto. These teachers were selected based on the criterion of seniority in the profession, specifically teachers whose professional career is relatively long, of about twenty years.

It was concluded that despite presenting in their speeches identifying features that are already consolidated, the majority of the respondents are being confronted with their forms of identification within their profession. They consider that the meanings ascribed to their profession today are not the same as those which they recognized and adopted at the beginning of their careers. Thus, we assess the emphasis that the study of the professional identity should be based on a relational and contextual perspective in space and time.

Oral Sessions II > A4

# AGRO FOOD I



## Effect of fresh and composted spent coffee grounds on lettuce photosynthetic capacity

**R. Cruz, S. Cunha, and S. Casal**

REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

Coffee is one of the most popular beverages worldwide. Coffee processing chain, however, leads to the accumulation of diverse organic residues, including spent-coffee grounds. Increasing efforts are being made to implement strategies to enhance their commercial value.

Therefore, a greenhouse pot experiment was conducted, using spent coffee as soil amendment, to evaluate the impact on carotenoids and chlorophylls content in lettuce (*Lactuca sativa* L. var. *capitata*). Fresh spent coffee grounds were collected in several coffee shops and used either as is (FSCG) or after being composted during 3 months with equal volumes of straw and fresh grass (CSCG) at Lipor. Five mixtures were prepared with standard vegetable soil: from 2.5% to 20% for FSCG and from 5% to 30% for CSCG, all on a volume basis, using plain vegetable soil as control (0%).

Plants were collected and analyzed for chlorophylls and carotenoids (lutein and  $\beta$ -carotene) by HPLC-PDA. The method encompasses lipid extraction of fresh samples (0.5g), by the classic Folch method, with  $\beta$ -apo-8'-carotenal as internal standard, followed by HPLC separation (Gilson), using ethyl acetate/methanol/water/0.05% triethylamine gradient, in a reversed-phase column (Phenomenex Luna; 250 x 4,60mm; 5 $\mu$ m) and phoydiode array detection (Varian ProStar) at 412 nm and 440 nm for chlorophylls and carotenoids, respectively.

Generally, the presence of spent coffee, either fresh or composted, increased the content of all analyzed pigments. Regarding FSCG, lutein and  $\beta$ -carotene highest levels were achieved with 15% ( $7.44 \pm 0.67$  mg/100g and  $5.70 \pm 1.23$  mg/100g, fresh weight) when compared to control samples (with  $3.99 \pm 0.80$  mg/100g and  $3.31 \pm 0.67$  mg/100g, respectively), while chlorophyll *a* was higher with 20% ( $17.33 \pm 3.14$  mg/100g vs  $11.28 \pm 2.29$  mg/100g). Concerning CSCG, lutein highest amounts were achieved at 10% ( $6.83 \pm 1.15$  mg/100g), as was chlorophyll *a* ( $19.04 \pm 3.06$  mg/100g), while  $\beta$ -carotene increased up to 15% ( $5.26 \pm 0.73$  mg/100g).

These data support that plants nutritional features, regarding these bioactive compounds, can be improved, but their increased content might represent a signaling plant stress, requiring further studies on other parameters. Knowing that spent coffee grounds obtained after beverage extraction are still rich in bioactive compounds, caffeine included, further studies on the mechanisms involved are mandatory in order to verify the feasibility of this agricultural practice, increasing within domestic agriculture.

Acknowledgments: This work was developed with funding from the Project FCT PTDC/AGR-AAM/102447/2008, co-financed by COMPETE/FEDER/OE. Rebeca Cruz thanks to FCT for the scholarship within this project.

## Antioxidant activity in minimally processed Bimi<sup>®</sup> Broccoli under different sanitizing techniques throughout shelf-life

A. C. Formica-Oliveira<sup>1,2</sup>; G. B. Martínez-Hernández<sup>1,3</sup>; P. Gomez<sup>3</sup>; F. Artés<sup>1,3</sup>; L. M. Cunha<sup>2</sup>; F. Artés-Hernández<sup>1,3\*</sup>

<sup>1</sup> Postharvest and Refrigeration Group, Department of Food Engineering, Technical University of Cartagena, Murcia, Spain. Tel: +34-968-325509; Fax: +34-968-325433. \*E-mail: [fr.artes-hdez@upct.es](mailto:fr.artes-hdez@upct.es). Web site: [www.upct.es/gpostref](http://www.upct.es/gpostref)

<sup>2</sup> REQUIMTE/DGAOT, Faculty of Sciences, University of Porto, Portugal

<sup>3</sup> Institute of Plant Biotechnology, Technical University of Cartagena, Murcia. Campus Muralla del Mar. 30202 Cartagena, Murcia, Spain

In the present days, consumers are faced with a diverse variety of minimally processed fruits and vegetables, where sensorial and nutritional quality plays a major role on marketing those products [1]. Broccoli is one of the most popular and highly perishable vegetables, and an important contributor of antioxidants in the diet. Although chlorine is the most widely sanitizer used in fresh-cut vegetables, it has some disadvantages that lead to research on alternative treatments [2]. This study shows the total antioxidant capacity changes during the shelf life in Bimi<sup>®</sup> broccoli (*Brassica oleracea* Italica x Alboglabra) after alternative sanitizing techniques. Four treatments were assayed (high oxygen active modified atmosphere packaging (MAP) – HO; a combination of neutral electrolyzed water and high oxygen MAP - NEW+HO; UV-C radiation plus high oxygen MAP- UV+HO; and the triple combination - NEW+UV-C+HO). As control, 100 ppm NaClO was used as sanitizer in the washing step. After fresh-cut processing, Bimi<sup>®</sup> was stored at 5°C in darkness and total antioxidant capacity under modified FRAP assay [3], was analysed on processing day and after 5, 9, 15 and 19 days at 5°C. Sanitizing treatments reduced the initial total antioxidant capacity of 479.6 mg ascorbic acid equivalent units (AAE) kg<sup>-1</sup> fw recording NEW+HO the lowest values with 307.8 mg AAE kg<sup>-1</sup> fw on 5<sup>th</sup> day. UV+HO responded with the maximum total antioxidant capacity after 5 days of storage (737.8 mg AAE kg<sup>-1</sup> fw), nonetheless presented the higher decrease after 19 days (353.1 mg AAE kg<sup>-1</sup> fw). On the other hand, HO registered initial antioxidant activity of 495.4 mg AAE kg<sup>-1</sup> fw, yet showed the lowest reduction after 19 days (326.2 mg AAE kg<sup>-1</sup> fw). Control samples registered the greatest total antioxidant content (456.5 mg AAE kg<sup>-1</sup> fw) after 19 days. All treatments reduced the total amount of antioxidant capacity after 9 days, but subsequently, an increased was found after 15 days at 5°C. As main conclusion, although all treatments had a similar trend, the combination of NEW+UV-C+HO could be remarked the best treatment for keeping the total antioxidant capacity at 5°C.

### References:

- [1] Ragaert, P., *et al.* (2004), *Consumer perception and choice of minimally processed vegetables and packaged fruits*, Food Quality and Preference, 15, 259–270.
- [2] Artés, F., *et al.* (2009), *Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities*, Postharvest Biology and Technology, 51(3), 287-296.
- [3] Benzie, I.F.F., Strain, J.J. (1996), *The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”*: The FRAP assay, Analytical biochemistry, 239, 70-76.

# Anthocyanin content and radical-scavenging activity of strawberries, jams and red fruit juices: influence of storage conditions

**M.T. Soares<sup>1</sup>, L.F. Amaro<sup>2</sup>, C. Pinho<sup>1</sup>, I.F. Almeida<sup>3</sup> and I.M.P.L.V.O. Ferreira<sup>1</sup>**

<sup>1</sup> REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

<sup>2</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal

<sup>3</sup> Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Red fruits consumption has increased in recent years due to its polyphenolic composition and their possible health benefits as antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic. Anthocyanins are the most important group of polyphenols in red fruits, however, these pigments are not stable and change their composition by storage conditions and thermal processing [1].

The aim of the present work was to compare the degradation of anthocyanins of strawberries (cv. Camarosa) and strawberry jam, and their radical-scavenging activity. The stability of these pigments was evaluated through different storage conditions (time, temperature, and light). In addition, anthocyanin composition and radical-scavenging activity commercial red fruit juices with similar composition and different types of package were compared.

Qualitative and quantitative analysis of these pigments were achieved by high-performance liquid chromatography with diode array detection (HPLC-DAD). The radical-scavenging activity for all analyzed samples was evaluated with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and expressed as IC50.

The HPLC analyses of strawberry anthocyanin contents indicated a degradation of 15%, after two months of storage of at -8 °C. Strawberry jam samples (Gourmet commercial samples without additives addition) stored with and without light exposure at room temperature, during 9 months showed almost complete degradation of anthocyanins. Less degradation was observed in refrigerated samples store at 3°C. Strawberry jams store at -8 °C preserved their anthocyanin content, thus the storage temperature was the most relevant parameter to guarantee the stability of anthocyanin strawberry jam samples.

Commercial red fruit juices from both Tetrapack® package and glass bottle were analyzed. An efficient separation of 11 anthocyanin peaks was obtained by HPLC, and results demonstrated that qualitative and quantitative anthocyanin profile was similar. The presence of food additives guarantees the stability of these products regardless the type of package and the storage temperature.

Higher radical-scavenging activity was observed for strawberry fresh fruit than for strawberry jams. During storage the radical-scavenging activity of strawberry jams decreased, except for samples store at -8°C. Commercial red fruit juices showed higher antiradical activity compared with both fresh strawberries and strawberry jam. The possible explanation for these results could be due to the addition of ascorbic acid as antioxidant by food industry to delay the oxidation process.

References:

[1] Queiroz, F., Oliveira, C., Pinho, O., Ferreira, I.M.P.L.V.O. (2009), *Degradation of Anthocyanins and Anthocyanidins in Blueberry Jams/Stuffed Fish*, Journal of Agricultural and Food Chemistry, 57, 10712–10717.

# Standardization of antioxidant capacity assessment through microchemical methods using a novel kinetic matching approach

**M. A. Maia, L. M. Magalhães, L. Barreiros, S. Reis, M. A. Segundo**

REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal

The antioxidant capacity of food samples is commonly assessed by chemical methods based on electron transfer reactions, such as Folin-Ciocalteu (F-C), cupric ion reducing antioxidant capacity (CUPRAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS<sup>•+</sup>) assays. The major limitation of these assays relies on the fact that the antioxidant capacity values estimated depend on the analysis time selected because the oxidation kinetic behavior of samples are different from those obtained for classical standard compounds (Trolox or ascorbic acid). Thus, in order to attain reliable results for the total antioxidant capacity, the analytical measurements should be taken at endpoint conditions concerning the redox reaction established between sample components and oxidizing species. This issue implies that these procedures are time-consuming and not suitable for routine/screening purposes. Moreover, the standard compound used also limit the comparison of data between different methodologies and works [1,2].

In this context, we propose here a novel kinetic matching approach to foster rapid assessment of endpoint antioxidant capacity, associated to the conversion of results into equivalents of a common standard compound, as a universal way for expression of results. The methodology proposed was applied to methods based on different chemistries (Folin-Ciocalteu (F-C), CUPRAC, DPPH<sup>•</sup> and ABTS<sup>•+</sup> assays) and red wines ( $n = 40$ ) were chosen as a model of complex food sample. Results showed that, for all methods, there was no statistical difference between results attained by kinetic matching approach (after <10 min of reaction) and that at endpoint conditions (after 60 to 300 min). The repeatability and the reproducibility of the kinetic matching approach was <4.5%, for all antioxidant assays. The sample throughput increases from <18 (endpoint measurements) to >108 h<sup>-1</sup> using the proposed kinetic approach. Moreover, we have established here a way of converting results to equivalents of a single standard, providing values independent of its kinetic profile, by using the ratio between calibration sensitivities performed at endpoint conditions.

## References:

- [1] Magalhães, L.M., Segundo, M.A., Reis, S., Lima, J.L.F.C. (2008), *Methodological aspects about in vitro evaluation of antioxidant properties*, *Analytica Chimica Acta*, 613, 1-19.
- [2] Nenadis, N.; Lazaridou, O.; Tsimidou, M. Z. (2007) *Use of reference compounds in antioxidant activity assessment*, *Journal of Agricultural and Food Chemistry*, 55, 5452-5460.

## Acknowledgements:

L.M. Magalhães thanks FSE and MCTES for the financial support through the POPH-QREN program.

# On the development of reduced salt bread through the incorporation of aromatic herbs and spices

**I. Gomes<sup>1</sup> and L. M. Cunha<sup>2,\*</sup>**

<sup>1</sup>MSc Consumer Science and Nutrition, Faculty of Sciences and Faculty of Food and Nutrition Sciences, University of Porto, Portugal.

<sup>2</sup>REQUIMTE/DGAOT, Faculty of Sciences, University of Porto, Portugal

\*Corresponding author: lmcunha@fc.up.pt

One third of all global deaths can be attributed to cardiovascular disease (CVD). Elevated blood pressure is a major, modifiable, causal factor of CVD, and salt (sodium chloride) is the primary cause of raised blood pressure. The main source of salt in the diet is processed foods (about 70–75% of the total intake), with cereal and cereal products contributing 30% of overall intake. As relatively moderate restrictions in salt intake have the potential to reduce average blood pressure, reducing salt in bread formulations may substantially reduce the burden of morbidity and mortality from CVD at a population level [1]. The main objective of this work was to test the effect of incorporating different flavour enhancers in bread dough on final loaf quality. 24 different recipes were tested, through the combination of 3 salt levels: 4, 8 and 16 g of salt (NaCl) per kg of added flour and 8 extra ingredients intended for flavour enhancement [amount per kg of flour]: none - control, soya sauce [10 mL], olive oil [10mL], lemon juice [10 mL], thyme infusion [7.8 g / L of water – replaces water], thyme leaves [5 g], powdered garlic [2 g] or cinnamon [2 g]. Quality was evaluated in terms of texture – with an Universal Testing Machine (Instron) with a 25 N load cell and a 12 mm diameter flat plunger, compressing up to 40 % strain, a 25 mm thick slice of bread loaf, at a speed of 100 mm/ min, colour – with a colorimeter (Minolta CR400) and final volume – measure through displacement of whole flax seeds, and a consumer discussion group (n = 5) was setup to make an exploratory evaluation of the perceived quality. Results show that for the control formulation elasticity modulus and maximum stress at 25 % strain increased with decreasing salt content, which is in accordance with the literature [1]. Nevertheless, this effect is only replicated with the use of soya sauce, inverted with the use of lemon juice, presenting a central maximum when using thyme infusion and presenting a minimum value at the intermediate salt level (8 g / kg) for all the other recipes. Loafs with lower salt content had a whiter crumb (measured through  $L^*$ ). Darker loafs with less volume were achieved when using thyme (infusion or leaves) and cinnamon, while whiter loafs with more volume were obtained when using olive oil, soya sauce, or lemon juice. General appreciation reveals that the different ingredients used as flavour enhancers yield better results at the lowest salt level, when compared with the intermediate level. Use of thyme infusion is preferred over thyme leaves, both being described with herbal notes. Olive oil leads to a softer loaf and cinnamon to a sweet and clearly identifiable taste. Main conclusion of this exploratory work is that the majority of the enhancers yield promising results for the use as salt replacers in bread loaf formulation.

References:

[1] Lynch, E.J., Dal Bello, F., Sheehan, E.M., Cashman, K.D. and Arendt, E.K. (2009), *Fundamental studies on the reduction of salt on dough and bread characteristics*, Food Research International, 42 (7), pp 885-891.

# Effect of the baking process on DNA degradation of conventional and transgenic maize bread

**Telmo J.R. Fernandes, M. Beatriz P.P. Oliveira, Isabel Mafra\***

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto  
(isabel.mafra@ff.up.pt)

Maize plays a major role in nutrition in many countries all over the world and is the second most important transgenic crop with the highest number of authorised genetically modified (GM) events (21) for food and feed in the EU [1]. *Broa* is a Portuguese traditional maize bread highly consumed, especially in the north and central regions of Portugal, still playing an important economic and social role in rural communities of the country [2].

Despite the recognised advantages of DNA analysis, especially compared with protein-based techniques, they are currently developed only for raw plant materials. Food processing can affect DNA recovery and integrity, affecting the efficiency of (polymerase chain reaction) PCR amplification and, consequently, the estimation of GMO content. Cooking and baking are essentially heat processes that may break down DNA whereas the thermal treatment with temperatures over 200°C, considerably reduces the size of the extracted DNA fragments [3,4].

The main goal of the present work was to assess the effect of the baking process in the detection and quantification of GM maize in maize bread along the production. Three different maize breads were prepared in a bakery according to the traditional method of preparation. DNA was extracted using the Wizard method. Yield and purity of DNA were assessed by spectrophotometry, while amplifiability was evaluated by targeting the endogenous *invertase* gene of maize. Event and construction-specific PCR primers were used to detect MON810 and TC1507 events and two types of PCR assays were applied for each GM event tested: qualitative PCR for the specific detection and real-time PCR with TaqMan probes to quantify the GMO content.

The results revealed that it was possible to detect and quantify the maize *invertase* gene and the events MON810 and TC1507 along the production of maize bread and in all parts of the final cooked breads. However, as expected, some degradation of DNA was noticed, which might have contributed to the differences found between the estimated GM maize contents in two of the breads. These results represent a great achievement for the traceability of GMO in the food chain.

## References:

- [1] James, C. (2010). Global Status of Commercialized Biotech/GM Crops: 2010. *ISAAA Brief No.42*. ISAAA: Ithaca, New York.
- [2] Brites, C., Trigo, M. J., Santos, C., Collar, C., Rosell, C. (2010). *Maize-Based Gluten-Free Bread: Influence of Processing Parameters on Sensory and Instrumental Quality*, Food Bioprocess Technol, 3, 707–715
- [3] Engel, K-H., Moreano, F., Ehlert, A., Busch, U. (2006), *Quantification of DNA from genetically modified organisms in composite and processed food*, Trends Food Sci & Technol, 17, 490-497.
- [4] Gryson, N. (2009). *Effect of food processing on plant DNA degradation and PCR-based GMO analysis: a review*, Anal and Bioanal Chem, 396, 2003-2022.

Oral Sessions II > A5

# Engineering I



# Modelling and simulation of a catalytic reactor for hydrogen production via methane autothermal reforming

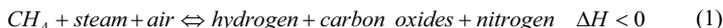
**C. Brandão<sup>1</sup> and Luis M. Madeira<sup>1,2</sup>**

<sup>1</sup> Chemical Engineering Department, Faculty of Engineering, University of Porto  
Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

<sup>2</sup> LEPAE - Laboratory for Process, Environmental and Energy Engineering, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

The interest in hydrogen has increased by the chemical, petrochemical and petroleum industries because of the escalation of global energy needs, which directs efforts to find alternative energies sources [1, 2].

Methane autothermal reforming (ATR), illustrated in equation 1, is the combination of steam reforming and partial oxidation. The combination of these two processes allows the use of enthalpy released by the oxidation reaction as a source of energy in the steam reforming reaction [3] so that the process can occur without external energy supply and with a better reactor temperature control. This way autothermal reforming systems can be more compact, and inherently more practical for small-scale and portable applications like fuel cells [2].



In this work a fixed bed reactor model has been developed and implemented in *Matlab* software for studying the methane autothermal reforming process. The influence of various parameters on the process efficiency has been studied, as well as on the temperature profile along the reactor length. Particular attention was given to the operation temperature and feed pressure effect in the methane conversion and on the species concentration profiles; the methane: oxygen: water ratio feed effect on products concentration and particularly on hydrogen production was also analyzed. The one-dimensional heterogeneous model (HT1) was studied using different approaches, with increasing level of complexity: i) isothermal and isobaric reactor with constant velocity and without axial dispersion; ii) isothermal and isobaric reactor with constant velocity and with axial dispersion; iii) non-isothermal and non-isobaric reactor with variable velocity and without axial dispersion; iv) non-isothermal, non-isobaric reactor with variable velocity and with axial dispersion.

Results obtained with the modeling work can be very useful for better understanding the process, and to better design methane ATR reactors for hydrogen production.

References:

- [1] Halabi, M.H., Croon, M.H.J.M. de, Schaaf, J. van der (2008), *Modeling and analysis of autothermal reforming of methane to hydrogen in a fixed bed reformer*. Chemical Engineering Journal, 137, 568-578.
- [2] Zhai, X., Ding, S., Cheng, Y., Jin, Y., Cheng, Y. (2010), *CFD simulation with detailed chemistry of steam reforming of methane for hydrogen production in an integrated micro-reactor*. International Journal of Hydrogen Energy, 35, 5383-5392.
- [3] Li, B., Maruyama, K., Nurunnabi, M., Kunimori, K., Tomishige, K. (2004), *Temperature profiles of alumina-supported noble metal catalysts in autothermal reforming of methane*. Applied Catalysis A:General, 275, 157-172 (2004).

## Purification Methods of Biodiesel from Animal Fats

T. M. Mata<sup>1a</sup>, A.C. Melo<sup>1</sup>, V.F.M. Silva<sup>2a</sup>, N. S. Caetano<sup>2\*</sup>

<sup>a</sup>LEPAE – Laboratory for Process, Environmental and Energy Engineering.

<sup>1</sup>Department of Chemical Engineering, Faculty of Engineering, University of Porto (FEUP), R. Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal.

<sup>2</sup>Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto (IPP), R. Dr. António Bernardino de Almeida, s/n, 4200-072 Porto, Portugal.

Recently, low-cost feedstocks for biodiesel, such as waste frying oils and animal fats [1,2], byproducts of the food-processing industries that cannot be used for human food purposes, have increasingly drawn interest. The traditional destination of animal fats was the production of soaps and pet food formulations [3]. However, as a result of bovine spongiform encephalopathy (BSE), part of these applications has been lost and newer alternative uses have been developed, such as converting them into biodiesel. This not only represents a new application for a lower cost and environmental friendly feedstock but also contributes for solving a waste management problem.

Beef tallow methyl esters (TMEs), pork lard methyl esters (LMEs), and chicken fat methyl esters (CMEs) were produced, purified, and characterized to evaluate their quality and compare two purification methods: (1) conventional neutralization, water washing, and drying and (2) purification using cationic exchange resins. The use of cationic exchange R for biodiesel purification claims to reduce the water consumption and avoid wastewaters, simplifying the process and reducing production costs. Also, B20 blends [20 % biodiesel (v/v) mixed with petroleum diesel] were characterized and evaluated. The conventional alkali-catalyzed transesterification process was used, with methanol as the reagent and KOH as the catalyst, yielding 76.8, 90.8, and 91.5 % (w/w) CME, TME, and LME, respectively. The ester content of these biodiesels was below 96.5 % (w/w), and the kinematic viscosity was high (ranging between 4.84 and 6.86 mm<sup>2</sup>/s), which poses restrictions to their use as fuel in vehicle engines, especially in low-temperature climates.

Although it is not possible to use 100% biodiesel produced from these animal fats, blends of 20 % biodiesel are viable with some advantages, such as the improved cold-flow properties [cold filter plugging point (CFPP) below 6 °C], lower kinematic viscosity (from 3.10 to 3.28 mm<sup>2</sup>/s), and higher heating value of the mixture (about 44.6 MJ/kg). Results also show that the resin purification helps to reduce biodiesel acidity and kinematic viscosity, while conventional water washing followed by adsorbent drying and filtration gives better results regarding water and alkaline metal (Na + K) content.

[1] Sabudak, T.; Yildiz, M. (2010), *Biodiesel production from waste frying oils and its quality control*. Waste Manage. 30, 799-803.

[2] Araújo, B. Q.; Nunes, R. C. R.; Moura, C. V. R.; Moura, E. M.; Citó, A. M. G. L.; Santos Júnior, J. R. (2010), *Synthesis and characterization of beef tallow biodiesel*. Energy Fuels 24, 4476-4480.

[3] Lefferts, L. Y.; Kucharski, M.; McKenzie, S.; Walker, P. (2007), *Feed for Food-Producing Animals: A Resource on Ingredients, the Industry, and Regulation*; The Johns Hopkins Center for a Livable Future, Bloomberg School of Public Health: Baltimore, MD.

## Treatment of textile effluents in a continuous packed-bed reactor by a Fenton-like process

**Isabel Mesquita<sup>1</sup>, Luís C. Matos<sup>1</sup>, Filipa Duarte<sup>1,2</sup>, F. J. Maldonado-Hódar<sup>3</sup>, Adélio Mendes<sup>1,2</sup>, Luis M. Madeira<sup>1,2</sup>.**

<sup>1</sup> Chemical Engineering Department, Faculty of Engineering, University of Porto  
Rua Dr. Roberto Frias, 4200-465 Porto, Portugal, <sup>2</sup> LEPAE- Laboratory for Process, Environmental and Energy Engineering, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal, <sup>3</sup> Department of Inorganic Chemistry, Faculty of Sciences – University of Granada, Spain.

The quality of water is of great concern in modern society. In order to achieve the legal standards that allow its reutilization or discharge, some treatment methods must be used. The synthetic dyes used in textile and food industries cause visual pollution and changes in biological cycles, affecting the photosynthesis process [1].

The Advanced Oxidation Processes (AOP) have shown to be excellent methods for treating dye-containing wastewaters. One of these AOPs is the Fenton's reagent, which has advantages such as high efficiency in discoloration, simplicity in the oxidation of a wide range of contaminants, being also simple and inexpensive [2]. This method is based on the generation of hydroxyl radicals (HO<sup>•</sup>) that attack non-selectively numerous organic molecules. The radicals are produced by the catalytic decomposition of H<sub>2</sub>O<sub>2</sub> by a transition metal like Fe (II) ions in acid medium. However, strategies are required for recovering the iron catalyst, which should not be present in the final effluent. One approach is to use supported catalysts, also allowing to operate continuously (e.g. in a packed-bed reactor).

In this work, oxidation with a Fenton-like process of a dye solution was carried out in an open packed-bed. The column was provided with a water jacket and the solutions (dye and H<sub>2</sub>O<sub>2</sub>) were stored in a thermostatic bath for a better temperature control. Activated carbon Norit RX 3 Extra was impregnated with ferrous sulphate and used as catalyst (7 wt.% of iron) [2]. The decolourisation of the model compound used (azo-dye Chicago Sky Blue - CSB) was followed continuously by spectrophotometry at the column outlet; mineralization was assessed by analysis of the total organic carbon (TOC), while the degree of leaching was determined from the effluent iron concentration by atomic absorption spectrophotometry.

The effect of the main operating conditions was analyzed in detail. In particular, the increase of the temperature leads to a higher removal of the dye and an increased mineralization. On the other hand, it also increases the iron leaching, but the values observed at the reactor outlet were below 0.4 ppm, thus complying with EU limits of 2 ppm. It was possible to reach, at steady-state, a dye conversion of 88 %, with a TOC removal of ca. 45 %, being the reactor operated at 50 °C, pH = 3,  $W_{\text{cat}}/Q = 4.1 \text{ g} \cdot \text{min} \cdot \text{mL}^{-1}$  ( $W_{\text{cat}}$  is the mass of catalyst used and Q the total feed flow rate) and a H<sub>2</sub>O<sub>2</sub> feed concentration of 2.25 mM (for a CSB feed concentration of 0.012 mM). The same performance was reached in three consecutive cycles, demonstrating a promising stability of the packed bed reactor.

[1] Kunz, A., Peralta-Zamora, P., Moraes, S.G. and Durán, N., "New tendencies on textile effluent treatment", *Química Nova* 25 (2002) 78-82.

[2] F. Duarte, F.J. Maldonado-Hódar, A.F. Pérez-Cadenas, L.M. Madeira, Fenton-like degradation of azo-dye Orange II catalyzed by transition metals on carbon aerogels, *Appl. Catal. B* 85 (2009) 139-147.

# Functionalized silica nanoparticles with thermo- and photochromic dyes for textile application

**L.S. Ribeiro<sup>1</sup>, T. Pinto<sup>2</sup>, A. Monteiro<sup>2</sup>, O.S.G.P. Soares<sup>1</sup>, C. Pereira<sup>2</sup>,  
C. Freire<sup>2</sup>, M.F.R. Pereira<sup>1</sup>**

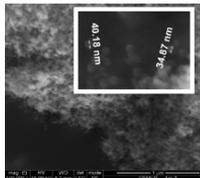
<sup>1</sup>Laboratory of Catalysis and Materials (LCM), Associated Laboratory LSRE/LCM, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup>REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

The coating of fabrics with nanoparticles is a promising technology for the textile industry in order to improve the performance and functionality of textiles, originating products able to react to different conditions. Nanotechnology allows the incorporation of new features providing highly durable products, relatively to conventional methods. Materials functionalized with thermo- and photochromic dyes have attracted considerable attention in both science and industry since their incorporation onto fabrics can impart fancy colour-changing effects and sensing environmental changes to the fabrics [1].

In this work, silica nanoparticles were functionalized with thermo or photochromic dyes by two different strategies: post-grafting and *in situ* co-condensation [2]. Different types of silica nanomaterials were prepared under alkaline conditions in the presence of a surfactant, functionalized with an organosilane and immobilized into textile substrates.

The morphology, particle size, chemical composition and textural properties of the silica particles were characterized by SEM, N<sub>2</sub> adsorption-desorption isotherms at -196 °C, FTIR and TG/DSC. The silica nanoparticles prepared with and without dye present spherical shape and diameters of 20-40 nm (by SEM, Fig. 1). From nitrogen adsorption-desorption at -196 °C a type IV isotherm with a clear hysteresis characteristic of materials with cylindrical pores is observed.



The vibration bands due to the organic component of the functionalized silica are clearly seen in the IR spectra. The decomposition temperatures vary from 283 to 328 °C and the weight losses are in the range of 10-70%. The prepared materials were successfully immobilized into textile substrates and present colour changes when submitted to different conditions, and some resistance to washing.

Fig.1: SEM micrograph of a sample synthesized with surfactant and organosilane.

## Acknowledgments:

Financial support was in part provided by FEDER (Portugal) and FCT, through project PTDC/CTM/108820/ 2008, and by Projecto de Investigação Científica na Pré-Graduação 2010, U.P. and Santander Totta. A.M and C.P acknowledge their grants by FCT. The authors thank Horquim for supplying some of the dyes used in this work.

## References:

- [1] Cheng, T., Lin, T., Brady, R. and Wang, X. (2008), *Photochromic Fabrics with Improved Durability and Photochromic Performance*, *Fibers and Polymers*, 9 (5), 521-526.
- [2] Kobler, J., Möller, K. and Bein, T. (2008), *Colloidal Suspensions of Functionalized Mesoporous Silica Nanoparticles*, *ACSNano*, 2 (4), 791-799.

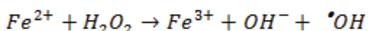
# Modelling of the Fenton Process for the Degradation of Paraquat Pesticide in Water with Artificial Neural Networks

**Nuno Cardoso, Mónica Santos, Arminda Alves, Luís M. Madeira and Fernando Martins**

Chemical Engineering Department, Faculty of Engineering, University of Porto, Portugal.

With the introduction of intensive agriculture, man needed to better protect their crops, so they started using large amounts of pesticides and herbicides, such as paraquat. This component isn't innocuous to human health and can be accumulated in water courses. Efficient methods are therefore required to eliminate paraquat (and other pesticides) from water / wastewater.

The advanced oxidation processes, such as the Fenton one, are able to eliminate the pesticides. During the Fenton's process the organic compounds are oxidized by the hydroxyl radical species formed in the reaction between hydrogen peroxide and a ferrous salt (catalyst):



The hydroxyl radicals react in a non selective way with the organic compounds, oxidizing them to less threatening compounds.

The modelling of this process is very important, e.g. for determining the best conditions to perform the oxidation. The phenomenological modelling approach is very complex, having too many mathematical expressions (e.g. system of dozens of differential equations for a batch reactor operation) and variables. To mitigate this problem, this work presents the application of Artificial Neural Networks (ANN) as a modelling tool. An ANN is a mathematical structure that imitates the biological neural system. This tool can learn from the provided data, by training the network, yielding a model that describes the process. With this computational methodology only input/output data is needed (for the present case temperature, initial concentration of the oxidant -  $H_2O_2$  -, initial concentration of the catalyst - ferrous salt -, initial concentration of the pollutant - paraquat -, pH and time of reaction as inputs, and the concentration of the pollutant as function of the time as output). Usually, the ANN structures comprise three layers (a first layer receiving the input data, an intermediate/hidden layer with several nodes capturing the non-linear characteristics of the phenomena and an output layer that predict the output variables).

In this work it was studied the effects of the number of nodes and their transfer functions of the intermediate layer to achieve the ANN with the best performance. The Neural Network Toolbox of the Matlab was used for these calculations.

It was concluded that the ANN modelling technique is able to obtain models to predict the concentration of paraquat as function of the time for experiments carried out in a batch reactor for different experimental conditions with good agreements.

## Immobilization of commercial laccase on modified spent grain

**A.M. Silva<sup>1</sup>, A.P.M. Tavares<sup>1</sup>, C. Rocha<sup>2</sup>, R. Cristóvão<sup>1</sup>, J.A. Teixeira<sup>3</sup> and E.A. Macedo<sup>1</sup>**

<sup>1</sup>Laboratory of Separation and Reaction Engineering (LSRE), <sup>2</sup>REQUIMTE, Department of Chemical Engineering, FEUP, Portugal

<sup>3</sup>IBB, Centre of Biological Engineering, University of Minho, Portugal

Enzymes are biocatalysts with excellent properties such as high activity, selectivity and specificity, which may permit to perform the most complex biochemical processes. Immobilization is the most important technique used as powerful tool to improve almost all the enzyme properties and reduction of inhibition. Laccases (EC 1.10.3.2) belong to multi copper oxidases that can catalyse the oxidation of various phenolic substrates. Due to their capability of oxidation catalysis, laccases are attracting increasing attention as potential industrial enzymes in various applications such as decontamination of environmental toxic compounds. Spent grain (SG) consists on rests of cereals and is the most important by-product of the brewing industry, which makes it economically attractive to use as a carrier. In Portugal, SG is a disposal material of the Unicer group (beer producer) with no associated costs. Laccase immobilization on spent grain can enhance the roles of enzymes in industry while opening the door for novel applications.

Commercial laccase from Novozymes was immobilized on SG using two strategies: adsorption and covalent binding (by adding glycidol (GSG) and ethylenediamine (EAGSG) with chemically modified SG or digested SG (DSG)). Several conditions of enzyme immobilization (pH, contact time, enzyme concentration) on the different carriers were optimised using ABTS as substrate, and the potentiality of the biocatalyst sets was also characterized.

The optimal immobilization conditions were: pH 7.0, laccase concentration 0.005 g/mL and contact time 3.5 h for SG; and pH 10.0, contact time 24h at 4 °C for DSG. Enzyme immobilization on DSG led to the best enzyme activities, better than the immobilization on SG or modified DGS as can be seen in Fig. 1. This can be explained by the changes on the molecular structure of the support, as confirmed by FTIR analysis.

Thermal stability presented a good stability for SG at 70°C. On the other hand, DSG has a higher operational stability than SG along 10 reaction cycles. DSG also remains stable when stored for large periods (47 days).

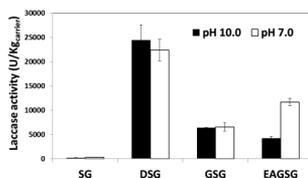
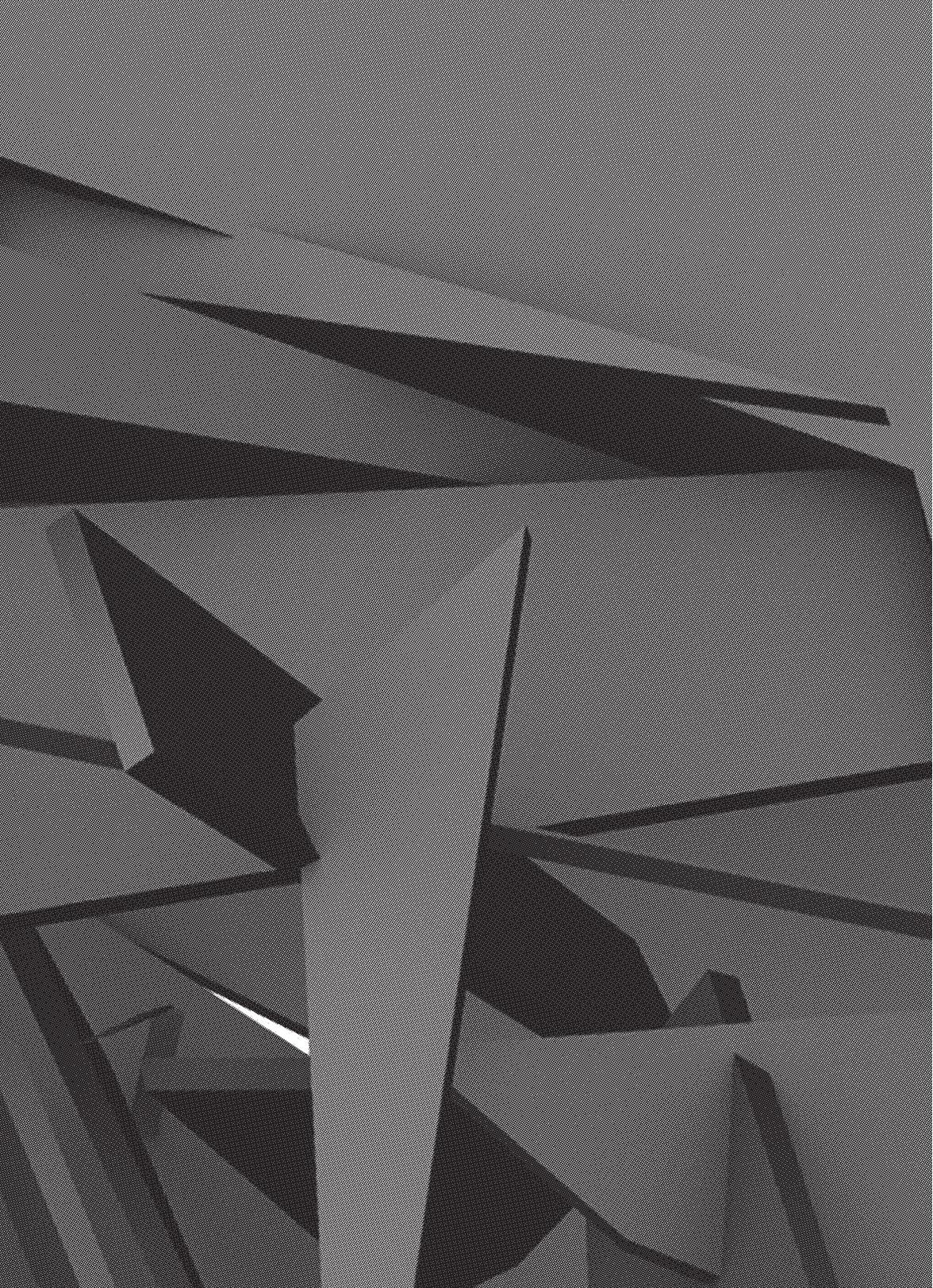


Figure 1 - Effect of spent grain modification: DSG, GSG and EAGSG on laccase activity.

### Acknowledgments:

A. Silva thanks FCT for the Scholarship (BII/LAB/0020/2009).





# Oral Sessions III



Oral Sessions III > A1

# Clinical Care



# **Efficacy of progressive muscle relaxation technique in chronic musculoskeletal pain and depression in institutionalized elderly adults**

**F. I. Nakau<sup>1</sup>, A. A. Peterson<sup>2</sup>, C. B. dos Santos<sup>3</sup>, E. C. de Carvalho<sup>4</sup>**

<sup>1</sup> Undergraduate student (BSN) at University of São Paulo at Ribeirão Preto College of Nursing (EERP-USP). Scientific Initiation Scholar – National Council for Scientific and Technological Development (CNPq).

<sup>2</sup> RN.<sup>3</sup> Associate Professor at EERP-USP.<sup>4</sup> Full Professor at EERP-USP.

Chronic pain is common in institutionalized people, preventing the accomplishment of tasks, causing physical discomfort and depression [1]. This study assessed whether the Progressive Muscle Relaxation Technique (PMRT), modified [2] is effective for reducing chronic musculoskeletal pain and depression in institutionalized elderly adults.

All residents of a long-term institution, older than 60 years, not bedridden, who reported chronic musculoskeletal pain and depression, without sign of dementia and clinical conditions to take part in the study were invited to participate. They were informed about the study and invited to take the Mini Mental State Examination, in the absence of signs of dementia, the Scale of Yesavage was applied. Subjects with signs of depression participated in the study, and were assessed with the Faces Pain Rating Scale, ECOG and physiological parameters. In follow, the RMP was performed, followed by assessments of pain, depression, and physiological parameters.

Of the 63 elderly adults, 15 subjects were included, with an average age of 74.13 years, 60% male. The differences in vital signs (blood pressure, pulse rate and respiration) between the pre and post-relaxation stages were not statistically significant, except the temperature ( $p=0.026$ ), which increased after the technique, data which is compatible with data found in literature [3]. Muscle tension at different locations showed no differences. Pain ( $p=0.008$ ) and depression ( $p=0.003$ ) showed a statistically significant reduction in their average values after relaxation. Our findings corroborate the results obtained by Paula and Carvalho, who also found reduction in the level of pain. Arnsstein studies (2000) [1] observed that severe chronic pain contributes to the development of depression. Because of pain, patients end up triggering severe stress, suffering and loss in the quality of life.

The Progressive Muscle Relaxation Technique used was effective in reducing chronic musculoskeletal pain and depression in studied institutionalized elderly adults.

## References:

- [1] Arnstein, P. (2000), *The mediation of disability by self efficacy in different samples of chronic pain patients*. Disability & Rehabilitation, 22(17), 794-801.
- [2] Paula, A. A. D. D., Carvalho, E. C. D. and Santos, C. B. D. (2002), *The use of the "Progressive Muscle Relaxation" technique for pain relief in gynecology and obstetrics*. Ribeirão Preto: Rev. Latino-Am. Enfermagem, Sept./Oct, 2002, 10(5).
- [3] Nosow, V. and Peniche, A. C. G. (2007), *The effect of calatonia on anxiety level of surgical outpatients*. São Paulo: Acta paul. Enfermagem, Apr./June 2007, 20(2).

## Health Promotion Lifestyle Profile II Scale (HPLP-II) Portuguese version

**F. Pires<sup>1</sup> T. Martins<sup>2</sup> F. Araújo<sup>3</sup>**

<sup>1</sup> Department of Master of Science in Nursing, Faculty of ICBAS, University of Porto, Portugal.

<sup>2</sup> Department of Nurse, Faculty of ESEP, University of Porto, Portugal

<sup>3</sup> Department of Nurse, Faculty of ESEP, University of Porto, Portugal

In order to enjoy a longer and healthier life it is imperative to adopt and promote appropriate lifestyles [1]. In the last years, efforts focusing the evaluation of population lifestyles and promotion of public health have increased and instruments of research, particularly in nursing, have become important tools to assess them. In Portugal, there are not many reports of such research interventions performed by nurses to nurses.

The aim of this study is to improve the instruments of research, contributing to the creation of tools that will guide health professional practices.

This research provides a methodological study, based on the translation and validation of the Health Promotion Lifestyle Profile II scale (HPLP-II) from Walker, Sechrist and Pender (1987) for the Portuguese population. The studied population was first and third years students, of Nursing and Engineering courses, from Porto and Funchal.

The translation process has been completed with the approval of the authors of the scale, and at this moment we are in the process of data collection.

We propose to present the preliminary data of the study.

[1] Walker, S. N. (2012), *web site of University of Nebraska Medical Center: Profile Susan Walker, EdD, RN, FAAN.*

Link: [[http://app1.unmc.edu/nursing/conweb/view\\_profile.cfm?lev1=facstf&lev2=fac&lev3=facswalker&PubStat=%28none%29&web=pub](http://app1.unmc.edu/nursing/conweb/view_profile.cfm?lev1=facstf&lev2=fac&lev3=facswalker&PubStat=%28none%29&web=pub)]

# Translation and cultural adaptation of the Portuguese version of Patient Assessment of Chronic Illness Care: PACIC-Portugal

**C. A. P. Landim**<sup>1</sup>, **M. M. Martins**<sup>2</sup>, **C. Paúl**<sup>3</sup> and **C. R. S. Teixeira**<sup>4</sup>

<sup>1</sup> Institute of Biomedical Science Abel Salazar, University of Porto, Portugal.

<sup>2</sup> Coordinator Professor of Higher School of Nursing of Porto, Portugal.

<sup>3</sup> Department of Behavioural Sciences, Institute of Biomedical Science Abel Salazar, University of Porto, Portugal.

<sup>4</sup> Associate Professor of Department of General and Specialized Nursing, University of Sao Paulo School of Nursing, Ribeirao Preto, Sao Paulo, Brazil.

**Introduction:** The Chronic Care Model (CCM), developed by Wagner *et al.* [1], at MacColl Institute for Healthcare Innovation at Group Health Research Institute, Seattle, Washington, United States, is one approach to improving chronic illness care, including diabetes *mellitus* (DM), that is being used increasingly to assess and improve care. The CCM is based on evidence-based practices and reviews of the literature on effective care. The Patient Assessment of Chronic Illness Care (PACIC) is a 20-item questionnaire assessing the implementation of the CCM from the patient perspective that focuses on the receipt of patient-centered care and self-management behaviors [2].

**Objective:** To translate and conduct the cultural adaptation of the Portuguese version of the PACIC in Portuguese patients with DM.

**Methods:** Methodological study, whose adaptation process cultural is being included: forward translation, expert committee, back-translation and cognitive interview [3]. The study will address a sample of 150 Portuguese patients with DM, according to inclusion/exclusion criteria, between February, March and April 2012, in a Endocrinology Service of a University Hospital in Porto, Portugal in 2012.

**Results:** It is expected that the former will reveal good acceptance of the translated version of the instrument, which participants will consider items of easy understanding. After analysis of the psychometric properties and completion of the validation process, the instrument will become available to Portuguese researchers, enabling its comparison with other cultures.

**Conclusions:** It is expected that a culturally adapted instrument in the Portuguese context with a focus on quality of care will be able provide support for health staff and managers in the planning the health care of patients with chronic illness, especially DM.

**Keywords:** Chronic Disease. Diabetes Mellitus. Validation Studies.

## References:

[1] Wagner, E. H., *et al.* (2001), *Quality improvement in chronic illness care: A collaborative approach*. Joint Commission Journal of Quality, 27 (2): 63-80.

[2] Glasgow, R. E., *et al.* (2005), *Development and validation of Patient Assessment of Chronic Illness Care (PACIC)*. Medical Care, 43 (5): 436-444.

[3] World Health Organization (WHO) (2011), *Management of substance abuse: process of translation and adaptation of instruments*. Geneva: WHO, 2011. Available at: [http://www.who.int/substance\\_abuse/research\\_tools/translation/en/](http://www.who.int/substance_abuse/research_tools/translation/en/). Accessed Dec 22, 2011.

## **What is the relevance of percutaneous endoscopic gastrostomy on the survival of patients with amyotrophic lateral sclerosis?**

**M. J. Pena<sup>1</sup>, P. Ravasco<sup>2</sup>, M. Machado<sup>2</sup>, A. Pinto<sup>3</sup>, S. Pinto<sup>3</sup>, L. Rocha<sup>3</sup>, M. de Carvalho<sup>3,4</sup> and H. C. Pinto<sup>2</sup>**

<sup>1</sup>Student at Faculty of Nutrition Sciences of University of Porto, Portugal.

<sup>2</sup>Nutrition and Metabolism Unit, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Portugal.

<sup>3</sup>Neuromuscular Unit, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Portugal.

<sup>4</sup>Institute of Physiology, Faculty of Medicine, University of Lisbon, Portugal.

**Introduction:** Percutaneous endoscopic gastrostomy (PEG) is a standard procedure for feeding dysphagic amyotrophic lateral sclerosis (ALS) patients. Nevertheless, the effect of prognostic factors influencing survival after PEG remains unclear. The aim of the study is to evaluate the prognostic value of several clinical features on survival after PEG placement.

**Methods:** This study investigated 151 patients with ALS, in whom a PEG was inserted over the last 16 years in our Center. Survival curves were determined by Kaplan-Meier and the analysis of potential prognostic factors was performed by Cox Regression Model.

**Results:** The overall median survival was 32 months, longer in spinal-onset disease patients: 42 vs 29 months in bulbar-onset patients ( $p < 0.001$ ); median survival after PEG placement was 8 months, similar in both bulbar- and spinal-onset patients, 7.9 vs 7.1 months, respectively. Thirteen percent of patients died within one month after PEG placement; this short-term survival was influenced by low forced vital capacity (FVC < 50%). In a multivariate analysis, only older age at disease onset was independently associated with poor outcome after PEG placement.

**Conclusions:** Survival after PEG placement was similar in bulbar and spinal-onset patients, suggesting that the latter were in a more advanced stage at the time of PEG placement. Low FVC was associated with higher risk of short-term mortality. Older age at disease onset was associated with poorer outcome in bulbar-onset patients. Younger bulbar-onset patients are those who benefited most from PEG.

# Bringing the Portuguese Primary Care Health Records to the Cloud using ASTM E2369-05 Continuity of Care Record

Alexandre Santos<sup>1</sup>, Ricardo Correia<sup>1</sup>

<sup>1</sup> Department of Health Information and Decision Sciences, Faculty of Medicine, University of Porto, Portugal.

The aim of this work is to integrate the clinical information stored in each Portuguese NHS Primary Care Centre into a wide network, building a tool that can export and import the clinical information to a XML document according to the ASTM E2369-05 Continuity of Care Record Standard and display the information on browser in a readable way using a XSLT style sheet. Each Primary Care Centre stores their patients' medical records on Oracle databases. These records include information such as their immunizations, prescriptions, medical exams and other data regarding primary care. This information is only available in the Centre where it is stored and there is no integration with other centres, hospitals or other healthcare providers. Although there is a network that interconnects all these facilities, there is no integration or interoperability of these systems.

The method chosen was to export the clinical information stored in the database to a XML document instead of creating database links between sites. Using this approach, the database creates a XML snapshot of the patient's health situation, making it possible to use this information on a multitude of systems, such as Personal Health Records, and storing it even on the patients' usb drives or mobile devices. To assure interoperability, it was chosen a standard that maps all the information regarding primary care on a XML file - the ASTM E2369-05. The clinical concepts and semantics stored on the databases are mapped to the XML elements described on the ASTM E2369-05 documentation and XSD. The Portuguese Primary Care Centres store their information on Oracle 7.3 and 9.0 databases that are shipped with JVMs, versions 1.1 and 1.2. To minimize costs and be able to run this tool, we have chosen to use Java 1.2 as the programming language and store all the code on the 9.0 database itself, running it on the embedded JVM from the Oracle database. The export tool is called from a PL/SQL stored procedure that is visible to the web by the configuration of a Database Access Descriptor and the use of the `mod_plsql` module through an Apache HTTP Server.

The ASTM E2369-05 can map all information that is stored in Portuguese Primary Care Centres databases. The Java version available on the Oracle database made the use of APIs and tools available freely on the Internet for producing and parsing XML files impossible, leaving no alternative other than the development of the tools from scratch.

Despite the antique versions of the Oracle databases and the JVM available on the Oracle database it is possible to generate XML files according to the Continuity of Care Record Standard. The use of Java as programming language makes possible the use of the Oracle server itself as an application server, allowing the migration of this solution to other platforms and the expansion of its capabilities. The use of a XSLT style sheet gives us the opportunity to display the PHR on a web browser making it humanly readable. Such work allows the recovery of clinical data that is stored on servers running databases from the late 80's making it available to a variety of health systems, from central repositories in large hospitals to personal-health records websites.

## Risk factors associated with one-year mortality of extremely premature newborns in Portugal

**A. Januário<sup>1,2</sup>, S. Gouveia<sup>2</sup>, J. Pinto da Costa<sup>1,2</sup>, M. I. Sá<sup>3</sup>, A. Almeida<sup>3</sup>,  
C. Carvalho<sup>3</sup>, J. P. Saraiva<sup>3</sup>, M. Fonte<sup>3</sup>, P. Soares<sup>3</sup>**

<sup>1</sup> Departamento de Matemática, Faculdade de Ciências, Universidade do Porto, Portugal

<sup>2</sup> Gabinete de Estatística, Modelação e Aplicações Computacionais, CMUP, Portugal

<sup>3</sup> Unidade Maternidade de Júlio Dinis – Centro Hospitalar do Porto (MJD), Portugal

The decreasing mortality rate of premature newborns results from recent improvements in perinatal obstetric and neonatal care. This study aims to identify the risk factors associated with one-year mortality in Portuguese extremely premature newborns (<27 weeks of gestational age, when average is 40). The data was collected from 205 cases followed up at MJD from 2000 to 2009. **In this preliminary study**, the mortality odds ratio was estimated for each protocol related factor from binary logistic regression (Table 1, [1]). Simple regression **(a)** indicated that lower mortality is associated with use of Antenatal Steroids and non-Vaginal Delivery, in accordance with [2]. In Portugal, non-Intubation and Birth at MJD also diminished the risk of death. Because Gestational Age (GA) determines the protocol procedures, the OR was adjusted for GA **(b)** and, of all factors, only Birth at MJD was related with a lower mortality risk. Birth at MJD continues to be significant in the multivariate analysis **(c)**, besides non-Intubation and increasing GA with OR 0.37(0.25,0.55). This result indicates that even when adjusted for the use of protocol interventions and GA, birth at MJD is still associated with a reduced risk, implying that the survival advantage of birth at MJD was not associated with the studied factors and remains to be explained. Updated information on premature Portuguese newborns is expected from the results of this study, with inherent repercussion in clinical practice.

**Table 1:** Mortality odds ratio (OR) and 95% Confidence Intervals for each protocol related factor, where OR<1 indicates decrease risk of mortality.

	All n=167*	Dead n=87	(a) Crude	(b) Adjusted	(c) Multivariate
Pregnancy Surveillance	155	79	<b>0.52</b> (0.15,1.8)	<b>1.12</b> (0.27,4.6)	-----
Antenatal Steroids	142	68	<b>0.29</b> (0.11,0.77)	<b>0.42</b> (0.14,1.24)	-----
Vaginal Delivery	71	45	<b>2.23</b> (1.19,4.17)	<b>1.15</b> (0.55,2.39)	-----
Iatrogenic Delivery	20	10	<b>0.91</b> (0.36,2.31)	<b>1.13</b> (0.4,3.16)	-----
Intubation	119	68	<b>2.04</b> (1.03,4.03)	<b>2.04</b> (0.96,4.33)	<b>2.27</b> (1.04,4.99)
Surfactant	150	81	<b>2.15</b> (0.76,6.12)	<b>2.34</b> (0.75,7.28)	-----
Birth at MJD	146	70	<b>0.22</b> (0.07,0.68)	<b>0.23</b> (0.07,0.77)	<b>0.21</b> (0.06,0.71)
Epoch (2007-2009)	36	21	<b>1.22</b> (0.53,2.82)	<b>1.17</b> (0.45,3)	
(2003-2006)	73	35	<b>0.8</b> (0.4,1.6)	<b>0.85</b> (0.4,1.81)	-----
(2000-2002)	58	31	<b>1</b> [Reference]	<b>1</b> [Reference]	

Binary Logistic Regression: (a) simple; (b) adjusted for GA; (c) multivariate with stepwise procedure including GA; \* =205 cases excluding stillbirths, delivery room death and missing values.

### References:

- [1] Hosmer, D. and Lemeshow, S. (2000), *Applied Logistic Regression*, John Wiley and Sons.  
 [2] The Express Group Members (2009), *One-Year Survival of Extremely Preterm Infants After Active Perinatal Care In Sweden*, JAMA, 301(21), 2225-2233.

Oral Sessions III > A2

# **Psychology & Education Sciences III**



## **Deaf Space: education for which Urbanity? – Paths of investigation**

**Tiago Salge Araújo**<sup>1</sup>

<sup>1</sup>Faculty of Psychology and Education Science of University of Porto - FPCEUP

“Space”, beyond being just the essential concept and analysis category for Geography, is above all a dimension of our lives that take us directly to the sensations, experiences and senses dimension. When we think about “space” we are inevitably tempted to think about its subjective (re) production and, even more than that, about its (re) interpretation. The daily “space”, although it might not seem to be, is not static and fixed; the way we understand it is determined by our experiences, and by what we know and believe. The interpretation of the living space and of its indissociable pair, time, is also related to our senses: vision, olfaction, hearing, tact and taste; it is impossible to not associate time-space to certain smell, sounds, tastes, textures, colors.

Therefore, we start discussing the role of the “space”, especially how it is related with the autonomy and empowerment of the deaf person, the responsibility of the parents and professionals and which spaces emerge from this rhizome. In general terms, what guide us in this research can be expressed through the following questions: how the deaf persons build their conception of time and space? How exclusive and particular is this specific interpretation of time and space? Which “spaces” are created from their necessities?

Those are some of the questions we try to answer in our research, developed as a master thesis, and here we will explore some of its theoretical and methodological aspects, mainly the narrative-biographical interviews, in order to discuss and understand the differences of time-space perceptions among the deaf persons, what create and (re) invent spaces and “urbanities” according to their needs, either in the school or in the cities, for example.

# The transition to a management function: Experiences of socialization and occupational stress in new position

**D. Rodrigues<sup>1</sup> and F. Jordão<sup>2</sup>**

<sup>1,2</sup> Psychology Centre of Porto University, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

The transition to a new management role often leads to feelings of insecurity and occupational stress (Uen, Wu & Huang, 2009), which can be minimized by the organizational socialization processes adopted (Chiavenato, 2004). We have developed an investigation with the main objective to describe the organizational socialization process and the occupational stress experience of individuals who moved recently to a management function in a specific organization. In a more specific way, we intend to answer to the main following research questions: How are characterized the organizational socialization process of the transition to a management role, in the organization under study? What are the events that induce job stress of individuals who have recently taken a management function in the same organization?

We have developed a qualitative study, using the method of multiple cases study (Yin, 2009) and the chosen technique of data collection was the interview in the semi-structured form in which is used the critical incident technique (Flanagan, 1954).

The results show that there is no socialization tactics implemented by this organization and in the description that the participants make of their experience of socialization they highlight especially the importance of interpersonal relationships. Participants related the difficulty that involves managing a team of employees of different ages and the less positive reaction of the team about the transition to a management role of an employee. Participants also emphasized the importance of developing new competencies, such as technical competencies and management skills that allow them to adequately perform their new role. With regard to the occupational stress, the main sources of stress identified by these individuals are related to the accomplishment of new tasks and deadlines. At an organizational level, the participants highlight consequences related to the organization and quality of work and at an individual level, they highlight learning, motivation but also headaches and fatigue.

We propose for future research, the study of socialization experiences in other organizations and the development of knowledge about occupational stress associated with specific situations of work role transitions.

## References:

- [1] Uen, J., Wu, T & Huang, H. (2009). Young manager's interpersonal stress and its relationship to management development practices: an exploratory study. *International Journal of Training and Development*
- [2] Chiavenato, I. (2004). *Gestão de Pessoas* (2ª Ed.). Rio de Janeiro: Elsevier Editora
- [3] Yin, R. K. (2009). *Applications of Case Study Research* (4th Ed). Thousand Oaks, California: SAGE Publications Inc.
- [4] Flanagan, J. C. (1954). The critical incident technique. *Psychological bulletin*, 51(4), 327-358.

# The work-family articulation experienced by Portuguese women managers: a source of occupational stress?

**M. Gonçalves<sup>1</sup> and F. Jordão<sup>2</sup>**

<sup>1,2</sup> Psychology Centre of Porto University, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Actually men and women face the challenge of balance work and family domains in a satisfactory manner, which has been difficult due to several changes, occurred in families and to increasing demands in work domain [1,2]. Management is the professional sector that is more likely to induce stress associated to work-family articulation in their professional's cause of the demands and characteristics of this activity [3].

The main goal of this study is to understand how woman managers live work-family articulation, which is translated into the following research questions: (1) Is management perceived as a stressful function? (2) Is work-family articulation perceived as an occupational stressor by female managers? If so, which management characteristics are considered by these female managers which influence positively and negatively this articulation? (3) What models of work-family articulation are used by female managers and what strategies they use to manage these domains? We have developed a descriptive, exploratory study with a qualitative approach [4] using the method of multiple cases [5] The semi-structured interview was the technique used for collecting information and the content analysis the technique to analyze data from interviews [6]. The results of this study show that these female managers perceived their function as stressful and that work-family articulation is understood as a source of occupational stress in which workload is the characteristic that has more influence on that articulation. Female managers live their relationship between these two domains through spillover and use the family support as a main strategy to manage the family with the professional sphere. We propose studies with a larger number of cases for a deeper analyze about the work-family articulation, both in female and male managers in Portugal. We also suggest the development of studies about the real effectiveness of "family friendly" policies of human resources management.

## References:

- [1] Roos, P., Trigg M. & Hartman M. (2006). Changing families/changing communities. Work, family and community in transition. *Community, Work and Family*, 9, 2, 197-224.
- [2] Dolcos, S. & Daley (2009). Work Pressure, Workplace Social Resources, and Work-Family Conflict: The Tale of Two Sectors. *International Journal of Stress Management*, 16, 4, 291-311.
- [3] Knudsen, K. (2009). Striking a different balance Work-family conflict for female and male managers in a Scandinavian context. *Gender in Management: An International Journal*, 24, 4, 252-269.
- [4] Sampieri, R. H., Collado, C. F. & Lucio, P. B. (2006). Metodologia de pesquisa (3ª Edição). São Paulo: McGraw-Hill.
- [5] Yin, R. K. (2009). *Case study research. Design and methods* (4th Ed). Thousand Oaks, California: SAGE Publications, Inc.
- [6] Bardin, L. (1977). *Análise de Conteúdo*. Lisboa: Edições 70.

# The influence of athletes' motivational goals on pre-game beliefs

Magalhães, C.<sup>1</sup> and Lemos, M. S.<sup>1</sup>

<sup>1</sup> Faculty of Psychology and Education, University of Porto

In the area of motivation on achievement contexts, there is a lively debate about the debilitating effects or overcoming in competition situations. The study and analyze of these effects becomes essential to understand the psychological processes involved in moments of evaluation and competition.

Most of this debate and research focuses on the school context, however, also in sport, the athletes are confronted daily with feelings of success and failure. In fact, the slogan of the Olympic Games (*Citius, Altius, Fortius* - "The faster, higher, stronger" proposed by Baron Pierre of Coubertin in 1894) power to overcome this character in times of pressure, evaluation, competitiveness and social comparison.

The achievement goal approach (Dweck, 1986; Nicholls, 1984) is the foundation for much of the recent research on motivation in sport contexts. In particular, Elliot (2003) describes 4 types of achievement goals (Table 1).

Type of Goal	Description
Mastery-approach goals	Focusing on the acquisition of absolute competence
Mastery-avoidance goals	Avoiding intrapersonal incompetence
Performance-approach	Demonstrating better performance than others
Performance-avoidance goals	Avoiding performing worse than others

**Table 1.** Description of 4 types of achievement goals

These different types of achievement goals have been associated with adaptive and debilitating patterns of motivational cognitions, feelings, and behavior in achievement settings.

Within a larger research project on sport motivation the study presented here was conducted with a senior female volleyball team (n=13) playing at the Portuguese first division and had two main aims: (1) to identify the athlete's achievement goal orientations, and (2) to analyze the association between the athlete's goal orientations and their specific motivational beliefs for particular games. The athlete's goal orientations were assessed at the beginning of the season using the Achievement Goal Questionnaire of Sport (Conroy et al., 2003). The athletes' situational specific motivational beliefs for 15 games were assessed before the beginning of each game, using an adapted version of the "On-line Motivation Questionnaire" (Boekaerts, 2002). The questionnaire focus on the athlete's pre-game anticipatory and preparatory beliefs that may affect game performance, such as expectancies, perceived game difficulty, importance, and intended effort expenditure.

The athletes' goals were moderately to strongly associated with various motivational variables. For example the results showed significant relations between Mastery approach goals and effort, evidencing a strong link between the athletes' goal to improve their competence and their willingness to strive.

## References:

- [1] Conroy, D., Elliot, A., & Hofer, S. (2003). A 2x2 Achievement Goals Questionnaire for Sport: Evidence for Factorial Invariance, Temporal Stability, and External Validity. *Journal of Sport & Exercise Psychology*, 25, 456-476.
- [2] Boekaerts, M. (2002). The on-line motivation questionnaire: A self-report instrument to assess students' context sensitivity. In P. Pintrich (Ed.), *New directions in measures and methods*. Vol.12 (pp. 77-120). Oxford: Elsevier.

# Contributions from Community Psychology for the fulfilment of a culture of justice: Implementation and evaluation of a family empowerment project

Ana P. Silva Sampaio M.D., Carlos Gonçalves Ph.D.

Faculty of Psychology and Educational Sciences of University of Porto, Portugal.

The aim of the study is to explore the hypothesis of effectiveness of a family empowerment project in the field of Community Psychology. The intervention occurs with a group of mothers living in a social housing neighborhood in the suburbs of Oporto.

The design of the intervention embodies the project methodology and collaborative action research methodology, with the objective of adjusting the practices to the needs of the target population<sup>1</sup>. Using qualitative changing indicators, our results show different patterns in the families' interactions<sup>2</sup>, before and after the intervention. These results suggest the project efficiency, illustrating the possibility of an effective methodology and ethical attitude in the work field with populations with similar characteristics.

The study conclusions focus on the theoretical and methodological relevance of an ethical approach specific to the Community Psychology with objective implications to the psychological practice<sup>3</sup>. This study conclusions alert to the importance of conceptualizing, implementing and evaluating interventions that don't circumscribe to the intra-individual alone, but are founded on the historical and social dimensions where the human individuality is fulfilled: the community<sup>4</sup>.

It also highlights that the success of an empowerment intervention depends on the ability to establish trust relations between professionals and citizens<sup>3</sup>, as the intervention doesn't occur **to** the others, **besides** the others, or **instead** of the others, but only acquires meaning in a shared process **with** the others<sup>4</sup>. Once only from the promotion of autonomy, legitimacy and effectiveness are conferred to the interventions towards the empowerment of communities.

## References:

- [1] Menezes, I. (2007). *Intervenção comunitária*. Porto: Livpsic/Legis Editora.
- [2] Vidal, A. S. (2000). Debunking the hero: Ethical responsibility in community intervention. In J. Ornelas, *Actas do II Congresso Europeu de Psicologia Comunitária* (pp. 243-265). Lisboa: Instituto Superior de Psicologia Aplicada.
- [3] Rojano, R. (2004). The Practice of Community Family Therapy. *Family Process*, 43(1), 59-77.
- [4] Martín-Baró, I. (1996). O papel do psicólogo. *Estudos de Psicologia*, 2(1), 7-27.

# **Incorporation and management of ambivalent belongings: a comprehensive approach to the identifications with profession expressed by junior psychiatrists**

**Nádia Costa**<sup>1</sup>

<sup>1</sup> Master in Sociology, Faculty of Arts, University of Porto, Portugal.

Psychiatry constitutes a privileged subject of inquiry within the domain of Social Sciences. Approached from different angles and questionings, this specific field plays a strong presence in collective imagination, which grants to it an unique and distinct symbolic connotation among the other existing medical domains. The defiant exponent summoned by Psychiatry lies in the nature of the social – and sociological – dilemmas to which we are brought back by its social practice and institutional set-up.

Beginning from those aspects and suggestions this study aims to approach the subjective professional experience that is produced in the field of Psychiatry, in order to understand the representations displayed by junior psychiatrists regarding their knowledge, their professional practices and their work environments. Theoretically and conceptually, we contemplate this questioning in the light of the processes and mechanisms of identification with Psychiatry thereby, pursuing to understand the sense of belonging expressed towards this occupation, and the social recognition given by it. The issue is based on a multidimensional theoretical perspective that places the profession in its double diachronic and synchronous axis.

Supported by a qualitative methodological approach, the research's framework sought to achieve a double valence of discovery and construction, and resulted in a methodological work staged in two phases, with differentiated goals and procedures. We obtained a qualitative treatment of the interviews, organized in the exposition of a double statement – biographical and relational – regarding the belongings expressed by psychiatrists until the age of thirty-five towards their professional occupation.

The belongings and the self-image as medical specialists revealed in these voices, show that «being a psychiatrist» implies the incorporation and the management of the *structural ambivalence* by which Psychiatry is portrayed. In spite of being specialists whose skills are exogenous to the domain of medical practices, the respondents assume however, a position that predicts the integration in the field of Medicine which, in addition, reflects the scientific, political, and institutional changes that are occurring within the mental health domain.

Oral Sessions III > A3

# Literature & Language Studies



## Visual Poetry in Portugal (1915-1977): Fields of Expression

R. Reis<sup>1</sup>

<sup>1</sup> Department of Heritage Studies, Faculty of Arts, University of Porto, Portugal.

In 1915, with "Manucure" Mário de Sá Carneiro creates the first visual poem of the Portuguese twentieth century, three years before of Apollinaire's *calligrammes*. The evolution of Portuguese visual poetry continues with the work of Almada Negreiros, the dimensional poetry of António Pedro and with the surrealist artists, achieving some autonomy with the experimental poetry movement in the 60's. Influenced by the Brazilian movement of Concret Poetry, the Portuguese experimental poets will develop new areas of expression, making the poem something to *view* instead of something to *read*.

This project wanted to understand the evolution of the Portuguese visual poetry in its different expressions, since the beginning of the Century until the late 70's, when poetry leaves the book pages occupying the wall of the galleries. We also wanted to examine the work of various artists such as Sá-Carneiro, Almada, António Pedro, Cesariny, Ana Hatherly and E. M. de Melo e Castro, and some artists that, despite not being visual poets, approached visual poetry: Amadeo, Eurico Gonçalves, António Sena or João Vieira.

Thus, we concluded that visual poetry is a presence since the Classical antiquity as an attempt to multiply poetic and aesthetic possibilities. It will also be used as a way to literary, artistic and political subversion, assuming several fields of expression, from literature to performance or video art.

**Daniel Faria and the Sísifo's complex.**  
**Brief considerations about the cyclic process of poetry**

**P. Lino**<sup>1</sup>

<sup>1</sup> Faculty of Letters, University of Porto, Portugal.

<sup>2</sup> Use as many lines as required to include all author's affiliations (Times New Roman, 11pt).

Starting from the difficult combination between silence/listening and word/writing present in the poetic work of Daniel Faria (1971-1999), the study "Daniel Faria and the Sísifo's complex" is a quest about the cyclic process that roundly identifies all of his work. Just as Sísifo, the poet searches divine perfection and, just as Sísifo, he ends, as the Human that he is, far from it. Aware of their condition, and because they ambioned contact with the absolute, they both repeat the gesture: going up to and coming down from the skies. Thus, Daniel Faria creates a repetitive circle, in which, at first, we can recognize the perseverance of someone who tremendously wishes for the summit, then his self-destruction and, at last, death as the only alternative.

# INTERSUBJECTIVITY AND INTERPRETATIVE COOPERATION IN UMBERTO ECO AND STANLEY FISH

**José António C. Baptista**

<sup>1</sup> Master of Literary Theory, Department of Portuguese and Romanic Studies of the Faculdade de Letras da Universidade do Porto

Researcher in CITCEM, Faculdade de Letras da Universidade do Porto

This paper aims to point out the way the idea of interpretative cooperation which governs the content of the postulated theories of interpretation either by Umberto Eco or Stanley Fish requires consideration of Husserl's seminal notions such as "intersubjectivity" and "intercommunication".

It is noted, therefore, a condition that installs these theories in the traditional demarcation line that, rather than postulate an irrefutable inseparability between the *topos* of Aesthetics and Theory, always outlined the interpenetration and mutual contamination of the specificity of each one of these domains.

If it true that Aesthetics and Theory are meant to be situated in opposite fields, even by the way they are associated to different states of objectivity in the relation between the reader and the aesthetic object, it is a fact as well that the literary text establishes the possibility of several interpretation acts. These interpretation acts are unified by "intersubjectivity" and "intercommunication" and are simultaneously aesthetic and theoretical by two reasons: on the one hand, Umberto Eco, similarly to the Kantian's aesthetic judgment process, refers the act of interpretation both as something rational and pleasurable, on the other hand Stanley Fish postulates the interpretation act as a non-objective *factum* derived from the actuation of a community of individuals regulated by a common knowledge. Moreover, if Fish asserts that the true knowledge of a text can only arise as an intersubjective knowledge, which consists in the unity of nature by a group of intelligent beings in relation of "intropy" and communicability", in Umberto Eco, this same connection between the text and his reader makes unclear the difference between the aesthetic formal moment and the moment of logical explanation of content.

References:

[1] Eco, Umberto – *Os Limites da Interpretação*, Lisboa, Difel, 1992.

– *Interpretação e Sobreinterpretação*, Editorial Presença, Lisboa, 1993.

– *Lector in Fabula*, trad. de Mário Brito, Lisboa, Editorial Presença, 1993.

[2] FISH, Stanley – *Is there a text in this class?: the authority of interpretive communities*, Massachusetts, Harvard University Press, 1980.

[3] HUSSERL, Edmund – *Zur Phänomenologie der Intersubjektivität: texte aus dem Nachlass*, Den Haag, Martinus Nijhoff, 1973.

## Violência da Fruição entre Sade e Salò

Maria Inês Castro e Silva<sup>1</sup>

<sup>1</sup>Faculdade de Letras, Universidade do Porto, Portugal.

The book *The Hundred and Twenty Days of Sodom or the School of Libertinage* (1785), by Marquis de Sade, and the film *Salò or The Hundred and Twenty Days of Sodom* (1975), by Pier Paolo Pasolini open the small and hidden door of horror that can trap the viewer that wants to see without commitment.

Sade's narrative takes place in only one setting – Silling's Castle – where several children and several libertines are locked up. Sade's pages show many different sexual skills and perversions, accompanied by constant devotion to sacrilege in a world where there seems to be no place for punishment. The speeches of the condemned and the presence of a fierce voyeurism have its culmination in the erotic movement as a movement of death.

The film that we intend to analyze is considered one of the great films of Pier Paolo Pasolini, who places the scene in the context of Fascist Italy and broadens its horizons to the entire scope of World War II. Having, in the background, the interpretation of the book by the Marquis de Sade, Pasolini further encompasses the denunciation of all the humiliation caused by the war. The characters embody the roles constructed by Sade, but now carrying on their shoulders the sacrificial weight of a great war.

Bearing in mind the relationship established between these two objects of art, we want to reflect not only on the thin line that separates love from death, through the analysis of the eroticism and the grotesque, but also on the transformations carried out by the cinema when it aims at re-working a literary work of great importance such as *The Hundred and Twenty Days of Sodom or the School of Libertinage*.

# 'O, Sir, I did not look so low'. Dislocations in Shakespeare's *The Comedy of Errors*

Tiago Sousa Garcia<sup>1</sup>

<sup>1</sup> Faculdade de Letras da Universidade do Porto

For centuries, criticism had little to say about *The Comedy of Errors*. It was considered to be Shakespeare's very first work, and its obedience to the classical unities rendered it an easy target for easy dismissal. It was a funny play, and that was all. Not even close to later comedies, not even in the same league as the tragedies and histories. Albeit recognising that it had a limited influence in western literature, it is my aim to prove that *The Comedy of Errors* has the qualities and depth of a superior work of art, and it should be regarded as such.

To do this, I will read the play through a conceptual framework of *dislocations and displacements*, that is to say, I will discuss deviations from an expected path, not limiting myself to spatial dislocations but extending my attention to ontological dislocations as well.

In a longer version of this essay I analyse multiple types of dislocations, namely social, religious, monetary, body, source, genre and stage dislocations. Here, however, I will focus on the two types that I consider crucial to a clearer understanding of *The Comedy of Errors*: *identity dislocations* and *travel dislocations*. The first type concerns the core of the farcical plot and relates to the numerous cases of mistaken identities – my analysis will try to make clear how this has ontological implications to the characters' own sense of self and reality. The second type concerns the romantic framework and, as I will try to prove, its vital necessity to the whole plot.

I will conclude that such an understanding of *The Comedy of Errors* contributes to a richer understanding of the play, helping to fight in the process, a centuries-old critical tradition that insists in a linear and simplistic analysis of one of Shakespeare's first known pieces.

## **Machado's story and the voices in the discursive representation of the social in nineteenth century**

**Maria da Paz de Freitas e Sousa**

Estudante de Pós Graduação em Estudos Literários - UERN

Machadiano Tale and the discursive voices in the representation of the social in the twenty first century have as scientific objective investigation about the discursive corpus of the Brazilian realist literature of the writer Joaquim Maria Machado de Assis (1839-1908), about eighteenth century social thematic. In the study, we have as investigation two machadiano tales: "The psychiatrist" and "Father against mother", in what the study will look for the cutting of social thought of that epoch. In what the tale The psychiatrist, discussion is around madness' historical thematic and psychiatry in the begin of Republic and Imperial Rio de Janeiro. Already in the Father against mother tale, we'll see the historical thematic of recreation and black slavery of the abolitionist Brazil, besides patriarchal power on black feminine character condition, this one (woman) silenced by coercion and political power of social. Machadiana literature's study will enter in dialog with literary criticism's studies, but it also will look for discusses of scientific slopes of language, as analysis of the French speech as the critical history in the slope of social thought of that epoch. The work look for together language science mainly to Brazilian literature's studies and Brazilian literary criticism that Machadiana literature produced social and real discusses of eighteenth century and peripheral Brazil's history and social thought of political stamp and ideological interest. But, that the representation of the social peripheral, writer Machado de Assis' speech to point to voices, discursive corpus and memoirs of the history of Rio de Janeiro and the situations that it was around the society. With that, analysis of the French speech in the slope of the thought of Foucault will contribute to the representation of the power-knowledge and the slope of the historicity through built thoughts by the historical cientificity of the man and the society.

Key-words: Machadiano tale; Discursive voices of social; Discusses of peripheral marginalization of Rio de Janeiro.

## A Rule Based System of Portuguese Verbal Forms

J. Barbosa<sup>1</sup>, S. Costa<sup>2</sup>, A. Francisco<sup>3</sup>, L. Matos<sup>2</sup>, N. Moreira<sup>3</sup>, R. Reis<sup>3</sup>, J. Veloso<sup>1,2</sup>

<sup>1</sup>Linguistic Centre of University of Porto, Portugal

<sup>2</sup>Faculty of Arts, University of Porto, Portugal

<sup>3</sup>Department of Computer Science, Faculty of Sciences, University of Porto, Portugal.

The socio-linguistic analysis proposed in the IJUP 2010 Project “Análise Social e Linguística das «Memórias do Trabalho»” required a computational tool for recognition of verbal forms in a plain text corpus, especially when searching passive or completive constructions. To pursue this task, a verb scanner must be used. The morpho-syntactic tagger [7] chosen for the project (due to its quality) did not provide tagged verbal forms, and the analysed automatic verbal conjugators were either inaccurate, or not possible to use for our purpose (e.g. were not open source or provided only rudimentary Web interfaces) [5,6,7].

Though complex, Portuguese verbal inflection system is quite regular. A simple tense form is composed by four constituents: Radical (Rad), Theme Vowel (VT), Tense/Mood Morpheme (MT) and Person Morpheme (MP), by this order. VT, MT and MP may or not occur.

Given a tense form, preview the ending morphemes, and vice-versa, or to identify a verbal form by its ending is usually a trivial task [1,2,3], since morphological irregularities occurred, with a few exceptions, only in the Radical. Yet, dealing with plain text, the transcripts of oral interviews that constitute the corpus to be analysed [8], the scanner must recognize not only morphological irregularities but also orthographic “irregularities”, like diacritics, special characters, or orthographic modifications due to morphophonemic phenomena. Specific rules had to be created to preview such “irregularities”. Based on these rules, we developed a new automatic verbal conjugator. The accuracy of this new tool was compared with two available web systems [5,6] and our analysis led to the conclusion that both systems had also several linguistic faults.

### References:

- [1] Cunha, C. & Cintra, L.L. (1988), *Nova gramática do Português Contemporâneo*, 5ª ed. Lisboa: Edições João Sá da Costa.
- [2] Mateus, M.H. et alii (eds), (2003), *Gramática da Língua Portuguesa*, 5ª ed. Rev. e aumentada. edn. Lisboa: Caminho.
- [3] Nogueira, R.S. (1986), *Dicionário de verbos portugueses conjugados*, 8ª ed. Lisboa: Clássica Editora.
- [4] Portal da Língua Portuguesa, <http://www.portaldalinguaportuguesa.org/>, Acesso: 10/1/2012.
- [5] Conjuga-me, <http://www.conjuga-me.net/>. Acesso: 10/1/2012.
- [6] LX-Center: Language Resources and Technology for Portuguese, <http://lxcenter.di.fc.ul.pt/>. Acesso: 10/1/2012.
- [7] Gconjugue, <http://sites.google.com/site/jalvesaq/gconjugue>, Acesso: 10/1/2012.
- [8] CDI- Centro de Documentação e Informação sobre o Movimento Operário e Popular do Porto, UPP, <http://cdi.upp.pt>.



Oral Sessions III > A4

# AGRO FOOD II



## **Influence of charcoal type on polycyclic aromatic hydrocarbon formation in barbequed muscle foods**

**P. Novo<sup>1</sup>, O. Viegas <sup>1,2</sup>, O. Pinho<sup>1,2</sup>, I.M.P.L.V.O. Ferreira<sup>1\*</sup>**

<sup>1</sup> REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Portugal

<sup>2</sup> Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Portugal.

Polycyclic aromatic hydrocarbons (PAHs) are an important class of toxicological compounds formed during incomplete combustion of organic matter. They are widely spread in the environment and human exposure to PAHs is unavoidable. Their presence in the environment contribute to accidental contamination of many raw foods, additionally, the traditional preservation practice of food with wood smoke or food cooked over an open flame induces PAHs generation and their presence in foodstuffs. Consequently, the major source of human exposure is attributable to the diet. The presence of PAHs in food is a matter of concern and requires continuous monitoring. Studies concerning the levels of grilled or barbecued food are scarce or quantified only Benzo[*a*]Pyrene alone or few PAHs [1].

The main objective of this study was to select the safer charcoal, concerning PAHs formation in grilled muscle foods. With this propose 15 PAHs, selected from the 16 US-EPA were quantified in meat and fish samples grilled with two different types of charcoal under standard temperature conditions.

Two similar garden-type (35 cm width, 52 cm length, and 15 cm height) grills were fuelled with two different types of charcoal: the traditional “wood charcoal”, and another called “ecological charcoal” from 100% coconut shell. Aiming to keep the temperature next to the grid at 200 ° C the distance to the heat source was selected depending on the type of charcoal. The grilling time was 18 min for beef and 23 min for salmon until well-done cooked, golden colour for salmon and moderately browned for beef. Samples were turned once during grilling at half the total cooking time.

PAHs were extracted with n-hexane and sonication, followed by solid-phase extraction (SPE) and separated by HPLC with fluorescence detection. Method validation included study of linear range, limits of detection and limits of quantification, precision and accuracy [2].

The results indicate significant differences concerning PAHs formation in salmon samples. Higher levels were quantified in salmon samples grilled with traditional charcoal. Apparently, the flameless characteristic of ecological charcoal is responsible for lower level of PAHs, despite of the high lipid content of salmon. Concerning beef samples lower levels of PAHs were quantified and no significant differences were observed between composition of samples grilled with traditional and ecological charcoal.

References:

[1] Phillips, D.H. (1999), *Polycyclic aromatic hydrocarbons in the diet*, Mutation Research, 443, 139-147.

[2] O. Viegas, P. Novo, O. Pinho, I.M.P.L.V.O. Ferreira: *A comparison of the extraction procedures and quantification methods for the chromatographic determination of polycyclic aromatic hydrocarbons in charcoal grilled meat and fish*. Talanta (in press)

# Analysis of Bisphenol A and B in canned tuna combining acetonitrile dispersion and dispersive liquid-liquid microextraction followed by gas chromatography

**C. Cunha, S.C. Cunha, and J.O. Fernandes**

REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal. Email: Sara.cunha@ff.up.pt

Bisphenol A (BPA) is a monomer used in the manufacture of polycarbonate and epoxy resins. It can be found in a diverse range of products including returnable beverage bottles, infant feeding bottles, tableware (plates and mugs) and food cans. Small amounts of BPA can potentially leach out from food containers into foodstuffs and beverages and therefore be ingested. This issue became of increasing concern since BPA shown to be an estrogenic compound besides its toxicity. In the European Union BPA is permitted for use in food containers with a specific migration limit of 0.6 mg/kg [1].

Current analytical methods devoted to the analysis of BPA and bisphenol B (BPB) in solid foodstuff encompass a traditional scheme, i.e., liquid-liquid extraction followed by a cleanup/enrichment step by solid phase extraction or evaporation before final quantification by liquid chromatography with ultraviolet or fluorescence detection or by gas chromatography coupled to mass spectrometry. Though sensitive, these procedures are still time consuming and are not fitted for high throughput analysis.

A new GC-MS method was developed for the simultaneous analysis of BPA and BPB in canned tuna. The sample preparation was limited to a simple liquid partitioning with acetonitrile and salts (QuEChERS), the analytes were concentrated in tetrachloroethylene by the dispersive liquid-liquid microextraction (DLLME). Acetonitrile which was the carrier of analytes from the QuEChERS extraction was used as disperser solvent of the DLLME procedure. The accurate determination of analytes was achieved by employed a deuterated BPA<sub>d16</sub> labeled as internal standard. The method performance was evaluated for a concentration range from 0.5 to 20 µg kg<sup>-1</sup>. Satisfactory recoveries and within- and between -day precisions were obtained for the 3 concentrations levels spiked that cover the whole work range. This reliable method has been successfully applied in a mini-survey of canned tuna of Portuguese origin.

Acknowledgments:

This research was supported by grant from the FCT project "PTDC/AGR-ALI/101583/2008" and COMPETE FSE/FEDER/OE and "PEst-C/EQB/LA0006/2011". S.C.C. is grateful to "POPH-QREN-Tipologia 4.2, Fundo Social Europeu e Fundo Nacional MCTES".

References:

- [1] Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intending to come into contact with foodstuffs.
- [2] Cunha, S.C., Almeida, C., Mendes, E. and Fernandes, J.O. (2011), *Simultaneous determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid-liquid micro-extraction and heart-cutting multidimensional gas chromatography-mass spectrometry*, Food Add. Cont. A, 28, 513-526.

# Sea-lamprey nutritional profile and reference intake values for human consumption during reproductive migration

**M. J. Araújo<sup>1,2</sup>, R. Ozório<sup>2</sup>, R. J. B. Bessa<sup>3,4</sup>, C. Antunes<sup>2,5,6</sup>**

<sup>1</sup> Institute for the Biomedical Sciences Abel Salazar, Porto University. Largo Prof. Abel Salazar, 2, 4099-003 Porto

<sup>2</sup> Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/UP). Rua dos Bragas, 289, 4050-123 Porto

<sup>3</sup> Univ Tecn Lisbon, Fac Med Vet, CIISA, Av. da Universidade Técnica, 1300 - 477 Lisboa

<sup>4</sup> INRB, Unidade Invest Prod, Fonte Boa, Vale Santarém, 2005-048 Santarém

<sup>5</sup> Gallaecia Superior School. Largo das Oliveiras, 4920-275 Vila Nova de Cerveira

<sup>6</sup> Aquamuseu do Rio Minho. Parque do Castelinho, 4920-290 Vila Nova de Cerveira

The sea lamprey (*Petromyzon marinus* Linnaeus, 1758) is an “Agnatha” species and the largest of all lamprey species. This species is consumed in Portugal, Spain and France. The sea lamprey is angled seasonally along international section of Minho River (NW Iberian Peninsula), and lamprey fisheries contribute for the income of nearly 1000 fishermen, as this species is highly priced. This species stop feeding when reproductive migration starts, thus, body energy reserves are expected to be mobilized over this stage. The present work aimed primarily to characterize the nutrient profile of lampreys caught at three different sampling points along Minho river: (1) downstream river mouth, (2) 30 km upstream river mouth and (3) 65 km upstream river mouth. Body composition (dry matter, crude lipids, crude protein, gross energy and fatty acid content) was evaluated for different tissues and were presented in wet weight basis (WW). In addition, correlations between changes in nutritional profile and endogenous nutrient mobilization to meet the metabolic costs during gonads maturation were discussed.

The results pointed significant reduction of energy levels on males carcass ( $11.1 \pm 0.9$   $9.1 \pm 0.4$   $\text{Kj.g}^{-1}$  downstream and upstream respectively,  $p=0.02$ ) mainly caused by a very significant decrease of lipid levels ( $19.4 \pm 2.3$  and  $12.6 \pm 1.2$  % WW,  $p=0.006$ ) during reproductive migration. In the females, such decrease was not found on carcass, but it was observed at liver ( $16.7 \pm 3.9$  and  $4.0 \pm 1.7$  %,  $p<0.05$ ), pointing its importance as energy source during migration. In terms of reference intake values (RIV<sup>[1]</sup>, per 100 g of portion), the total lipids from downstream carcasses contribute to  $25.6 \pm 4.6$  % of RIV. Mono-unsaturated fatty acids are the larger fraction ( $54.9$ - $56.4$  % carcass); however saturated fatty acids highly contribute to RIV ( $35.6 \pm 7.3$  % of RIV). As a consequence, only  $12.7 \pm 1.3$  % of RIV energy was accomplished.

The current study showed a dynamic nutrient mobilization in sea-lamprey tissues to provide energy for the migration and possibly in preparation for the reproduction. In addition, sea lampreys may easily contribute to accomplish energy and lipid RIV levels for human consumption.

## References:

[1] EFSA [European Food Safety Authority], 2009. *Scientific Opinion of the Panel on Dietetic products, Nutrition and Allergies on a request from European Commission on the review of labelling reference intake values for selected nutritional elements*. The EFSA Journal, 1008 : 1-14.

## Determination of the sodium content in vegetable soups served at public institutions' canteens to different age groups

C. Gonçalves<sup>1</sup>, G. Silva<sup>1</sup>, O. Pinho<sup>1</sup>, S. Camelo<sup>1</sup>, L.F. Amaro<sup>1</sup>, V. Teixeira<sup>1,2</sup>, P. Padrão<sup>1,3</sup>, P. Moreira<sup>1,2,3</sup>

<sup>1</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

<sup>2</sup> Research Centre in Physical Activity, Health and Leisure, University of Porto, Portugal.

<sup>3</sup> Institute of Public Health, University of Porto, Portugal.

Cardiovascular diseases are a major cause of death and disability in developed countries, accounting for 39% of all deaths [1]. One of the most important factors associated with their onset is the high sodium intake, which leads to the development of high blood pressure [2]. High salt intakes are associated with the consumption of processed foods and meals prepared outside home [1].

Modern lifestyle takes, on an ongoing basis, the consumer to eat out and away from home, from one to all day meals, and this may be associated with an increased risk of higher energy intake and to obesity [3]. The inclusion of vegetable soup may be important as its consumption is negatively associated with obesity [4], but its high levels of sodium raised some concern recently [5].

This study aims to quantify the content of sodium present in vegetable soups served in Portuguese public institutions' canteens, including kindergartens, elementary schools and nursing homes.

Soups without salt and with added salt were collected from kindergartens (n=110), elementary schools (n=450) and nursing homes (n=28) and sodium contents were determined by flame photometry.

Sodium contents of soups without added salt ranged from 0.13 to 216.63 mg/100g, in nursing homes, and 0.93 to 284.02 mg/100g, in kindergartens. Sodium content in soups with added salt ranged from 124.71 mg/100g to 429.04 mg/100g, in nursing homes, from 36.58 mg/100g to 409.53 mg/100g, in elementary schools, and from 63.23 mg/100g to 438 mg/100g, in kindergartens.

Considering an average serving of 300g, the estimated average sodium intake from a soup alone may be up to 459-850mg, representing 31 to 54% of the sodium adequate daily intake, becoming a major contributor to the high sodium intakes reported in developed countries. As most of the sodium comes from added salt during cooking processes, intervention strategies should be directed to raise awareness among food handlers and chefs about limiting salt content in different foods, as well as educational strategies directed for the consumer, in order to maintain acceptability of foods with reduced sodium content.

### References

- [1] Ferlay, J., Autier P, Boniol M, Heanue M, Colombet M, Boyle P. (2006) *Estimates of the cancer incidence and mortality in Europe in 2006*. Annals of Oncology, 18(3), pp. 581-592.
- [2] He, F.J., MacGregor, G.A. (2002) *Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health*. Journal of Human Hypertension, 16, pp. 761-770
- [3] Orfanos, P., Naska, A., Trichopoulos, D., Slimani, N., Ferrari, P., van Bakel, M., et al. (2007). *Eating out of home and its correlates in 10 European countries. The European Prospective Investigation into Cancer and Nutrition (EPIC) study*. Public Health Nutr, 10(12), pp. 1515-1525.
- [4] Maureen, K.S., Leann, L.B., Liane, S.R., Barbara, J.R. (2011). *Serving large portions of vegetable soup at the start of a meal affected children's energy and vegetable intake*. Appetite, 57, pp. 213-219
- [5] Conceição, R., Mendes, E., Casal, S. *Salt amounts in Oporto ready-to-eat soups*. (2011) Porto: REQUIMTE, Faculdade de Farmácia, Laboratório de Bromatologia e Hidrologia

## Valorisation of food private labels: exploring consumer perceptions

**A. Mendonça<sup>1</sup>, A.P. Moura<sup>2</sup>, R.C. Lima<sup>3</sup> and L.M. Cunha<sup>1,\*</sup>**

<sup>1</sup>REQUIMTE/DGAOT, Faculty of Science, University of Porto, Portugal

<sup>2</sup>REQUIMTE-UP/DCEt, Universidade Aberta, Porto, Portugal

<sup>3</sup>Sense Test, Lda, Vila Nova de Gaia, Portugal

\*Corresponding author: [lmcunha@fc.up.pt](mailto:lmcunha@fc.up.pt)

Growth of private label brands (PLBs) is quite impressive in food and grocery segments. Initially, PLBs were considered as cheap alternatives offered in generic categories, today they are a part of well-defined retail mix strategies. At the national context, PLBs present an increasing market: recent data [1] shows an 8 % growth in value, with a market share increase up to 36.6 %. This exploratory paper aims to investigate consumer's perception of PLBs and explore factors that influence the purchase of such PLBs. Two focus groups were conducted, with female participants responsible for purchasing and for preparation of family meals. Groups were segregated by labour situation and education level, between unemployed with lower education – group 1 and employed higher education– group 2, thus accomplishing a larger distance between groups. Participants were asked to perform a free-listing of known PLBs and to self-report their PLBs food shopping during the last two weeks before the focus group session. Participants also discussed perceptions and attitudes towards PLBs, as well as criteria used during their food purchase. There were 8 participants in each group. Participants from focus group 1 recall from memory an average of  $6.0 \pm 1.6$  PLBs, against  $5.1 \pm 1.8$  from group 2. Additionally, participants from group 1 reported buying an average of  $5.9 \pm 3.6$  PLBs food items per week, while at group 2 buying of PLB's food products was at  $4.9 \pm 3.7$  items per week. Generally, participants associated PLBs to cheap alternatives widespread through different food categories. Participants from group 1 considered PLBs' packages less attractive than corresponding packages of manufacturer's brands and complained about perceived absence of PLBs' product origin. Both focus groups agreed about the fairness of PLBs' price, when considering offered quality. Nevertheless, this rational is a function of the product category: for olive oil the perceived quality of offered PLBs' products is less than expected and for some PLBs' yogurts quality was referred as superior than that of matching manufacturer's brands. In order to increase buying frequency for PLBs' food products, these must have a standard quality at an attractive price level, and must offer the same chances to complaint, according to participants of focus group 2. In sum, this exploratory research clearly brings forth the importance of price as an attribute influencing consumers' acceptance of PLBs. Nevertheless, the quality of products offered play an equal a role among "value money segment" consumers, in the sense that these consumers expect certain quality standards. As a result, quality at affordable prices may be the guideline for PLBs in the food sector, while incorporating additional services, such as consumer information.

References:

[1] Kantar Worldpanel Portugal (2011), *Shopper soberano na crise*, Kantar Worldpanel, Lisbon. [available at: <http://kantarworldpanelportugal.com/?p=870>]

## Seasonality in human diet: from production to nutritional status

**A. André<sup>1</sup>, S. Rodrigues<sup>2</sup>**

<sup>1</sup> Department of Public Health, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> Department of Nutrition, Faculty of Nutrition, University of Porto, Portugal.

The seasons determine the availability of food that dominates the life of most none equatorial species. For humans, the influence of seasonality begins in the food production, since the vast majority of plant species and many species of animals used for human consumption are subject to an "agricultural calendar" imposed by the succession of the seasons, modifying the availability for sale, acquisition or, ultimately, food consumption and its consequences on nutritional status.

The aim of the present study was to identify and discuss the relevant literature about the implications of seasonality in the human diet.

A systematic literature review, using the search terms "season\* AND (food supply OR food access OR food availability OR food consumption)" was performed in PubMed, ISI and Scopus databases, subsequently completed with snowball search in Google Scholar.

The results showed that it is generally accepted that the consumption of foods "in the season" is more advantageous, as arguments showing the lowest price, best organoleptic characteristics (specially taste) and nutritional value optimized, which are also factors that influence the household purchase and availability of food.

In developed countries it is possible to produce and market food "out of season" using agro-industrial techniques more or less sophisticated, but most of the fresh food produced, sold and purchased by households in any country is still subject to seasonal changes.

Although scarce, there are studies that showed clear seasonal influence on purchase of foods such as fruits and vegetables, even in urban areas and less developed countries.

Much more studies have showed marked seasonality in food consumption, which may be different depending on the social group, economic status and culture, and this is higher in less developed countries, where most families depend on the production of food for home consumption.

Not all of the different foods consumed are reflected in differences in energy and nutrient intakes, but some recent studies showed that there was clear seasonality in energy and nutrient intakes, and that may have greater or lesser impact on nutritional status according to the country and / or group studied. Again, there were the least developed countries and the poorest which showed higher energy and nutritional changes during the year, with higher impact on nutritional status.

Research on the influence of seasonality covers diverse aspects of the issue: from production and sale to purchase and household availability, and ultimately, to consume and individual nutritional status. However future research should focus on how seasons influence household food purchase and availability, as there are few studies on this area, but is also need more research with focus directly on the influence of seasons on food consumption.

Oral Sessions III > A5

# Engineering II



# Fault Localization using Dynamic Code Coverage

**A. Perez<sup>1</sup>, A. Riboira<sup>1</sup>, R. Abreu<sup>1</sup>**

<sup>1</sup> Department of Informatics Engineering, Faculty of Engineering, University of Porto, Portugal.

In software development, a large amount of resources is spent in the debugging phase [1]. This process of detecting, locating and fixing faults in the source code is not trivial and rather error-prone. In fact, even experienced developers are wrong almost 90% of the time in their initial guess while trying to identify the cause of a behavior that deviates from the intended one [2].

In order to improve the debugging efficiency, this process needs to be automated. Some effort was already made to automatically assist the detecting and locating phases. This led to the creation of automatic fault localization tools, namely Zoltar [3] and Tarantula [4]. The tools instrument the source code to obtain code coverage traces for each test, which are then analyzed to return a list of potential faulty locations. To improve the exploration and intuitiveness of that list, an Eclipse [5] plugin was also developed – GZoltar [6] – that adds fault localization functionality to an integrated development environment, with several visualization options.

Although these tools can be helpful, they do not scale. These tools need to instrument a large portion of the project at the line of code level so that an analysis can be performed. This is acceptable for small software applications, but impractical for large, real-world projects that contain hundreds of thousands of lines of code.

A new approach to this problem would be to avoid as much as possible the line of code level of instrumentation detail, while still using the proven techniques [3] that these fault localization tools implement, using dynamic code coverage.

The dynamic code coverage method consists of coarsening the granularity of the instrumentation, obtaining only coverage traces for large components, and running the same fault localization analysis detailed previously. This would return a list of potential faulty components. After that, the components most likely to contain a fault would be re-instrumented, with a finer-grained detail. The process will then loop until a list of the lines of code most likely to contain a fault was reached.

The objective of this work is to implement a working prototype of this dynamic code coverage approach in GZoltar, and evaluate its performance improvement. Another important goal is to minimize the impact of the debugging phase in the software project's resources.

## References:

- [1] G. Tasse. The economic impacts of inadequate infrastructure for software testing. National Institute of Standards and Technology RTI Project, 2002.
- [2] Andrew J. Ko and Brad A. Myers. Debugging Reinvented : Asking and Answering Why and Why Not Questions about Program Behavior. ICSE '08.
- [3] Tom Janssen, Rui Abreu, and Arjan J. C. van Gemund. Zoltar: A toolset for automatic fault localization. ASE '09.
- [4] James A. Jones, Mary Jean Harrold, and John Stasko. Visualization of test information to assist fault localization. ICSE '02.
- [5] Eclipse Integrated Development Environment. <http://www.eclipse.org/>.
- [6] André Riboira and Rui Abreu. The GZoltar project: a graphical debugger interface. TAIC PART'10.

# RZoltar: a plug-in Eclipse for Regression Testing

**J. Campos<sup>1</sup>, R. Abreu<sup>1</sup>**

<sup>1</sup> Department of Informatics Engineering, Faculty of Engineering, University of Porto, Portugal.

Software development is an iterative process: there are new requirements, some need to be reworked, removed, implement new features, etc. The best existing bug prediction techniques in the literature predict that modifications of original software introduce bugs in 78% of times [1]. So, testing is in general the main method to determine whether the software deviates from the specified behavior (requirements) or not.

The process of retesting software to establish that the modified program still work correctly on all test cases used to test the original program is know as *regression testing* [2]. To reduce the effort of regression testing several approaches have been studied: test suite minimization, selection, and prioritization. Techniques to reduce such effort are provided in an Eclipse [3] plug-in, coined GZoltar [4], proposed by us previously to alleviate the debugging effort.

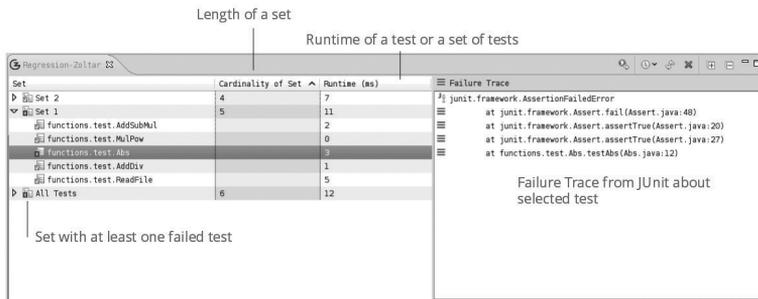


Figure 1 – Eclipse Plug-in RZoltar View

GZoltar is a graphical visualization which represents the system under test, and indicates which components have higher failure probability. The plug-in was extended with yet another view, RZoltar (see Figure 1), which is an interface to interact with regression suite tests: calculate the set with minimal cardinality or minimal runtime, execute the selected suite, etc. With these two views: GZoltar and RZoltar, in the same Eclipse plug-in, we create an ecosystem where user can interact between debugging and testing in real time.

References:

- [1] Sunghun Kim, E. James Whitehead, Jr., and Yi Zhang. Classifying software changes: Clean or buggy? IEEE Transactions on Software Engineering, 34:181–196, March 2008.
- [2] F. I. Vokolos and P. G. Frankl. Empirical evaluation of the textual differencing regression testing technique. In Proceedings of the International Conference on Software Maintenance, ICSM '98, pages 44, Washington, DC, USA, 1998. IEEE Computer Society.
- [3] <http://www.eclipse.org>
- [4] André Ribeiro, Rui Abreu. The GZoltar project: A graphical debugger interface. In Proceedings of the 5<sup>th</sup> international academic and industrial conference on Testing – practice and research techniques, TAIC PART'10, pages 215-218, Windsor, UK, 2010.

# Driving Coach: a Smartphone Application to Evaluate and Teach Driving Efficient Patterns

**Rui Araújo, Ângela Igreja, Ricardo de Castro, Rui Esteves Araújo**

Faculty of Engineering, University of Porto, Portugal

Nowadays, the efficient utilization of energy resources associated with the transportation of people and goods has been of great concern among government agencies, industry and society in general. The increasing costs of fossil fuels, together with environmental problems and atmospheric pollution, has encouraged the development and marketing of energy efficient vehicles, using strategies such as: (i) reducing the rolling resistance of tires, (ii) reducing the aerodynamic drag factor of the vehicle, (iii) increase the energy efficiency of the vehicle powertrain (engine, transmission, etc.), (iv) hybridization of the vehicle through the electric propulsion, etc..

In spite of these technical improvements, the efficiency of the vehicle will be always dependent on the driver's behavior. For example, recent studies by Toyota show that driving style could affect the vehicle's energy consumption up to 20%[1,2]. Although the drivers have been alerted for these issues during their driving lessons[3], practical tools to monitor and classify your style of driving from the point of view of energy consumption are still needed, and also to help identifying the incorrect behavior in real time. To fill this gap we propose the application "Driving Coach" capable of advising and teaching the driver to follow more efficient driving strategies.

The main features of the "Driving Coach" application are: (i) collect and process the state of the vehicle (speed, consumption, acceleration,...) by sensors embedded in the smartphone (eg GPS) and complemented with measurements taken from the internal network of the vehicle (through the interface OBD-II), (ii) Based on data acquired in the previous point, will be done an evaluation of driving performed in real time. During the trip, if suitable, the application will suggest the driver ways to reduce energy consumption of the vehicle, (iii) At the end of the trip, the application allows access to a report about the performance of the driver during the journey. This report can be exported for visualization on a computer or shared on social networks.

## References:

- [1] H. Xi, T. Ying, and H. Xingui, "An Intelligent Multifeature Statistical Approach for the Discrimination of Driving Conditions of a Hybrid Electric Vehicle," Intelligent Transportation Systems, IEEE Transactions on, vol. 12, no. 2, pp. 453-465, 2011.
- [2] T. J. Barlow, S. Latham, I. S. McCrae, and P. G. Boulter, "A reference book of vehicle driving cycles for use in the measurements of road vehicles emissions," TRL Limited, Tech. Rep., 2009.
- [3] "Overview on the Status of Ecodriving Integration in Driver Education and Testing," Ecodrive Project, Tech. Rep., 2010

# Lightweight Approach to Automatic Error Detection Using Program Invariants

**J. Santos<sup>1</sup>, R. Abreu<sup>1</sup>**

<sup>1</sup> Department of Informatics Engineering, Faculty of Engineering, University of Porto, Portugal.

Despite all efforts made during the development phase to test the application thoroughly, errors always creep to the operational phase. To fix these errors, a debugging phase is required, where the faults are located and fixed. This, however, is a costly process [1] and locating faults can be a very difficult task. Automating the process of locating the root cause of observed failures is one of the possible ways to reduce the cost of debugging.

In order to address this issue, tools like Zoltar [2] were developed. Through the use of generic invariants, also known as screeners, it is possible to predict the location of a faulty code segment, using Spectrum-based Fault Localization [3]. These fault screeners are software constructs that detect errors on the values of variables [4].

However this method presents some obstacles to its use on real applications. Using a fault screener on all occurrences of every variable creates an immense overhead. To reduce this overhead, it is necessary to decrease the number of instrumented points on the code. To achieve this, a possible solution would be to discover what are the *collar variables* of the system and only monitor those variables. According to Tim Menzies [5], *collar variables* are the key variables of the system that truly influence its behavior.

Another situation faced when using screeners is the accuracy of the results given by the screeners. Fault screeners are prone to produce erroneous results. These results can either be false positives, when an unexistent error is detected, or false negatives, when no error is detected in the presence of one. This happens because of the training screeners must go through during testing of the development phase. Since each passed test increases the range of values that the screener allows, the number of tests affects the rate of false positives and false negatives [3]. So, it is necessary to know the training a screener needs, to provide the most accurate results.

With this work it is expected the implementation of a lightweight prototype capable of detecting the *collar variables* of a system under test and evaluating when the instrumented screeners for those variables have received enough training to be used. By achieving these goals, it is expected to make the use of fault screeners for automatic software fault localization a viable option.

## References:

- [1] Gregory Tassej. The economic impacts of inadequate infrastructure for software testing. National Institute of Standards and Technology RTI Project, 2002.
- [2] Tom Janssen, Rui Abreu, and Arjan J. C. van Gemund. Zoltar: A toolset for automatic fault localization. In Proceedings of ASE '09, pages 662–664.
- [3] Rui Abreu, Alberto González, Peter Zoetewij, and Arjan J.C. van Gemund. Automatic software fault localization using generic program invariants. In Proceedings of SAC'08, pages 712–717, 2008.
- [4] Paul Racunas, Kypros Constantinides, Srilatha Manne, and Shubhendu S Mukherjee. Perturbation-based Fault Screening. In Proceedings of HPCA'07, pages 169–180, 2007.
- [5] Tim Menzies, David Owen, and Julian Richardson. The strangest thing about software. *Computer*, 40(1):54–60, 2007.

# Facebook Filter Plugin

**L. Gonçalves<sup>1</sup>, E. Rodrigues<sup>1</sup>, T. Silva<sup>2</sup>, P. Abreu<sup>2</sup>**

<sup>1</sup> Department of Informatics Engineering, Faculty of Engineering University of Porto, Portugal.

<sup>2</sup> Shortcut, Portugal

The huge supply of information content in the Internet and its continuous growth makes the selection process more difficult for end users. Many filtering systems [1] have been created to help users wade through the large amount of information, but they proved ineffective, not just because of the information overload, but also because of the type and quality of the information deemed relevant to users.

This particular problem is being experienced by Facebook users. They see their Facebook page incessantly populated with irrelevant content, which significantly hinders reading what is indeed relevant for them. Facebook has made a first attempt at solving this problem by creating their own filtering system, which sorts the user stream based on some notion of relevance. However, their approach is not very effective since it often misses to identify the posts which are indeed the most relevant to users.

Concerned with this problem, our research focused on developing a more effective filtering system, capable of finding the real user preferences. Recent studies point to the existence of an user profile that can be personal and selective, which becomes more efficient and responsive when it is built in a filtering system. So choosing the best features for a user profile is crucial on a filtering system.

The result of our research is the Facebook Filter Plugin, a web-service that uses four different APIs that work together to build a content filtering system. It uses Facebook API, Google Prediction API, Google Cloud Storage and Google Chrome Extensions.

Google Prediction API is a service that enables the creation of "smarter" applications. This intelligence comes from capabilities of pattern combinations and machine learning to analyze data that is repeated on a recurring basis and predict possible future results. In other words, it predicts new results through historical data obtained by the user. To use this service we also need Google Cloud Storage which is a RESTful service for storing and accessing out data in Google's infrastructure [2].

The application runs on the browser as a Google Chrome extension. It adds some changes to Facebook page to "activate" the true application features. The filtering system relies on the user's "Like" history to train a personalized model. The system keeps adapting the model by receiving further "Like" or "Dislike" signals from the user, which in turns keeps improving the relevance of the filtered content.

References:

[1] Toon De Pessemier, Tom Deryckere, and Luc Martens. Context aware recommendations for user-generated content on a social network site. In Proceedings of the seventh european conference on European interactive television conference, EuroITV '09, pages 133–136, New York, NY, USA, 2009. ACM.

[2] <http://code.google.com/intl/pt-PT/apis/predict/>

# The Mathematics Behind Google - PageRank Algorithm

C. Furtado<sup>1</sup>, N. Moreira<sup>1</sup>, A. P. Rocha<sup>1,2</sup>

<sup>1</sup> Departamento de Matemática, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

<sup>2</sup> CMUP, Centro de Matemática da Universidade do Porto, Porto, Portugal

PageRank is an assignment of importance to pages based on the hyperlink structure of the Web. It is fundamental to current conceptions of Web search [2]. The pagerank value explains why pages precede others in the Google search. Larry Page and Sergey Brin developed an algorithm, named PageRank Algorithm, with high performance in the web search. This algorithm made Google such an efficient search engine!

Behind PageRank Algorithm there are many interesting mathematical aspects. Our goal with this work is to present some modeling aspects of the web, pagerank and its efficient computing. The model for PageRank uses the hyperlink structure of the web to build a Markov chain with transition probability matrix  $P$  [1]. Pages in the web are organized according to its pagerank value. Hence, when we submit a query in Google™ the links that appear are organized by pagerank (Fig.1).



Figure1: A simple model for the url FCUP with 3 pages and respective pagerank value

Regardless of the method for filling in and storing the entries of  $P$ , PageRank is determined by computing the stationary solution  $\pi^T$  of the associated chain. That vector  $\pi^T$  can be found determining the dominant eigenvector of  $P$  [1].

In our work we present a version of PageRank algorithm in Matlab™ that can calculate pagerank in real time using power method.

Finally, we will present some current research trends in this area.

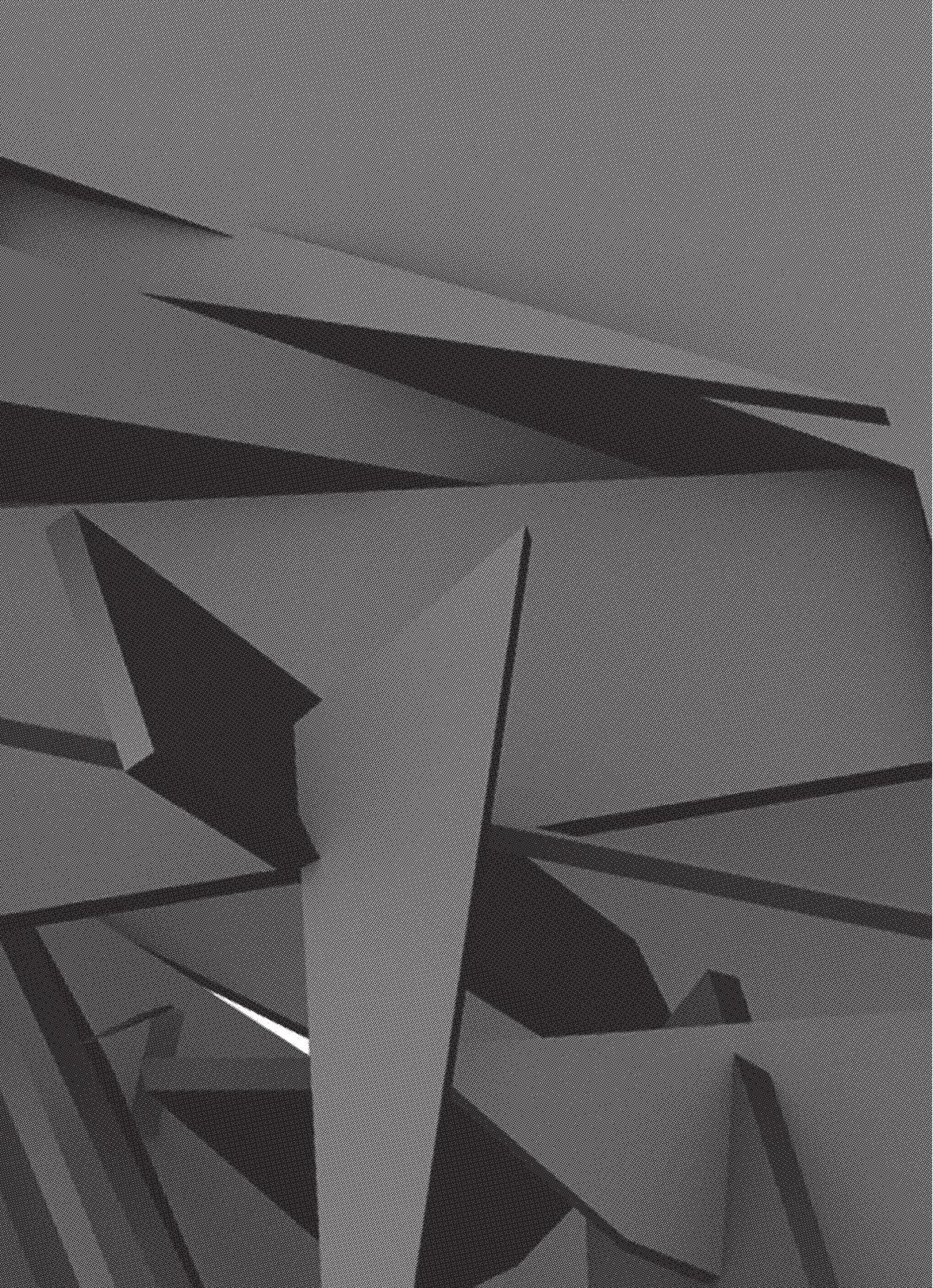
## References:

[1] A. Langville, C. Meyer (2006). *Google's PageRank Beyond – The science of search engine Rankings*, Pincetonwon University Press, New Jersey

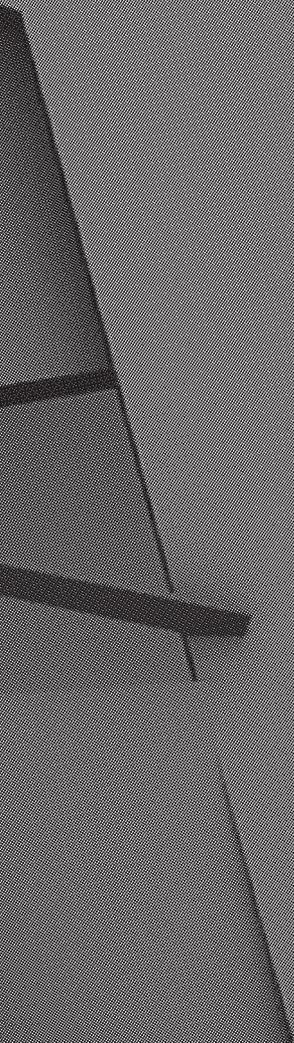
[2] S. Brin, L. Page, R. et.al . *The PageRank Citation Ranking: Bringing Order to the Web*

[3] [www.mathworks.com/moler/index.html](http://www.mathworks.com/moler/index.html)





# Oral Sessions IV





Oral Sessions IV > A1

# Endocrinology & Metabolism



## Obesity: the role of melanocortins in rat adipose tissue

**B. Sousa-Pinto<sup>1</sup>, L. Gonçalves<sup>1</sup>, B. Almeida<sup>1</sup>, A.R. Rodrigues<sup>1</sup>, I. Tomada<sup>1</sup>, D. Neves<sup>1</sup> and A.M. Gouveia<sup>1,2</sup>**

<sup>1</sup>Department of Experimental Biology, Faculty of Medicine and IBMC of University of Porto,

<sup>2</sup>Faculty of Nutrition and Food Sciences of University of Porto

The melanocortin system is composed by several melanocortins (adrenocorticotrophic hormone, alpha, beta and gamma melanocyte - stimulating hormone) that act through five subtypes of receptors (MC1R-MC5R). It is well known its role on energy homeostasis and, particularly, the melanocortins expressed at the central nervous system are extensively involved in the regulation of food intake and body weight. However, the role of melanocortins in peripheral tissues, such as adipose tissue, remains to be clarified. There is evidence that intravenous administration of melanocortins reduces food intake, by a central nervous system independent mechanism [1]. This and other findings suggest the involvement of the melanocortin system in the regulation of energy balance at peripheral tissues. The study of the mechanisms through which this system operates is thus of great importance, considering the perspective of development of novel pharmacological therapeutics, vital for treatment of diseases like obesity.

The first aim of this project was to evaluate the expression of the five melanocortin receptors (MCRs) in rat adipose tissue, since there is no consensus about which MCRs are expressed in both human and rat adipose tissue. Qualitative RT-PCR experiments revealed that the five subtypes of melanocortins receptors are present in rat adipose tissue (previous studies only described the expression of MC4R and MC5R [2]). Further, we intent to evaluate the influence of a high fat diet (HFD, 45% fat content) in the MCRs expression levels in subcutaneous, mesenteric, peri-renal, and gonadal rat adipose tissues. Real-time PCR analysis revealed that MC2R level was higher in peri-renal than in mesenteric or subcutaneous adipose tissue from rats fed with the control diet (4% fat content). However, no statistically differences were observed in MC2R expression between HFD treated rats and controls. These results suggest that the fat content in diet does not influence the expression of MC2R in rat adipose tissue. Real-time PCR analysis of the other receptors is still in progress.

### References:

- [1] Trivedi, P. *et al* (2003), *Exploring the site of anorectic action of peripherally administered synthetic melanocortin peptide MT-II in rats*. Brain Res. 977, 221–230.
- [2] Hoggard, N, *et al* (2004), *Regulation of adipose tissue leptin secretion by alpha-melanocyte-stimulating hormone and agouti-related protein: further evidence of an interaction between leptin and the melanocortin signalling system*. JMolEndocrinol. 32(1):145-53.

## Ghrelin decreases the IOP in an acute glaucoma model

**P Pereira-Silva<sup>1</sup>; M Tavares Silva<sup>1</sup>; S Azevedo-Pinto<sup>1</sup>; J. Araujo<sup>1,2</sup>; A Rocha Sousa<sup>1,2</sup>**

<sup>1</sup> Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> Department of Ophthalmology, Hospital São João, Porto, Portugal.

### Introduction

Ghrelin and des-acyl-ghrelin are two peptides derived from the pro-ghrelin. Ghrelin, an acylated 28 aminoacid peptide, is the endogenous ligand of the growth hormone somatosecretagogues receptor-type 1a (GHSR-1a). Des-acyl-ghrelin lacks the acyl group and does not bind GHSR-1a. In the eye, ghrelin promotes the relaxation of the iris' sphincter muscle independently from GHSR-1a and dependently on prostaglandins. On the iris' dilator muscle ghrelin promotes relaxation, mediated by the GHSR-1a. Ghrelin's aqueous humour levels were determined in patients with glaucoma. Glaucoma consists on an optic nerve damage, appearing subsequently to an increased intraocular pressure (IOP) in the majority of cases, being ghrelin's aqueous humour levels significantly decreased in glaucoma patients.

The aim of this study was to evaluate the effect of the ghrelin and des-acyl ghrelin in the intraocular pressure in an animal model of acute glaucoma.

### Methods

The first part of the experimental protocol was the calibration of the Tonovet® tonometer, used to measure the IOP. The external calibration involved the cannulation of the eye's anterior segment with a 25G needle, connected to a pressure transducer and to a 0,9% NaCl reservoir, which enabled us to alter the IOP. IOP values varied from 5 to 60 mmHg. For each pressure level, IOP was measured both with the pressure transducer and the Tonovet tonometer.

In the second part of the protocol acute glaucoma was induced in rabbit and in rat animal models through an intravitreal injection of 20% NaCl (50µL). In rabbits, we studied the effects of ghrelin ( $10^{-4}$ M, n=6), in the presence or absence of L-NAME (a NO synthase inhibitor; 150mg/Kg; 500 µL; n=11) or ketorolac (a COX inhibitor; 30 mg/mL; 500µL; n=7). Des-acyl-ghrelin's effect ( $10^{-4}$ M, n=7) was also evaluated. In the rat, only ghrelin's ( $10^{-4}$ M, n=8) effect has been studied yet. All the results were compared to a control group which did not receive ghrelin or des-acyl-ghrelin.

### Results

The real IOP was compared to the tonometer measurements, and we concluded that there is a linear correlation ( $Tonovet = -0,331 + 0,750 \text{ real}$ ) between the two variables, being that the tonometer underestimated the real IOP. Our results showed that ghrelin promotes a decrease in IOP of  $20,8 \pm 5,0$  mmHg ( $47,9 \pm 11,6\%$ ), in rabbits, and of  $12,2 \pm 3,5$  mmHg ( $41,4 \pm 12,2\%$ ) in rats, while des-acyl-ghrelin did not significantly change IOP. When ketorolac or L-NAME were added, ghrelin's hypotensive effect was completely blunted.

### Conclusion

Ghrelin promotes a significant and sustained decrease of the intraocular pressure in acute glaucoma models. This effect is independent of GHSR-1a and dependent on NO and prostaglandins.

# Chronic ingestion of high-fat diet and energy restriction modulates expression of VEGF, VEGFR2, angiopoietins and Tie2 in aged rat myocardium.

**D. Fernandes<sup>1</sup>, I. Tomada<sup>1</sup>, H. Almeida<sup>1</sup>, D. Neves<sup>1</sup>**

<sup>1</sup>Department of Experimental Biology, Faculty of Medicine and IBMC, Universidade do Porto, Portugal.

Regular intake of hyperlipidic diet and obesity induce endothelial dysfunction that always precedes the onset of atherosclerosis and cardiovascular diseases (CVD). Furthermore, the association of regular high-fat food intake with advanced age seems to be particularly hazardous, considering that CVD constitute a leading cause of death in the aged population. Indeed, both factors contribute independently to structural and molecular modifications of the heart, such as fibrosis, and deregulation of expression of angiogenic factors and their specific receptors [1,2]. Herewith we aimed to characterize the cellular organization of the heart and the expression pattern of vascular endothelial growth factor (VEGF), angiopoietin 1 (Ang1), angiopoietin 2 (Ang2) and receptors VEGFR2 and Tie2 in young and aged rats under high-fat diet and energy restriction.

Adult male Sprague-Dawley rats (8-wks old) were divided into three groups (n=15): a control group (C); a group of rats fed with hyperlipidic diet (HF) and another subjected to energy restriction (ER). In each group, 5 rats were sacrificed when ages 6, 12 and 18 months. Dual-labelling immunofluorescence detection of the endothelial protein PECAM (platelet/endothelial cell adhesion molecule) and  $\alpha$ -actin, VEGF and VEGFR2, Ang1 or Ang2 and Tie2 were observed in an Apotome fluorescence microscope. Besides, we performed morphometrical analysis of smooth muscle content in the myocardium after immunohistochemical detection of  $\alpha$ -actin. Semiquantification of VEGF and VEGFR2 was made by Western blotting.

The expression of PECAM was restricted to the endothelium and  $\alpha$ -actin to smooth muscle layer of myocardium's blood vessels in all experimental groups. VEGF and VEGFR2 were detected in endothelium and in lower extension in the cardiomyocytes. It was verified that VEGF expression was significantly reduced in 18-months rats under consumption of high-fat diet compared with age-matched group, and that VEGFR2 expression significantly decreased during aging. Expression of Tie2/Ang1 was observed in the cardiomyocytes in all groups and co-localization of Tie2 and Ang2 was detected in endothelial cells and cardiomyocytes. Morphometrical study concerning the perivascular smooth muscle layer demonstrated a significant decrease in the treated groups compared with controls.

Overall, the data suggest that aging and fat content of diet modulate vascular mechanisms of the heart; nevertheless, further molecular studies will be necessary to elucidate this hypothesis.

References:

- [1] Cordeiro, A.L., et al. (2010), *Characterization of the Expression of Ang1, Ang2, and Tie2 in the Corpus Cavernosum of the Rat during Aging*, *Microscopy and Microanalysis*, 16, 699-709.
- [2] Ferrara, N., et al. (2003), *The biology of VEGF and its receptors*, *Nature Medicine*, 9, 669-676.

## Effect of body position on girths in older adults

A. Sousa<sup>1</sup>, O.Sousa<sup>2</sup>, T. F. Amaral<sup>1</sup>

<sup>1</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

<sup>2</sup> Magalhães Lemos Hospital, E.P.E., Porto, Portugal.

Standards for anthropometrical assessment determine that anthropometric measurements must be taken on the subject assuming a standing position or, sometimes, a sitting position, although not always possible. The effect of body position on anthropometric measurements has not been studied. This is clinically relevant because it may lead to biased results of undernutrition screening tools. The main purpose of this study was to evaluate the effect of body position and symmetry on girth assessment.

A cross-sectional study was conducted on 102 older adults. Measurements were conducted while the subject was standing, lying down and also on both the right and left side of the body. Mini Nutritional Assessment Short-Form® screening tool was used to search for eventual score differences according to calf circumference and was assessed with the subject again standing and lying down.

Significant differences were found in regards to body position, though an exception was found for waist and mid-thigh ( $p < 0.001$ ). A high correlation ( $r > 0.90$ ) and high agreement ( $k > 0.79$ ) between the two body positions was found and no differences were found between right and left side of the body for all the body girths.

In conclusion, differences found between body positions do not have impact on nutritional screening for clinical purposes. Furthermore, these data confirm body symmetry for arm girth, calf circumference and mid-thigh girths.

## The Influence of Obesity and Gestational Weight Gain on the Newborn Weight in a Group of Woman with Gestational Diabetes

L. Braz<sup>1</sup>, F. Fonseca<sup>2</sup>, L.Figueiredo<sup>3</sup>

<sup>1</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

<sup>2,3</sup> Centro Hospitalar do Alto Ave, EPE /Service of Nutrition

The Gestational Diabetes is associated with numerous complications for mother and fetus. The previous weight before pregnancy and the gestational weight gain have been associated to the newborn weight.

**Objective:** Evaluate the association of the previous BMI and the weight gain during pregnancy with the newborn weight.

**Methodology:** In this retrospective study participated 257 pregnant women with gestational diabetes. It were collected sociodemographic and anthropometric data from mother and newborn. It was held multiple linear regressions to predict the newborn weight. The statistics analysis was done using SPSS 18®.

**Results:** Of the women evaluated, the following categories of BMI were analysed: normal weight (45%), overweight (33%) and obesity (22%). The average weight gain (Kg) in the groups was 10,7±4,2, 10,5±5,5 e 7,1±5,9 respectively, from which 39,3% and 35,5% of the women with overweight and obesity had a weigh gain above recommended. The macrosomia prevalence was 2%. The previous BMI ( $p<0,001$ ), weight gain ( $p<0,001$ ), gestational age on birth ( $p<0,001$ ) are predictive of the birth weight. Women with previous overweight and obesity have 1,4 and 3,5 times more chance to give birth to a newborn large for gestational age when in comparison to normal weight women. The weight gain above IOM recommendation, doubles the risk of newborn large for its gestational age, although with no significant statistically meaning.

**Conclusion:** The pre-pregnancy BMI and weight gain during pregnancy are predictive of newborn birth weight in women with gestational diabetes.

# DEDENDERA - An illness-cure journey for the female reproductive system and the voice through an artistic work

**F. Augusta<sup>1</sup>**

<sup>1</sup> Department of Sculpture, Faculty of Fine Arts, University of Porto, Portugal.

This artistic work has its focus on the feminine body, plenty of fluid and cellular memory.

Through fine art print studio methods, it establishes the crossing between anthropological studies and medicine, in the sense that it explores artistically how dealing positively-negatively with an awaiting circumstance (either personal or generational) weakens the basin organs such as the reproductive system, or strengthens it in a way that it enables women to overcome genealogical gynaecological problems.

For this work I've attended conferences, read different approaches from gynaecologists and therapists and even served at a birth house as a volunteer. It was also relevant the empirical experience as a doula apprentice as well as a singer since it also builds up a connection with the voice and how its colour is influenced in an endocrinal and physiological way, and how it is able to produce frequencies in order to nurture the basin back again.

I've worked over material from pathological anatomy, specifically on irregularities of the feminine reproductive device. The series culminate in the use of this sort of materials of complex character (Fig. 1).



Fig. 1

Oral Sessions IV > A2

# Environment I



# Comparative study of methodologies for assessing the hydromorphological quality of river systems

S. Teixeira<sup>1</sup>

<sup>1</sup>Department of Geosciences, Environment and Territorial Planning, Faculty of Sciences, University of Porto, Portugal.

The present study compared four methods used to assess the hydromorphological conditions of river systems, two European and two American.

The field results obtained with three of those methods – RHS<sup>1</sup>, HIDRI<sup>2</sup> and AVH<sup>3</sup> - were compared to evaluate which of them was the best adapted to the characteristics of rivers in northern Portugal.

Field work was conducted between February and May 2011, in five sampling points, in the rivers Âncora (São Lourenço da Montaria), Sousa (Senhora do Salto) e Leça (Ermesinde; Ribeira do Arquinho e Maia Ambiente).

From the comparison of the results, the AVH was considered the most adequate method. Based on this method, a new methodology, adapted to the characteristics of the region, is proposed, also including features from the other methods used in the study.

This study revealed that the methods discussed did not applied effectively to the sampled locations, because they contain features that cannot be found in the rivers in the North of Portugal, or because they are difficult to assess due to the lack of the necessary information.

The development of the initial form for the channel characterization was based on the concepts presented in the method of Rosgen (the fourth method used in the study, which does not require field work at this level of application), in order to obtain an overview of the channel features.

The ultimate goals of the new method are easiness of implementation by all observers, experienced or not; quick gathering of all the information needed; and the use of concepts that are easy to understand avoiding, as much as possible, ambiguities in the final classification.

References:

[1]Environment Agency (2003), River Habitat Survey in Britain and Ireland, Field Survey Guidance Manual: 2003 Version

[2] Agència Catalana de L'aigua. (2006). Protocol d'avaluació de la qualitat hidromorfològica dels rius.

[3] Barbour, M., Gerritsen, J., Snyder, B., & Stribling, J. (1999). *Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition.*

# Observation of the Douro River Plume Using Satellite Images

**D. Ayres-Sampaio<sup>1</sup>, J.C.B. da Silva<sup>1,2</sup>, and L. Bastos<sup>1,2</sup>**

<sup>1</sup> Department of Geosciences, Environment and Spatial Planning, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal.

River plumes are a mixture of fresh water and river sediment load, with some dilution caused by currents [1]. They play an important role in a marine ecosystem due to their capacity to deliver sediments, nutrients and pollutants to the adjacent coastal region [2]. Satellite observations of the optical properties of coastal surface waters can be used to distinguish turbid plume water from ambient water masses, particularly based on increased concentrations of suspended material in the plumes. Using satellite images, instead of traditional ship-based surveys, the extent of the plume can be monitored with synoptic and frequent overviews of the coastal zone leading to a more effective analysis of the temporal and spatial variability of the plume [2].

The main objective of this work is to develop a method to observe the temporal and spatial variability of the Douro river plume, off Portugal. We have used MODIS-Aqua normalized water-leaving radiance at 547 nm ( $nLw_{547}$ ) obtained from July 2002 to July 2011. The area of study was divided in three regions and a time series of the plume area has been created for each region. The plume area was evaluated through the sum of turbid pixels. A pixel was considered turbid if the  $nLw_{547}$  exceeded the threshold of  $1.3 \text{ mWcm}^{-2} \mu\text{m}^{-1} \text{ sr}^{-1}$  [2]. The time series of the plume area is compared with the Douro river water volume (at Crestuma dam) and wind speed.

Using this method, we found that the Douro river plume follows a pattern of temporal variation with two phases of visibility per year. In the middle of these two phases, the plume size is very low. However, at the end of 2002 and 2009 this pattern is not verified, probably due to the occurrence of a high water volume. Finally, we observe that when the water volume was low (reduced river outflow), the plume size was influenced by southerly winds.

We believe that the proposed methodology is a promising approach for the river plume size evaluation through satellite measurements, but further studies will be needed to reach conclusive results, and develop further the methodology presented here.

## References:

[1] Teodoro, A.C., Gonçalves H., Veloso-Gomes, F., and Gonçalves, J.A. (2009), *Modeling of the Douro River Plume Size, Obtained Through Image Segmentation of MERIS Data*, IEEE Geoscience and Remote Sensing Letters, 6 (1), 87-91.

[2] Valente, A.S., and da Silva, J.C.B. (2009), *On the observability of the fortnightly cycle of the Tagus estuary turbid plume using MODIS ocean colour images*, Journal of Marine Systems, 75 (1-2), 131-137.

# Linear alkylbenzene sulfonate (LAS) removal present in commercial laundry wastewater in anaerobic fluidized bed reactor

T.Z.Macedo<sup>1</sup>, J. K. Braga<sup>1</sup>, M. B.Varesche<sup>1</sup>

<sup>1</sup>Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo, Brazil.

The laundry is an important service sector in the commercial area and the surfactants are the major active ingredients of laundry detergents. Linear alkybenzene sulfonate (LAS) is the most synthetic surfactant produced worldwide. Although are witnessed a growing number of studies in seeking alternatives to the surfactants removal, they are still scarce. Thus, developing technologies which allow not only the removal but the anaerobic surfactants mineralization in commercial laundry wastewater treatment justifies the relevance of this research.

The fluidized bed reactor was made of acrylic, 100 cm in length (L) and 4 cm internal diameter (D) and a total volume of 1256 mL. The hydraulic detention time (HDT) was 18 hours for 174 days and the temperature was maintained at 30°C. The reactor was inoculated with sludge from a UASB reactor treating swine culture wastes and it was fed with synthetic substrate [1] plus laundry wastewater for concentrations of LAS in the range of 10 mg L<sup>-1</sup> and 30 mg L<sup>-1</sup>. The reactor behavior was accomplished by the following analyses: chemical oxygen demand (COD), pH, alkalinity, solids, sulfate [2], bicarbonate alkalinity [3], volatile acids [4], LAS [1]. The reactor was operated in two stages: stage I (10 mg L<sup>-1</sup> of LAS and 637 ± 80 mg L<sup>-1</sup> COD) and stage II (30 mg L<sup>-1</sup> of LAS and 723 ± 82 mg L<sup>-1</sup> of COD).

The removal efficiencies of organic matter and LAS were 84.7±9.6% and 74.6±14.1%, respectively, in stage I. In stage II it was obtained, respectively, 91.1±3.9% and 68.2±16.9%. Despite the increase in LAS influent concentration has not prejudiced the organic matter removal, the LAS removal efficiency significantly decreased (68.2±16.9% for LAS influent concentration of 23.9±5 mg L<sup>-1</sup>).

The reactor proved highly efficient in the removal of organic matter, analyzed in terms of filtered COD, which was not influenced by presence of LAS. Thus, the anaerobic fluidized bed reactor containing immobilized biomass can be considered a feasible reactor configuration for treating real wastewater with LAS.

## References:

- [1] Duarte, I.C.S., Oliveira, L.L., Buzzini, A.P., Adorno, M.A.T., Varesche, M.B.A. development of a Method by HPLC to Determine LAS and its Application in Anaerobic Reactors. *J. Braz. Chem. Soc.*, 117, 7, p. 1360-1367, 2006.
- [2] APHA/AWWA/Water Environment Federation. (2005) Standard Methods for the Examination of water and wastewater. 20th ed. Washington, DC, USA.
- [3] Dilallo, R.; Albertson, E. (1961). Volatile acids by direct titration. *Journal WPCF*, **33**, pp. 356-365.
- [4] Moraes, E. M., Adorno M. A. T., Zaiat M. E Foresti E. (2000). *Determinação de Ácidos Voláteis por Cromatografia Gasosa em Efluentes de Reatores Anaeróbios Tratando Resíduos Líquidos e Sólidos* In: VI Oficina e Seminário Latino-Americano de Digestão Anaeróbia, 2000, Recife - PE. Anais da VI Oficina e Seminário Latino-Americano de Digestão Anaeróbia. Recife – PE. Ed. Universitária - UFPE, 2:235-238.

## Single and binary metal oxide catalysts for VOC abatement

**X. Chen**<sup>1,2</sup>, **S.A.C. Carabineiro**<sup>2</sup>, **S.S.T Bastos**<sup>2</sup>, **J.J.M. Órfão**<sup>2</sup>, **M.F.R. Pereira**<sup>2</sup>, **J.L. Figueiredo**<sup>2</sup>

<sup>1</sup> School of Industrial Engineering, Metropolia University of Applied Sciences, Finland

<sup>2</sup> Laboratório de Catálise e Materiais, Laboratório Associado LSRE/LCM, Faculdade de Engenharia, Universidade do Porto, 4200-465 Porto, Portugal

Volatile organic compounds (VOCs) are important environmental pollutants originated from petroleum refineries, fuel storage and loading operations, painting and printing operations, and motor vehicles [1,2]. Catalytic oxidation is an environmental friendly technology for VOC abatement that requires low temperatures (around 250-500 °C) and causes less NO<sub>x</sub> formation [1,2].

The purpose of this work is to prepare metal oxides for the catalytic oxidation of volatile organic compounds. Single and mixed oxides were prepared by an exotemplating method [1-3]. Two carbon materials: carbon xerogel (CX) prepared by a previously described method [4] and commercial activated carbon NORIT ROX 0.8 (AC), were used as templates in the synthesis of copper, iron and nickel oxides. A thermal evaporation method (EM) was additionally used for the synthesis of mixed oxides [5]. All synthesized samples were characterized by thermogravimetry and differential scanning calorimetry, N<sub>2</sub> adsorption at -196 °C and temperature programmed reduction. The materials obtained were tested for VOC (ethyl acetate) oxidation.

The mixed oxides prepared by EM were very effective in the oxidation of ethyl acetate. Co-Ce 2:1 EM, for example, showed the best performance with 100% conversion to CO<sub>2</sub> attained at 225 °C. Cu-Ce prepared by exotemplating with CX, the best catalyst prepared by this method, catalyzed the oxidation reaction to full conversion at about 240 °C. In terms of single oxides, CuO showed the best performance. Nevertheless, the exotemplating procedure did not lead to more active Cu, Fe and Ni oxide catalysts, in comparison to their commercial counterparts.

**Acknowledgements:** Funding from IAESTE (PT/2011/59) and APIET (for XC), FCT and FEDER in the framework of Program COMPETE (Project PEst-C/EQB/LA0020/2011), and CIENCIA 2007 program (for SACC).

References:

- [1] Bastos, S., Órfão, J., Freitas, M., Pereira, M. and Figueiredo, J. (2009), *Manganese Oxide Catalysts Synthesized by Exotemplating for the Total Oxidation of Ethanol*. Applied Catalysis B: Environmental 93, no. 1-2: 30-37.
- [2] Bastos, S., Carabineiro, S.; Órfão, J.; Pereira, M.; Delgado, J. and Figueiredo, J. (2012), *Total oxidation of ethyl acetate, ethanol and toluene catalyzed by exotemplated manganese and cerium oxides loaded with gold*, Catalysis Today 180, n°1: 148– 154.
- [3] Schwickardi, M., Johann, T., Schmidt, W. and Schüth, F. (2002), *High-Surface-Area Oxides Obtained by an Activated Carbon Route*, Chemistry of Materials 14, no. 9: 3913-19.
- [4] Job, N.; Pirard, R.; Marien, J. and Pirard, J. (2004), *Porous carbon xerogels with texture tailored by pH control during sol-gel process*, Carbon 42, no. 3: 619–628.
- [5] Solsona, B., Garcia, T., Aylón, E., Dejoz, A., Vázquez, I., Agouram, S., Davies, T. and Taylor, S. (2011), *Promoting the Activity and Selectivity of High Surface Area Ni-Ce-O Mixed Oxides by Gold Deposition for VOC Catalytic Combustion*, Chemical Engineering Journal 175: 271-278.

# Treatment of wastewater resulting from washing of phytopharmaceutical plastic containers using combined biological and solar-driven advanced oxidation processes

**A. Ferreira<sup>1</sup>, F. Moreira<sup>1</sup>, C. Silva<sup>1</sup>, L. Matos<sup>1</sup>, R. Teixeira<sup>1</sup>, S. Reis<sup>2</sup>, E. Sousa<sup>2</sup>, V. Vilar<sup>1</sup> and R. Boaventura<sup>1</sup>**

<sup>1</sup> Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup> Faculdade de Farmácia da Universidade do Porto, Portugal.

Currently, one of the main worldwide concerns is the growth of surface water and groundwater pollution. In particular, water contamination by pesticides is a severe problem due to their high solubility in water, low-sorption affinity to soils, high toxicity, high chemical stability, potential for bio-accumulation and low biodegradability. The risk to aquatic organisms and the human being is prominent.

Among the different approaches that promote pesticide degradation, and considering their bio-recalcitrant character, advanced oxidation processes (AOPs) have been recognized as especially efficient when compared to conventional technologies [1]. AOPs are chemical processes characterized by the production of hydroxyl radicals ( $\text{HO}^\bullet$ ), which are extremely reactive and unselective oxidants.

In Portugal, the management of the empty phytopharmaceutical plastic containers, resulting from application in agriculture, is accomplished by VALORFITO. The collection of empty containers focus only on the primary containers of plant protection products, classified as hazardous wastes. In 2010, there were 416 operational reception spots and around 221 tons of containers were collected. The current plastic containers treatment is performed in EGEO-SISAV-CIRVER facilities, where the containers are screened, shredded into small pieces, granulated and washed using a steam system. The washed plastics are recycled and the hazard sludge produced is disposed in a landfill.

This study focuses on the development of an alternative treatment solution for the phytopharmaceutical plastic containers, in order to avoid the production of hazardous sludge and further disposal in landfills. This treatment consists of washing the shredded plastic containers and treating the resulting wastewater contaminated with pesticides combining a biological treatment, performed in an immobilized biomass reactor (IBR), with an AOP in heterogeneous ( $\text{TiO}_2/\text{UV}$  or  $\text{TiO}_2/\text{H}_2\text{O}_2/\text{UV}$ , both with and without acidification) or homogeneous ( $\text{UV}$ ,  $\text{H}_2\text{O}_2/\text{UV}$ ,  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{UV}$  or  $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ ) phase. The photocatalytic treatment took place in a solar pilot plant with compound parabolic collectors (CPCs). This process leads to the degradation of pesticides and consequent enhancement of wastewater biodegradability, allowing its further treatment in an aerated biological reactor, so that the achieved final effluent may comply with the discharge limits imposed by the Portuguese Legislation.

References:

[1] Devipriya, S. and Yesodharan, S. (2005), *Photocatalytic degradation of pesticide contaminants in water*, Solar Energy Materials and Solar Cells, 86 (3), 309-348.

IAREN - Instituto da Água da Região Norte, supplied all the human resources and equipment's needed for the identification and quantification of the pesticides and its degradation by-products.

# Determination of copper in freshwaters based on reversed flow injection analysis using a liquid core waveguide capillary cell

**P.S. Peixoto<sup>1</sup> and I.V. Tóth<sup>2</sup>**

<sup>1</sup> Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

<sup>2</sup> REQUIMTE/ Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

Copper is an essential metal to aquatic life that is generally present in surface waters. Besides naturally occurring concentrations of copper (reported from 0.50 to 30 µg/L) in freshwater systems, copper is identified as an anthropogenic pollutant and it may become toxic to some forms of aquatic life at higher concentrations [1]. Thus, copper analysis in estuarine and coastal water is relevant.

As the copper is present in low concentrations in natural water, a variety of techniques have been used to quantify trace dissolved copper concentrations, like voltammetry, inductively-coupled plasma atomic emission, flame atomic absorption spectrometry and electrothermal atomic absorption spectrometry [2]. However, most of these methods are not applicable for in situ monitoring of this analyte.

In this work, a simple, rapid and sensitive reverse flow injection analysis (rFIA) coupled with a 1 m liquid core waveguide capillary cell (LWCC) was used to determine copper in estuarine water samples. The spectrophotometric determination of copper was based on the colorimetric reaction with bathocuproine [3]. The different physical and chemical parameters of the flow system were selected to provide maximum sensitivity and good repeatability (CV < 5%). The influence of salinity variation on the determination was evaluated and the analytical characteristics of the LWCC flow cell were compared to a conventional U-shaped flow cell.

The detection and quantification limit of copper for LWCC were respectively, 0.90 µg/L and 1.30 µg/L and the relative standard deviation (n=5) in the analysis of fresh water certified reference materials was < 2%.

Acknowledgements:

I.V. Tóth thanks FSE and MCTES (Ministério da Ciência, Tecnologia e Ensino Superior) for the financial support through the POPH-QREN program and through grant PEst-C/EQB/LA0006/2011.

References:

- [1] U.S. Environmental Protection Agency, Office of Water (2007) *Aquatic life ambient freshwater quality criteria – copper*, Office of Science and Technology, Washington, DC.
- [2] Fifield, F.W. and Haines, P.J. (Eds.) (2000) *Environmental Analytical Chemistry*, Blackwell Science Ltd, Oxford.
- [3] Smith, G.F. and Wilkins, D.H. (1953), *Determination of copper in iron*, *Analytical Chemistry*, 25, 510-511.

Oral Sessions IV > A3

# Geography & Sociology



## The effect of green spaces' (bio) structure on climatic parameters

**S. Velho<sup>1</sup>, A. Monteiro<sup>2</sup> and H. Madureira<sup>3</sup>**

<sup>1</sup> Researcher of PTDC/SAU-ESA/73016/2006-FLUP/FMUP, Member of ISPUP (Public Health Institute of Porto University), Master Student of Geographic Information System and Territory management

<sup>2</sup> Department of Geography, Faculty of Letters, University of Porto, Portugal, Researcher of PTDC/SAU-ESA/73016/2006-FLUP/FMUP, Member of ISPUP (Public Health Institute of Porto University), Member of CITTA (Research Centre of Territory, Transport and Environment).

<sup>3</sup> Department of Geography, Faculty of Letters, University of Porto, Portugal, Researcher of PTDC/SAU-ESA/73016/2006-FLUP/FMUP, Member of ISPUP (Public Health Institute of Porto University), Member of CEGOT (Study Centre of Geography and Territory Management)

The way of vegetation composes a space, since its organization, structure and morphology, has effects on several climatic variables that are felt in a given space. This allows creating different climate contexts on a micro scale which, in turn, manifest themselves in bioclimatic comfort, conditioning the choices of a space for active or passive leisure.

This presentation rises from a multidisciplinary project that begun in 2011 [1], which integrates various scientific areas such as landscape architecture and applied climatology.

The project had as one of the main objectives understand the effect of vegetation's organization and structure in some climatic variables which act on the bioclimatic comfort, particularly in a green open space. In this case were Serralves' gardens, which by their hypsometric, extent and morphology revealed as a good laboratory for study. It's about some of these results that this exposition will focus.

To obtain the climatic data there were performed itinerant measurements for the following variables: temperature, relative humidity and wind speed, on two routes that extend through the Serralves' gardens. Seven fixed sensors were also installed to allow the acquisition of continuous data for temperature and relative humidity.

The analysis through the use of multivariate statistics and bioclimatic indexes calculation allowed us to recognize different contexts bioclimatic under the effect of the presence / absence of vegetation and shadow / light spaces. This result reinforces the importance attached to green open spaces as climate regulators, through the directly or indirectly relationship which establish with the effects of climate change. Considering this, green open spaces contribute to the human being adaptation to the climatic changes, increasing his bioclimatic comfort and urban quality of life.

[1] Projectos Pluridisciplinares | Concurso 2010. Projecto "O efeito da Bioestrutura dos espaços verdes no conforto bioclimático" (código 164)  
([http://ijup.up.pt/pluridisciplinares/documentos/Categoria\\_Geral.pdf](http://ijup.up.pt/pluridisciplinares/documentos/Categoria_Geral.pdf)).

# **The optimization of the spatial arrangement of the green spaces in the areas of urban expansion as a strategy for the design of a system of greenways**

**A. Queirós**<sup>1</sup>

<sup>1</sup> Master in Risks, Cities and Territorial Planning, Faculty of Letters, University of Porto, Portugal.

This paper focus on two main goals: devising a strategy for designing a system of greenways in the area considered “City of Gaia” in the Município of Vila Nova de Gaia and the implementation of the design of a greenway subsystem based on optimizing the spatial arrangement of the green spaces in the areas of urban expansion.

By categorizing and analyzing the territorial characteristics it was achieved the design of a greenway system. This track features five greenways, three of which are connected to form a subsystem. Using this subsystem, calculations were performed in order to determine the areas of urban expansion to be used for green space. The design was aided by the remaining green areas in the territory, as well as the spaces that, by their function or type, were consistent with those of greenways. This work climaxes with the creation of a conceptual project for the subsystem of greenways, including the proposal of spatial organization of the areas of urban expansion and its connections, and in connecting it with other components of the territory form greenways.

# **Tourism and Leisure Activity in Nature, The Framework Programs in Finance**

**Lopes, Marco Alexandre Marques**<sup>1</sup>

<sup>1</sup> Department of Geography, Faculty of Arts, University of Porto, Portugal.

Tourism is recognized as a competitive activity with economical potential in our country. Its relevance as a growth and development trigger is remarkable, as well in the National, as in the Regional and in the Local scale.

Given such strong impact in the economy's competitiveness, Tourism is contemplated in several documents of National Strategy and Policy.

Tourism, in general, and Nature Tourism in particular, establish a very close tie with the territories, its resources, and therefore, with environmental preservation and valorization. This territorial approach, confides to the municipalities a double role of promoting local development and managing land planning.

The aim of this project is to analyze the political and legal framework of Nature Tourism, through the several National, Regional and Local Policy documents, particularly the fund structures available in the period regulated by the European program QREN 2007/2013.

This research is based on life experience in work environment, and in the analysis of different market actors' perspective, as well as governments', consulters', professionals' and associations' perspective of which will be the future role of the municipalities in the development of Nature Tourism.

The study case is the Valongo Municipality, which, for its urban characteristics allied to its natural conditions, is very suitable for the development of Nature Tourism activities, and therefore, Valongo is a municipality with particular touristic potential in this field.

## Quality of life in urban context

**D. Ribeiro**<sup>1</sup>

<sup>1</sup> Department of Geography, Faculty of Letters, University of Porto, Portugal.

This work addresses the issue of quality of urban life under the Master Course in "Risks, Cities and Planning" branch "Urban Policies" and a stage in Porto Vivo, SRU.

Conceptually this work is based on the conceptual approach of Allardt (1993) and the notion of well-being on the dimensions Being, Having and Loving. In analytical terms the methodological approach developed is inspired by Hancock (2000) when, he conceptualizes the quality of life in quantitative and qualitative dimensions, which are developed individually or collectively.

In the subjective dimension (qualitative) analyzes the perceptions of individuals living in urban areas. In the analysis method, we analyzed 1007 questionnaires, which represent the population living in different urban contexts in Portugal. This analysis is mainly focussed on the urban amenities in the vicinity of place of residence (environmental conditions, equipment and services, transport and mobility, housing and work, public space and urban image, citizenship and governance). The analysis provides important information for the reflection of urban policies in different urban contexts.

In the objective dimension we have created an information system for the city of Oporto, which allowed us to assess the quality of life around two levels: the individual and collective (here selected housing). Thus cross individual characteristics with the territorial conditions.

The work showed territorial patterns capable of supporting urban regeneration policies.

The chosen conceptual framework and methodological approach developed shows virtually able to support monitoring and evaluation of urban policies.

# Porto and Vila Nova de Gaia, near the Douro City, identity and opportunity management of public spaces

M. Rocha Ribeiro<sup>1</sup>

<sup>1</sup> Department of Geography, Faculty of Letters, University of Porto, Portugal.

The theme was selected – the waterfronts and public spaces – because it has a major role in the competitiveness strategies of cities that are associated with urban regeneration processes. The banks of Douro river – with the cities of Porto and Vila Nova de Gaia as landscape – were chosen to develop the theme.

The aims of this study are to relate additional disciplinary areas, like urban planning and urban management, in a waterfront landscape (specifically in the cities of Porto and Vila Nova de Gaia) and also discourse about the importance and opportunity to implement a Geographic Information System (GIS) as a tool to support decisions in terms of public spaces management.

The question is to demonstrate the ability of GIS tool to monitor, evaluate and manage the appropriation of public space and its suitability for obtaining spatially referenced data, particularly on how people interact with urban space in their daily life, which means, in their journeys through streets, squares, gardens and other public spaces compositions.

The purpose is to create a management model based on multivariate analysis on how the public space is used. The model will standardize the behaviours of users and get to know the favourable conditions to the occurrence of urban life, thereby contributing to taking the decisions best suited to reversing the negative trends that happens during the enjoyment of public space, promoting, in this way, better management.

Considering the aim of contributing to the public space better management based on GIS technologies, this work studies the issues of waterfronts and public spaces, correlates the context of the study area of Porto and Vila Nova de Gaia along the banks of the Douro river and also outlook the build of a management model based on their practice and knowledge of the conditions that determine the variation of the intensity of certain patterns of urban life that occur in public space.

The purpose of formal creation of a methodology for developing a GIS applied to the public spaces of Porto and Gaia, alongside the Douro river, sets up an opportunity to know which variables that determine the best conditions for the occurrence of urban life in the public spaces of these waterfronts, enabling a consistent and effective management.

**Keywords:** Oporto, Vila Nova de Gaia, historic center, public space, waterfronts, urban renewal, GIS.



# The historic center of cities in the dynamics: The historic centre of Oporto

M. Esperança de Carvalho<sup>1</sup>,

<sup>1</sup> Department of Geography, Faculty of Letters, University of Porto, Portugal.

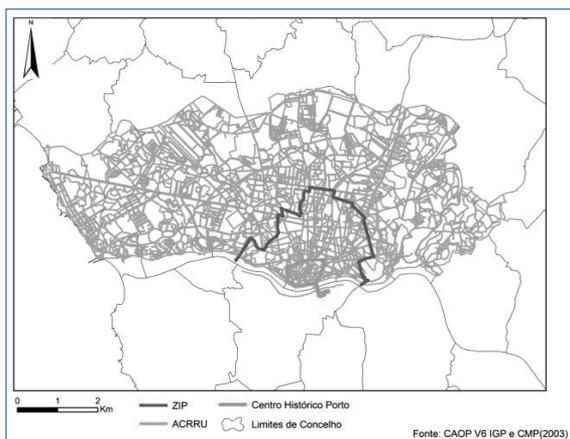
Cities dynamics as well as cities competitiveness requires a cohesive, harmonious and entering developing of its territory. In several cities, historic centers are viewed as a nucleons with limitations to a full development. These limitations transcend the urban aspects, being sometimes the reflection of an evolutionary framework of several generations of policies, programs and visions about the role of historic centers and the way how to live with their heritage.

Currently, urban policies are focused on rehabilitation strategies, giving them value. We intend to analyze the evolution of policies regarding the promotion of the built heritage in general, and the historical centers in particular. The guidelines for distinguished urban planning thoughts from an crono-urban analysis since the XIX century are presented in this work.

Due to its historical, cultural and patrimonial characteristics, the Oporto Historical Centre arises as an example of considerable dimension having suffered stratified moments of occupation till the present. Therefore its study reveals as an inviting challenge.

Finally, the Oporto Historic Centre not only reflects the features, events and issues listed above, as underline the applicability of the framework of urban policies and programs targeting urban regeneration. Moreover, at the same time it makes clear is important to implement other measures in the future, in order to extend / improve the efficiency of existing policies and programs.

**Keywords:** Historical Centre, Urban Policies, Urban Programs, Urban Renewal, Porto, Historical Centre.



## Housing cooperatives and the crisis – Which Answers?

Ana Natálio<sup>1</sup>, Bruno Rocha<sup>1</sup>, Fátima Matos<sup>2</sup>

<sup>1</sup> Departamento de Geografia da Faculdade de Letras, Universidade do Porto

<sup>2</sup> Departamento de Geografia da FLUP, Centro de Estudos em Geografia e Ordenamento do Território.

The economic housing cooperatives have a long history promoting social housing. The State's policy to support the cooperatives has had "ups and downs", a situation that has had consequences on its production. Nevertheless, and despite the constraints they had to face, many cooperatives managed to survive.

During the crisis in the housing sector, to which cooperatives are not exempt, the aim of this communication is to understand the effects of the crisis in this sector and to identify the innovative responses that have been promoted in order to face and mitigate its effects.

This communication is based on the analysis of three case-studies – Sete Bicas, CETA and SACHE Cooperatives – and it uses the method of directly interviewing the cooperatives' directors to collect the information.

To achieve the goal of this communication, we proceeded to prepare a brief theoretical framework which discusses the genesis and history of housing cooperatives in Portuguese territory. We considered important to make a brief characterization of our three case-studies, through the junction of the existing documentation and the information provided by the ones responsible. We conducted a direct interview to three of the cooperatives' directors so we could achieve the objectives outlined for this study.

The situation that we live in large urban centers, together with the aim of our study, allowed us to conduct the interviews in order to better understand the performance of cooperatives to face the current reality and what structural reforms are considered to be implemented. Thus, the analysis of the interviews allowed us to rank the problematic situations indicated by each of the three directors interviewed, as well as highlight some innovative processes and responses that are being run.

The valuable contribution started by the CHE's in the seventies in our country is undeniable, and during the troubled times we are experiencing, it is essential to harness all the expertise that is common to it. By privileging urban regeneration logic, stimulating leasing, construction and management of senior and geriatric housing, establishing more cohesive partnerships between the CHE's, Local Authorities and Central Administration, we will find, surely, strategies aimed to develop more effective action mechanisms for the cities' politics. It is necessary to face the crisis as a vehicle for achieving economic and environmental sustainability, as we believe it is in times of crisis that great solutions and new ideas are born.

MATOS, Fátima Loureiro de (2001) A habitação no Grande Porto uma perspectiva geográfica da evolução do mercado e da qualidade habitacional desde finais do séc. XIX até ao final do milénio. Dissertação de douramento, Universidade do Porto, Porto.

<http://www.setebicas.com/> [Acedido em 18 de Maio de 2011].

<http://www.ceta.pt/> [Acedido em 18 de Maio de 2011].

<http://sache.pt/> [Acedido em 18 de Maio de 2011].



Oral Sessions IV > A4

# Biological Sciences I



# Leonese dialects in Portugal: linguistic-genetic relationships through Y chromosome analysis

**S. Marques<sup>1,2</sup>, M.J. Prata<sup>1,2</sup>, L. Gusmão<sup>1</sup>, A. Amorim<sup>1,2</sup>, L. Alvarez<sup>1</sup>**

<sup>1</sup> Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Portugal.

<sup>2</sup> Department of Biology, Faculty of Sciences of the University of Porto, Portugal.

Although Portugal is well recognized for its linguistic homogeneity, an exception does exist - the Leonese dialects located in the North-Eastern region of the country. Among these, the Mirandês, spoken in the Miranda do Douro municipality, is the most notorious and spread. Some historical processes had an important role for the establishment of the Leonese dialects in the area, mainly the entry of population from the kingdom of Leon, which in the 13<sup>th</sup> century resettled the region. Conversely, another theory suggests that the language was already established in the area, due to a past cultural assimilation. Despite historical events, the intrinsic geographic isolation of the region, until recent times, is the main reason for the current persistence of the Mirandês [1]. The previous considerations made the Mirandês language and the Mirandês speakers a case study that has been continuously analyzed from a linguistic and historical point of view. Therefore, this project aims at exploring a new perspective, analyzing the correlation between spatial distribution of the dialects and genetic composition of the speakers.

As part of a major project that intends to genetically characterize the populations of these regions, this work is centered in the analysis of the male specific region of Y chromosome (MSY). Due to the distinctive characteristics of the Y chromosome, the study of the MSY provides the genetic composition of a given population from the perspective of paternal lineages. Specifically, a set of 27 binary markers were hierarchically typed with the SNaPshot technique, as previously described [2]. Complementarily, 17 Y-STR loci were also analyzed (AmpF1STR Yfiler).

The analyzed Y-SNPs reveal 10 different haplogroups and, as expected, a high frequency of the haplogroup R\* (M173), the most common in Western Europe populations. Inside the R\* (M173) haplogroup, the R1b (M269) presented the higher frequency (60%). Other haplogroups were also detected in significant proportions, namely J (12f2a), I (M170) and T (M70) found at high frequencies in Jewish populations [3]. To further detail the haplogroup characterization, Y-STRs were also considered. In conclusion, while these preliminary results point out a slightly different composition in the population genetic background in this particular area, we hope that further analysis will allow us to bridge the gap between linguistic affiliation and genetics.

## References:

- [1] Alves, AB. (2007). *Palavras de Identidade da Terra de Miranda*. Publicações Pena Perfeita, Porto.
- [2] Gusmão A, et al. (2008). A Perspective on the History of the Iberian Gypsies Provided by Phylogeographic Analysis of Y Chromosome Lineages. *Annals of Human Genetics*; 72:215-27.
- [3] Nogueiro I, et al. (2010). Phylogeographic Analysis of Paternal Lineages in NE Portuguese Jewish Communities. *American Journal of Physical Anthropology*; 141:373-381.

# The New European Standard Set of Forensic Genetic Markers in the Portuguese Population

**F. Melo<sup>1,2</sup>, C. Alves<sup>2</sup> and A. Amorim<sup>1,2</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> IPATIMUP, Institute of Pathology and Molecular Immunology of the University of Porto, Portugal

Human STR (Short Tandem Repeat) genotyping for forensic analyses (kinship investigations and genetic profiling) and for population studies is currently done with commercially available autosomal STR multiplex kits, the majority of which amplify a minimum of 15 markers in a single PCR (Polymerase Chain Reaction). Although this number is usually sufficient for solving simple kinship cases (e.g. paternity tests), our genetic identification laboratory (at IPATIMUP) applies, in routine casework samples, two STR kits: Identifiler (Applied Biosystems) and Powerplex 16 (Promega). Together they amplify a total of 17 STRs and share 13 between them. This strategy not only increases the informativeness of the analyses (when compared to only 15 STRs from one kit), but also serves as a means of quality control assurance in the laboratory. However, in situations as in deficient paternity cases (where access to the alleged father is not possible) or in other complex kinships, additional genetic information is usually necessary in order to obtain a sounder conclusion of the relationships involved. The recent adoption of new European genetic markers, ESS (European Standard Set of loci), for European Union DNA data exchange, has led commercial companies to develop new multiplex kits which include the recommended five new markers, namely D1S1656, D2S441, D10S1248, D12S391 and D22S1045. Kits including the ESS loci, with increased PCR performance and sensitivity, are available from Applied Biosystems (e.g. NGM kit) and Promega (e.g. ESX kit), and most recently from Qiagen who has entered the forensic genetics market with very interesting alternatives, like the ESS Plus kit. Therefore, it is worth re-thinking the laboratory's strategy by answering both the necessity of more informativeness and evolution to the current European standards. Adoption of one of these new kits will not only increase the number of STR markers analysed but should also allow for better analysis of degraded DNA samples since three of these five markers are mini-STRs (amplicons < 200bp).

Application of a new commercial kit and new STR markers in routine casework implies validating its performance and undertaking a genetic population study. Since it is the first time that these five markers were genotyped, two commercial kits were used, namely the NGM kit and the recently launched ESS Plus, to test for genotype concordance (it is expected that primer pairs are different between kits).

A total of 246 unrelated donors living in Portugal were typed. Allele frequencies and parameters of forensic interest were estimated for the 5 new loci and no deviations from Hardy-Weinberg equilibrium were observed. Comparison of our sample with other European samples available in the literature (e.g. Spain, Italy and Germany) revealed no significant allele frequency differences. Also, no genotyping inconsistencies were observed between ESS Plus and NGM kits.

This study reveals that the five new ESS markers are suitable for application in our laboratory's routine casework.

## Insights into South-American colonization through mtDNA analysis in Colombian populations

**C. Xavier**<sup>1,2</sup>, **J. Builles**<sup>3,4</sup>, **V. Gomes**<sup>1</sup>, **J.M. Ospino**<sup>3</sup>, **A. Amorim**<sup>1,2</sup>, **L. Gusmão**<sup>1</sup>, and **A. Goios**<sup>1</sup>

<sup>1</sup>IPATIMUP, Institute of Pathology and Molecular Immunology of the University of Porto, Portugal.

<sup>2</sup>Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>3</sup>Laboratorio Genes Ltda, Medellín, Colombia.

<sup>4</sup>Instituto de Biología, Universidad de Antioquia, Medellín, Colombia

America was the last continent to be colonized by humans and it has been suggested that the first inhabitants arrived this continent at least 15000 years before present. The number of migrations and the dispersion routes through North America are still not clarified: while some studies propose a unique colonization route by land through Beringia and an ice-free corridor towards the south, others indicate the possibility of a sea-route entrance via Siberia and the Pacific coast.

The peopling of South America is even more controversial due to the persisting doubts on the number and relevance of the migrations associated with the dispersion of native populations in this subcontinent. However, the most accepted hypothesis is that the entrance was made through Colombia and then subdivided into two different routes, one following the Andes chain and the other into the Amazonian plains. Five centuries ago, when people from the old world, such as Europeans and lately the Africans, arrived in Colombia, the territory was already colonized by several ethnic groups of various linguistic families.

Aiming to add some clues on the above mentioned issues, studies with lineage DNA markers such as mitochondrial DNA (mtDNA) and Y chromosome have been performed. Lineage markers allow tracing back the history of populations because they are transmitted without recombination to the descendants; these lineages are grouped into haplogroups, distinguished by specific polymorphisms that tend to be geographically restricted. In the present study we analyzed the mtDNA of two populations in Colombia – Emberá-Chami (highland) and Cauca (lowland) – in order to determine the ancestry of the observed lineages, including those belonging to European and African haplogroups that could be explained by recent migrations, during and after the colonial period. Based on the analysis of the Native American haplogroups in both populations, we also intended to perceive if there are differences that could indicate different migrations towards the South of the continent.

The mtDNA control region was sequenced for 98 samples from the two populations (38 Emberá and 60 Cauca) and compared with the revised Cambridge Reference Sequence. Haplogroup frequencies were calculated and phylogenetic analyses were performed.

The majority of haplogroups found in both Colombian populations are typically Native American (A, B, C and D), which contrast with Y-Chromosome data for Cauca group, suggesting that the preferred mode of miscegenation was through Native American women and non-Native American men. Furthermore, our preliminary results show that there are differences between the two populations, which may result from distinct ancient courses.

# Development of Notch-based polymeric biomaterials as a platform to study Notch Pathway

**P Costa<sup>1,2</sup>, J Galante<sup>3</sup>, MA Barbosa<sup>1,2,4</sup>, ID Bernstein<sup>5</sup>, MCL Martins<sup>1</sup>, RM Gonçalves<sup>1</sup>**

<sup>1</sup>INEB, NEWTherapies Group, Porto, Portugal; <sup>2</sup>Faculty of Engineering, University of Porto; <sup>3</sup>Instituto Superior Técnico, Technical University of Lisbon; <sup>4</sup>Institute of Biomedical Sciences Abel Salazar, University of Porto; <sup>5</sup>Fred Hutchinson Cancer Research Center, Seattle, USA

The Notch signaling pathway represents an essential mechanism activated during fetal development that also regulates stem cell behavior [1, 2]. In mammals, Notch transmembranar receptors (Notch-1, -2, -3 and -4) can detect and bind specific ligands, namely Jagged-1 and -2, Delta-1, -3 and -4 [1]. After ligand-receptor binding, subunits from the receptors are cleaved and act in the nucleus as transcription factor for specific genes [3]. The establishment of platforms designed at the nanoscale to mimic the stem cell niche could give important insights on the generation of diverse cell types ex vivo. The present work aims to develop innovative polymeric substrates to control Notch signaling activation, envisaging future clinical applications.

Poly-(2-hydroxyethyl methacrylate), pHEMA, discs were synthesized and activated by N,N'-carbonyldiimidazole (CDI) at different concentrations (0; 0.03; 0.3; 3 and 30 mg/mL) and functionalized with an antibody F(ab'')<sub>2</sub> anti-human IgG-Fc specific fragment (Ab) in order to assure a correct orientation of the chimeric Notch ligand Delta-1-extIgG (Dll-1). The biofunctionality of Dll-1-pHEMA discs was evaluated at the protein level by flow cytometry in a T-cell lymphoblastic leukemia (T-ALL) cell line encoding the enhanced green fluorescent protein (EGFP) reporter gene.

The presence of Ab was confirmed by X-ray Photoelectron Spectroscopy (XPS), Fourier Transform Infrared Spectroscopy with attenuated total reflectance (FTIR-ATR) and protein radiolabeling (using <sup>125</sup>I), being as higher as the CDI concentration increased. The amount of immobilized Ab ranged from 4.4±2.7 to 37.1±9.3 ng/cm<sup>2</sup>.

After binding Dll-1 on Ab-pHEMA, the surfaces were shown to trigger Notch signaling on TALL-1-rbs-EGFP cells. Although only a low percentage of cells were activated suggested low cell-ligand binding on the surfaces (% GFP<sup>+</sup> cells), the GFP signaling (mean fluorescence intensity) increase from 2 to 3 times when Dll-1 was not bound. Different ligand concentrations suggest different signaling levels, being the highest signaling level obtained for the surfaces activated with 0.3 mg/ml of CDI. This suggests that ligand orientation is more important than its density on the surface [1].

Overall, these results show a new biomaterial-based approach to control signaling levels of Notch. A dose-dependence of ligand orientation was suggested at the protein level. These substrates revealed themselves as a promising tool in the control and modulation of the Notch pathway with variable intensities, in diverse cellular systems.

## References

- [1] Gonçalves RM et al. (2009). Induction of notch signaling by immobilization of jagged-1 on self-assembled monolayers. *Biomaterials* **30**, 6879-6887
- [2] Galante, JNR (2010). *Development of Notch Signaling Biomaterials as a tool to control stem cell behavior* – Master's Degree dissertation in Biomedical Engineering, IST.
- [3] Chiba S (2006). Concise review: notch signaling in stem cell systems. *Stem Cells* 2006; 24:2437-47.

# Sequence diversity analysis of the human mtDNA control region: comparative analysis in two populations from the Portuguese-Spanish border

M. Silva<sup>1,2</sup>, M.J. Prata<sup>1,2</sup> and L. Alvarez<sup>2</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Portugal.

The present work can be enclosed in a main project titled “Leonese dialects in Portugal: a genetic approach to a historical-linguistic issue”. In this context, the province of Zamora represents a very interesting case-study population to evaluate the impact of relatively recent historical events in the genetic structure of human populations. The province can be subdivided into six regions. According to a previous study, one of this regions, Aliste, shows a strong affinity with the neighbor Portuguese municipality of Bragança [1].

Although most of the studies in the Population Genetic field rely only on data from the HVRI region of the mitochondrial DNA molecule (mtDNA), complete information should be obtained studying the entire control region (D-loop) [2]. In this sense, this study aims to analyze the entire D-loop sequence of 214 samples from the different regions of Zamora, in order to define the haplogroup composition and determine the usefulness of HVRII and HVRIII in phylogeographic analysis.

Sequences for the mtDNA control region have been obtained for the majority of the DNA samples using for the PCR amplification an mtDNA specific set of primers [3].

The results will be analyzed with appropriated software (Arlequin ver. 3.5.4) [4] and compared with data already available for the HVRI. Preliminary tests show higher gene diversity when studying the entire control region –  $0.9967 \pm 0.0064$  against  $0.9579 \pm 0.0203$ . In addition, results will be crossed with those from Miranda do Douro [5]. The analysis will then be extended further along the mtDNA molecule (completely sequenced) for some samples. This information is expected to provide new perspectives on the relationships between populations located in the Portuguese-Spanish border.

References:

- [1] Alvarez, L. et al. (2010), *Mitochondrial DNA patterns in the Iberian Northern plateau: Population dynamics and substructure of Zamora province*, Am J Phys Anthropol, 142, 531-539.
- [2] Van Oven, M. and Kayser, M. (2009), *Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation*, Human Mutation, 30 (2), 386-394.
- [3] Ramos, A. et al (2009), *Human mitochondrial DNA complete amplification and sequencing: a new validated primer set that prevents nuclear DNA sequences of mitochondrial origin co-amplification*, Electrophoresis, 30, 1585-1593.
- [4] Excoffier, L. et al (2005), *Arlequin ver. 3.0: An integrated software package for population genetics data analysis*, Evolutionary Bioinformatics, online,(1), pp. 47-50.
- [5] Cánovas, Q.M. (2011), *Patrón de linajes femeninos en la población de habla mirandesa (Mirando do Douro, Noreste de Portugal)*, thesis for Master in Human Biology, Universitat Autònoma de Barcelona.

## Current perspectives on the post-translational modulation of Prrx11 function

**R. Soares-dos-Reis<sup>1,2</sup>, A.S. Pessoa<sup>1,2</sup>, I. Regadas<sup>1,2</sup>, M. Falcão<sup>1,2</sup>, F.A. Monteiro<sup>1,2</sup>, B. Manadas<sup>3</sup>, C. Reguenga<sup>1,2</sup> and D. Lima<sup>1,2</sup>**

<sup>1</sup> Department of Experimental Biology, Faculty of Medicine, University of Porto, Portugal

<sup>2</sup> Institute for Molecular and Cell Biology, University of Porto, Portugal.

<sup>3</sup> Centre for Neuroscience and Cell Biology, University of Coimbra, Portugal

During the development of the nociceptive system, several key-players, mainly basic helix-loop-helix and homeodomain transcription factors, have been identified. These control cell differentiation, migration and connectivity. Prrx11 is one of such factors and it is thought to play a major role in the accurate establishment of the dorsal root ganglion (DRG) – spinal cord (SC) connection. Knockout mice studies have shown anatomical changes, especially at the superficial laminae of the dorsal SC, together with significant decreases in sensitivity to painful stimuli. When analyzed by immunoblotting, Prrx11 presents a multiple-band pattern, which is highly suggestive of post-translational modification [1-2].

This multiple-band pattern was studied through different approaches. Using embryonic SC extracts, we showed that the band pattern was eliminated by incubation with a phosphatase and that the upper bands had higher affinity for  $Ga^{3+}$ , thus establishing that Prrx11 is phosphorylated in vivo. 2D-electrophoresis time course analysis of Prrx11-expressing tissues ensued, revealing a trend towards less phosphorylated states as development progresses.

Afterwards, several Prrx11 truncated constructs were used to transfect ND7/23 (a neuronal-derived cell line) cells, and analyzed by 1D and 2D-electrophoresis, mapping Prrx11 phosphorylated regions. Phosphorylation occurs mainly in clusters, one near the N-terminus (the DNA-binding domain), and the other near the C-terminus (site of the OAR domain). These constructs were further characterized by DNA-binding and luciferase reporter assays showing that the N-terminus is sufficient for transcriptional activity and the C-terminus acts as a regulatory domain. A particular construct, bearing a T38AT39A mutation at a putatively phosphorylated site, exhibits reduced transcriptional activity, while maintaining DNA-binding. Furthermore, through large scale Prrx11 immunopurification and ulterior LC-MS/MS analysis, S119 and S238 were identified as *bona fide* phosphorylation sites, consistent with the previous mapping data. Lastly, co-expression of Prrx11 and Tlx3, which our group has recently identified as an interactor of Prrx11, leads to hyper-phosphorylation of Prrx11, seen by immunoblotting.

Taken together, these data establish the case for phosphorylation as an important modulator of Prrx11 function. It also puts forward the hypothesis that the Prrx11-Tlx3 interaction may mediate the developmental changes in Prrx11 phosphorylation pattern.

### References:

- [1] Caspary, T. and Anderson K.V. (2003) *Patterning cell types in the dorsal spinal cord: what the mouse mutants say*. Nature reviews Neuroscience, 4, 289-297.
- [2] Rebelo, S., Reguenga, C., Osorio L., et al. (2007) *DRG11 immunohistochemical expression during embryonic development in the mouse*. Developmental Dynamics, 236, 2653-2660.

# Glial Cell Development and Function in the *Drosophila* eye

**R.Traquete<sup>1</sup>, L. Tavares<sup>1</sup>, N. Amaral<sup>2</sup>, T. Martins<sup>1</sup>, J. Relvas<sup>1</sup>, P. Pereira<sup>1</sup>**

<sup>1</sup> Institute for Molecular and Cell Biology, University of Porto, Portugal.

<sup>2</sup> Centre of Genomic Regulation, Barcelona, Spain

Neurons and glia are the two major cell types constituting any complex nervous system, with the latter comprising 90% of the cells in mammalian brain. Although over the last years many studies revealed glial cells as being crucial regulators of nervous system development, function and health, these have not been studied as intensively as neurons. Therefore, our knowledge regarding glial cell development and specific function is still limited.

The fruit-fly, *Drosophila melanogaster*, is an ideal model organism to study glial cell functions both at the cellular and tissue level. This is due to the conservation of many aspects of glial cell biology across the animal kingdom, and because there are powerful molecular-genetics tools that can be easily explored in this organism. In particular, the *Drosophila* compound eye is ideally suited to study basic glial cell biology, as flies can survive without it and gene function in this organ can be manipulated easily and specifically in an unprecedented manner [1].

Thus, our aim is twofold: firstly to use the *Drosophila* visual system to characterise and understand some of the mechanisms regulating axon-glia interactions that are regulated by known molecules involved in cytoskeleton dynamics and signaling pathways; and secondly to identify and fully characterise the function of uncharacterised genes in developmental axonal wrapping and neuron-glia interactions. The uncharacterised genes that are being tested have been identified by proteomic analysis in mammalian nervous systems (J. Relvas, unpublished data).

The genes encoding our proteins of interest were therefore ablated specifically in glial cells with different dsRNA lines and the resulting phenotype analysed by immunofluorescence. Given that almost all known proteins in *Drosophila* have an associated dsRNA available, downregulation of candidate genes is readily achieved. To do so we used the GAL4/UAS tool [2], which enabled to target expression in a variety of temporal and spatial fashions.

Preliminary evidence suggests that some of the molecules identified, such as integrins ( $\beta$ -PS1) and RhoGTPases (Rho1 and Rac-2), might have an important role during glial cells development and function, namely at the level of cellular proliferation and migration. A thorough evaluation of these phenotypes will allow a better understanding of the pathways that are affected, and how these signaling modules are interconnected during development, therefore providing novel insights in glial cell biology.

## References

- [1] Silies, M., Yuva-Aydemir, Y., Fransdóttir, SR., Klambt, C. (2010) The eye imaginal disc as a model to study the coordination of neuronal and glial development. *Fly (Austin)* 4(1): 71-9
- [2] Duffy, JB. (2002) GAL4 System in *Drosophila*: A fly geneticist's Swiss Army Knife. *Genesis*. 34(1-2): 1-15.



Oral Sessions IV > A5

# Engineering III



# GUITAR: Graphical User Interface Tool For Automata Representation

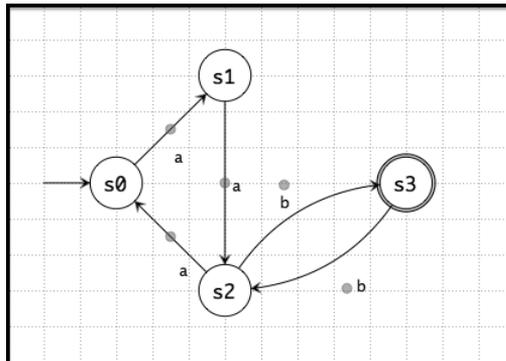
**Rizó Isrof, Nelma Moreira, Rogério Reis<sup>1</sup>**

<sup>1</sup> Department of Computer Science, Faculty of Science, University of Porto, Portugal.

GUITAR is an ongoing project which aims to create a software tool for automata editing, manipulation and visualization [INFORUM]. Although the application specially focuses on finite automata diagrams, it also supports other types of graphs and data and computation representations, such as digraphs, multigraphs, pushdown automata and Turing machines. It intends to include automatic and assisted diagram drawing, interactive editing, algorithm animation, and import/export filters.

GUITAR is implemented using the Qt graphical framework which guarantees a solid foundation for an efficient, extensible and cross-platform application architecture. It has support for rich styling of the diagrams that represent automata; it is possible to fully customize the appearance of the objects on the canvas and export it in the most standard formats. However, GUITAR intentionally provides a very limited set of functionalities for automata manipulation – only basic structural operations are allowed, such as adding and removing graph elements and defining their attributes. For the advanced manipulation and analysis of the automata, and the regular languages behind them, GUITAR relies on the FAdo library. The FAdo system[FAdo], also developed at Department of Computer Science by Rogério Reis and Nelma Moreira, aims to provide an open source extensible high-performance software library for the symbolic manipulation of automata and other models of computation.

The central motivation of the project is supply an easy to deploy system to assist the work with finite automata and to encourage the pedagogical use of GUITAR.



References:

[INFORUM] André Almeida, Nelma Moreira, Rogério Reis. (2011), *GUITar and Fagoo*.

[FAdo] Nelma Moreira, Rogérios Reis. (2005), *Interactive manipulation of regular objects with FAdo*.

# Clock synchronization using servo-clock<sup>1</sup>

A. Costa Lima<sup>†</sup> and H. Marques<sup>†</sup>

<sup>†</sup> Dep. of Electrical and Computers Eng., Faculty of Engineering, University of Porto, Portugal.

*Servo* is the designation for a technique or device that provides control through the use of feedback, i.e., closed loop. This approach can be used to synchronize clocks between computers, with a master clock providing the reference to the slave(s) that synchronize with it acting on their own pace. Our objective is to study the behavior of a possible servo-clock implementation with respect to its parameters.

The master clock can be modeled by a linear function that grows monotonically with a certain rate and offset that might vary over time [1]. The slave(s) adjust their rate to keep up with the reference variations. The slave rate, at a given synchronization instant  $s_n$ , is defined by equation (1) where  $C_p(s_n)$  and  $C_m(s_n)$  are the slave and master clock amplitudes at time  $s_n$ , respectively,  $\Delta$  is the network delay,  $k$  is the clock reactivity factor and  $T_s$  is the sync interval. The rate  $\rho_n$  is further truncated to  $[\rho_{min}; \rho_{max}]$ . The slave clock  $C_p(t)$  at a time  $t$  is then given by equation (2).

$$\rho_n = 1 - \frac{C_p(s_n) - (C_m(s_n) + \Delta)}{k \cdot T_s} \quad (1) \quad C_p(t) = C_p(s_n) + \rho_n \cdot (t - s_n) \quad (2)$$

In a distributed system, the parameter  $\Delta$  is obtained by measuring the round-trip delay (RTD) including processing overheads. Precision  $\delta$  is given by the steady-state error.

The implementation was done in software using Java in a client-server scenario, using TCP sockets, where the server provides the reference and the client is the slave. The client requests the instantaneous reference amplitude, computes the new clock rate and a new sync point is established. The relations  $k \geq 1$  and  $k \cdot T_s \geq T_r$  must be verified, where  $T_r$  is the server request interval, in order to minimize steady-state oscillation amplitude.

Figure 1 shows rate correction with  $T_r = T_s = 0.5$  seconds,  $k = 2$  and master clock (blue) rate is unitary and constant with zero offset. Steady-state is achieved approximately at 8 seconds and is directly proportional to  $k$ . The growth rate is bounded to the interval  $[0.7; 10.0]$ .

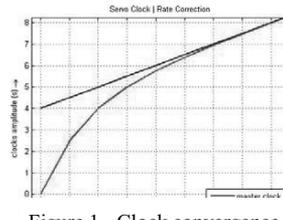


Figure 1 - Clock convergence

Results show that precision  $\delta$  and network delay  $\Delta$  are in the order of  $10^{-4}$  and  $10^{-3}$  seconds, respectively, when the server and client communicate through a wireless LAN. In our presentation we will show how our servo implementation is sensitive to critical parameters variation such as  $T_r$ ,  $T_s$ ,  $k$  and growth bound,  $\rho_{min}$  and  $\rho_{max}$ . We will also show how the precision is influenced by the network delay — processing overheads and the delay intrinsic to the network. Processes running in background may also affect overall performance, but we were not able to assess that accurately.

[1] Luis Almeida, *Clock Synchronization*, lecture notes of the Distributed Systems course, MIEEC, University of Porto, November 2011.

<sup>1</sup> This work was developed within the scope of the Distributed Systems of MIEEC.

# Implementation of a Real-Time Database with Sockets<sup>2</sup>

C. Brito<sup>2</sup> and R. Ferreira<sup>2</sup>

<sup>2</sup> Dep. of Electrical and Computer Eng., Faculty of Engineering, University of Porto.

Real-time databases [1] (RTDB) contain data with limited temporal validity, e.g. sensor readings. Thus, updating and reading such data must meet given deadlines otherwise an onset of problematic aftermath might occur. In this work we explored the implementation of an RTDB with replication for fault-tolerance and lower access delays [2] using socket communications that simplify implementation and allow abstracting the RTDB from the producers/consumers. The system gives priority to the producers while still allowing the consumers a chance to read the freshest data.

To test our system we applied multiple queries to the RTDB concurrently with high frequencies and observed the respective latencies and jitter. An example test began with just one RTDB instance and then a replica was added at run-time so we could analyze/compare the impact in performance. The latency results are displayed in Figure 1. Initially, with only one RTDB the latency is occasionally very high and later, after the mark, with the addition of a replica, the latency and jitter lowers considerably.

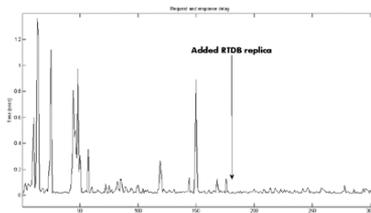


Fig. 2 - MATLAB plot of the jitter in data query latencies

The results allowed us to conclude that using multiple replicas in different machines reduces latency thus maintaining the temporal validity of the data. Multiple replicas however are only necessary if there are enough concurrent accesses to the RTDB. Therefore, in a system where the extent of accesses are known and limited and the deadlines of the data are firm, it is possible to compute the optimal number of replicas to be used to achieve the best performance/cost ratio.

In our presentation we will show more results confirming that using multiple RTDB replicas in different machines allows for faster and more stable query responses thus helping in maintaining the temporal validity of the data. We will also elaborate on the suitability of the sockets interface for the replicated RTDB implementation.

References:

- [1] Bestavros, A., Lin, K. and Sang Hyuk Son (1997), *Real-Time Database Systems*, Kluwer Academic Press, U.S.
- [2] Tanenbaum, A.S. and van Steen, M. (2007), *Distributed Systems – Chapter 7*, Prentice Hall, U.S.

<sup>2</sup> This project was developed in scope of the course on Distributed Systems of the MIEEC.

# Economic Dispatch of Pumped Hydro Storage interconnected with Thermal and Wind energy – An Educational Tool

**J. Gonçalves<sup>1</sup> and H. Leite<sup>2</sup>**

<sup>1</sup> Student of MSc in the Department of Electrical and Computers Engineering, Faculty of Engineering of University of Porto, Portugal.

<sup>2</sup> Is with INESC TEC and Faculty of Engineering of University of Porto, Portugal.

Economic Dispatch allocates generation changes of a power system among generator units to achieve optimum area economy. As part of the generation management system, Economic Dispatch utilizes power flow algorithms in order to determine the optimal generation and generation levels of individual units are calculated and dispatched in order to meet the load demand at minimal costs. Consideration is given to the fact that the cost of generation is on the day-ahead market. The systems are geographically spread out, and transmission losses are dependent on the generation pattern.

This paper introduces an Economic Dispatched software tool for education, research and training in power engineering studies.

A general description of the interactive procedure implemented in MATLAB follows. All execution procedures start with entering case study parameters and culminate in the display of Economic Dispatch of the thermal units and pumped hydro storage for the next 24h. The prices of the Thermal unit are from the day ahead market (Iberian market) and the prices from the wind come from 2010 “feed-in tariff” in Portugal. Figure 1 shows the dispatch economic results:

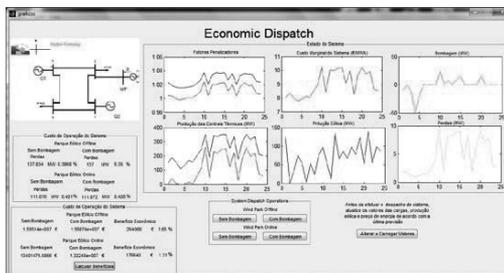


Figure 3 – The Economic Dispatch Results display

The algorithm and the display can stress the economical importance of the pumped hydro storage with various scenarios of wind energy accommodation.

## References:

- [1] Zeng Ming; Wu Zhi-fu; Liu Bao-hua; Yuan De; Lu Ye; , "An Economic Dispatching Model of Hydro-Therm System Under Market Environment and its Application," *Transmission and Distribution Conference and Exhibition: Asia and Pacific, 2005 IEEE/PES*, vol., no., pp.1-7, 2005
- [2] Kennedy, T.; Mabuca, E. M.; , "Dispatch of Pumped Hydro Storage on an Interconnected Hydrothermal System," *Power Apparatus and Systems, IEEE Transactions on*, vol.84, no.6, pp.446-457, June 1965
- [3] Shihong Huang; Distanto, D.; , "On Practice-Oriented Software Engineering Education," *Software Engineering Education and Training Workshops, 2006. CSEETW '06. 19th Conference on*, vol., no., pp. 15, 19-21 April 2006

# BIOSWIM - Validation of Textile Electrodes for Measuring ECG

**P. Fonseca**<sup>1,2,3</sup>, **M. Borgonovo-Santos**<sup>2,3</sup>, **A. Catarino**<sup>5</sup>, **J. A. Duarte**<sup>4</sup>, **M. Correia**<sup>1,3,6</sup>, and **J.P. Vilas-Boas**<sup>2,3</sup>

<sup>1</sup> Faculty of Engineering, University of Porto, Portugal;

<sup>2</sup> Faculty of Sports, CIF12D, University of Porto, Portugal;

<sup>3</sup> Porto Biomechanics Laboratory, University of Porto, Portugal;

<sup>4</sup> Faculty of Sports, CIAFEL, University of Porto, Portugal;

<sup>5</sup> Department of Textile Engineering, University of Minho, Portugal;

<sup>6</sup> INESC TEC (formerly INESC Porto), Porto, Portugal.

Currently there are several wearable health solutions available, with integrated electrocardiographic (ECG) systems [1, 2], but most of them use conventional Ag-AgCl electrodes, which entails several drawbacks. This kind of electrodes are typically single-use, can be very uncomfortable, and their conductive gel has a tendency to cause skin irritation and even bacterial growth [3]. Textile electrodes have been presented as an alternative because they are reusable, washable, can be integrated into a garment and some of them do not require conductive gel. The BIOSWIM project aims to create a sensory garment that allows the measurement of physiological and biomechanical parameters of swimming and other sport and physical activity (or rest) modes, in order to monitor athlete's performance. This work aims to validate the use of BIOSWIM's ECG textile electrodes at rest. The signals recorded with the textile and the Ag-AgCl electrodes were compared using morphological, time and frequency analysis.

Fifteen healthy subjects (6 male, 9 female) with  $23\pm 3,6$  years, body mass of  $66\pm 9,8$  kg and height of  $1,69\pm 9,3$  m participated in the study. Both textile (stainless steel with  $26\text{ cm}^2$  of sensory area) and Ag-AgCl electrodes were placed simultaneously on the body and near to each other. Recording was made with BIOPAC MP100 and an amplification system (gain of 1100). Each individual was measured for 5 minutes. Signal processing was made after acquisition and included DC component removal, a pass-band of 1-100Hz and a 50Hz notch filter.

The signals recorded with textile electrodes show less noise than the conventional electrodes, while easily allowing to observe all the major waves of the ECG signal. This may be due to the greater sensory area of the textile electrodes. Welch periodogram revealed a similar power distribution of both signals in the frequency domain. The R-R intervals Bland-Altman tests showed there was a 96% agreement between the signals.

All these results seem to indicate that the textile electrodes are reliable to be used as an alternative when measuring ECG at rest. Further dynamic tests will be conducted to verify noise levels from motion artifacts as well as tests in water.

References:

[1] Paradiso, R., G. Loriga, and N. Taccini, *A wearable health care system based on knitted integrated sensors*. Information Technology in Biomedicine, IEEE Transactions on, 2005. **9**(3): p. 337-344.

[2] Di Rienzo, M., et al., *Textile Technology for the Vital Signs Monitoring in Telemedicine and Extreme Environments*. Information Technology in Biomedicine, IEEE Transactions on, 2010. **14**(3): p. 711-717.

[3] Marozas, V., S. Daukantas, and A. Lukosevicius, *A comparison of conductive textile-based and silver/silver-chloride gel electrodes in exercise electrocardiogram recordings*. Journal of Electrocardiology, 2011. **44**(2): p. e57-e58.

## Image Filer: a semi-automatic image classifier

Silvestre Lacerda<sup>1</sup>, António Marques<sup>2</sup>, Nelma Moreira<sup>2</sup>, Rogério Reis<sup>2</sup>

<sup>1</sup> CIE, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

<sup>2</sup> Department of Computer Science, Faculty of Sciences, University of Porto, Portugal.

In this work, we present a software system - Image Filer - that allows the annotation, classification, manipulation, and archive of digital images (digitalized documents, photographs, etc.). Besides the metadata associated with technical (or physical) image information our aim is to extract and associate content information to the images.

Image Filer is able to:

- ♣ Load, manipulate, and store digital images;
- ♣ Organize them into collections, which can also be manipulated;
- ♣ Annotate a document or a part of it with plain text or predefined keywords;
- ♣ Interface to an external optical character recognition tool (OCR) to perform automatic text recognition from the document;
- ♣ Integrate the service of a face recognizer to identify faces and associate them to identities, which can be also created or edited;
- ♣ Search upon the data stored, by plain text, keywords or associated persons;
- ♣ Import and export according to the standard ANSI/NISO Z39.87-2006 [6]
- ♣ Run on Windows OS and Linux based OS;

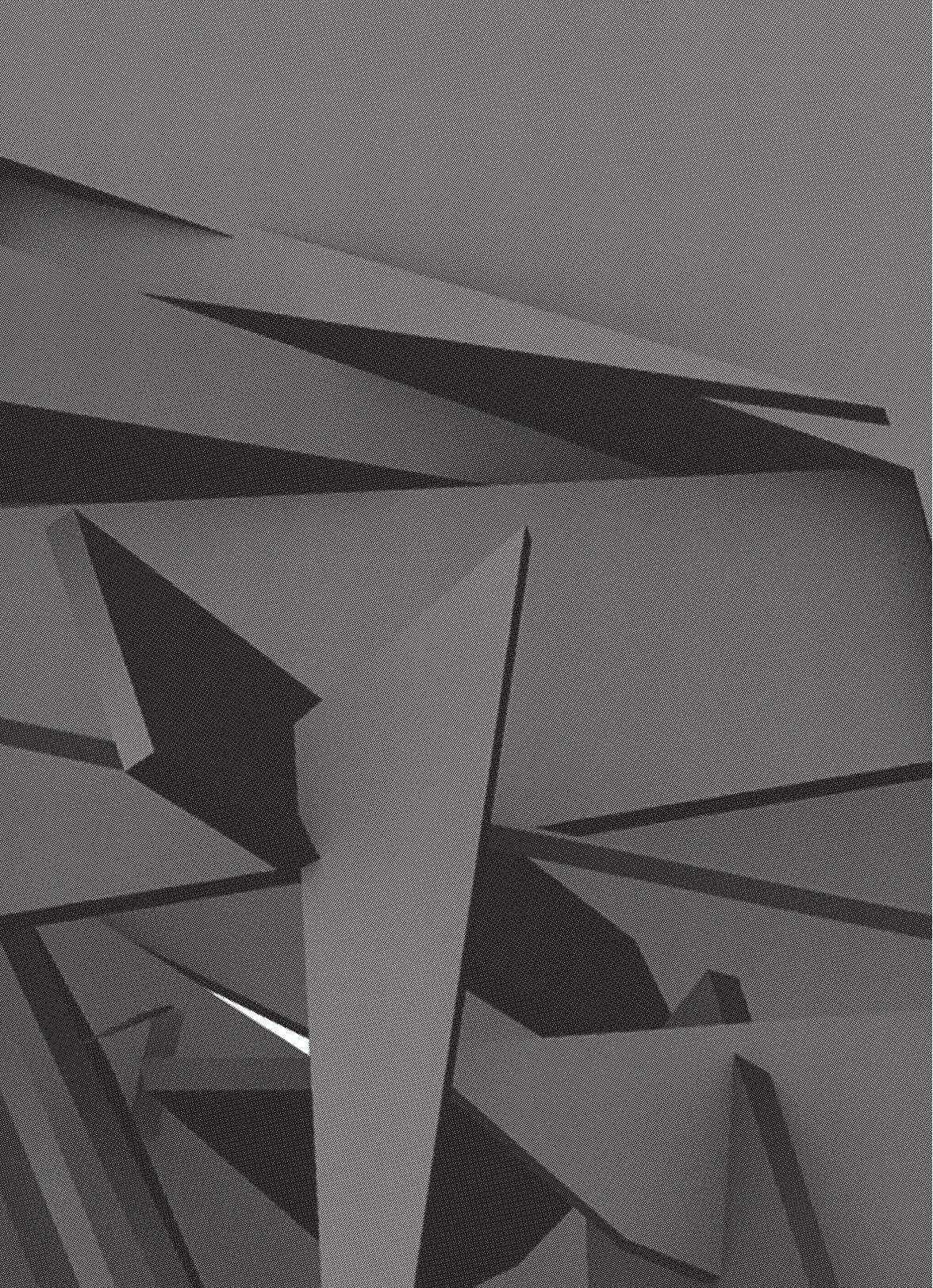
The system was built upon Eclipse IDE with Pydev plugin, using the Python programming language and Qt as graphic library [1,2,3]. Tesseract [4], is used as an OCR tool, and ExifTool [5], is used to manipulate image metadata. PDF documents are manipulated with pyPdf [7].

This application has already been tested, since early stages of development, with digitalized documents from “CDI-Centro de Documentação e Informação sobre o Movimento Operário e Popular do Porto” [7]. It is part of IJUP 2010 project 162, “Análise Social e Linguística das memórias do Trabalho”.

References:

- [1] Eclipse. <http://www.eclipse.org/>. Access: 10/1/2012.
- [2] Python Programming Language. <http://python.org/>. Access: 10/1/2012.
- [3] Qt. <http://qt.nokia.com/products/>. Access: 10/1/2012.
- [4] Tesseract, Google. <http://code.google.com/p/tesseract-ocr/>. Access: 10/1/2012.
- [5] ExifTool. <http://owl.phy.queensu.ca/~phil/exiftool/>. Access: 10/1/2012.
- [6] NISO. <http://www.niso.org/>. Access: 10/1/2012.
- [7] PyPdf. <http://pybrary.net/pyPdf/>. Access: 10/1/2012.
- [8] CDI-UPP, Universidade Popular do Porto, <http://cdi.upp.pt>. Access: 10/1/2012.





# Oral Sessions V



Oral Sessions V > A1

# Innovation in Health



## Development and characterization of lipid nanoparticles as delivery systems for anticellulite active compounds

**A. Alves<sup>1\*</sup>, M. Lúcio<sup>1</sup>, S. Martins<sup>2</sup>, D. Ferreira<sup>2</sup>, José L.F.C. Lima<sup>1</sup>, Salette Reis<sup>1</sup>**

<sup>1</sup>REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Portugal

<sup>2</sup>Laboratório de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade do Porto, Portugal  
*\*nitinhaves@gmail.com*

Cellulite is a metabolic disorder that affects more than 85% of the women and causes modifications in skin topography, evident by its orange peel appearance. There is still no effective treatment for cellulite and thus the development of new and more effective therapies is required. With this purpose, we aimed to develop lipid nanoparticles (solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC)) with the ability to incorporate and deliver anti-cellulite active compounds (namely caffeine, retinol and coenzyme A), into the dermis and subcutaneous tissue of the cellulite-prone areas and to further stimulate the adipocytes lipolysis.

The first part of this work consisted in a systematic study of development, characterization and optimization of the preparation process of the lipid nanoparticles, followed by a time course evaluation of several important parameters that may influence the shelf stability of the formulation developed. Anti-cellulite active compounds: caffeine, retinol and coenzyme A were encapsulated in the nanoparticles prepared by ultrasonication method. Lipid nanoparticles showed mean particle sizes below 200 nm, as desired to achieve the dermis and the fat layer of the skin, and zeta potential values above  $|-30 \text{ mV}|$ , which indicates a good physical stability. Over a month of storage little variations in these parameters were observed, which indicates that all nanoformulations are stable in storage. Cryo-SEM measurements showed that all the lipid nanoparticles exhibit a spherical shape and a smooth surface independently of their composition. Differential scanning calorimetry (DSC) studies revealed that the crystal ordering and density of NLC decreases in comparison to SLN, as expected. A high loading efficiency (above 95%) was achieved for all the active compounds over a month. Controlled release assays in physiological conditions demonstrated that nanoparticles loaded with caffeine yield a prolonged release, as desired for anti-cellulite therapy. Rheological tests performed after incorporation of the nanoparticles in a cosmetic gel showed that all the designed formulations had a non-Newtonian, pseudoplastic, thixotropic behavior, suggesting that they are suitable for dermal anti-cellulite active compounds' delivery.

In conclusion, the developed novel formulation consisting of a cosmetic gel containing lipid nanoparticles loaded with three anticellulite active compounds presents several optimal characteristics, namely size and charge stability, and a favorable rheological behavior suggesting a promising therapy for cellulite.

Acknowledgements: The authors are grateful to Dr. Daniela Silva (CEMUP, UP) for expert help with scanning electron microscopy. The authors also acknowledge U.Porto and Santander Totta for financial support through the pluridisciplinary projects "Lipid nanocarriers for dermal and transdermal drug delivery intending the treatment of cellulite by inducing adipocytes lipolysis"

## Development of innovative lipophilic hydroxycinnamic antioxidants: a solution to tackle mitochondrial oxidative damage

**J. Teixeira<sup>1,3</sup>, P. Soares<sup>1</sup>, S. Benfeito<sup>1</sup>, C. Oliveira<sup>1</sup>, A. Gaspar<sup>1</sup>, J. Garrido<sup>1,2</sup>, M. P. Murphy<sup>3</sup>, F. Borges<sup>1</sup>**

(1) CIQ/ Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; (2) Departamento de Engenharia Química, Instituto Superior de Engenharia do Porto, IPP, Porto, Portugal; (3) MRC Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Cambridge, UK.  
[jcsteixeira8@gmail.com](mailto:jcsteixeira8@gmail.com); [fborges@fc.up.pt](mailto:fborges@fc.up.pt)

There is evidence that several of major clinical disorders such as cancer, heart failure, diabetes, obesity, stroke and neurodegenerative diseases are associated or linked to mitochondrial dysfunctions and oxidative stress. Mitochondria consume nearly 85% to 90% of the cell's oxygen to support oxidative phosphorylation by harnessing oxidized fuel for ATP synthesis. The oxidative phosphorylation is the major source of energy supply, but due to elevated rate of oxygen consumption it is also the major source of toxic endogenous free radicals. Consequently, the selective inhibition of mitochondrial oxidative damage and the development of mitochondriotropic antioxidants are considered at the moment a promising therapeutic strategy. This type of compounds could be looked as potent and selective agents throughout specific targeting the mitochondria in oxidative stress-related diseases.

The *human diet* contains a vast number of dietary *phenolic* compounds of which hydroxycinnamic acids represents one class that have been intensively investigated due to their antioxidant value. For that reason in the present project new lipophilic mitochondriotropic antioxidants have been developed using cinnamic acid as template. Briefly, the natural hydrophilic antioxidant caffeic acid and its dimethoxylated analogue were attached to an aliphatic lipophilic carbon chain containing a triphenylphosphonium (TPP) cation through expedite synthetic strategies.

The data obtained so far revealed that the mitochondriotropic antioxidant (TPP-OH) is significantly more hydrophobic than caffeic acid although it has similar antioxidant activity and redox potential as demonstrated by measurement of DPPH/ABTS radical scavenging and electrochemical properties. TPP-OH and its dimethoxylated analogue (TPP-OCH<sub>3</sub>) are rapidly taken up for mitochondria driven by the membrane potential. The abilities of these compounds to prevent lipid peroxidation in mitochondria exposed to ferrous chloride, hydrogen peroxide and ascorbate, were also evaluated. As expected, only TPP-OH has the ability to prevent lipid peroxidation in isolated rat liver mitochondria, an effect that was not exhibited by caffeic acid despite the presence of molecules with the same antioxidant moiety. The toxicity of newly mitochondria-targeted antioxidant was assessed in mouse myoblast cell line C2C12. Only, TPP-OH protected cells against H<sub>2</sub>O<sub>2</sub> and linoleic acid hydroperoxide-induced oxidative stress.

As mitochondrial oxidative damage is associated with a large number of clinical disorders, TPP-OH may be a useful lead that could be added to the family of mitochondria-targeted antioxidants that can decrease mitochondrial oxidative damage.

Financial support : IPG12 2009/10 and PTDC/QUI-QUI/113687/2009 (FCT, FEDER, QREN and COMPETE) Patente 2012100003813

# Synthesis of Dual Inhibitors of Tumor Cell Growth and of P-Glycoprotein

**F. Castro**<sup>1,2</sup>, **A. Palmeira**<sup>1,2,3</sup>, **M. H. Vasconcelos**<sup>3,4</sup>, **M. Pinto**<sup>1,2</sup>, and **E. Sousa**<sup>1,2</sup>

<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP)

<sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal

<sup>3</sup>Cancer Drug Resistance Group, IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal, Rua Dr Roberto Frias s/n, 4200-465 Porto

<sup>4</sup>Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, 4050-047, Porto, Portugal

P-glycoprotein (Pgp), which is involved in multidrug resistance, represents one important obstacle to cancer treatment, and is an attractive therapeutic target since its inhibition could boost the effectiveness of chemotherapy. However, the Pgp inhibitors used until now have failed when tested in clinical trials, mainly due to limitations in potency, specificity and interactions with anticancer drugs [1].

The design of new Pgp inhibitors with concomitant antitumor activity was developed by the research group CEQUIMED-UP [2]. From a series of thioxanthenes previously obtained, the following compounds 1-(piperidin-1-yl)-4-propoxy-9H-thioxanthen-9-one (**TX34**), 1-[2-(1H-benzimidazol-2-yl)ethanamine]-4-propoxy-9H-thioxanthen-9-one (**TX48**) and 1-(4-acetyl-piperazin-1-yl)-4-propoxy-9H-thioxanthen-9-one (**TX53**) revealed to be potent inhibitors of Pgp with concomitant antitumor activity in a leukemia cell line (K562) (Fig. 1).

The main objective of this work was to investigate the best reaction conditions for the synthesis of **TX34**, **TX48** and **TX53** (summarized in Table 1.). **TX34**, **TX48** and **TX53** were obtained by copper and palladium-catalyzed aromatic nucleophilic substitutions

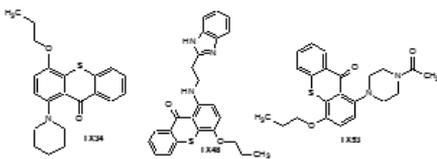


Fig. 1. Structures of thioxanthenes synthesized

Table 1. Reagents and conditions used in the synthesis of thioxanthenes

	Catalyst	Base	Solvent	Heating Source	Yield
<b>TX34</b>	CuO	K <sub>2</sub> CO <sub>3</sub>	MeOH	oven, 100°C	20%*
<b>TX48</b>	Pd(dppf)Cl <sub>2</sub> .CH <sub>2</sub> Cl <sub>2</sub>	Cs <sub>2</sub> CO <sub>3</sub>	NMP:H <sub>2</sub> O	oven, 100°C	7.1%
<b>TX53</b>	Pd(dppf)Cl <sub>2</sub> .CH <sub>2</sub> Cl <sub>2</sub>	K <sub>2</sub> CO <sub>3</sub>	NMP	Microwaves	8.7%

\* after conversion of **TX34** into **TX34.HCl** by the addition of HCl/Et<sub>2</sub>O

The structure elucidation of the thioxanthenes was achieved based on spectroscopic methods (IR and <sup>1</sup>H NMR). **TX34**, **TX48** and **TX53** will be further investigated for their cellular and molecular mechanisms of action, using human tumor cell lines.

[1] Higgins, C.F. (2007) *Multiple Molecular Mechanisms for Multidrug Resistance Transporters*. Nature, 446, 749. [2] Palmeira, A., Vasconcelos, M.H., Paiva, A., Fernandes, M.X., Pinto, M. and Sousa, E. (2012). *Design of dual inhibitors of P-glycoprotein and tumor cell growth: (re)discovering thioxanthenes*. Biochemical Pharmacology, 83, 57–68.

To U.Porto/Santander Totta and to FCT project CEQUIMED-Pest-OE/SAU/UI4040/2011 and PTDC/SAU-FAR/110848/2009, FEDER through COMPETE projects FCOMP-01-0124-FEDER-011057 for financial support.

# SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPIONPs) ARE TOXIC TO HUMAN NEURONAL CELLS IN VITRO

**Rita Azevedo<sup>1</sup>, Sónia Fraga<sup>1</sup>, Arturo Lopez-Quintela<sup>2</sup>, José Rivas<sup>3</sup>, Paulo Freitas<sup>3</sup>, Maria de Lourdes Bastos<sup>1</sup>, Félix Carvalho<sup>1</sup>**

<sup>1</sup>REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Pharmacy of Faculty, University of Porto, Porto, Portugal; <sup>2</sup>University of Santiago de Compostela, Spain; <sup>3</sup>International Iberian Nanotechnology Laboratory, Braga, Portugal.

Superparamagnetic iron oxide nanoparticles (SPIONPs) have earned the interest of the biomedical community and are promising in a variety of applications such as magnetic resonance imaging (MRI), site-directed drug and gene delivery and targeted destruction of tumour tissues through hyperthermia [1, 2]. In the event of their biomedical applications, SPIONPs can reach the Central Nervous System (CNS) [3] but knowledge about their putative neurological effects is still scarce. Therefore, the present study is aimed to determine the neurotoxic potential of iron oxide (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles (NPs) in neuron-like SH-SY5Y cells, a well-established *in vitro* cell model for neurotoxicity studies.

Uncoated and polyacrylic acid (PAA)-coated Fe<sub>3</sub>O<sub>4</sub> nanoparticles (10 nm) were synthesized and characterized at the International Iberian Nanotechnology Laboratory (INL). SH-SY5Y cells were cultured in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin B and 0.1 mM nonessential amino acids. To induce differentiation, cells were grown for 7 days in the presence of 10 µM retinoic acid. Cells were then exposed for 4 to 24 hours to increasing concentrations of the nanoparticles (5 to 100 µg/mL) prepared in DMEM with 3% FBS. Cytotoxicity was evaluated by the neutral red and propidium iodide uptake assays. Production of reactive oxygen species (ROS) was determined fluorometrically using the DCFH-DA assay.

Exposure to uncoated-Fe<sub>3</sub>O<sub>4</sub> NPs, for 4 and 24 hours, resulted in a concentration-dependent decrease in cell viability. The highest tested concentrations of these NPs (50 and 100 µg/mL) increased the production of ROS. At the same time-points, PAA-Fe<sub>3</sub>O<sub>4</sub> NPs also decreased cell viability in a concentration-dependent manner, though no alteration of basal ROS levels were detected.

Our results indicate that acute exposure to uncoated-Fe<sub>3</sub>O<sub>4</sub> NPs is associated with generation of ROS leading to a decrease in cell viability. Functionalization of Fe<sub>3</sub>O<sub>4</sub> NPs with the PAA polymer only partially prevented the toxic effects induced by the uncoated-Fe<sub>3</sub>O<sub>4</sub> NPs, which suggests that functionalization still needs to be improved to protect neuronal cells from the harmful effects of SPIONPs.

This work has been supported by the Portuguese Foundation for Science and Technology (FCT) through grant PEst-C/EQB/LA0006/2011.

- [1] Huber, D.L. (2005), *Synthesis, properties, and applications of iron nanoparticles*, Small, 1(5), p. 482-501.
- [2] Gupta, A.K. (2005), *Synthesis and surface engineering of iron oxide nanoparticles for biomedical application*, Biomaterials, 26(18), p. 3995-4021.
- [3] Simko, M. (2010), *Risks from accidental exposures to engineered nanoparticles and neurological health effects: a critical review*, Particle Fibre Toxicology, 7, p. 42.

# Fiber optical tweezers for single cell manipulation: numerical analysis and experimental implementation

**A. Ribeiro**<sup>1,2</sup>, **A. Guerreiro**<sup>1,2</sup>, **P. A. S. Jorge**<sup>2</sup>

<sup>1</sup> Department of Physics and Astronomy, Faculty of Science, University of Porto, Portugal.

<sup>2</sup> INESC Porto, Rua do Campo Alegre, 687. 4169 007 Porto, Portugal.

In 1986, Arthur Ashkin, first demonstrated optical trapping of dielectric particles by a single-beam gradient force [1]. This method has been developed for optical tweezers technology in the fields of physics, biology and chemistry. Through optical trapping it is possible to capture and manipulate, for example, particles or cells in a nearly non-invasively process [2].

Usually optical tweezers require bulky structures, which are expensive, complex and have a limited application in several environments. The implementation of optical tweezers based on single optical fibers is capable of turning this device into a miniaturized and handy diagnostic tool, suitable for many applications, like *in vivo* biological operations [3].

In our project, fiber optical tweezers were achieved by microlensed optical fiber tips. Experimentally, this was accomplished either through chemical etching processes or by in-fiber photo-polymerization techniques.

In order to analyze the force on particles caused by the light beam, some computational simulations are being developed and used as an optimization tool. Based on them it is possible to determine the optimal operational parameters both for the fiber tip or the surrounding media. For instance optimal curvature ratio, refractive index, and characteristics of the fluid environment in which the experience is executed can be evaluated. The numerical analysis is being performed using the Finite Difference Time Domain Technique.

With the experimental and theoretical tools developed, the improvement of fiber optical tweezers and their application to the study of red blood cells is in progress.

## References:

- [1] Ashkin, A., Dziedzic, J. M., Bjorkholm, J.E. and Chu, S. (1986), *Observation of a single- beam gradient force optical trap for dielectric particles*, Opt Lett., 11, 288-290.
- [2] Wei, M.T., Yang, K.T., Karmenyan, A. and Chiou, A. (2006), *Three-dimensional optical force on a Chinese hamster ovary cell in a fiber-optical dual-beam trap*, Opt. Express, 14 (7), 3056-3064.
- [3] Minzioni, P., Bragheri, F., Liberale, C., Fabrizio, E. and Cristiani, I. (2008), *A Novel Approach to Fiber-Optic Tweezers: Numerical Analysis of the Trapping Efficiency*, IEEE Journal of Selected Topics in Quantum Electronics, 14 (1), 151-157.

# Radioluminescent fiber dosimeter for dosimetry in brachytherapy and external radiotherapy

**S. Chiquita<sup>1</sup>, N. Sooraj Hussain<sup>1,2</sup>, João Coelho<sup>3</sup>, C. C. Rosa<sup>1,2</sup>**

<sup>1</sup> INESC Porto, Optoelectronics and Electronics Systems Unit, Porto, Portugal

<sup>2</sup> Department of Physics and Astronomy, Faculty of Sciences, University of Porto, Portugal

<sup>3</sup> Department of Metallurgical and Materials Engineering, Faculty of Engineering, University of Porto, Portugal

Radiotherapy procedures use ionizing radiation to destroy cancer cells while minimizing the absorbed dose - energy imparted by ionizing radiation to matter - by nearby healthy tissues. The measurement of absorbed dose within a patient - *in vivo* dosimetry - allows one to compare dose measurements with dose values calculated by the treatment planning system, to ensure that the prescribed dose to the target volume is within certain limits. Although some real-time dosimeters are already available in the market, more research is needed to effectively implement real-time dosimetry routinely in radiotherapy, such as fiber optic dosimeter [1-2].

In this work [3] radioluminescent materials were tested as promising radioluminescent materials for real-time optical dosimetry. A testing prototype was developed and radioluminescent glasses produced at INESC-Porto were tested at IPO-Porto under brachytherapy treatment conditions with irradiation from an Iridium (<sup>192</sup>Ir) source. A phantom has been assembled and used for irradiation of various radioluminescent materials coupled to optical fibers (see figure 1). The scintillating samples were BCF-60 (Saint-Gobain) and rare earth doped glasses. The radioluminescent signal was detected and acquired with a DAQ (NI) controlled with Labview. According to the results a neodymium doped glass, which showed infrared radioluminescence response, may be used for future applications in dosimetry. The prototype tested at IPO-Porto proved to be valuable as an additional tool for brachytherapy treatment quality control. It is expected to develop low cost dosimeter for *in vivo* real-time dosimetry in brachytherapy, based on the developed prototype.

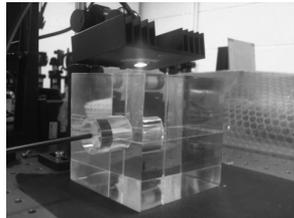


Fig. 1. Assembled phantom used for testing radioluminescent materials.

**Acknowledgments:** I would like to acknowledge FCT for providing financial support to this project (FIBDOSE: PTDC/SAU-BEB/108644/2008), to INESC-Porto (559/BI\_B1D/11), and to Brachytherapy Service at IPO-Porto.

**References:**

- [1] Andersen, C. E. et al. Characterization of a fiber-coupled Al<sub>2</sub>O<sub>3</sub>: C luminescence dosimetry system for online *in vivo* dose verification during <sup>192</sup>Ir brachytherapy. *Medical physics*, v. 36, n. 3, p. 708, 2009.
- [2] Suchowerska, N. et al. A fibre optic dosimeter customised for brachytherapy. *Radiation Measurements*, v. 42, n. 4-5, p. 929-932, 2007.
- [3] Samuel Chiquita, University of Porto, M. Sc. Thesis, “Dosímetro radioluminescente em fibra ótica para braquiterapia e radioterapia externa”, 2011.

Oral Sessions V > A2

# Environment II



## Characterization of soils in an area of prescribed fire

**N. Santos<sup>1</sup>, M.J. Šmid<sup>2</sup>, A.M. Castro<sup>2</sup>, J. Espinha Marques<sup>1</sup>, J.P. Meixedo<sup>2</sup>,  
J. Góis<sup>3</sup>, H. I. Chaminé<sup>4</sup>, A. Vivas<sup>5</sup>, E. Carvalho<sup>5</sup>**

<sup>1</sup> Faculty of Sciences (DGAOT) and Centre of Geology of the University of Porto, Portugal

<sup>2</sup> School of Engineering (ISEP), Polytechnic of Porto and Centre CIGAR|UP, Portugal

<sup>3</sup> CIGAR – Faculty of Engineering, University of Porto, Portugal

<sup>4</sup> School of Engineering (ISEP), Polytechnic of Porto and Centre GeoBioTec|UA, Portugal

<sup>5</sup> AFN - Forestry Services, Portugal

The prescribed fire is a technique that is often used, it has several advantages. Pedological and hydropedological techniques were tested to assess the prescribed fire changes may cause in soils. This work was performed in Tresminas area (Vila Pouca de Aguiar, Northern Portugal), during February and March 2011.

In the present study we applied several techniques. For the field sampling was followed the ISO 10381-1<sup>[1]</sup>, ISO 10381-2<sup>[2]</sup>, and FAO rules <sup>[3]</sup>, as well as were used a grid with 17 points for measuring the soil parameters. During the fire, we have tried to check, with the assistance of the Portuguese Forestry Authority, some important parameters such as, the propagation speed, the size of the flame front and the intensity of energy emitted per unit area. Before the fire, was collected carefully soil disturbed and undisturbed samples for laboratory analysis, and measured soil water content; we also have placed four sets of thermocouples for measuring soil temperature. After the fire, were collected the thermocouples and new soil samples; the water content were measured in the soil and collected ashes.

In the laboratory, after preparing and sieving the samples, were determined the soil particle size. The soil pH and electrical conductivity in water was also determined. The total carbon (TC) and inorganic carbon (IC)<sup>[4]</sup> was measured by a Shimadzu TOC-Vcsn.

The water content in soil has not varied significantly before and after the fire, as well as soil pH and soil electrical conductivity. The TC and IC did not change, which was expected, since the fire not overcome the 200° C. Through the various parameters, we determined that the prescribed fire didn't affect the soil. The low temperature of the fire and its rapid implementation that lead to the possible adverse effects caused by the wild fire didn't occurred.

### References:

[1] ISO 10381-1:2002. Soil quality – Sampling - Guidance on the design of sampling programs

[2] ISO 10381-2:2002. Soil quality – Sampling – Guidance on sampling techniques

[3] FAO (1988). *Soil Map of the World. Revised Legend*. World Soil Resources Report 60. FAO, Rome.

[4] ISO 10694:1995. Soil quality – Determination of organic and total carbon after dry combustion

## **Biodegradation of petroleum hydrocarbons in estuarine sediments: influence of metals**

**Raquel Almeida<sup>1</sup>, Ana P. Mucha<sup>1</sup>, Catarina Teixeira<sup>1,2</sup>, Adriano A. Bordalo<sup>1,2</sup>, and C. Marisa R. Almeida<sup>1</sup>**

<sup>1</sup> CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal

<sup>2</sup> Laboratório de Hidrobiologia, Instituto de Ciências Biomédicas, Universidade do Porto (ICBAS-UP), Largo Professor Abel Salazar, 2, 4099-003, Porto, Portugal

Estuaries are often considered sinks for contaminants, being ranked as the most anthropogenically degraded habitat types on Earth [1]. The preservation and clean-up of these ecosystems is paramount and bioremediation is a valuable non-invasive option. However, the simultaneous presence of different types of pollutants, namely organic and inorganic contaminants, can interfere with this technique.

In this work, we investigated the effect of Cd, Cu and Pb, on the biodegradation of petroleum hydrocarbons in estuarine sediments, under laboratory conditions. Two types of non-vegetated sediment, a sandy and a muddy one, were collected in the River Lima estuary (NW Portugal) and spiked with crude oil and each of the metals at concentrations selected according to the ERM (Effects Range-Median), i.e. concentrations suspected of causing adverse biological effects [2]. Spiked sediments were left in the dark under constant agitation for 15 days, after which crude oil biodegradation was evaluated. Total petroleum hydrocarbons (TPHs) concentrations were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) after their extraction by sonication and metal contents were determined by atomic absorption spectrometry (AAS). Microbial abundance was also estimated, total cell counts (TCC) were enumerated by DAPI staining and culturable hydrocarbons degraders were determined using a modified most probable number (MPN) protocol. Microbial community structure was assessed using Automated rRNA Intergenic Spacer Analysis (ARISA).

The obtained results showed that microbial communities from estuarine sediments had the potential to degrade petroleum hydrocarbons, with a maximum of 32% degradation obtained for sandy sediments. Both crude oil and metals changed the microbial community structure, with a more pronounced effect observed for Cu. Also, among the studied metals, only Cu displayed measurable deleterious effect on the hydrocarbons degradation process. Both degradation potential and metal influence varied with sediment characteristics probably due to differences in contaminant bioavailability.

### References:

[1] Lytle, JS; Lytle, TF (2001) Use of plants for toxicity assessment of estuarine ecosystems. *Environmental Toxicology and Chemistry* 20, 68-83.

[2] Long, ER; MacDonald, DD; Smith, SL; Calder, F. (1995) Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management* 19, 81-97.

**Acknowledgments:** This work was partially funded by Fundação para a Ciência e Tecnologia (FCT) through project PHYTOBIO (PTDC/MAR/099140/2008) and REEQ/304/QUI/2005 re-equipment project.

## Development of resistance to agricultural antifungal compounds - emergence of multidrug cross resistance among the human fungal pathogens *Candida*, *Cryptococcus* and *Aspergillus*.

**Pedro Tavares<sup>1</sup>, João Maia<sup>1</sup>, Sofia Farinha<sup>1</sup>, Isabel Faria Ramos<sup>1</sup>, Elisabete Ricardo<sup>1,2</sup>, Isabel Marcos Miranda<sup>1,2</sup>, Cidália Pina-Vaz<sup>1,2,3</sup> and Acácio Gonçalves Rodrigues<sup>1,2,4</sup>**

<sup>1</sup> Department of Microbiology, Faculty of Medicine, University of Porto, Portugal; <sup>2</sup> Cardiovascular Research & Development Unit; Faculty of Medicine, University of Porto, Portugal; <sup>3</sup> Department of Microbiology, Hospital S. João, Porto, Portugal; <sup>4</sup> Burn Unit, Department of Plastic and Reconstructive Surgery, Hospital S. João, Porto, Portugal

Clinically relevant fungal infections have become more prevalent during the past two decades. However, resistance to antifungal drugs has become a major clinical problem. Antifungals of classes similar to those used in human therapy are widely used in agriculture, a field where resistance to antifungals is also becoming an increasing problem. Among antifungal agents used for crop protection, the azoles are widely used (e.g. propiconazole, prochloraz, imazalil) in the European Union and its use has gradually increased during recent years. Antifungal exposure is associated to the development of resistance. In addition, there is the possibility of development of cross-resistance to drugs of the same class. The acquisition of resistance in nature may thus result in a significant, yet undetermined, impact in veterinary and human health.

The main goal of this work was the exposure of clinical and environmental strains to antifungal prochloraz (PCZ): *Candida albicans*, *C. parapsilosis*, *C. glabrata*, *Cryptococcus neoformans* and *Aspergillus fumigatus* with susceptible phenotype to clinical azoles. Afterwards, MIC will be determined for different antifungals used in agriculture (prochloraz, propiconazole, imazalil, benalaxyl, cyprodil, azoxystrobin) and for antifungals used in the clinical setting (fluconazole, voriconazole, posaconazole). Acquired mechanisms of resistance will be clarified molecularly. Minimal inhibitory concentrations (MIC) were determined for PCZ for the species mentioned above, according to the Clinical Laboratory Standards Institute (CLSI) M27-A3 protocol (for *Candida* and *Cryptococcus*) and to M38-A protocol (for *Aspergillus*). Briefly, a single, randomly selected colony from a fresh 24h culture in Sabouraud agar medium was incubated in sub-inhibitory concentrations of PCZ (see table 1 for details). The cultures were incubated at 35°C, 150 rpm. Every 24h, the culture was refreshed. At each sub-culture, an aliquot was stored at -70°C in 40% glycerol. MIC of PCZ was re-determined according to CLSI protocols once a week.

An induction of resistance was evident to PCZ with cross-resistance to clinical azoles.

**Minimal Inhibitory Concentrations (MIC) for prochloraz (PCZ)**

Clinical Strains	MIC PCZ (µg/ml)
<i>C. albicans</i>	0.5
<i>C. glabrata</i>	2.0
<i>C. parapsilosis</i>	4.0
<i>C. neoformans A</i>	2.0
<i>C. neoformans B</i>	2.0
<i>A. fumigatus A</i>	0.125
<i>A. fumigatus B</i>	0.25

# Effects of a metalaxyl photocatalytic-treated solution on biomarkers of oxidative stress in *Solanum nigrum* L. plants

S. B. Neves<sup>1,2</sup>, A. M.T. Silva<sup>3</sup>, J. Teixeira<sup>1,2</sup> and F. Fidalgo<sup>1,2</sup>

<sup>1</sup>Center for Biodiversity, Functional & Integrative Genomics (BioFIG)

<sup>2</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

<sup>3</sup>Laboratório de Catálise e Materiais (LCM), Laboratório Associado LSRE/LCM, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Portugal

In the last decades, the risk of pesticide pollution in watercourses has grown exponentially with agriculture intensification, demanding pesticides and fertilizers to improve yield and crop quality. Metalaxyl is a systemic fungicide widely used in agriculture and has a tendency to accumulate in soils and groundwater, but it can be successfully degraded by the photo-Fenton process [1]. In fact, the studies of Silva *et al.* [1] suggested that the integration of the photo-Fenton treatment, as chemical primary treatment, followed by *Solanum nigrum* L. plants biological remediation (as post-treatment) can be used for the remediation of waste waters contaminated with concentrations of metalaxyl as high as 150 mg/L. *S. nigrum* is a pioneer plant species growing worldwide in several polluted sites and was reported to phytoremediate several heavy metals and others hazardous compounds from soils, revealing a significant phytoremediation potential.

In the present study, to assess the effects that a metalaxyl photocatalytic-treated solution could have on *S. nigrum* development, some physiological parameters related to the plant antioxidant system, namely lipid peroxidation, free proline and photosynthetic pigments levels were evaluated. *S. nigrum* seedlings were grown hydroponically in a mixture of vermiculite:perlite (2:1) watered with a metalaxyl photo-Fenton-treated solution supplemented with a commercial liquid fertilizer, and were maintained in a plant growth chamber, at a constant temperature of 23°C and under a 16 light/8h dark photoperiodic regime. After one month of treatment, at least 4 plants from each growth condition (control and treated) were collected, frozen and grinded under liquid N<sub>2</sub> and stored at -80 °C until used. The data obtained in this study revealed that the treated solution caused in both roots and shoots an increase in lipid peroxidation, while a decrease in the free proline levels was quantified. No difference in the total chlorophyll and carotenoids content was detected in exposed plants compared to controls. These preliminary results reveal that although some symptoms of oxidative stress were observed in both roots and shoots of *S. nigrum* plants treated with metalaxyl photocatalytic-treated solution, a deeper knowledge about antioxidant defense system of plants will be necessary in order to evaluate the usefulness of the photo-Fenton process plus *S. nigrum* L. weed plants integrated process for the abatement of highly concentrated metalaxyl on waste waters.

Acknowledgements: The financial support from the University of Porto (projeto pluridisciplinar PhotoWeed), with the contribution of Santander Totta, is gratefully acknowledged. Syngenta Agro S.A. Portugal is acknowledged for providing Ridomil®. AMTS acknowledges financial support from POCI/N010/2006.

## References

[1] Silva et al. (2012), Chemical Engineering Journal (in press).

# ***Solanum nigrum* L. as a tool to remediate Cr(VI)-polluted sites – the contribution of its metallothioneins**

**N. Verde, F. Fidalgo, J. Teixeira**

Center for Biodiversity, Functional & Integrative Genomics (BioFIG)  
Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n,  
4169-007 Porto, Portugal

Due to careless and non-conscientious dumping, many toxic substances end up in the environment, such as Chromium-IV (Cr(VI)), which is an extremely harmful carcinogenic [1]. Metallothioneins (MTs) are cysteine-rich proteins capable of binding with different metals reducing their toxic activity. MTs seem to be related with metal homeostasis and detoxification of metals and organic compounds, but their role is still to be fully understood [2]. In this study, the resistance to Cr(VI) and its remediation by the Cd/Zn hipperaccumulator *Solanum nigrum* were investigated in order to discover if this plant species can be used as a to clean up Cr(VI)-contaminated sites and to further comprehend the role of MTs in plant heavy metal metabolism. Plants were grown in a vermiculite:perlite (2:1) substrate for one month in three different groups. The control plants (C) were supplemented with a commercial fertilizer, only. The shock treatment (St) plants were supplemented with the same fertilizer for three weeks, and during the fourth week potassium dichromate ( $K_2Cr_2O_7$ ), in a concentration of 12 mg/L, was also added. In the prolonged treatment (Pt), plants were supplemented with the same fertilizer plus  $K_2Cr_2O_7$  in a concentration of 1.5 mg/L the whole time. In the Pt group, the roots and shoots' length were reduced in 66% and 50%, respectively, but no reduction was detected with the St plants. Plants from both groups suffered a decrease in chlorophyll accumulation, with a 20% decrease in St plants and of 10% in Pt plants being registered. Lipid peroxidation analysis showed that St plants accumulated 2x as much malondialdehyde (MDA) in roots but 4x less in shoots. The Pt group accumulated 1.6x more MDA in roots and 2x less in shoots. Proline analysis in St plants showed a 3x-increased accumulation in roots and 2x in shoots, while Pt plants suffered a decrease in 12% and 10% in roots and shoots, respectively. Regarding the MTs' mRNA accumulation analysis, no MT2b mRNAs were detected in any treatment or organs analyzed. MT2a is expressed in both control tissues and it increases in both tissues in the treated plants. MT2c mRNAs were detected only in the shoot tissues of both treated plants, suggesting that this MT is specifically induced by Cr(VI). In contrast, MT2c+d analysis suggests that MT2d is present both in control and in the shock treatment-derived tissues, but not on the prolonged treatment-derived ones. MT3c transcripts were only detected in shoot control tissues, while all the MT3 were detected in both control tissues and with a slight increase in the shock treatment shoot tissues.

## References:

- [1] Panda, S.K. and Choudhury, S. (2005), *Chromium stress in plants*, Brazilian Journal of Plant Physiology, 17 95-102
- [2] Palmiter R.D. (1998), *The elusive function of metallothioneins*. Proc Natl Acad Sci USA 95: 8428–8430

The financial support from the University of Porto (projeto pluridisciplinar Metallochromium), with the contribution of Santander Totta, is gratefully acknowledged.

# The influence of physical and economical factors in conservation of *Caesalpinia echinata*, on Bahia South Region

**N. Dantas<sup>1</sup>, D. Lobão<sup>2</sup> and M. Moreau<sup>3</sup>**

<sup>1</sup>State University of Santa Cruz

<sup>2</sup>CEPLAC - Executive Committee of the Plan's Cocoa Crop

<sup>3</sup> Department of Agricultural and Environmental Sciences - State University of Santa Cruz

The Brazil is widely known by your rich biodiversity. In the coastal region on south of Bahia we found the Atlantic Forest, that occupy all Brazilian coast. It is considered a Hot Spot, presents high degree of endemism. So this ecosystem has, specifically, a big biodiversity that can be justified for a lot of factors how the latitudinal and altitudinal variation (SANTOS 2008, p. 2). The richness of species too can be explained because the Atlantic Forest occupies different climatic zones and does interface with various others biomes, how the Caatinga, Cerrado and Pampas. To work was chosen the species *Caesalpinia echinata*, commonly known by Pau-brasil, because this species can express the history of natural resources exploration on Brazil, it was the first resource explored. On Bahia's South Region, the practice of agroforestry system, known by *Cabruca*, made possible the conservation of Pau-brasil, and other species.

The team of Pau-Brasil program, composed by technical staff of the CEPLAC, in several field visits identified and did the georeferencing of the specie. They found 1525 matrices of pau-brasil (Fig. 1). The data were worked on ArcGIS 9.2, where was done the relation of species occurrence with physical, environmental and economical factors. Some of data, was found accessible for free on IBGE website, where could to do the download of the *shape* file and the metadata, that was the basemap used..

Figura 1

Brazil wood is locate on the boards of Pliopleistoceno Group, which arise naturally poor soils, however it is a climax plants, its occurrence has latitudinal variation of 5 ° 39 'S to 23 ° S, which encompasses the tropical climatic types Af, Am, As and Aw, and altitudinal variation between 10 and 320m(LORENZI,1998).

The results show that most matrices occurs where have more planted areas. Part of the matrices, specifically 841, has occurred in Itamaraju town, that have big planted areas, large spaces for *Cabruca*, and too have two national ecological parks. Another factor that can explain the conservation is because the species variation of all matrices found there is the Brazil wood sheet of orange(pau-brasil folha de laranja), that is endemic and until recently was unknow, just being off the list of woods with great commercial value.The *Cabruca* system has shown efficient on productive conservation, serving how a ecological corridor. However this agroforestry system has lost space for the monoculture system, what represent big lost in ambiental terms.

References

[1] Lorenzi, H. *Árvores Brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil*, Vol 2, 2 ed- Nova Odessa, SP: Editora Plantarum, 1998.

[2] Lobão, D. E. *Agroecossistema Cacauero Da Bahia: Cacau- cabruca e fragmentos florestais na conservação de espécies arbóreas*. Disponível em: < [www.fcav.unesp.br/download/pgtrabs/pv/d/2524.pdf](http://www.fcav.unesp.br/download/pgtrabs/pv/d/2524.pdf) > Acesso em: 10/07/2011.

[3] dos Santos, N. D. *Hepáticas da Mata Atlântica do estado do Rio de Janeiro: diversidade, fitogeografia e conservação*. Disponível em:< [http://www.jbrj.gov.br/enbt/posgraduacao/resumos/2008/nivea\\_dos\\_santos.pdf](http://www.jbrj.gov.br/enbt/posgraduacao/resumos/2008/nivea_dos_santos.pdf) > Acesso em 10/07/2012.

Oral Sessions V > A3

# Virtualities



## A transitory complex abstraction

**M.Oliveira<sup>1</sup>, G.Furtado<sup>2</sup>**

<sup>1</sup> Faculty of Architecture, University of Porto, Portugal

This research, on Architectural Theory and History, focus on the influence of aesthetics in architecture, throughout the twentieth century. The research attempts to synthesize three ideologies that led to specific oscillations - the ideology of "Abstraction" related to the modern period, "Complexity" related to the postmodern period, and "Transitory" related to the contemporary period.

Twentieth century's civilization introduced the ideology of modernity (and a "modern" idea derived from the Latin *modernus* that means "new" as opposed to "old"), based on the mechanical processes, the rationalist and functionalist logic, and an utopian impulse supported by science, industrial technology, and belief in progress. It is close related to the scientific and artistic developments of this period - through the discovery of "relativity theory" to the intense speculation of "Abstraction" in arts (by Kandinsky among others). Abstraction meant a "*a general idea*"[1], and relates to concepts like "concrete", non-representative basic forms, thinking, reflection. The modern period led to the so-call postmodern shift, a counter-culture process, in Saussure's idea of "*diference*", characterized by hybridity and plurality. It followed the post war period, and discoveries such as Quantum Physics and later the DNA. The postmodern "condition", was defined by Lyotard, influenced trough Structuralism (Barthes et al), and later by post-Structuralism (Derrida et al). From this, emerges the ideology of "Complexity", which definition is often tied to the concept of a "*state of being formed of many parts*"[2]; and it relates to chaos theory, systems or reality of the complex. Furthermore, the Contemporary arised with the aforementioned epistemological mutation (modern to postmodern), was also tied to the sedimentation of a new techno-cultural order (S), and and the consensus on "*fluidity*" (Deleuze). From this context, appears the idea of "Transitory"; and it means "*to go over, to pass*"[3], relating to concepts such as simultaneity, mobility, temporary, transition, movement or action.

In short, the present presentation, attempts to speculate and understand the cultural changes of modernity, and aims to evolve towards a operative epistemological attitude, attempting to decode the twentieth century's sequence of aesthetical ideologies that marked architecture.

[1] Hornby, A.S. (2010), *Oxford Advanced Learner's Dictionary of Current English*, Oxford university press 2010, pp. 9

[2] Idem, Ibidem, pp. 306

[3] Idem, Ibidem, pp. 1646

# Designing Serendipity

R. Melo<sup>1</sup>

<sup>1</sup> Masters in Multimedia, Faculty of Engineering, University of Porto, Portugal

This ongoing project envisions the exploration of the concept of Serendipity within network communications and information visualization as a mechanism for an enhanced interaction experience.

With the continual increase of information gathered through user-created networks and the escalating optimization of search engines in regards to users' web navigation patterns (and the possible limitations that arise with such networks and optimizations), this study will examine how designing towards serendipity might expand the network discovery possibilities as well as help circumvent some of the limitations of the current principal methods of information retrieval on the web.

By analyzing the serendipity process[1], detailing its inherited mechanisms and reviewing available literature of attempts at activity promote serendipitous discoveries[2], we hope to be able to precise how it can be applied into user experience design, in regards to network communication, as well as to provide solutions in regards to the increased observable problem of cyberbalkanization [7].

For this, the research focus will be to the discovery of the impact of careful information visualization techniques, as well as research in user interface design as vehicles towards the creation and discoverability of valuable connections made buy the user and their interaction with content, while not simply increasing the level of apparently random inputs, but rather actively promoting relevant, and new, information.

This investigation purposes to elaborate on the hypothesis of serendipity as a privileged and worthwhile concept in network communication, which, in turn might translate into the creation of a series of good practices as well as evidences, theoretical and empirical, towards the value of designing with serendipity in mind.

It is our hope that, by exploring the concept of serendipity, we might help to recover the limitlessness and diversity of the web, breaking through content bubbles and to help to the creation and discovery of meaningful patterns between different, and relevant, media objects.

## References:

- [1] Erdelez, S. (1999) Information encountering: It's more than just bumping into information, Bulletin of the American Society for Information Science.
- [2] Foster, A. & Ford, N. (2003) Serendipity and information seeking: An empirical study, J. Documentation.
- [3] Van Alstyne, M. and Brynjolfsson, E. (1997), Electronic Communities: Global Village or Cyberbalkans?, MIT Sloan School, Cambridge.

# Machinima: player-as-producer paradigm

Sandra Araújo<sup>1</sup>

<sup>1</sup> Faculty of Fine Arts, University of Porto, Portugal

Machinima are visual narratives “created by recording events and performances (filmmaking) with artistically created characters moved over time (animation) within an adjustable environment (3D game technology platform or engine)” [1].

When a computer game is released today, it is as much a set of design tools as a finished game design. PC game developers are routinely releasing their development tools for experimentation and play; that is, play with technology and play with animation, stories, graphics and movies as much as play with games [2].

Machinima is part of an artistic appropriation practice that combines art and videogames, emergent from a network-based participatory culture. Often associated to MOD art, which implies the modification of a video game engine to create something new, machinimartists merge their gameplay performance and technical expertise as players-producers. Nevertheless, machinima is a non-interactive MOD form that can be analysed through the notion of remediation [3], i.e. the appropriation of content of one media of art from another.

## References:

- [1] Marino, P. (2004), *The art of machinima: 3D game-based filmmaking*, Paraglyph, Scottsdale, AZ, pp. 1.
- [2] Lowood, H. (2007), *High-Performance Play: The Making of Machinima*, in Clarke, A. and Mitchell G., “Videogames and Art”, Intellect Books, Bristol, pp. 59-79.
- [3] Bolter, J.D. and Grusin, R. (1999), *Remediation: Understanding new media*, The MIT Press, Cambridge, MA.

## MNEMOSINE

**D.Monteiro, G. Madeira, J. Ferreira and M. Carvalho**

Faculty of Architecture, University of Porto, Portugal.  
Faculty of Fine Arts, University of Porto, Portugal

The denomination of this Project arises from the proximity and the good relationship which has existed between both faculties through the years. The present nostalgia follows a period when the arts were enjoyed in unison and then became slightly forgotten as time passed by. Thus, only the memory of a past which leaves behind it light fragments of its existence has resisted. With this project, we wish to create room for meetings between students from the Faculty of Fine Arts and students from the Faculty of Architecture, presenting works produced in both faculties, while at the same time, promoting the possibility of working together.

Our main objective is based on raising students' awareness to the potential apprehensions that may come up during the projection of the two different areas and in the way in which these can be inserted into the perspective of personal work.

This approximation of students would be achieved through thematic exhibitions related to the faculties, conferences, and a workshop proposing a conjoint piece of work between their students.

In the sphere of this initiative for an young researchers at University of Porto, we herewith wish to propose the execution of an exhibition in the Galeria dos Leões, in the building of the rectory of the University of Porto, which would include the participation of three students from each faculty, with Professor Graciela Machado (FBAUP) and Professor Gonçalo Furtado (FAUP) as commissioners for both faculties.

This exhibition would function as a prototype which would mark the beginning of our project, the first contact between students from both faculties, hoping to develop in these students an interest that could hopefully lead to the possibility of taking part in future projects.

Oral Sessions V > A4

# **Biological Sciences II**



## Modulation of *Streptomyces natalensis* secondary metabolism: evidence of cross-talk between quorum-sensing and oxidative stress

S. M. Silva<sup>1,2,3</sup>, C. L. Santos<sup>1</sup>, S. D. S. Pires<sup>1,3</sup>, T. Beites<sup>1,2</sup>, M. V. Mendes<sup>1</sup>

<sup>1</sup>IBMC - Institute for Molecular and Cell Biology, University of Porto. <sup>2</sup>Faculty of Sciences, University of Porto. <sup>3</sup>ICBAS - Institute for the Biomedical Sciences Abel Salazar, University of Porto

*Streptomyces* are filamentous soil-dwelling bacteria well known for their ability to produce a wide range of secondary metabolites of great scientific and economic significance. The biosynthesis of these compounds is under the control of intricate networks which are influenced by a wide variety of environmental and physiological stimuli. Amongst them, the influence of oxidative stress on the regulation of secondary metabolism has been previously addressed and is a main subject of study in our laboratory. In addition, quorum-sensing, a bacterial communication mechanism based on the production and release of molecular signals, also aroused our interest since it is closely related with the production of secondary metabolites and at the same time seems to be associated with the oxidative stress adaptive response. In this context, we aimed to unveil and characterize the putative interactions of quorum-sensing and oxidative stress response in *Streptomyces*, as well as their effects in the secondary metabolism.

The model organism used in this study was *Streptomyces natalensis*, which produces pimaricin, an important antifungal widely used in the food industry (E235) and also in the treatment of fungal keratitis. In order to evaluate the effects of a redox imbalance, a knock-out mutant for *oxyR* was constructed and characterized (*S. natalensis* CAM.03). OxyR is known to regulate the transcription of the alkyl hydroperoxidase system (AhpCD), which is involved in H<sub>2</sub>O<sub>2</sub> detoxification in *Streptomyces* spp. The results showed that the total catalase activity of the *S. natalensis* CAM.03 along culture growth was significantly higher than that of the wild-type, presumably to compensate the low transcriptional levels of AhpCD. Moreover, *S. natalensis* CAM.03 presented higher levels of intracellular H<sub>2</sub>O<sub>2</sub> during the beginning of the exponential growth phase, which likely led to the early induction of KatA1 (a monofunctional catalase previously shown to be inducible by H<sub>2</sub>O<sub>2</sub>) [1]. Moreover, in terms of pimaricin production, this mutant presents an overproducing phenotype. In agreement, the transcription profile of genes related to pimaricin biosynthesis was also higher in the *S. natalensis* CAM.03. In order to clarify the putative interactions between quorum-sensing and the oxidative stress response, the influence of the supernatants of the two strains (where quorum-sensing molecules accumulate) on the catalase activity was analysed. The results demonstrated that the mutant supernatant has the ability to alter the catalytic activity profile of the wild-type strain. Moreover, the transcription profile of genes related to quorum-sensing revealed that the *oxyR* deletion affected its transcription. In summary, our results showed that, beyond the oxidative stress response, OxyR modifies the external medium inducible properties and the transcription of quorum-sensing related genes, which suggests that the oxidative stress response and QS are indeed correlated in *S. natalensis* and both interfere in the production of pimaricin.

1. Beites, T., S.D. Pires, C.L. Santos, H. Osorio, P. Moradas-Ferreira and M.V. Mendes (2011). Crosstalk between ROS homeostasis and secondary metabolism in *S. natalensis* ATCC 27448: modulation of pimaricin production by intracellular ROS. *PLoS One*, **6**(11): p. e27472.

## Role of border-like cells in defence against microorganisms in *Arabidopsis thaliana* roots

D. Silveira<sup>1</sup>, N. Góis<sup>1</sup>, M. Costa<sup>1,2</sup>, S. Nobre<sup>1,2</sup>, L.G. Pereira<sup>1,2</sup> and S. Coimbra<sup>1,2</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> BioFIG, Centre for Biodiversity, Functional and Integrative Genomics.

Plant innate immunity is based on an ancient system of molecules that defend the host against infection. Defence relies on the capability of each cell to recognize the presence of pathogens and activate downstream responses [1]. One common strategy that pathogens use to overcome the first line of defence of plants is secreting polygalacturonases (PG), enzymes that degrade pectic cell wall polysaccharides. These enzymes, secreted in the beginning of the infection, depolymerize the main component of the wall pectins by cleaving the  $\alpha$ -1,4 glycosidic bonds between galacturonic acid units [1].

PGs are secreted by a wide range of parasites such as fungi, bacteria, nematodes and insects [1] so, many plants developed strategies to combat them, and so far the most efficient strategy known are the Polygalacturonase Inhibiting Proteins (PGIPs) which is the subject of our work. PGIPs are Leucine-rich repeat proteins (LRR) that recognize and inhibit fungal polygalacturonases [2].

Many of the referred parasites infect plants through roots and it is known that in several species, roots release a large number of border cells into the rhizosphere, which are believed to play a key role in root development and health [3]. However in *Arabidopsis thaliana* and other BRASSICACEAE, roots release cells in layers which stay alive for several hours. In order to evaluate if border-like cells act in defence response in *Arabidopsis*, we will use transgenic plants that overexpress PGIPs in border like-cells.

These transgenic plants will be obtained by transformation with DNA constructions which contain either the PGIP1 or the PGIP2 gene sequences under the influence of a specific border-like cell promoter, MDK4-20 [4].

The role of border-like cells in defence will be then evaluated by eliciting the transformed plants with soil pathogens.

### References:

- [1] Di Matteo A., Bonivento D., Tsernoglou D., Federici L., Cervone F. (2006), Polygalacturonase-inhibiting protein (PGIP) in plant defence: a structural view, *Phytochemistry*, 67, 528–533.
- [2] Federici L., Di Matteo A., Fernandez-Recio J., Tsernoglou D., Cervone F. (2006), Polygalacturonase inhibiting proteins: players in plant innate immunity?, *TRENDS in Plant Science*, Vol.11 (2), 65-70.
- [3] Vicré M., Santaella C., Blanchet S., Gateau A., Driouich A. (2005), *Root Border-Like Cells of Arabidopsis. Microscopical Characterization and Role in the Interaction with Rhizobacteria*. *Plant Physiology*, 138 (2), 998-1008.
- [4] Lilley CJ., Wang D., Atkinson HJ., Urwin PE. (2011), *Effective delivery of a nematode-repellent peptide using a root-cap-specific promoter*. *Plant Biotechnology Journal*, 9, 151-161.

## The role of the plant cell wall in the regulation of protein sorting

**M. Figueiredo<sup>1</sup>, C. Pereira<sup>1</sup>, S. Pereira<sup>1</sup> and J. Pissarra<sup>1</sup>**

<sup>1</sup> BioFig - Centre for Biodiversity, Functional and Integrative Genomics, Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, s/nº, 4169-007, Porto

The cell wall is a plant cell compartment with unique characteristics and dynamic structure, which plays an important role on cell growth and division, cell shape and defence. Despite the long lasting discussion, the cell wall is believed to be an important intermediate of protein sorting to and from this compartment. In the last few years, there has been an outburst of data regarding this issue, even though most of it has shown to be contradictory, hampering the definition of the importance of the cell wall in the trafficking regulation. Aspartic proteinases cardosins A and B from *Cynara cardunculus* have been extensively characterized at our lab, and will be used as models in this study. In the flower tissues of *C. cardunculus*, these proteinases are known to have different destinations, being cardosin A accumulated in the vacuole and cardosin B secreted.

However, both cardosins were shown to accumulate in the vacuole when expressed in tobacco cells. Interestingly, a secretion assay on tobacco protoplasts expressing cardosin B showed secretion of the protein. The aim of this work was to unveil the effects of the presence/absence of cell wall in the regulation of protein sorting, namely in vacuolar trafficking. Secretion assays were repeated for cardosin B and also for cardosin A, confirming that the two proteins are indeed secreted upon removal of the cell wall. Based on this preliminary data, we believe that the cell wall is somehow involved in cardosins vacuolar accumulation. To confirm our hypothesis, cell wall regeneration methods are currently being done to evaluate if the proteins are redirected to vacuole while the cell wall is synthesized.

# Molecular characterization of *Bet v 1* – homologues and *profilin* from pollen of *Quercus spp.*

C. Bernardo<sup>1</sup> and M.I. Amorim<sup>1,2</sup>

<sup>1</sup> Department of Biology; Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Environment and Society – CGUP

The *profilins* are small proteins expressed in eukaryotic cells involved in cell growth, cytokinesis, membrane traffic and cell motility. These ubiquitous proteins of approximately 15 KDa are encoded by one or few genes in most animals, fungi and protists, whereas in plants there are several genes that code for *profilin* isoforms [1 - 2].

The *Bet v 1 - homologues* are proteins with low molecular weight of 17 KDa expressed preferentially in tree pollen from *Fagales* and belong to the family of pathogenesis related proteins. *Bet v 1 - homologues* and *profilin* from tree pollen of the order *Fagales* are potential allergens [3 - 4].

In this work, we analyzed the transcripts from pollen *profilin* and *Bet v 1 - homologues* for the different *Quercus spp.* (*Q. suber*; *Q. rubra*; *Q. robur*; *Q. faginea*) by RT-PCR. The different amplicons were purified and cloned into the vector pGEM ®-T Easy and sequenced. The *Bet v 1* genes from *Q. suber* were cloned and sequenced. It was observed the presence of introns of varying size in genome sequences of *Bet v 1*. The aligned sequences were translated and used to construct the phylogenetic tree, using Neighbor Joining method from MEGA 4.0 software. The phylogenetic relationship based on *Bet v 1 – homologues* coding sequences of *Q. robur* and *Q. faginea* showed that they were present in the same cluster, and further away from the other *Quercus* sequences. Furthermore, it was constructed predicted 3D structures of *profilins* and *Bet v 1 – homologues* proteins and studied their physicochemical properties with yasara and prosite software.

[1] Hauser, M., et al. (2010), *Panallergens and their impact on the allergic patient*, Allergy Asthma Clin. Immunol. 6 (1), 1-14.

[2] Krishnan, K. and Moens, P. D. J. (2009), *Structure and functions of profilins*, Biophys. Rev., 1: 71-81.

[3] Musidłowska-Persson, A., Alm, R., & Emanuelsson, C. (2007). Cloning and sequencing of the *Bet v 1*-homologous allergen Fra a 1 in strawberry (*Fragaria ananassa*) shows the presence of an intron and little variability in amino acid sequence. *Mol Immunol*, 44(6), 1245-1252.

[4] Schenk, M. F., Cordewener, J. H., America, A. H., Peters, J., Smulders, M. J., & Gilissen, L. J. (2011). Proteomic analysis of the major birch allergen *Bet v 1* predicts allergenicity for 15 birch species. *J Proteomics*, 74, 1290-1300.

# Evolutionary Genomics of The Hedgehog Gene Family in Metazoans: Identification of The Desert Hedgehog Gene on Avian Genomes

**Joana Pereira<sup>1</sup>, Warren E. Johnson<sup>2</sup>, Stephen J. O'Brien<sup>2</sup>, Vitor Vasconcelos<sup>1,3</sup>, Agostinho Antunes<sup>1,2,3</sup>**

<sup>1</sup> CIMAR/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Portugal

<sup>2</sup> Laboratory of Genomic Diversity, National Cancer Institute, Frederick, USA

<sup>3</sup> Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

The Hedgehog (Hh) gene family codifies a class of secreted proteins composed of two active domains that act as signalling molecules during embryo development (e.g. development of the nervous and skeletal systems and the formation of the testis cord) [1]. The vertebrate Hh genes evolved by ancient duplications of the ancestral invertebrate Hh gene: while only one Hh gene is found typically in invertebrate genomes, vertebrates have three, each with different functions in distinct tissues (Sonic hedgehog – Shh; Indian hedgehog – Ihh; and Desert hedgehog – Dhh), which likely favoured the increased complexity of vertebrates and their successful diversification [2]. However, when the vertebrate members of the Hh genes family are browsed over the avian genomes available to date on the GenBank and Ensemble databases no evidence of the Dhh gene is found.

In this study, we used detailed comparative genomic and synteny analyses to characterize the Hh family on avian genomes and understand why no Dhh gene is found in the bird genome assemblies available to date. The analysis of the Dhh gene synteny on the lizard genome using the Genomicus 64.01 server [3] shows that this gene forms a conserved cluster with the LMBR1L and RHEBL1 genes, which are also not found on the current avian genomes assemblies. When the scaffold where these three genes are found on the lizard genome is compared with the avian genomes, we find that it has strong homologies with several regions of the avian ChUn\_random sequence, suggesting that if the Dhh and LMBR1L and RHEBL1 genes are present on avian genomes, they may be located on a microchromosome: very small chromosomes, rich in gene content and CpG islands, that are difficult to sequence and map and are usually assigned to the avian ChUn\_random or even absent of the current genome assemblies [4].

## References:

- [1] Burglin, T.R. (2008), *The Hedgehog protein family*. Genome Biology, 9, pp. 241.
- [2] Ingham, P.W. and McMahon, A.P. (2001), *Hedgehog signaling in animal development: paradigms and principles*. Genes and Development, 15, pp. 3059-3087.
- [3] Muffato, M., Louis, A., Poisnel, C.E. and Croliius, H.R. (2010), *Genomicus: a database and a browser to study gene synteny in modern and ancestral genomes*. Bioinformatics, 26, pp. 1119-1121.
- [4] Burt, D.W. (2002), *Origin and evolution of avian microchromosomes*. Cytogenetics and Genome Research, 96, pp. 97-112.

# Species identification and authentication of hare (*Lepus*) meat by the use of the mitochondrial *cytb* gene

**C.G. Santos<sup>1,2</sup>, V.S. Melo<sup>1,2</sup>, J. Costa<sup>1</sup>, J.S. Amaral<sup>1,2</sup>, M.B.P.P. Oliveira<sup>1</sup>, I. Mafra<sup>1\*</sup>**

<sup>1</sup>REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto,

<sup>2</sup>Polytechnic Institute of Bragança \*E-mail: [isabel.mafra@ff.up.pt](mailto:isabel.mafra@ff.up.pt)

Nowadays, consumers are increasingly concerned with issues of food safety and authenticity. In particular, game meat has been much appreciated by consumers for their exotic flavor and texture, low in fat and cholesterol as well as by the absence of steroids or other drugs. Food authenticity assessment is important in that it avoids unfair competition among producers and allows consumers to have accurate information about the products they purchase. Therefore, it is important to ensure that species of high commercial value declared are not replaced by other species of lesser value [1].

The present work was part of a project aiming to assess the authenticity of “Alheiras de caça” based on the development of species-specific polymerase chain reaction (PCR) techniques to detect game meat species, including hare meat. Since there were no reports in the literature concerning hare meat identification, this work aimed to propose for the first time a PCR technique able to specifically detect this species.

Mitochondrial *cytochrome b (cytb)* gene was used to design species-specific primers for hare detection [2]. The new primers were assessed for their specificity to *Lepus* species and the PCR amplification was optimized to detect 0.01% of hare meat in pork meat. To confirm the identity of the PCR products obtained, the fragments were sequenced. Performing a BLAST for the obtained sequences, the results showed a 100% of homology for *Lepus granatensis*, while primers matched also *L. europaeus* and *L. capensis*. Additionally, with the proposed new primers, we developed a novel methodology based on real-time PCR with the new intercalating EvaGreen dye. This technique proved to be fast and specific for the identification of hare with the sensitivity of 1 pg of hare DNA. The application of the developed species-specific PCR to 18 commercial samples of “Alheiras de caça” showed that hare meat was absent in all samples, even in one that was labeled as containing this meat. It can be concluded that the proposed new primers can be used by both species-specific end-point PCR or real-time PCR to accurately authenticate hare meat in food products.

## Acknowledgments:

The authors acknowledge the financial support of U. Porto/ Santander Totta “Projectos pluridisciplinares 2010”. Joana Costa is grateful to FCT PhD grant (SFRH/BD/64523/2009) financed by POPH-QREN (subsidized by FSE and MCTES).

## References:

- [1] Fajardo, V.; González, I.; Rojas, M.; García, T.; Martín, R. (2010). A review of current PCR-based methodologies for the authentication of meats from game animal species. *Food Science and Technology* 21: 408-421.
- [2] Santos, C.G.; Melo, V.S.; Amaral, J.S.; Estevinho, L.; Oliveira, M.B.P.P.; Mafra, I. (2012). Identification of hare meat by a species-specific marker of mitochondrial origin. *Meat Science* 90: 836-841.

## Assessment of the origin of the Iberian populations of *Podarcis sicula* using mitochondrial markers

**I. Rocha<sup>1</sup>, D. Salvi<sup>1</sup> and Miguel A. Carretero<sup>1</sup>**

<sup>1</sup> CIBIO, University of Porto, Portugal.

Biological invasions are very problematic and currently are the major cause of biodiversity loss. The multiple consequences can range from ecological level to economic/social aspects. The Italian wall lizard, *Podarcis sicula*, is one of the reptile species most widely introduced, from the United States to Turkey. In Iberian Peninsula and adjacent islands, there are four populations known to be introduced. The estimated age of these introductions is variable. While Menorca populations date back to the Middle Age, Noja (Cantabria) and Almería populations are considered to be introduced in the late XIX century or early XX century and that in Lisbon would be even more recent (end of 90's). In spite this knowledge, the geographic origin still unknown, except for Menorca population whose origin probably is Sicily-Sardinia. Tracing the origin of introduced populations is essential to understand the source and the pathway of the species dispersal and hence to set conservation policies aimed at preventing new introductions. Using molecular markers for detecting genealogical affinities between these introduced populations and those from the native range, we want to identify the geographic origin of four introduced populations of *Podarcis sicula* from all the known Iberian Peninsula (Lisbon, Almería and Cantabria) and Menorca Island.

Total genomic DNA was extracted from tail tips following the standard saline method. A fragment of the mitochondrial gene cytochrome b was amplified by PCR using the primers GludG-L and cb3H. In order to infer the relationships between the Iberian samples and those from the native range of *P. sicula* analyzed by Podnar et al. (2005)[1], a Maximum Likelihood (ML) phylogenetic analysis was carried out employing the HKY + G model of evolution (selected by Mega 5) under the Bayesian Information Criterion. Node support was calculated over 1000 bootstraps replicates.

A total of 40 haplotypes were analysed, 6 of which are from the introduced populations in study. Lizards from Cantabria are closely related to lizards from Tuscany. Also lizards from Lisbon clustered together with Tuscanian samples but also showed a slight divergence from them. Almería and Menorca populations are related with the Sicula haploclade (distributed in Sicily and Sardinia) with one haplotype from Menorca being identical to the Menorcan haplotype found by Podnar et al. (2005) [1].

So, it was identified multiple origins for the Iberian introduced populations. In the case of Lisbon and Cantabria, the populations are probably from near Tuscany and Tuscany, respectively. Both Menorca and Almería lizards are from Sicily (Sardinia) and finally, La Rioja population is from Calabria. These multiple origins suggest different pathways and routes, which are important to understand in order to delineate strategies to control new introductions are needed, namely major control in source and the education of people and governments to the biological introduction problematic.

References:

[1] Podnar, M., Mayer, W. & Tvrtković, N. 2005. Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Molecular Ecology*, 14: 575-588



Oral Sessions V > A5

# Astronomy



# GPGPU N-Body Cosmological Particle-Particle Simulations

**D. Oliveira<sup>1,2</sup>, A. da Silva<sup>2</sup>, A. R. Fernandes<sup>3</sup>**

<sup>1</sup> FEUP - Universidade do Porto, Rua Dr. Roberto Frias, s/n 4200-465 Porto, Portugal

<sup>2</sup> Centro de Astrofísica, Universidade do Porto, Rua das Estrelas, 4150-764 Porto, Portugal

<sup>3</sup> Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal

We explore the new emerging technique of GPGPU (General-Purpose computing on Graphics Processing Units) and its low-cost commodity high computing capacity, in the context of N-Body cosmological simulations.

We have implemented a standard Particle-Particle (PP), direct summation solver in the style of the well-known Hydra [2] cosmological simulation code in a GPGPU, GLSL over OpenGL 2.1 environment. This results in a GPU hardware vendor independent, platform-agnostic, complete PP direct-summation code with all steps and the full simulation iterative loop running on the graphics hardware in its entirety – fully leveraging the performance potential of the GPGPU framework.

This endeavour required the reengineering and recasting of the known algorithm in a parallel, vectorial form in order to fully exploit the GPU hardware.

Therefore, a novel implementation of the traditional PP algorithm was developed to address the particularities of this vector processing paradigm, opposed to the standard sequential processing based one.

We perform an analysis of the performance gains of this novel approach, compared to a “classical” implementation of the same algorithm in a traditional x86 architecture CPU.

We show that by using a GPGPU approach in PP direct-summation simulations, we transform the algorithm runtime scaling from  $O(N^2)$  to  $O(\sim N)$  for the surveyed problem sizes, making large, formerly impractical, PP simulations a feasible option.

We highlight that our code was implemented without 'if' conditional statements and also, relinquishes the need to transfer data to or from the host, whilst iterating through our numerical pipeline.

Our architecture allows to bypass possible processing pipeline stalls and the expensive memory transfer operations that would seriously hamper performance and, most importantly, the full leverage of the benefits of the GPGPU paradigm.

References:

[1] D. Göddeke. Gpgpu-basic math tutorial. Universität Dortmund, 2005

[2] G. Yepes. Cosmological numerical simulations. 1997

[3] H. Couchman et al. Hydra: an Adaptive-Mesh Implementation of P3M-SPH. 1995

[4] S. Kay. Modelling Properties of Galaxies and Clusters. University of Durham, 2000

# Spectral Synthesis using the Metropolis-Hastings algorithm

**J. P. Moura<sup>1,2</sup>, J. M. Gomes<sup>2</sup>, P. Papaderos<sup>2</sup>**

<sup>1</sup> Department of Physics and Astronomy, Faculty of Sciences, University of Porto, rua do Campo Alegre s/n, 4169-007 Porto, Portugal. [jpmoura@alunos.fc.up.pt](mailto:jpmoura@alunos.fc.up.pt)

<sup>2</sup> Centro de Astrofísica da Universidade do Porto, University of Porto, rua das Estrelas, 4150-762 Porto, Portugal.

Semi-Empirical Population Synthesis (SEPS) is a technique used in Astrophysics in which the electromagnetic spectrum from an observed galaxy is decomposed in terms of base elements, i.e., individual stars or stellar clusters with known physical properties, such as age and chemical composition, to derive their Star Formation Histories (SFH) [1]. Our SEPS model is based on the assumption that galaxies can be described as linear combinations of Simple Stellar Populations (SSPs). These are theoretical stellar clusters (BC03 models [2]) born at the same time and with the same initial element composition.

More complex physics such as extinction (due to gas and dust) or line-of-sight velocity dispersion (due to stellar motions) were previously corrected. Also, the most prominent emission lines or spurious pixels were masked out.

We have implemented an optimization code written in C++ to fulfill the task of finding the best match between the observed and modeled spectrum, subject to some boundary constraints. This program uses an adaptation of the Metropolis-Hastings algorithm [3] with a Simulated Annealing procedure, introducing a gradual decrease in the temperature while iterating.

First we tested the reliability of our code through fictitious noiseless data using previously known SFHs. Then we created more realistic test galaxies with signal-to-noise equal to 10, 20 and 30 around the wavelength of 4000 Å. Both tests showed that it is possible to reliably recover the SFHs but the degeneracies tend to get higher when the signal-to-noise ratio decreases. The mean stellar age and metallicity errors are of order of 0.1 dex. Finally, we have applied our C++ code to data from the Sloan Digital Sky Survey to retrieve the description of stellar populations from real galaxies. We see that the code performs extremely well with reduced  $\chi^2$  of the order of 1. The main results can be summarized: Early Type (Passive) galaxies tend to harbor old stellar populations whilst Late Type (Spiral) galaxies tend to harbor young and intermediate ones.

Acknowledgements: J. M. Gomes monitored this project in the framework of the PEEC program (FCUP). J. M. Gomes is supported by a Post-Doctoral grant and P. Papaderos by a Ciência 2008 contract, both funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

References: [1] Gomes, J. M. *Síntese Espectral de Galáxias: Modelos de Populações Estelares Simples e Compostas, Ajustes, Calibrações e Aplicações*. Universidade Federal de Santa Catarina (2009). [2] Bruzual, G., Charlot, S. *Stellar population synthesis at the resolution of 2003*. Monthly Notices of the Royal Astronomy Society 344, 4 (2003); [3] Metropolis, N., Rosenbluth, A. W., Rosenbluth, M. N., Teller, A. H., and Teller, E. *Equation of State Calculations by Fast Computing Machines*. Journal of Chemical Physics 21, 1087 (1953);

## PCA tools for probing dark energy with varying couplings

**P. O. J. Pedrosa<sup>1,2</sup>, L. Amendola<sup>3</sup>, A. C. O. Leite<sup>1,2</sup>, C. J. A. P. Martins<sup>2</sup>, N. J. Nunes<sup>3</sup>, and A. Seganti<sup>4</sup>**

<sup>1</sup>Faculdade de Ciências, Universidade do Porto, Porto, Portugal

<sup>2</sup>Centro de Astrofísica, Universidade do Porto, Porto, Portugal

<sup>3</sup>Institut für Theoretische Physik, Universität Heidelberg, Heidelberg, Germany

<sup>4</sup>Dipartimento di Fisica, Università di Roma "La Sapienza", Roma, Italy

Understanding the origin of dark energy is currently one of the deepest enigmas of theoretical cosmology and fundamental physics. Recent advances in instrumentation will soon provide us with the opportunity to characterize its properties with unprecedented accuracy.

We discuss methods, introduced in [1] and based on Principal Component Analysis techniques, for reconstructing the dark energy equation of state and constraining its evolution, using a combination of Type Ia supernovae at low redshift and spectroscopic measurements of varying fundamental couplings at higher redshifts.

It will be shown how the addition of the measurements of varying couplings can realize the prospect of a detailed characterization of dark energy properties all the way up to redshift 4. A related contribution (A. C. O. Leite et al.) discusses the application of these methods to datasets obtained with two ESO spectrographs (ESPRESSO and CODEX) in which CAUP is actively involved.

This work was done in the context of the FCT-DAAD cooperation grant 'The Dark Side of the Universe' (reference 441.00 Alemanha), with additional support from project PTDC/FIS/111725/2009 from FCT, Portugal.

The work of CM is funded by a Ciência2007 Research Contract, funded by FCT/MCTES (Portugal) and POPH/FSE (EC), and is also partially supported by grant PTDC/CTE-AST/098604/2008. NJN is supported by Deutsche Forschungsgemeinschaft (project TRR33) and is also partially supported by grants CERN/FP/109381/2009 and PTDC/FIS/102742/2008. The work of PP was partially funded by grant CAUP- 09/2009-BII.

[1] Amendola L., Leite A. C. O., Martins C. J. A. P., Nunes N. J., Pedrosa P. O. J. and Seganti A. (2011), Variation of fundamental parameters and dark energy. A principal component approach. Phys. Rev. D (submitted), arXiv:1109.6793 [astro-ph.CO].

## Probing dark energy with ESPRESSO and CODEX

**A. C. O. Leite**<sup>1,2</sup>, **L. Amendola**<sup>3</sup>, **P.O.J. Pedrosa**<sup>1,2</sup>, **C.J.A.P. Martins**<sup>2</sup>, **N.J. Nunes**<sup>3</sup>,  
and **A. Seganti**<sup>4</sup>

<sup>1</sup>Faculdade de Ciências, Universidade do Porto, Porto, Portugal

<sup>2</sup>Centro de Astrofísica, Universidade do Porto, Porto, Portugal

<sup>3</sup>Institut für Theoretische Physik, Universität Heidelberg, Heidelberg, Germany

<sup>4</sup>Dipartimento di Fisica, Università di Roma "La Sapienza", Roma, Italy

ESPRESSO for VLT (under construction) and CODEX (planned for the E-ELT) are two of a new generation of high-resolution ultra-stable spectrographs that will help shed light on the enigma of dark energy.

Using the PCA tools described in a related contribution (by P. O. J. Pedrosa) we have studied optimal ways of exploiting the spectroscopic measurements of varying constants up to  $z \sim 4$ , provided by both spectrographs, to reconstruct the evolution of the dark energy equation of state [1]

We have simulated several future datasets of fine-structure constant measurements (in the context of different theoretical scenarios), which differ in number and precision, and then analysed them in combination with luminosity measurements of Type Ia supernovae at low redshift.

It will be shown how the addition of these measurements allows the extension of the reconstruction procedure to much higher redshifts, possibly all the way up to  $z \sim 4$ . In fact we find that CODEX may, on its own, constrain the dark energy dynamics better than supernova measurements.

This work was done in the context of the FCT-DAAD cooperation grant 'The Dark Side of the Universe' (reference 441.00 Alemanha), with additional support from project PTDC/FIS/111725/2009 from FCT, Portugal.

The work of CM is funded by a Ciência2007 Research Contract, funded by FCT/MCTES (Portugal) and POPH/FSE (EC), and is also partially supported by grant PTDC/CTE-AST/098604/2008. NJN is supported by Deutsche Forschungsgemeinschaft (project TRR33) and is also partially supported by grants CERN/FP/109381/2009 and PTDC/FIS/102742/2008. The work of PP was partially funded by grant CAUP- 09/2009-BII.

Reference:

[1] Amendola, L., Leite, A. C. O., Martins, C. J. A. P., Nunes, N. J., Pedrosa, P. O. J. and Seganti A.(2011), *Variation of fundamental parameters and dark energy. A principal component approach*. Phys. Rev. D (submitted), arXiv:1109.6793 [astro-ph.CO].

# Scaling Properties of Domain Wall Networks

**A. M. M. Leite<sup>1,2</sup> and C. J. A. P. Martins<sup>2,3</sup>**

<sup>1</sup> Centro de Astrofísica, Universidade do Porto, Rua das Estrelas, 4150-762 Porto, Portugal

<sup>2</sup> Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal

<sup>3</sup> Department of Applied Mathematics and Theoretical Physics, Centre for Mathematical Sciences, University of Cambridge, Wilberforce Road, Cambridge CB3 0WA, United Kingdom

Phase transitions that are thought to have happened in the early Universe have a number of inevitable consequences, the most interesting of which is the formation of topological defects [1,2]. The literature on the subject has (for good reasons) focused on cosmic strings, but other defects can be of interest too. Domain walls, being the simplest defect (they can be described by a single scalar field) provide a simple test bed where one can study how several physical mechanisms influence defect evolution, and this knowledge can then be applied to other defects.

We took advantage of the continuous improvements in computing power to carry out a large set of high-resolution simulations of domain walls in two, three and four spatial dimensions, using the standard Press-Ryden-Spergel (PRS) algorithm [3]. One can also describe the broad macroscopic properties of these networks by an analytic model, in the same spirit of the model of Martins and Shellard for cosmic strings. Having carried simulations with various amounts of damping, we find strong support for the suggestion that the attractor solution for the evolution of these networks is a linear scaling solution. Our results [4] suggest that previous hints of deviations from this behavior may have been due to the limited dynamical range of those simulations. Moreover, we have used the results of the largest ( $1024^3$ ) of our simulations to provide a calibration for the velocity- dependent one-scale model for domain walls. We numerically determined the two free model parameters to have the values  $c_w = 0.5 \pm 0.2$  and  $k_w = 1.1 \pm 0.3$ .

Given the conceptual simplicity of the analytic model, we believe that the present numerical results support its validity, and suggest that it can be reliably used as a tool to study the cosmological consequences of these networks in quantitative detail. This combination of analytical and numerical techniques, leading to a detailed calibration of a model (which so far had only been carried out for cosmic strings) can in principle be extended to networks of domain walls with junctions.

References:

- [1] Kibble T. W. B. , J. Phys. A 9, 1387 (1976).
- [2] Vilenkin A. and Shellard E. P. S., Cosmic Strings and Other Topological Defects (Cambridge University Press,Cambridge, U.K., 1994).
- [3] Press W. H., Ryden B. S., and Spergel D. N., Astrophys. J. 347, 590 (1989).
- [4] Leite A. M. M. and Martins C. J. A. P. , Phys. Rev. D 84, 103523 (2011)

# Cosmic String Evolution with a Conserved Charge

**M. F. Oliveira**<sup>1,2</sup>; **A. Avgoustidis**<sup>3</sup> and **C. J. A. P. Martins**<sup>2</sup>

<sup>1</sup> Department of Physics and Astronomy, Faculty of Sciences, University of Porto

<sup>2</sup> Centre of Astrophysics, University of Porto

<sup>3</sup> School of Physics and Astronomy, University of Nottingham

Cosmic strings [1,2] are line-like topological defects that can be produced in cosmological phase transitions, and provide a valuable tool for constraining models of the early universe. Having a wide range of potential observational signals[3], which depend directly on their microphysical properties, they can be used to constrain high-energy physics parameters from cosmological observations. In particular, the magnitude of their observational signatures is mainly determined by the energy scale of the corresponding symmetry breaking transition.

Cosmic strings with degrees of freedom beyond the standard Abrikosov-Nielsen-Olesen or Nambu-Goto strings are ubiquitous in field theory as well as in models with extra dimensions, such as string theoretic brane inflation scenarios. Here we carry out an analytic study of a simplified version of one such cosmic string model. Specifically, we extend the velocity-dependent one-scale (VOS) string evolution model [4-7] to the case where there is a conserved microscopic charge on the string worldsheet. We find that whether the standard scale-invariant evolution of the network is preserved or destroyed due to the presence of the charge will crucially depend on the amount of damping and energy losses experienced by the network. We also discuss some consequences of our findings, the most important of which being that results derived in Minkowski space may not extend to the case of an expanding universe.

## References:

- [1] A. Vilenkin and E. P. S. Shellard, *Cosmic Strings and other Topological Defects* (Cambridge University Press, Cambridge, U.K., 1994).
- [2] M. Hindmarsh and T. Kibble, *Rept.Prog.Phys.* 58, 477 (1995), hep-ph/9411342
- [3] E. J. Copeland, L. Pogosian, and T. Vachaspati, *Class.Quant.Grav.* 28, 204009 (2011), 1105.0207.
- [4] C. J. A. P. Martins and E. P. S. Shellard, *Phys. Rev. D* 53, 575 (1996), hep-ph/9507335.
- [5] C. J. A. P. Martins and E. P. S. Shellard, *Phys. Rev. D* 54, 2535 (1996), hep-ph/9602271.
- [6] C. J. A. P. Martins and E. P. S. Shellard, *Phys. Rev. D* 65, 043514 (2002), hep-ph/0003298.
- [7] C. J. A. P. Martins, J. N. Moore, and E. P. S. Shellard, *Phys. Rev. Lett.* 92, 251601 (2004), hep-ph/0310255.

## Analytic Models for the Evolution of Semilocal String Networks

**A. S. Nunes<sup>1</sup>, A. Avgoustidis<sup>2</sup>, C.J.A.P. Martins<sup>1</sup> and J. Urrestilla<sup>3,4</sup>**

<sup>1</sup> Centro de Astrofísica, Universidade do Porto, Portugal.

<sup>2</sup> C.T.C., DAMPT, University of Cambridge, Cambridge, U.K.

<sup>3</sup> Department of theoretical Physics, University of Basque Country UPV-EHU, Spain.

<sup>4</sup> Department of Physics & Astronomy, University of Sussex, U. K.

The formations of topological defects networks in the early universe may have had an important role in the evolution of cosmologic structures. We revisit previously developed analytic models for defect evolution and adapt them appropriately for the study of semilocal string networks [1]. We also discuss in detail the evolution of individual semilocal segments, focusing on the phenomenology of segment growth, and also provide a preliminary comparison with existing numerical simulations.

We confirm the expectation that linear scaling is the attractor solution for a broad range of model parameters.

References:

[1] A. Nunes, A. Avgoustidis, C. Martins and J. Urrestilla, Phys.Rev. D84, 063504 (2011), 1107.2008.

# Constraints on the CMB temperature redshift dependence from SZ and distance measurements

A.M.R.V.L. Monteiro<sup>4,5</sup>, A. Avgoustidis<sup>1,2</sup>, G. Luzzi<sup>3</sup>, C.J.A.P. Martins<sup>4</sup>

<sup>1</sup> School of Physics and Astronomy, University of Nottingham, University Park, Nottingham NG7 2RD, England

<sup>2</sup> Centre for Theoretical Cosmology, DAMTP, CMS, Wilberforce Road, Cambridge CB3 0WA, England

<sup>3</sup> Laboratoire de l'Accélérateur Linéaire, Centre Scientifique d'Orsay, Bâtiment 200 – BP 34, 91898 Orsay cedex - France

<sup>4</sup> Centro de Astrofísica, Universidade do Porto, Rua das Estrelas, 4150-762 Porto, Portugal

<sup>5</sup> Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, 4169-007

The relation between redshift and the CMB temperature,  $T_{\text{CMB}}(z)=T_0(1+z)$  is a key prediction of standard cosmology, but is violated in many non-standard models. Constraining possible deviations to this law is an effective way to test the  $\Lambda$ CDM paradigm and search for hints of new physics. We present [1] state-of-the-art constraints, using both direct and indirect measurements. In particular, we point out that in models where photons can be created or destroyed, not only does the temperature-redshift relation change, but so does the distance duality relation, and these departures from the standard behaviour are related, providing us with an opportunity to improve constraints. We show that current datasets limit possible deviations of the form  $T_{\text{CMB}}(z)=T_0(1+z)^{1-\beta}$  to be  $\beta=0.004\pm 0.016$  up to a redshift  $z\sim 3$ . We also discuss how, with the next generation of space and ground-based experiments, these constraints can be improved by more than one order of magnitude.

This work was done in the context of the cooperation grant ‘Evolution and Astrophysical Consequences of Cosmic Strings and Superstrings’ (ref. B-13/10), funded by CRUP and The British Council. We also acknowledge the support of Fundação para Ciência e Tecnologia (FCT), Portugal, in the form of the grant PTDC/FIS/111725/2009. A.A. was supported by a CTC Postdoctoral Fellowship at DAMTP, University of Cambridge and in part by a Marie Curie IEF Fellowship at the University of Nottingham. The work of G.L. is funded by a CNRS Postdoctoral Fellowship at LAL, Centre Scientifique d’Orsay. The work of C.M. is funded by a Ciência2007 Research Contract, supported by FCT/MCTES (Portugal) and POPH/FSE (EC). The work of A.M.M. was partially funded by Grant No. CAUP-03/2011-BII.

References:

[1] <http://arxiv.org/abs/1112.1862>

## A stellar test of the physics of unification

**J.P.P. Vieira**<sup>1,2</sup>, **C.J.A.P. Martins**<sup>1</sup>, **M.J.P.F.G. Monteiro**<sup>1,2</sup>

<sup>1</sup> Centre of Astrophysics, University of Porto, Portugal.

<sup>2</sup> Department of Physics and Astronomy, Faculty of Sciences, University of Porto, Portugal.

It is impossible to teach, learn, or even talk about science without at least an intuitive notion of what a law of Nature is. Ever since the days of Isaac Newton, such laws have been associated with certain fundamental quantities (for example, Newton's constant,  $G$ ), which historically have been assumed to be universal constants. For that reason, we have come to refer to them as "fundamental constants". In recent years, however, such assumptions have been shaken by experimental evidence that these constants depend on an energy scale. These days, whether they vary with time as well is one of the major bones of contention among cosmologists [1,2], and efforts to address this problem have resulted in an array of theoretical models which rely on unification parameters ( $R$  and  $S$ ) whose bounds are yet unclear.

By introducing variations of the fine-structure constant ( $\alpha$ ) in the standard equations of stellar structure, it should be possible to assess how small variations of this constant must affect the structure of a star similar to our Sun, thus providing us with hints on what kinds of astrophysical tests can be carried out in order to shed some light on this issue. We have done so by computational means for the simple case of a polytropic star.

Using this approach, we have been able to estimate how key measurable parameters of a star's structure should differ from our own Sun's due to small differences in the value of  $\alpha$ . Comparison between these estimations and the current experimental uncertainty in the value of those stellar parameters has enabled us to establish bounds on variations of  $\alpha$  and the possible values of  $R$  and  $S$ .

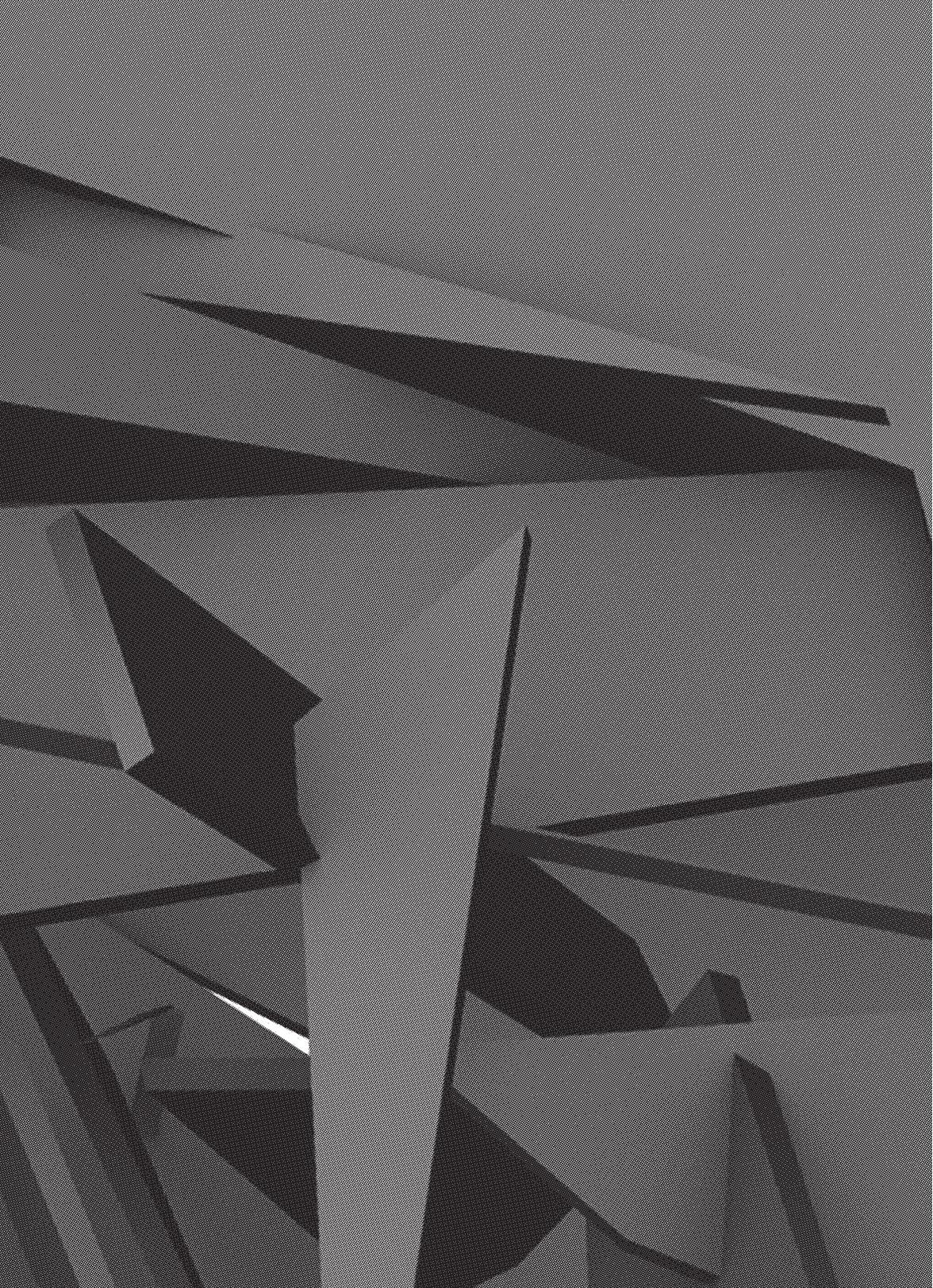
This means stellar tests of modern fundamental physics are a real possibility. Stars do have a word to say on the matter of the variation of fundamental constants, and it is our job to hear it out.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT, Portugal. The work of JPV was partially funded by grant CAUP-02/2011-BII. The work of CJM is supported by Ciência2007 Research Contract, funded by FCT/MCTES (Portugal) and POPH/FSE (EC). CJM and MJM are also partially supported by grant PTDC/CTE-AST/098604/2008.

References:

[1] M. T. Murphy et al., Lect. Notes Phys. 648, 131 (2004), astro-ph/0310318.

[2] R. Srikanand, H. Chand, P. Petitjean, and B. Aracil, Phys. Rev. Lett. 99, 239002 (2007).



# Oral Sessions VI



Oral Sessions VI > A1

# Molecular Medicine I



## CHARACTERIZATION OF THE QUERCETIN AND THE EPIGALLOCATECHIN GALLATE INHIBITORY EFFECTS UPON GLUCOSE UPTAKE BY BREAST CANCER CELLS

Isabel Araújo<sup>1</sup>, Tito Costa<sup>1</sup>, Liliana Moreira<sup>1</sup>, Ana Correia-Branco<sup>1</sup>, Ana Faria<sup>1,2,3</sup>,  
Fátima Martel<sup>1</sup> and Elisa Keating<sup>1</sup>

<sup>1</sup>Department of Biochemistry (U38-FCT), Faculty of Medicine; <sup>2</sup>Chemistry Investigation Centre (CIQ), Faculty of Sciences; <sup>3</sup>Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal

Quercetin (QUE) and epigallocatechin gallate (EGCG) were previously shown by our group to impair glucose uptake and metabolism by the MCF7 breast cancer cell line compromising both cell proliferative capacity and survival (unpublished data). So, the aim of the present work was to characterize the inhibitory effect of QUE and EGCG upon <sup>3</sup>H-DG uptake by MCF7 cells.

Since both QUE and EGCG are known to interact with estrogen receptors [1, 2] we tested whether their inhibitory effect upon glucose uptake were mediated by estrogen signaling. To do this we studied the effects of QUE and EGCG upon <sup>3</sup>H-2-deoxyglucose (DG) uptake by the estrogen receptor (ER)-negative MDA-MB-231 breast cancer cell line, and compared the findings to those previously obtained by our group in the ER-positive MCF7 cell line. We also searched for the involvement of PKA, PKC or calcium signaling pathways in the inhibitory effect of QUE and EGCG upon <sup>3</sup>H-DG uptake by MCF7 cells.

<sup>3</sup>H-DG uptake by MDA-MB-231 cells was time-dependent and saturable with transport kinetics similar to those observed in MCF7 cells. Also, very similarly to what had been observed in MCF7 cells, <sup>3</sup>H-DG uptake by MDA-MB-231 cells was: a) inhibited by cytochalasin B (10 and 50 µM) and by phloridzin (500 and 1000 µM); b) sodium-independent; c) slightly stimulated by insulin and d) potently and concentration-dependently inhibited by 26 min or 4 h exposure to QUE or EGCG. Moreover, we observed that the 26 min-inhibitory effect of QUE upon <sup>3</sup>H-DG uptake by MCF7 cells was completely abolished by the presence of H7 (10 µM, PKA and PKC inhibitor), H89 (10 µM, PKA inhibitor), and calmidazolium (50 µM; calcium/calmodulin inhibitor) and it was not affected by chelerythrine (0.1 µM; PKC inhibitor). On the other hand, the 26 min-inhibitory effect of EGCG upon <sup>3</sup>H-DG uptake by MCF7 cells was abolished in the presence of calmidazolium but was unaffected in the presence of H7, H89 or chelerythrine.

In conclusion, <sup>3</sup>H-DG uptake by ER-negative MDA-MB-231 cells is mediated by GLUTs (most probably by GLUT1 and/or GLUT4/12) not by SGLT1, and is very similar to that observed in MCF7 cells, suggesting that <sup>3</sup>H-DG uptake by breast cancer cells is itself estrogen-independent. Characterization of QUE and EGCG inhibitory effect upon <sup>3</sup>H-DG transport in MCF7 cells indicate that it does not seem to be strictly dependent on estrogen signaling, involving instead calcium signaling. Additionally, QUE-inhibitory effect was shown to be dependent on PKA activation.

[1] Farabegoli F et al. (2007) *Cancer Detect Prev* **31**(6):499-504.

[2] Moutsatsou P (2007) *Hormones (Athens)* **6**(3):173-93.

Supported by PTDC/SAU-OSM/102239/2008, SFRH/BPD/40170/2007 and IJUP/Santander/190/2010.

# Isoxanthohumol inhibits *in vivo* vascular proliferation and stabilization

**Duarte D., Costa R, Soares R. and Negrão R.**

Department of Biochemistry (U38/FCT), Faculty of Medicine, University of Porto, Portugal.

Angiogenesis and inflammation are two fundamental biological processes that act jointly towards the successful resolution of many situations, such as wound healing. In other circumstances though, they interact and contribute to the evolution of pathological conditions, like cancer, diabetes or obesity. Recently, our group described isoxanthohumol (IXN), a flavonoid of beer and the main xanthohumol metabolite, as an interesting angiostatic and anti-inflammatory molecule, using *in vitro* studies, the mouse matrigel plug and the rat wound healing assays [1]. The aim of this study was to evaluate IXN effects on specific *in vivo* angiogenic steps, on different time-points.

We used the neonatal retinal neovascularization model, where the vessels spread in a reproducible temporal and spatial pattern and can be easily imaged to study the interactions between endothelial cells (EC), mural cells and microglia, allowing a detailed visualization IXN action on different phases of angiogenesis [2].

Briefly, C57BL/6 mice pups were injected intraperitoneally with IXN or control (PBS), daily, until post-natal day (P)3, P4, P6 or P14. Then, pups were euthanised and eyes enucleated, fixed in 4% p-formaldehyde overnight, and retinas were dissected out and stained with FITC-conjugated isolectin,  $\alpha$ SMA, VEGFR-2 or PDGFR $\beta$ . Fluorescence and ApoTome microscopy was used and vascular quantification measurements performed.

By P5 and P7 time-points, IXN significantly decreased vascular proliferation and coverage by  $\alpha$ SMA-positive mural cells (i.e. pericytes and smooth muscle cells). These effects were not so evident at P4 and P15, which may be explained by the need of an initial accumulation of IXN and a latter pro-angiogenic compensatory effect due to anti-oxidant properties of the compound. At P5, vascular diameter, EC survival, arteriolar/venular differentiation and VEGFR-2 patterning were also significantly affected by the treatment. Furthermore, IXN led to a non-significant decrease on migration of EC (tip cells and filopodia) and an apparent absence of bridge cells (i.e. microglia) on the vascular fusion sites.

Further studies on the molecular targets and signaling pathways involved and a deep analysis on the uptake, bioavailability and toxicity of IXN are crucial for a better translational understanding of the molecule. So far, the accumulated evidence proposes IXN as an interesting anti-angiogenic compound that might have therapeutic and preventive applications in angiogenesis- and inflammation-associated diseases.

Supported by SFRH/BD/41888/2007 from FCT and projects PTDC/SAU-OSM/102083/2008 e PESt-OE/SAU/UI0038/2011.

[1] Negrão, R. et al (2010), *Angiogenesis and inflammation signaling are targets of beer polyphenols on vascular cells*, J Cell Biochem 111:1270-9.

[2] Gerhardt, H. et al. (2003). *VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia*. J Cell Biol 161, 1163-1177.

## Insight on transcriptional network that controls Prrxl1 expression in nociceptive neurons

**D. Martins, I. Regadas, M. Matos, F. Monteiro, D. Lima and C. Reguenga**

Department of Experimental Biology, Faculty of Medicine, University of Oporto, Portugal  
IBMC – Instituto de Biologia Molecular e Celular, University of Oporto, Portugal

Pain is a multi-sensory experience associated with potential tissue damage. Its perception depends on signaling the presence of noxious stimuli at the periphery through specialized small diameter neurons of the dorsal root ganglion (DRG) that synapse extensively with marginal zone projecting neurons and substantia gelatinosa interneurons in the spinal cord dorsal horn.

The paired-homeodomain transcription factor Prrxl1 (also known as Drg11) has a crucial role in the establishment of synaptic connections between DRG and spinal nociceptive neurons [1]. Nevertheless, our understanding of the transcriptional mechanisms that control the proper spatiotemporal expression of the Prrxl1 gene remains limited. Recent studies appointed Tlx3 and Lmx1b, other homeodomain transcription factors that highly co-localizes with Prrxl1, as fulcral upstream factors of Prrxl1 transcription regulation cascade, however little is known about the exact procedural dynamic of this transcriptional network [2,3].

In order to understand how the expression of Prrxl1 is regulated by Tlx3 and Lmx1b, the entire Prrxl1 promoter sequence was cloned in a luciferase-reporter vector and its activity was measured after overexpression of Prrxl1, Tlx3 and Lmx1b, alone or in combination with Prrxl1, in the DRG-derived cell line ND7/23 and in non-neuronal HeLa cells. Prrxl1 overexpression, *per se*, induces a slight decrease in the luciferase activity. Overexpression of Lmx1b led to a small induction (about 2 fold) of luciferase activity either in the ND7/23 or in non-neuronal HeLa cells. Overexpression of Tlx3 led to a 22-fold induction while Prrxl1 and Tlx3 simultaneous overexpression resulted in a 42-fold luciferase induction. On the other hand, Prrxl1 silencing reduced in three times the inductive effect produced by Tlx3 overexpression which pointed to a coordinated action of these transcription factors in the regulation of the Prrxl1 gene, much likely by the formation of heterocomplexes. This hypothesis was tested by co-immunoprecipitation assays, and an interaction between Prrxl1 and Tlx3 was detected. Prrxl1-Lmx1b complexes seem not to occur in ND7/23 cells.

Altogether these results ascribe an important role for Tlx3 in controlling Prrxl1 transcription, directly or in a molecular complex with Prrxl1 itself implying an autoregulation mechanism.

### References:

- [1] Zhou-Feng, C; Rebelo, S (2001); The Paired Homeodomain Protein DRG11 Is Required for the Projection of Cutaneous Sensory Afferent Fibers to the Dorsal Spinal Cord; *Neuron*, 31, 59-73;
- [2] Ding, Y; Jun, Y (2004); Lmx1b controls the differentiation and migration of the superficial dorsal horn neurons of the spinal cord; *Development*, 131, 3693-3703;
- [3] Xu, Y; Lopes, C (2008); Tlx1 and Tlx3 coordinate specification of dorsal horn pain modulatory peptidergic neurons; *Journal of Neuroscience*, 28(15): 4037–4046;

# Canine Osteoarthritis and Stem Cell Therapy: A Case Study

**M. M. Afonso<sup>1</sup> L. Gardel<sup>2</sup>**

<sup>1</sup> UP VET-Department of Veterinary Clinics and Cytogenetic Laboratory-Department of Microbiology, ICBAS – University of Porto; Biomechanics Laboratory, FADEUP - University of Porto.

<sup>2</sup> Assistant Professor of Surgery and Anaesthesiology- Department of Veterinary Clinics ICBAS - University of Porto; 3B's Research Group- Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine.

Osteoarthritis (OA) is a slowly progressive pathology in which degeneration of cartilage with associated osteophyte production occurs and it is usually caused by abnormal cartilage wear. [1] OA affects up to 20% of the world canine population [2,3,4] and the pain and lameness associated with the disease greatly affect the life quality of these animals. The fact that this is a chronic condition and that the conventional pharmacological therapies have adverse systemic effects associated with long-term administration of drugs is one of the reasons that researchers have been so interested in developing therapeutic alternatives that do not present these effects. [4,5,6] Stem Cell Therapy with subcutaneous adipose tissue cells has gained a lot of popularity and has revealed itself to be an extremely beneficial alternative to control clinical signs exhibited by affected dogs and has been proven to have an effect which is prolonged in time. [4,7] This therapy consists in the obtention of adipose tissue samples from the patient and on the posterior stem cell isolation, expansion with or without differentiation and application in the same animal (autologous therapy).

In this case-study, a large breed dog diagnosed with OA was treated with cellular therapy. An amount of  $1 \times 10^6$  stem cells originated from the adipose tissue were differentiated in chondroblasts and were applied percutaneously in the affected articulation. The clinical evolution was documented with owner questionnaires over the time, imagiology techniques (X-Rays) and gait analysis (kinematic computerized analysis). It was concluded that, for this animal, Stem Cell Therapy is an interesting alternative to long-term conventional treatments and that the life quality of this animal significantly improved after treatment.

## References:

[1] Piermattei, D.L., Flo G.L., DeCamp C.E. (2006), *Arthrology* in Piermattei, D.L., Flo G.L., DeCamp C.E., "Handbook of Small Animal Orthopedics and Fracture Repair", 4<sup>th</sup> edition, Saunders Elsevier, St Louis, pp. 185-215; [2] Roudebush, P. (2006), *Pain Management for Canine Osteoarthritis* in Proceedings of the North American Veterinary Conference, Orlando, January 7 – 11 2006, 6, pp. 1007-1010; [3] Fox, S.M. and Millis, D. (2010), *Osteoarthritis: The Disease* in Fox, S.M. and Millis, D., "Multimodal Management of Canine Osteoarthritis", Manson Publishing, London, pp. 24-29; [4] Guercio, A., Di Marco, P., Casella, S., Cannella, V., Rossotto, L., Purpari, G., Di Bella, S., Piccione, G. (2011), *Production of Canine Mesenchymal Stem Cells from Adipose Tissue and Application in Dogs with Chronic Osteoarthritis of the Humeroradial Joints*, Cell Biology International, 36 (2), 189-194; [5] Rychel, J.K. (2010), *Diagnosis and Treatment of Canine Osteoarthritis*, Topics in Companion Animal Medicine, 25 (1), 20-25; [6] Scott, H.: *Current Medical Therapies for Canine and Feline Osteoarthritis*, Veterinary Focus, 17 (3), 18-23; [7] Black, L., Gaynor, J., Gahring, D., Adams, C., Aron, D., Harman, S., Gingerich, D.A., Harman, R., *Effects of Adipose-derived Stem and Regenerative Cells in Dogs with Chronic Osteoarthritis of the Coxofemoral Joints: A Randomized, Double-blinded, Multicenter Controlled Trial*, Veterinary Therapeutics, 8 (4), 272-284

## Effects of glucosamine and chondroitin sulphate on human osteoclastogenesis

**K.A. Moniz<sup>1,2</sup>, M.H. Fernandes<sup>1</sup>, J. Costa-Rodrigues<sup>1</sup>**

<sup>1</sup> Laboratório de Farmacologia e Biocompatibilidade Celular, Faculdade de Medicina Dentária, Universidade do Porto, Portugal.

<sup>2</sup> Faculdade de Farmácia, Universidade do Porto, Portugal.

Glucosamine is a glucose derivative that is important for the synthesis of many glycosylated molecules, including the glycosaminoglycans present in cartilage. Due to this, it is used for the treatment of diseases such as osteoarthritis, where it induces the regeneration of damaged cartilage. Chondroitin sulphate is a glycosaminoglycan that is one of the major components of cartilage, contributing to its structure, function, lubrication and nutrition. Like glucosamine, chondroitin sulphate can be used as a therapeutic agent, promoting growth and regeneration of cartilage tissue. Since cartilage and bone tissues display some similarities, glucosamine and chondroitin sulphate might display as well some effects on bone tissue. However, this issue is poorly documented and the few published reports present some contradictory results. Furthermore, the studies were conducted mainly with osteoblastic cells, and almost no information is available regarding the effects of glucosamine and chondroitin sulphate on osteoclast development. The aim of this work was to characterize the cellular and molecular effects of these molecules, when used either isolated or combined, on human osteoclastogenesis.

Osteoclastic precursors were isolated from human peripheral blood. Cell cultures were treated with different concentrations (20, 100 and 500 µg/mL) of isolated or combined glucosamine and chondroitin sulphate. Cell cultures were characterized throughout a 21 day period for tartrate-resistant acid phosphatase (TRAP) activity, number of TRAP+ multinucleated cells, presence of cells with actin rings and expressing vitronectin and calcitonin receptors. Regarding the intracellular mechanisms involved in the osteoclastic cell response, cell cultures were treated with MEK, NFκB, PKC, MAPK, JNK and p38 signaling pathways inhibitors, and a PGE2 production blocker. In addition, the possibility of these molecules to induce apoptosis was also addressed.

Results showed that glucosamine stimulates osteoclast development, while an opposite effect was found for chondroitin sulphate-treated cell cultures. In the presence of both molecules, an increase on osteoclastogenesis was observed. The characterization of the intracellular processes involved revealed differences on the contribution of the analyzed signaling pathways, as well as on the apoptosis rate, in the different tested conditions.

Taken together, glucosamine and chondroitin sulphate have the ability to modulate human osteoclastogenesis. A detailed characterization of the involved mechanisms can open new perspectives in the utilization of such compounds in clinical contexts, particularly in diseases with involvement of the bone tissue.

## RKIP and CD147 expression as prognostic factors in squamous cell lip carcinoma

**A. C. B. Fonseca**<sup>1</sup>, **T. Capela**<sup>1</sup>, **C. Costa**<sup>2</sup>, **R. Reis**<sup>3</sup>, **A. Longatto-Filho**<sup>3</sup>, **L. Santos**<sup>1,2,4</sup>

<sup>1</sup> Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto.

<sup>2</sup> Fernando Pessoa University (UFP), Health Sciences Faculty.

<sup>3</sup> Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus of Gualtar.

<sup>4</sup> Francisco Gentil Portuguese Institute of Oncology, Porto.

Lip cancer is the second most frequent cancer between head and neck neoplasias and since it mainly is a visible lesion, its diagnostic is usually made earlier and due to that fact the patient's prognosis is generally good. However, the presence of lymphatic, vascular and perineural invasion substantially decrease the patient survival<sup>[1]</sup>. RKIP and CD147 are two proteins that have a role in the invasion and metastization processes<sup>[2,3]</sup>. RKIP expression is related with the decrease in tumor growth and proliferation and is higher in normal tissues than in tumors or metastasis<sup>[2]</sup>. Otherwise, CD147 is a protein that stimulates the basal membrane degradation and the development of a more aggressive phenotype for the tumors. It expression is higher in tumors than in normal tissues. The immunoexpression of these two proteins is related with patients' prognosis in different carcinomas<sup>[3]</sup>.

The main goals of the present work were the evaluation of RKIP and CD147 immunoexpression in lip squamous cell carcinoma of patients admitted and treated in IPO-Porto and also the comparison of proteins expression in different histological sites - normal tissue, tumor, invasion front and perineural invasion. Additionally, we related these expressions with the clinicopathological features and the prognostic of each patient. For the immunoexpression evaluation the immunohistochemistry technique was used and the results were analyzed by three independent and specialized persons.

It was possible to notice that RKIP expression either in tumor or in invasion front is related with the stage of the patients' tumor and it decreases in the site where the metastization starts compared with the tumors and normal tissue. On the other hand, in our study, CD147 expression is not related with any clinicalpathological feature of the patients although tumors express higher levels of CD147 than normal tissue. As observed for RKIP, the expression of CD147 is not related with the patients' prognosis.

### References:

[1] Casino, Á. R., Toledano, I. P., Jorge, J. F., Díez, J. M. O., Llobet, C. C., Prat, M. A. (2006), *Brachytherapy in lip cancer*, Braquiterapia en el cáncer de lábio, 11(3): 137-143.

[2] Zeng, L., Imamoto, A., Rosner, M. R. (2008), *Raf kinase inhibitory protein (RKIP): A physiological regulator and future therapeutic target*, Expert Opinion on Therapeutic Targets, 12(10): 1275-1287.

[3] Foda, H. D., Zucker S. (2001), *Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis*, Drug Discovery Today, 6(9): 478-482.

Oral Sessions VI > A2

# Legal Studies & Criminology I



# How do they defend themselves? Retailers perspective about shoplifting in two different contexts

Óscar Ramos<sup>1</sup> & Carla Cardoso<sup>1</sup>

<sup>1</sup> Department of Criminology, Faculty of Law, University of Porto, Portugal.

Currently, shoplifting is considered one of the biggest adversities in the retail sector and one of the major causes of loss and investment. According to the Global Retail Theft Barometer, total losses in Europe during 2011 were €36.281 millions, caused by shoplifters (45.7%), employee theft (30.2%), dishonest suppliers (6%) and internal errors (16.1%) [1].

This type of crime emerges from the interaction between the individual with a certain potential for offending, and the environment or situation which provides opportunities for offending [2]. According to the literature, these crimes have different temporal and spatial distributions and may vary depending on various factors, like store environment, type of products and implemented security systems [3]. *So, how can we prevent these crimes?* The problem has been addressed by situational crime prevention strategies that can include techniques such as CCTV, electronic alarm tags, security guards, sales staff attention, good lighting, reduction of blind spots and other design principles. Thus, we should look for an integrated solution between the traditional security methods and the current solutions that use design and store layout modifications to reduce the opportunities for crime [4].

The present work aims to compare the extension and impact of shoplifting, the use and perceived effectiveness of prevention measures, and the most common techniques used to commit theft in two different retail environments: the traditional town center and the shopping mall. To achieve that purpose, a self-report survey was performed to a sample of 108 store managers, addressing the problem in the Portuguese context. Preliminary results will be presented, comparing the differences and similarities of the variables referred above in these two different realities (town center and shopping mall).

Furthermore, this empirical research could provide key elements to develop and implement new solutions and more customized preventive strategies, taking into account the store environment.

## References:

- [1] Center for Retail Research (2011). *The Global Retail Theft Barometer*. England.
- [2] Farrington, D. P., Bowen, S., Buckle, A., Burns-Howell, T., Burrows, J., and Speed, M. (1993). An experiment on the prevention of shoplifting. In R. V. Clarke (Ed.), *Crime Prevention Studies* (pp. 93-119). Monsey, N.Y: Criminal Justice Press.
- [3] Nelson, A. L., Bromley, R. D. F. and Thomas, C. J. (1996). The geography of shoplifting in a British city: Evidence from Cardiff. *Geoforum*, 27(3), 409-423.
- [4] Carmel-Gilfilen, C. (2011). Advancing retail security design: Uncovering shoplifter perceptions of the physical environment. *Journal of Interior Design*, 36(2), 21-38.

## Self control or morality: what matters in antisocial behavior?

Carla Cardoso<sup>1</sup>, Josefina Castro<sup>2</sup>, Teresa Sousa<sup>3</sup>

<sup>1</sup>Department of Criminology, Faculty of Law, University of Porto, Portugal.

<sup>2</sup>Department of Criminology, Faculty of Law, University of Porto, Portugal.

<sup>3</sup>Department of Criminology, Faculty of Law, University of Porto, Portugal.

Self-control and morality are two important concepts in the study of crime and deviant behavior.

Self-control is an individual ability to control one's behavior in order to obtain some reward or avoid some punishment. Self-control is, to Gottfredson and Hirschi, the main factor behind criminal and disruptive behavior [1] and one of the most concepts ever tested in social sciences, especially in Criminology.

In turn, morality is a set of rules about what is wrong or right in particular circumstances. To Wikström morality is the prime factor in explaining misconducts [2].

For a long time, these two concepts were studied as two different and independent ideas. However, in the recent years, it has been a growing consensus about the importance of their integration: which of both matters in the explanation of deviant behavior? Both? Wikström and Antonaccio [3] are two examples of authors that are studying these possible relations between self-control, morality and misbehavior.

In this sense, the aim of this study is to contribute to understand the cross talk between self control, morality and deviant behavior.

To test these relationships, we used data from an anonymous self-reported survey in a sample of adolescents based in self-control and morality measures already tested (by Grasmick [4] and Wikström [2], respectively) and measures of deviant behavior.

Preliminary results will be presented and discussed in the light of General Theory of Crime [1] and Situational Action Theory [2].

[1]Gottfredson, M. R. & Hirschi, T. (1990). *A General Theory of Crime*. Stanford University Press. University Press.

[2]Wikström, P. H. & Svensson, R. (2010). When does self-control matter? The interaction between morality and self-control in crime causation. *European Journal of Criminology*, 7(5), 395-410.

[3]Antonaccio, O. & Tittle, C. R. (2008). Morality, self-control, and crime. *Criminology*, 46(2), 479-510.

[4] Grasmick, H., Tittle, C., Bursik, R. and Arneklev, B. (1993). Testing the core empirical implications of Gottfredson and Hirschi's general theory of crime. *Journal of Research in Crime and Delinquency* 30, 5–29.

## The protection model of juvenile delinquency in Portugal: A study from a justice facility

Ana Faria<sup>1</sup> & Josefina Castro<sup>2</sup>

<sup>1</sup> Master's student in Criminology, School of Criminology, Faculty of Law, University of Porto

<sup>2</sup> Assistant Professor, Master's Supervisor, School of Criminology, Faculty of Law, University of Porto.

The juvenile justice system in Portugal was begun in 1911 with the publication of the *Protection Law of Childhood*. His main feature of protectionism was successively accentuated with the subsequent juvenile justice reforms. The Decree-Law N. 314/78 (October 27, 1978) is seen as a maximalist example of the protection model in Europe. The tutelary measures (“protection, assistance and education measures”) were intended for minors in danger and juvenile offenders alike. All the problematic behaviors were considered as *symptoms* of maladjustment and this criterion dictated the nature and the (indeterminate) length of the measures applied. The measures were applied indistinctively in cases of child in danger or delinquent child and there were not means of defense or procedural guarantees for the youth [1].

The aim of the present study is to analyze how this legal model was enforced, focusing on the concrete practices and strategies of assessment and intervention implemented in justice facilities for minors and their underlying assumptions. Moreover, this study aspires to establish the relationship between those practices and the life trajectories of the youngsters under these measures. For that purpose, documental analysis of individual files was used. The preliminary results are presented and discussed in order to provide some key ideas about how law and institutional practices interact.

[1] Castro, J. (2010). “The punitive turn” – are there any points of resistance? An answer from the Portuguese experience. In F. Bailleau & Y. Cartuyvels (eds.). *The criminalization of youth: juvenile justice in Europe, Turkey and Canada*. Brussels: VUBPRESS

# Importance of Shame and Guilt in Moral Behavior

## A Systematic Review

Cláudia Isabel Costa<sup>1</sup>, Josefina Castro<sup>2</sup>

<sup>1</sup> Department of Criminology, Faculty of Law, University of Porto, Portugal.

<sup>2</sup> Department of Criminology, Faculty of Law, University of Porto, Portugal

This presentation aims explore the relevant research that has been contributed to the study of the role of moral emotions – *shame* and *guilt* - on the moral behavior. Tangney et al (2007) and Eisenberg (2000) refers that emotions like *guilt* and *shame* were important in the linkage between our moral standards and moral behaviour. When people consider behavioural alternatives they can anticipate emotional reactions and that can have a strong influence in the option chosen. By the way the central question is: What's the influence of *shame* and *guilt* on the prosocial and antisocial behavior?

Empirical studies of diverse samples clearly indicate that guilt-proneness is inversely related to antisocial and risky behaviour (Tangney et al, 2007). Tangney & Dearing 2002 (in Tangney et al, 2007) showed that children prone to shame-free guilt in the fifth grade were, in adolescence, less likely to be arrested, convicted, and incarcerated and Tibbetts (2003 in Tangney et al, 2007) found that college students' guilt-proneness was inversely related to self-reported criminal activity.

The definition of these moral emotions, how they could be measured and what research shows about their importance in antisocial and prosocial behavior will be presented. Finally the importance of the emotional side of moral development and the implications of it in intervention programs will be discussed. Most of the common moral interventions in childhood have focused on promoting children's cognitive development. It's necessary that children understand the emotional consequences of their own acts introduced a wide range of moral emotions, such as, guilt and shame, into educational practice in a systematic way (Malti & Latzko 2010).

### References:

[1] Tangney, J. P., Stuewig J., and Mashek D. J. (2007). Moral Emotions and Moral Behavior. *Annual Review of Psychology*. 8:345-372

[2] Eisenberg, N. (2000). Emotion, Regulation and Moral Development. *Annual Review of Psychology*. 51:665–697

[3] Malti, T., & Latzko, B. (2010). Children's moral emotions and moral cognition: Towards an integrative perspective. In B. Latzko & T. Malti (Eds.), *Children's moral emotions and moral cognition: Developmental and educational perspectives. New Directions for Child and Adolescent Development*, 129, 1–10. San Francisco: Jossey-Bass.

# Characterizing male intimate partner offenders

N. Azevedo<sup>1</sup>,

<sup>1</sup> School of Criminology, Faculty of Law, University of Porto, Portugal.

Domestic violence (DV) carries heavy social, economic and personal costs. Therefore, it is important to know better what kind of violent offenders in intimate partner relationships we have. In first place we define relevant concepts for the issue under research, after which we review the risk factors for the perpetration of intimate partner violence, being important to emphasize the contributions of Cattaneo e Goodman [1]; Riggs, Caulfield e Street [2]; Stith, Smith, Penn, Ward e Tritt [3]. In the second part of the paper, we present some empirical evidence, especially taking into account variables that literature refer as the most relevant in the issue.

**Sample:** We use a sample of 100 men condemned for DV crime and followed by the legal system (*Direcção Geral de Reinserção Social*), in North of Portugal, in the period 2009-2010. Some of those were integrated in a Batterer Intervention Program (BIP).

**Methodology:** Data was collected using documental sources (eg: social reports, criminal record, documents sent by the court, interview guide for DV cases, Spousal Assault Risk Assessment instrument and other documents that constitute the individual process).

It was used SPSS v.19 (Statistical Package for the Social Sciences) to analyze collected data and to describe the sample (mainly with descriptive statistics).

**Main results:** The average age is 46 years (SD= 11,744). Most of the offenders' family of origin has 3 to 6 members and low economic resources. About half the individuals reported having witnessed or been victims of violence in the family of origin.

Most cases have, only, the primary school (45%) and low economic resources. The alcohol abuse was mentioned in 73% of cases and, frequently, it is associated to other problems (psychological, employment, behavior, health, etc.). There are few cases of frequency of problem support/treatment. Most offenders have no criminal record (59%).

The presence of minors in the couple's discussions is frequent. Physical violence, insults and threats are the most common DV behaviors, sometimes using weapons. Most of offenders are violent only in the family context and in more than half the cases there have been at least one disruption in the relationship.

These findings have being the basis of my current research, related to variables or risk factors that can be "predictors" of intimate partner violence. More scientific research is need, to inform the public policies and achieve more accurate political decisions. BIP's should be well informed by the scientific studies and by the evidence based-practice.

References:

- [1] Cattaneo, L., & Goodman, L. (Abril de 2005). "Risk Factors for Reabuse in Intimate Partner violence: A Cross-Disciplinary Critical Review". *Trauma, Violence & Abuse*, pp. 141-175.
- [2] Riggs, D., Caulfield, M., & Street, A. (2000). "Risk for domestic violence: factors associated with perpetration and victimization". *Journal of clinical psychology*, 56(10), pp. 1289-316.
- [3] Stith, S., Smith, D., Penn, C., Ward, D. & Tritt, D. (2004). "Intimate partner physical abuse perpetration and victimization risk factors: A meta-analytic review". *Aggression and Violent Behavior*, 10(1), pp. 65-98.



Oral Sessions VI > A3

# History & Cultural Studies I



## **Data base for ethnological heritage**

**Pedro Dâmaso e Rui Teixeira**

Department of Heritage Studies (DCTP), Faculty of Arts, University of Porto, Portugal

The ethnological heritage – buildings, objects and oral tradition – has not been a frequent object of study in scientific and academic investigations. The national authorities have, so far, shown no interest for systematic register and organization in a data base with both broader and detailed criteria.

Given the former and considering that only local authorities have done some work in maintaining and registering the heritage, frequently lacking adequate scientific accuracy, the creation of an accurate and thorough inventory would be therefore very important.

The idea first came in to our minds when we started our own inventory of the traditional systems of grinding in the basins of rivers Leça and Pele for our master studies in Archeology. Since we had no knowledge of common method of inventory and registration of these heritage, we developed a set of procedures based on the studies of TIMS (The International Molinological Society), of IMC (Instituto dos Museus e da Conservação) as well as in the Works of Ernesto Veiga de Oliveira, Fernando Galhano, Benjamim Pereira and Jorge Dias.

We then developed a set of four forms that allow any user to examine/characterize buildings both generally (the building structure) and in detail (precise description of mills) including also data of the analyses of objects found in the site, and oral testimonies of locals.

These forms were in the foundation of our project of developing a date base for the mentioned heritage, which is being done with the collaboration of MAPoteca of the Faculty of Arts of University of Porto, namely with Miguel Nogueira. This data base will be read by geographical information systems and will allow investigators, not only to access data from our studies, but also to use it as a tool to register their owns investigations, thus building a unique and detailed data base on this subject with scientific quality.

Both data base and inventory code were thought not only to include water mills, but also to be applied to every category of ethnological heritage. The general code of the inventory was design with the goal to register all projects of investigation on ethnology both in Portugal and Europe, supplying an easy accessed tool, very intuitive and able to respond to every need of register of past and future investigations/inventories on this scientific area of studies.

In short with this data base it is already possible to: identify geographically the ethnological heritage; search the data base selecting specific characteristics; and in future, investigators will be able to identify ideal sites to develop projects of preservations and tourism enterprises; develop comparative studies at national and international levels and gather in one place all information on ethnological heritage.

## Representations of the East in *O Mundo Português* (1934-1947)

M. Couto

Faculty of Letters, University of Porto, Portugal.  
Master student of contemporary history between 2009 and 2011.

The work developed has focused, such as the title suggests, in the study of the oriental representations present in the magazine of colonial propaganda of the Estado Novo, published between 1934 and 1947, *O Mundo Português*.

Through the discourse analysis we seek to demonstrate how the Orientalism was a very useful “instrument” for the regimen, by the way he has “built” the East to legitimize an identity and an imperial destiny, which will have direct impact not in Asia but in Africa. So, a racist and prejudiced ideology was produced in order to demonstrate the civilizational and cultural superiority of Portugal contrasting the habitants of their colonies.

Thus, we developed and deepened the analysis of the multiple “realities” that the East acquires, as well as of all the discursive ambivalences that the oriental was subject in the magazine *O Mundo Português*. In fact, it is intended to show how is possible to resize, in the discursive and imagetic plan, history, cultures, identities and individuals. Is the Orientalism produced by the Estado Novo that constructs and reconstructs its East in the most convenient way for the regimen, varying from an observation that sees the influence and indelible presence of the Portuguese in the East, as it sees an East full of exotism and alterity. For the oriental the situation is the same. As much is practically “Portuguese”, result of the assimilation process that provides a raising of the cultural and civilizational standard that Portugal granted to it, as is a “primitive” that makes the most varied aberrations, fact which demonstrates its need of help from Portugal to leave the barbarity in which is installed.

## Making necklaces, weaving a network: indigenous women in Manaus

Ana Luísa Sertã<sup>1</sup>

<sup>1</sup> Department of Anthropology, Faculty of Philosophy, Letters and Human Sciences, University of São Paulo, Brazil.

The topic of this research is the indigenous presence in urban centers of Brazil, from the point of view of sociality, exploring craftwork as a central element of the relationships experienced by indigenous in the city of Manaus and outside it. During a two year fieldwork, the research passed from a focus on three indigenous women associations of different ethnic groups to a central interest on the Association of Sateré-Mawé Indigenous Women (AMISM). The research takes part on the group of works developed at the GEU/NAU – USP – Urban Ethnology Group of the Urban Anthropology Core from the University of São Paulo.

An analysis “from close by and inside” (Magnani, 2002), developed by the method of participant observation, was performed in the association and other spaces frequented by its members, as an attempt to accompany the social actors’ arrangements within the urban context, having as a reference the day-to-day dynamics. The production of data resulted also from semi-structured interviews with key-informants from the association.

The Association of Sateré-Mawé Indigenous Women offered access to an overview of the spatial occupation of the indigenous people in the capital city of Manaus, contributing to a comprehension of the new ethnic configurations in Manaus and its consequences on the arrangements of the urban setup. In this scenario, craftwork revealed itself as a key element for the maintenance of the association and the networks that its members develop between their indigenous homelands and the trails that they build within the urban space, just as it shows continuities and actualizations of “ways to do”. The Sateré-Mawé study case showed that the craftwork seems to triggers an expanded network that involves relatives in the city and at the indigenous homeland, indigenous people from other ethnic groups, NGO’s, businessmen, government representatives and also a fundamental non-human component, the seeds. A particular circuit of people and seeds goes beyond Manaus and includes the coming and going to and from the indigenous homeland, just as the recollection of seeds in the city, the participation in fairs and events, the meeting at friends’ houses or at the association and indigenous communities that were developed in the city. One of the aims of this research is to identify this circuit, a category developed by Prof. J. Magnani, and to purpose an analysis of the continuities and actualizations concerning the usage of seeds and craftwork techniques from the point of view offered by the concept “skills” as in the terms of T. Ingold, which takes knowledge as a group of inherited skills, modes to do, developed and taught in specific environmental contexts, and built from the practical and personal experience, in a mix of imitation and improvisation.

References:

[1] MAGNANI, J. G. C.. (2002), De perto e de dentro: notas para uma etnografia urbana. Revista Brasileira de Ciências Sociais, ANPOCS, vol 17 No 49.

[2] INGOLD, T. (2000). *Building, dwelling, living: How animals and people make themselves at home in the world. Essays on livelihood, dwelling and skill*. London, Routledge.

# Row Cemeteries and Tombs of Chiefs: the Franks and the Burial Archaeology in northern Gaul (V-VIII centuries)

**B. G. Bengozi**<sup>1</sup>

<sup>1</sup> Department of History, Faculty of Philosophy, Letters and Human Sciences, University of São Paulo, Brazil.

Several written sources and funerary artefacts were interpreted, since the nineteenth century, as “ethnic traces” of Germanic peoples in the Early Middle Ages. In this case, “row cemeteries” and “tombs of chiefs” were seen for a long time as evidences of the Frankish people in northern Gaul. Therefore, the present research aims to analyze two archaeological case studies on “row cemeteries” and “tombs of chiefs”, produced by European historians and archaeologists in the twentieth century. By means of the analysis of the sources, we sought to reflect on how these studies are included in discussions on the use of burial archaeology in the interpretation of ethnic identities and on the Frankish elite’s social organization during the Merovingian Period.

This research was based on analysis and comparisons from two archaeological case studies. The first case study was produced by Édouard Salin on the “row cemeteries”, while the second work was directed by Cécile Varéon and refers to an Exhibition catalogue on the “tombs of chiefs” excavated in Saint Dizier. We tried to understand how these studies were used to interpret the Franks as “ethnic entity”. Beyond the analysis of case studies, it was necessary to incorporate an auxiliary bibliography that allowed the expansions of discussion on the limitation of the concept of ethnogenesis associated with burial archaeology by means of ethnic identification of the Franks.

As previously mentioned, the funerary artefacts were interpreted as evidences to identify the presence of distinct ethnic groups in cemeteries. However, the most recent historiography has criticized this approach and brought new explanations for the existence of these graves, especially from a political point of view, as in the studies produced by Guy Halsall and Walter Pohl.

The readings for this research have demonstrated the relevance of debates on the archaeological case studies and of the criticism on the relationship between funerary artefacts and ethnicity in the Early Middle Ages. Therefore, we sought to demonstrate how the analysis of “row cemeteries” and “tombs of chiefs” have been interpreted and reinterpreted and how these interpretations have influenced the understanding on the Franks and on the role of ethnic identities during the mentioned period.

References:

[1] Salin, É. (1952), *La Civilisation Mérovingienne d'après les Sépultures, les Textes et le Laboratoire*, Vol. 2, A. and J. Picard, Paris.

[2] Varéon, C. (Dir.) (2009), *Nos Ancêtres, Les Barbares. Voyage autour de trois tombes de chefs francs*, Somogy, Saint-Dizier.

[3] Halsall, G. (1995), *Settlement and Social Organization: The Merovingian Region of Metz*, Cambridge University Press, Cambridge.

[4] Pohl, W. (1998), *Telling the Difference: Signs of Ethnic Identity*, in Pohl, W., and Helmut, R. (Eds.), “Strategies of Distinction: The Construction of Ethnic Communities, 300-800”, E. J. Brill, Leiden, pp. 17-69.

## **Prehistoric Spaces and Territories – metal analysis of Iron Age artifacts from Crasto de Palheiros - Murça (Vila Real – Portugal)**

**Ana Abrunhosa**<sup>1</sup>

<sup>1</sup> Master student in Archaeology, Faculty of Arts, University of Porto. Researcher at CEAUCP - Centro de Estudos Arqueológicos das Universidades de Coimbra e Porto

This presentation set up the first results of a research project directed by Professor Maria de Jesus Sanches and carried out within CEAUCP supported by the grant IJUP'10 – Projectos Pluridisciplinares – funded by Universidade do Porto/Santander Totta.

The project consisted on the study of metal artifacts from the I Iron Age archaeological site of Crasto de Palheiros – Murça (Vila Real, northern Portugal). Five copper alloy adornment objects within this collection were studied on an archaeometry perspective.

The study of the objects was developed by non-destructive methods at CEMUP – Centro de Estudo de Materiais da Universidade do Porto – using SEM-LVMSEM analysis and microvisualization to assess its elemental composition and to detect possible manufacturing marks that may distinguish those items in terms of regionalisms and craftsman ability. On this site there have been found items related to metallurgic technology like slag and crucibles that have not yet been analyzed.

Through physicochemical techniques available at the University we were looking if the differences in style reflect different traditions in manufacture. The morphology study seeking typological classification of the metal objects from Crasto de Palheiros had been made earlier by Dulcinea B. Pinto (2008). This work gave the necessary support to the definition of suitable spots on the surface of objects for detailed physicochemical analysis in association with micromorphology picturing with scanning electron microscopy (SEM).

Analysis of selected chemical elements in those spots showed that in most cases the metal alloy is of heterogeneous composition. Since the analyses are made in at the micrometre scale the results are not a real representation of the precise composition of the alloy or the whole object. So far this study was unable to confirm or revoke a proposed theory of local manufacturing. The results obtained allow us to verify that in all cases the used alloys took into account the technical and practical requirements of each object.

# Charcoal Analysis in Monte Mozinho - Preliminary results from sector A

Costa Vaz, F.<sup>1</sup> Tereso, J.<sup>2</sup>

<sup>1</sup> Department of Heritage Sciences and Techniques, Faculty of Arts, U.P.

<sup>2</sup> CIBIO, Research Center in Biodiversity and Genetic Resources, U.P.

In the last five years the archaeological site of Monte Mozinho, Penafiel, has been the place of a groundbreaking archaeobotanical study in the Northwest of the Iberian Peninsula. Until now, this study was mainly focused on the agricultural activities undertaken by the populations who inhabited the site from the 1<sup>st</sup> to the 4<sup>th</sup> century of this era [1]. The study which we'll be presenting aims to fill the information gap regarding the ecosystems that surrounded the settlement as well as to identify specific strategies of gathering and using firewood.

During the archaeological excavation, several samples of sediment were collected according to a previously determined method. The processing of these samples included bucked flotation for the separation of charcoal [2]. The next step consisted in the analysis of each bit of charcoal in order to identify its species with the help of anatomical atlases [e.g. 3]. The interpretation of the archaeobotanical data was then crossed with the information regarding the archaeological context of the samples.

Results from the seven samples show a substantial variety of species from different ecosystems. There is an overall predominance of *Castanea sativa* but, despite being native to this area, its role is still unclear. The same happens with the few *Prunus* sp. charcoals found. Several of the identified species come from riparian formations: *Populus*, *Salix*, *Ulmus minor*, *Fraxinus angustifolia* and possibly *Acer pseudoplatanus*. This latter could also be present in deciduous oak forests, testified by the presence of *Quercus* deciduous and eventually *Pinus Pinaster*. Maritime pine was found in one sample and data available for the region [4] suggests no real pine forests existed at this time. Two shrubby species of Leguminosae and the few *Erica* sp. charcoals demonstrate the presence of scrublands. Fragments of monocotyledon were also found.

Generally speaking, it is possible to imply that the inhabitants of Monte Mozinho at the 3<sup>rd</sup>-4<sup>th</sup> centuries recollected wood from distinct ecosystems such as deciduous forests, riparian formations and scrublands. Data made possible to question the role of chestnut in ancient landscapes and economy. Furthermore, dendrological evidences suggest sporadic wood recovering and storage to have occurred in this compartment.

[1] TERESO, J.; CARVALHO, T.; ALMEIDA DA SILVA, R.; RAMIL REGO, P. (2010) Cultivos e armazenagem em Monte Mozinho: dados preliminares. In: Bettencourt, A.; Alves, M.; Monteiro-Rodrigues, S. (Eds.), *Variações Paleoambientais e evolução antrópica no Quaternário do ocidente peninsular*. APEQ/CITCEM: p. 149-158.

[2] BUXÓ, R. (1997) *Arqueologia de las plantas*, Ed. Critica, Barcelona.

[3] SCHWEINGRUBER, F. (1990) *Anatomie Europäische Hölzer*, Ed. Haupt, Stuttgart.

[4] FIGUEIRAL, I. (1990). Le Nord-Ouest du Portugal et les Modifications de l'écosystème, du bronze final à l'époque romaine, d'après l'anthracolanalyse de sites archeologiques. PhD Thesis. Université des Sciences et Techniques du Languedoc

# Cooperation and Conflict(s). The fishing “*companhas*” in Mira’s coast in the first decades of the 19<sup>th</sup> century (1802 – 1830)

Ana Isabel Teixeira<sup>1</sup>

<sup>1</sup> Department of History and International Politics Studies, Faculty of Arts, University of Porto, Portugal.

In the scope of the Master’s Degree in History and Heritage studies, branch of Memory Construction, a research work was developed envisaging the understanding of a labouring universe and attempting to recognize and study the sociability means established in a sea work environment.

Nowadays, the importance attributed to the primary sector is narrowed, though it is more and more said that there is a return to the structuring basic sectors of the economy, such as fishing.

The time period - 1802-1830- in which the research is developed, is receded, but already reveals some difficulties concerning this activity. In light of the promulgation of charters and decrees falling upon fishing – that release the fishermen from their lord’s guardianship and establish their liberty of performing – the activity may consider some significant alterations that place it in a better position.

The fisheries are held at the coastline regions, where it is possible to see the men of the sea around their arts, maneuvering watercrafts in an intense sharing of knowledge and the fruit of labour. In a dynamic environment that seems to strengthen those involved, the personal bonds are merged in a perfect symbiosis with the labor relations. These bonds, that continue to manifest, are so close that they may influence the labor relations – the assigned tasks’ performance, the profit’s sharing, and the commitment to which they submit themselves when entering the sea collectivity.

Around the activity, fishing societies are created – Mira’s *companhas* – which congregate every element, that, duly enrolled as fishermen, contribute to it and relate to each other within it – passing knowledge to the newly registered and employing some members of the fisherman’s family, as labor reinforcement.

The detailed research was ruled by the readings concerning the subject, by the concepts’ definitions and by formulating problems and pre-indicators, moving on, in a second section, to the sources. The National Archive of Torre do Tombo, the Archive of the University of Coimbra and the Municipal Archive of Mira’s City Hall were given a substantial and profound analysis.

The research’s conclusions allowed for a different reading from the previously elaborated and opened doors to a subject that does not run out, not only given its different possible approaches, but also due to the its pertinence, in current days.

## References

[1] Amorim, Inês (2001), *Relações de trabalho e Gestão Pesqueira nos sécs. XVIII e XIX – a costa de Aveiro*, in Revista de História Económica e Social, Âncora Editora, Lisboa, pp.75-120

[2] Brito, Raquel S. de (1981), *Palheiro de Mira. Formação e declínio de um aglomerado de pescadores*, Instituto Nacional de Investigação Científica/Centro de Estudo Geográficos da Universidade de Lisboa, Lisboa

[3] Silva, A. A. Baldaque da (1891), *Estado actual das pescas em Portugal compreendendo a pesca marítima, fluvial e lacraste em todo o continente do reino*, Imprensa Nacional, Lisboa.



Oral Sessions VI > A4

# **Biological Sciences III**



## Evaluation of the conditions for photoconversion and imaging of Kaede and mEosFP by laser scanning confocal microscopy in transformed plant cells

V. Vieira<sup>1</sup>, C. Pereira<sup>1</sup>, P. Sampaio<sup>2</sup>, S. Pereira<sup>1</sup> and J. Pissarra<sup>1</sup>

<sup>1</sup>BioFig - Centre for Biodiversity, Functional and Integrative Genomics, Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, s/nº, 4169-007, Porto

<sup>2</sup>Instituto de Biologia Molecular e Celular (IBMC), Rua do Campo Alegre, 823, 4150-180 Porto

Fluorescent proteins (FPs) technology has revolutionized live cell imaging. When coupled to recent technical advances in widefield fluorescence and confocal microscopy, namely, multitracking laser control systems, the Green Fluorescent Protein (GFP) and its genetic derivatives have accomplished invaluable service in countless live-cell imaging experiments. Classic multicolored fluorescent protein methods are efficient in highlighting their target but are limited in their applications since their colors cannot be altered as and when required. The use of Kaede and mEos is a novel approach involving a single FP, by changing their color irreversibly from a green to a red fluorescent form upon exposure to violet-blue light. The conditions of photoconversion and imaging of these FPs in various optical configurations need to be explored in plant models. In this work, we propose to study the conditions for conversion and imaging of Kaede and mEosFP in *Nicotiana tabacum* leaf epidermis, under a transient expression system. mEos and Kaede fused with a Golgi marker – Sialyl-transferase (ST-Eos/ST-Kaede) were engineered and tested simultaneously with a mEos version fused with a signal peptide for endoplasmic Reticulum (SP-Eos). *Nicotiana tabacum* leaves were *Agrobacterium*-infiltrated with the three constructs and imaged with a laser scanning confocal microscope (Leica TCS SP2 AOBS, Leica Microsystems, Germany). The fluorescent proteins were excited with the 488 nm and 561 nm laser lines to detect, respectively, the green and red fluorescence emission, while a 405 nm laser line short-pulse promoted the photoconversion. In order to better understand the photoconversion efficiency in tobacco leaves and given the difficulties associated with this system (namely autofluorescence and multiple layers of cells), Bright Yellow-2 (BY-2) tobacco cells were also transformed by co-cultivation with *Agrobacterium tumefaciens* containing the SP-Eos fusion. Plant aspartic proteinases cardosin A and cardosin B have been extensively studied in our laboratory either in the native plant *Cynara cardunculus* or in genetic transformed models such as *Nicotiana tabacum* since they revealed to be excellent reporters to study intracellular trafficking and protein interactions due to different intracellular accumulation, even though they share high homology in terms of nucleotide and amino acid sequence. Therefore, fusions with mEos and Kaede were tested to investigate the use of photoconvertible fluorescent proteins as tools to explore the trafficking pathways of proteins in living cells. The results unequivocally demonstrate that, monomeric EosFP-based probes retain all the qualities of single-colored fluorescent proteins while providing the additional capability of photoconversion. Both green and red fluorescent forms of mEosFP are stable during prolonged live imaging allowing us to follow molecular phenomena in real time. Cardosin A-Kaede and cardosin B-Kaede expression confirmed the great potential of Kaede as an intracellular optical marker to differentially color and track an organelle and follow endomembrane dynamics over time.

# A methodology to assess fast-evolving domains on protein-coding genes

**R. Borges<sup>1,2</sup>, V. Vasconcelos<sup>1,2</sup> and A. Antunes<sup>1,2</sup>**

<sup>1</sup>CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental,  
Universidade do Porto, 4050-123 Porto, Portugal

<sup>2</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

Gene evolution can be achieved by comparing the number of non-synonymous versus synonymous mutations ( $\omega$ -ratio =  $dN/dS$ ). Fast evolving genes are more likely to accumulate non-synonymous mutations and so present generally higher  $\omega$ -ratio, while by contrast slowly evolving genes present lower omega-ratios [1]. Recent approaches in molecular evolution calculate the omega value for each site in the studied molecule and then the global omega values can be estimated [2]. However, it is common to obtain a variable omega value for each site of the protein, since not all of them are of the same functional or structural importance. We may also suspect for different rates of site evolution within the protein.

Thus, it is relevant to assess with a statistic significant level, whether a domain can be considered as fast-evolving domain comparatively to the other parts of the molecule. We propose a one-way analysis of variance (ANOVA), but very stringent assumptions need to be made, i.e. the must be normally distributed and data must be collected independently.

Here, we used three real case studies to highlight how difficult is to meet the ANOVA requirements. In order to solve this problem, we propose a non-parameter methodology to assess fast-evolving domains on protein sites. We show that  $\omega$ -ratio data are not normally distributed for the generality of genes, even for those evolving under positive selection. Additionally, we determine the exponential family as the most likely to represent the data and determine a statistic to compare two omega ratios assuming an exponential parametric model.

Our methodology revises the statistical foundation of the recurrent parameters used to understand gene/protein evolution – the omega ratio. With this approach we are able to test the selective constrains in protein domains, which can be of great interest to assess functional or structural implications in the protein function. Future research includes the expansion of the applicability of our method to test more complicated parametric models in more than two domains.

## References:

[1] Pál, C., Papp, B. and Lercher, M. J. (2006), *An integrated view of protein evolution*. Nature reviews, 7:337-48.

[2] Yang, Z. and Bielawski, J. (2000), *Statistical methods for detecting molecular adaptation*. Trends in ecology & evolution, 15:496-503.

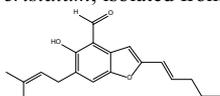
## HPLC analysis from the crude extract and metabolites isolated from the marine fungus *Eurotium cristatum*

**B. Macedo<sup>2</sup>, A.P. Almeida<sup>1,3</sup>, S. Cravo<sup>1,2</sup>, T. Dethoup<sup>4</sup>, R.T. Lima<sup>1,6</sup>, M. H. Vasconcelos<sup>1,5,6</sup>, M. Pinto<sup>1,2</sup>, A. Kijjoa<sup>7</sup>**

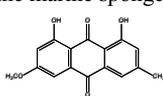
<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP)

<sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal; <sup>3</sup>- LAEQUIFAR, Universidade Severino Sombra, RJ, Brasil; <sup>4</sup>-Department of Plant Pathology Faculty of Agriculture, Kasetsart University, Bangkok, Thailand; <sup>5</sup>- Departamento de Ciências Biológicas, Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Portugal; <sup>6</sup>- Cancer Drug Resistance Group, IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal; <sup>7</sup>- Instituto de Ciências Biomédicas de Abel Salazar and CIIMAR, Universidade do Porto, Portugal.

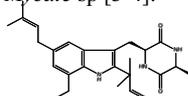
The oceanic environment has been a vast source of natural compounds covering a wide range of bioactivity [1]. The research and development in the pharmaceutical area relies on analytical data obtained using chromatographic techniques such as HPLC [2]. Our main goal is to study the chemistry and the antitumor effect of new secondary metabolites obtained from fungi from the Thailand sea, one of the richest in biodiversity in the world. In a previous work developed by our group, an ethyl acetate extract (ECE) obtained from the fungus *E. cristatum*, isolated from the marine sponge *Mycale* sp., was fractionated. Three compounds (1-3) were identified and the evaluation of their effects on the growth inhibition of three human tumour cell lines was verified [3]. In this work we describe the identification of a new isolated anthraquinone (4) and some preliminary results of the HPLC analysis (ECE and 1-4) [4]. Successive chromatographic purifications led to the isolation compound 4 and spectroscopic data indicated the chemical structure of erythroglaucin. The ECE and metabolites (1-4) were analyzed by RP-HPLC (GraceSmart RP-18; 250 mm x 4.6 mm, chromatogram obtained at 190 nm), using a flow-rate of 1.0 mL/min, with a gradient system starting with water-methanol (85:15) and going to methanol (100) in twenty-five minutes. The retention times of compounds 1-4 were determined. The HPLC method allowed a rapid identification of these metabolites using a small amount of substance, which could be of great value as an auxiliary tool in the identification of these compounds. To the best of our knowledge we are the first team to study the marine fungus *E. cristatum*, isolated from the marine sponge *Mycale* sp [3-4].



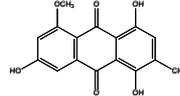
Benzaldehyde  
Derivative



Physcion



Echinulin



Erythroglaucin

### Reference

- [1] Blunt, J. W. et al. (2011), Natural Product Report, 28: 196-268.
- [2] Nielsen, K. F. and Smedsgaard, J. (2003), Journal of Chromatography A, 1002: 111-136.
- [3] Almeida, A.P. et al. (2010), Journal of Natural Pharmaceuticals, 1: 25-29.
- [4] Macedo, B. *Eurotium cristatum* isolated from the marine sponge *Mycale* sp.: Chemical and Biological Studies. Oporto: Faculty of Pharmacy from Oporto University; 2011. 121 p.

Acknowledgments: CEQUIMED-UP (PEst-OE/SAU/UI4040/2011), FERDER, POCl, U.Porto/Santander Totta, for financial support.

# Study of *Planctomycetes* pigments by a HPLC-DAD and LC-MS methodology

C. Moreira<sup>1,3</sup>, R. Airs<sup>2</sup>, C. Llewellyn<sup>2</sup> and O. Lage<sup>3</sup>

<sup>1</sup> Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Portugal.

<sup>2</sup> Plymouth Marine Laboratory, England.

<sup>3</sup> Department of Biology, Faculty of Science and CIIMAR University of Porto, Portugal.

*Planctomycetes* are a group of unique bacteria, with peptidoglycan-less cell walls, internal compartmentalization, budding reproduction and a complex life cycle. Nowadays, this group has aroused great importance in many biological scientific areas. However, up-to-now there are not any reports on literature about *Planctomycetes* pigmentation. They can be white or present orange and often pink coloration, pointing to the presence of carotenoids.

The aim of this work is to find out what are the carotenoids responsible for *Planctomycetes* pigmentation.

Several strains belonging to the *Planctomycetes* phylum were grown in M13 medium. The pigments were extracted from the biomass with methanol and the total extract containing the pigments of interest submitted to HPLC-DAD and LC-MS.

The analysis performed indicated that pink colored *Planctomycetes* should have in their constitution a group of carotenoids, three of them related to KG carotenoid (Fig. 1. A1) [1]. In orange Gr7 strain, potentially carotenoid crocetin and a related compound was found in their composition.

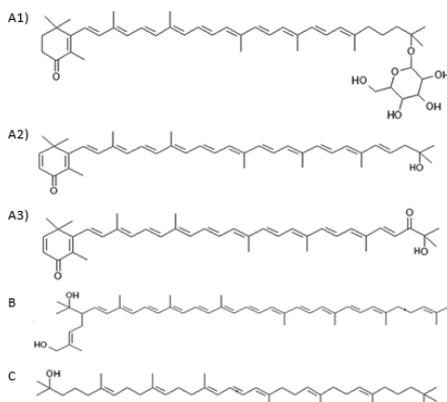


Figure 1: carotenoid structures found in pink colored *Planctomycetes*.

## References:

[1] George Britton, S.L.-J., Hanspeter Pfander, *Carotenoids Handbook*. 1<sup>st</sup> ed. 2004, Liverpool: Birkhäuser BioSciences.

# Real-time PCR technique to detect hazelnut as a potential hidden allergen in commercial chocolates

V.S. Melo<sup>1,2</sup>, C.G. Santos<sup>1,2</sup>, J. Costa<sup>1</sup>, M.B.P.P. Oliveira<sup>1</sup>, I. Mafra<sup>1\*</sup>

<sup>1</sup> REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto

<sup>2</sup> Polytechnic Institute of Bragança, \*E-mail: isabel.mafra@ff.up.pt

Allergic reactions caused by foods represent an emerging problem of food safety management. The reactions caused by ingestion of trace amounts of tree nuts, in particular hazelnut, are among the most dangerous because of their frequency and severity. In order to protect the health of allergic consumers, the ingredients that may cause adverse reactions must be correctly labeled and cross-contamination must be avoided [1]. Since the correct labeling of foods is the only means to protect allergic individuals, appropriate detection techniques of food allergens are needed. The use of DNA-based methods, namely Polymerase Chain Reaction (PCR), is a rather reliable option to detect trace amounts of a potential allergenic ingredient. However, complex food matrices, such as chocolate are rich in polyphenols and polysaccharides, which inhibit PCR amplification making the detection of trace amounts of tree nuts a difficult task [2].

The aim of this project was to detect hazelnut DNA in commercial chocolate samples by PCR and real-time PCR. To achieve this goal, reference chocolate mixtures containing known amounts of hazelnut were prepared and 25 chocolate samples were acquired in the market. Prior to this work, a comparative study of DNA extraction methods was performed, enabling to establish that the best protocol to obtain hazelnut DNA from chocolate was the NucleoSpin Food kit [3]. The detection of hazelnut was performed by species-specific PCR and TaqMan real-time PCR targeting the *hsp1* gene [4].

The optimization of both PCR techniques allowed detecting hazelnut down to the level of 0.005% (50 mg/kg) in chocolate. The application of PCR assays to commercial chocolate samples was effective, indicating some inconsistencies with labeling. Also, it was possible to detect traces of hazelnut in chocolates that were not labeled with hazelnut as an ingredient, but declared “may contain traces of...” On the other hand, some samples were excessively labeled since hazelnut was not detected. TaqMan real-time PCR confirmed the qualitative PCR results and showed its high potentiality for quantitative analysis of hazelnut allergens in a linear range of 0.005-10%.

Acknowledgments: Joana Costa is grateful to FCT PhD grant (SFRH/BD/64523/2009) financed by POPH-QREN (subsidized by FSE and MCTES).

## References:

- [1] Arlorio, M.; Cereti, E.; Coisson, J.D.; Travaglia, F.; Martelli, A. (2007). Detection of hazelnut (*Corylus* spp.) in processed foods using real-time PCR. *Food Control* 18: 140-148.
- [2] D’Andrea, M.; Coisson, J.D.; Locatelli, M.; Garino, C.; Cereti, E.; Arlorio, M. (2011). Validating allergen coding genes (Cor a 1, Cor a 8, Cor a 14) as target sequences for hazelnut detection via Real-Time PCR. *Food Chem* 124: 1164-1171.
- [3] Costa, J.; Melo, V.S.; Santos, C.G.; Mafra, I.; Estevinho, L.; Oliveira, M.B.P.P. Assessing DNA extraction methods for the detection of tree nut allergens in chocolates (submitted).
- [4] Píknová, L.; Pangallo, D.; Kuchta, T. (2008). A novel real-time polymerase chain reaction (PCR) method for the detection of hazelnuts in food. *Eur Food Res Technol* 226: 1155-1158.

# Bacterial discrimination by ATR-FTIR spectroscopy: towards a faster diagnosis of infection and a more targeted antimicrobial therapy

**Raúl G. Saraiva<sup>1</sup>, João Lopes<sup>2</sup>, Paula Gameiro<sup>1</sup>, Maria J. Feio<sup>1</sup>**

<sup>1</sup> REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Porto, Portugal

<sup>2</sup> REQUIMTE, Departamento de Química, Faculdade de Farmácia, Porto, Portugal

Common modern techniques for bacteria identification, though rather sensitive and consistent, are either too specific (*e.g.* species-specific PCR; immunoassays), requiring preliminary analysis to avoid a trial and error approach or, on the contrary, so broad that demand subsequent testing in order to give a definitive result (*e.g.* broad-range PCR), leading to an excessive amount of time required for the diagnosis of infections [1, 2].

The potential of infrared spectroscopy for bacterial discrimination has been studied since the 1950s, as it is able to establish spectral fingerprint-like patterns of intact bacteria [3]. With the development of attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, there is a great promise of using this technique as a diagnostic tool, namely in food microbiology for routine epidemiological investigation of foodborne bacteria [4].

In this work, several *E. coli* porin mutants are analysed by ATR-FTIR spectroscopy, in an attempt to establish the sensitivity of this technique. Indeed, it seems to be possible to distinguish *E. coli* strains that vary solely in their envelope composition, so a diagnosis by ATR-FTIR spectroscopy not only allows the identification of the pathogen, but also returns important structural information that can be used to further target the antimicrobial therapy for better results.

## References:

- [1] Willey, J. M., *et al.* (2008). *Prescott, Harley, and Klein's Microbiology*, McGraw-Hill Higher Education, New York.
- [2] Schabereiter-Gurtner, C., *et al.* (2008). Evaluation of a protocol for molecular broad-range diagnosis of culture-negative bacterial infections in clinical routine diagnosis. *Journal of Applied Microbiology* 104, 1228-1237.
- [3] Levine, S., *et al.* (1953). Infrared spectrophotometry of enteric bacteria. *Journal of Bacteriology* 65, 10-5.
- [4] Davis, R. and Mauer, L. J. (2010). Fourier transform infrared (FT-IR) spectroscopy: A rapid tool for detection and analysis of foodborne pathogenic bacteria. In *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology* (Méndez-Vilas, A., ed.), Vol. 2. Formatex Research Center.

## Polimerase chain reaction as a tool for diet studies of crustaceans

**A. Matos<sup>1</sup>, E. Ferreira<sup>1</sup>, S. Costa-Dias<sup>2</sup>, J. Campos<sup>2</sup> and C. Moreira<sup>2</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup>CIMAR/ CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Portugal.

In this work we used genetic tools to study the diet of crustaceans, specifically the shore crab *Carcinus maenas* Linnaeus 1758 and the brown shrimp *Crangon crangon* Linnaeus 1758. Both crustaceans are common epibenthic species in European shallow coastal waters, including estuaries, from as far north as Norway to Morocco in the south limit. Crabs' native distribution extends further south to Mauritania; yet this species is highly invasive and is now present almost worldwide. Due to their generally high abundance, these crustaceans play a relevant role in the ecosystem functioning both as preys and predators. However, because they grind the preys into pieces, hardly any remain can be identified in the stomachs. Therefore, other indirect analysis, such as the genetic studies of stomach contents, can be a useful tool for diet investigations.

We started by doing an extensive literature review on diet studies of these species and on genetic studies of their preys, followed by field sampling to collect the biological material (*C. crangon*, *C. maenas* and their prey). Since both species are opportunistic predators, preying upon 67 different species, we decided to restrict the studies to common potential preys in Minho estuary (the common goby *Pomatoschistus microps* (Krøyer, 1838) and the ragworm *Nereis diversicolor* (O.F. Müller, 1776)), and to two commercially important species, the flounder *Platichthys flesus* (Linnaeus, 1758) and the peppery furrow shell *Scrobicularia plana* (da Costa, 1778).

The laboratory work consisted first on sample sorting, preservation in ethanol 80% and biometry. For a total of 389 shrimps and 127 crabs, the stomach was removed, classified according to its fullness (0 for empty; 1 for up to half full; and 2 for full), and its content roughly inspected and preserved in ethanol 80%. Then, the DNA extraction (PureLink™ Genomic DNA, Invitrogen) was conducted to prey samples (positive control) and to stomach content samples. It was followed by PCR amplification with specific primers for each prey (already developed), electrophoresis in agarose gel, and data treatment.

Preliminary results allowed us to confirm the most appropriate methods to sequence the potential prey (positive controls), which were then applied to stomach content samples. Up to now, we were able to confirm the presence of *P. microps* in the stomachs of both crustaceans. We conclude that this technique can be useful to study the crustaceans' diets as long as the potential preys are known by previous analysis of stomach contents.



Oral Sessions VI > A5

# Architecture I



# Public Space in Oporto Social Quartes

## A Conceptual Plan to Space and Social Rehabilitation

Ana Reis Loureiro<sup>1</sup>, Isabel Martinho da Silva<sup>2</sup>

<sup>1</sup> Master Student, Department of Geosciences, Environment and Planning, Faculty of Science, University of Porto, Portugal.

<sup>2</sup> CIBIO (Research Center in Biodiversity and Genetic Resources), Landscape Ecology Group, University of Porto, Portugal.

All over Oporto we find social quarters which public space is lack of identity, function and space design. These problems generate lack of identification with these public spaces, that results in misappropriation, as they are not used neither acknowledge creating non-places<sup>1</sup>, residual spaces that don't have any contribution to the improvement of their users' life quality or social problems.

This research has as study object the public spaces in six Oporto social quarters and it aims to understand their dynamic and experience in a way to develop a general intervention strategy to their spatial and social rehabilitation designing places that improves the sense of identification and belonging.

Based on spatial, social and expansion analysis of these quarters, and through the identification of common spatial typologies, a conceptual model is developed, applicable to these diverse public spaces typologies. This model suggests a series of guidelines in terms of public space's hierarchy and design strategies in order to promote the social contact and the community spirit of these sensitive and needed groups.

That model leads to a proposal that applies the conceived guidelines proving their application in the ambition that they could be applied to other neighborhoods and, this way, improve the interventional role of public spaces in the communities of Oporto social quarters.

In conclusion, there is a long way to go in terms of social and urban inclusion of these housing units, but the improvement of their public spaces is an important step in the reconstruction of their community sense and in the inhabitants' well-being. The importance in the creation of well design spaces that fulfill the needs of their users is underestimated and the role of the public spaces in the social structure of a urban community is, often, neglected. This study also allows us to conclude that a guide model, that leads to the creation of well design identity and belonging places in this fragile communities, is urgent and that translates the strong role of urbanism in social matters.

References:

[1] O'Beirne, E., 2006. *Mapping the Non-Lieu in Marc Augé's Writings*. In O'Beirne ed. Forum For Modern Language Studies, vol. 42, No. 1, 2006. Oxford University Press, pp. 38 – 50.

# Proposal of Rehabilitation and Maintenance for Copan Building's Façades

C. Paim<sup>1</sup>, F. Oliveira<sup>2</sup>

<sup>1</sup> Graduate Student, Faculty of Architecture and Urbanism, University of São Paulo, Brasil.

<sup>2</sup> Associate Professor of Department of Technology of Architecture Faculty of Architecture and Urbanism, University of São Paulo, Brasil.

Copan Building is one of the most important icons of modern architecture in São Paulo. Designed by Oscar Niemeyer in the mid 1950s, the building currently presents need to be rehabilitated, most evidenced by its façades. The rehabilitation of a building with such proportions, almost 140 meters long and 118 meters high, must be thought in a systematic way in order to prevent future difficulties during the process.

The main objective of this research was to make a survey of pathological manifestations of the façades of Copan building, and proposing the appropriate methods of rehabilitation among with a plan of maintenance to guarantee its performance by the years.

It was necessary to study all the original drawings to redraw the elevations corresponding to the façades of Copan building actually. Copan's façades were analysed by a survey of pathological manifestations. Then the data obtained *in loco* was registered in a Map of Damages [1-2]. It was possible to identify the main damages, its probable causes and at last, the rehabilitation methods for Copan building were proposed.

This research concluded that the current situation of Copan's façades requires immediate intervention, not only because of its historical value, but mostly to guarantee better conditions to its users and adequate service life to the building.

Beyond that it was observed the need of an accurate planning, a project of rehabilitation associated with a plan of maintenance, preventing future issues and making sure the intervention would be efficient and lasting.

## References:

[1] TINOCO, Jorge Eduardo Lucena (2009). Mapa de danos: recomendações básicas. Textos para discussão – série 2: Gestão de restauro. Centro de Estudos avançados de Conservação Integrada. Olinda. Available in <http://www.ceci-br.org/ceci/br/noticias/519-textos-para-discussao.html>

[2] BARTHEL, C. (2009). O papel do mapa de danos na conservação do patrimônio arquitetônico. Fundação do patrimônio histórico e artístico de Pernambuco. Recife.

GALVÃO, Walter José Ferreira (2007). COPAN/SP: A trajetória de um mega empreendimento, da concepção ao uso: estudo compreensivo do processo com base na avaliação pós-ocupação. São Paulo. Master's degree. Available in <http://www.teses.usp.br/teses/disponiveis/16/16132/tde-19092007-121207-pt-br.php>

## Methodology for assessing the state of disrepair of the building

A. Alves<sup>1</sup>, M. Vale<sup>2</sup>

<sup>1</sup> Faculty of Architecture, University of Porto, Portugal

<sup>2</sup> Faculty of Architecture, University of Porto, Portugal

Rehabilitation is nowadays a major theme of the cities and it is extremely important the discussion of the rehabilitation actions that have to be taken in historical centres or in urban centres. Rehabilitating why? How much is important the rehabilitation? How deeply is necessary to rehabilitate? These are certainly the questions putted by this work.

The analyses of the building and of the urban zone is essential in order to know the real needs and weaknesses of the buildings, as well as to identify and characterize them and its anomalies in order to find rehabilitation solutions that contribute to increase its useful life.

To achieve these goals, this work presents a methodology that permits to characterize the built environment and at the same time attribute the conservation level of each functional element and of the general conservation level of the building.

With the achievement of the conservation level of the building, by weighting the results obtained for each one of its functional elements, it is possible to estimate the level of rehabilitation that is needed.

Through the characterization of the building and the identification of its anomalies, as well as from its conservation level, it is possible to define rehabilitation strategies, especially when we talk about a set of buildings. Also from these data it is possible to define methodologies of action throughout the characterization of constructive solutions of similar situations of rehabilitation.

Frequently the factors that contribute to the rising prices of rehabilitation works are the lack of adequate solutions for the real problems that must be solved, due to reduced knowledge of the building. Also for this reason its correct characterization and conservation level is essential.

The methodology test was done in the urban centre of Águeda, where two examples were chosen, according to the characteristics desired by the town council, as pilot projects. Through the study of these two examples, it was possible to do a first test of the general methodology. In spite of this it is stated that it would be interesting and essential to extend this study to the buildings of an area of the city, to assess the real potentialities of this tool. But, due to the inherent characteristics of a Master thesis of this Miqr, it is not possible to do such a large work.

## **BETWEEN PUBLIC AND PRIVATE. The Domestic Architecture of Marques da Silva at Oporto**

**R. Alegria**<sup>1</sup>

<sup>1</sup> Department of Architecture, Faculty of Architecture, University of Porto, Portugal.

Talk about houses, although its individuality, requires simultaneously a reflection of a time, a moral and a society. Exploring the domestic housing evolution, the transformations occurred between the XVII and XX century determine new concepts that remain until nowadays. The progressive affirmation of the man as an individual, exalts the idea of private life, embodied in the home concept. In this sense, a new all value was attributed to the interior conception and to the comfort notions, which concerns to the home concept the aim of experience a new sociability in the family isolation.

Analysing the Portuguese housing context during the beginning of the XX century, the architect Marques da Silva emerges as an important character who contributed for the national architectural development. His projects, designed for the bourgeoisie, reflect the architect ability to update and essay his knowledge about the domestic space.

Following the theoretical research, the study of ten housing projects of Marques da Silva at Oporto pretend to explain all the particularities that define a new space conception based on the public and private confluence in the housing projects. Taken the intimate preservation as a fundamental value, the study focus on the home internal organization where a reformulation of the articulation and spatial distribution qualifies the relations between the multiple actors, controlling the different level of sociability at home. We pretend to understand how this live organism works and which elements contributed for an elaborated spatial conception, able to answer to all the requirements of a social class who pretend to assert itself through this architectural element, as on the other hand, it wants to find its refuge at home. The space and his programme start to be thought according to the modern culture and *home*, as a spatial dispositive, assume itself as the intersection between functionality, comfort and representation.

According to the bourgeois domestic project between the XIX and XX centuries, the architect becomes the theatre director, capable of leading the role of different characters - family and guests - acting on a stage that is home.

## Housing and Extreme Poverty UTPMP – a chance for a solution

**Lara Ferreira**<sup>1</sup>, investigation tutored by **Rui Ramos**<sup>1</sup>

<sup>1</sup> Faculty of Architecture, University of Porto, Portugal

The problem of housing has serious implications in urban growth and territorial inhabitation. Distribution and access to a proper habitat lead us to question the share of responsibility of architects and planners on this matter.

In fact, the problem is far from ceasing to be a current theme. Present in all societies at all times, the issue of housing as a social problem has emerged over times. This alerts us to the constant need to create solutions in this area.

This paper describes an attempt of solution to the housing problem. Um Teto Para Meu País (UTPMP), a Non-Governmental Organization (NGO) created in 1997 in Chile and established in 19 countries of Latin America, aims to eradicate extreme poverty in that Continent. It acts in human settlements through participatory interventions, in collaboration with local populations, university students and the general society.

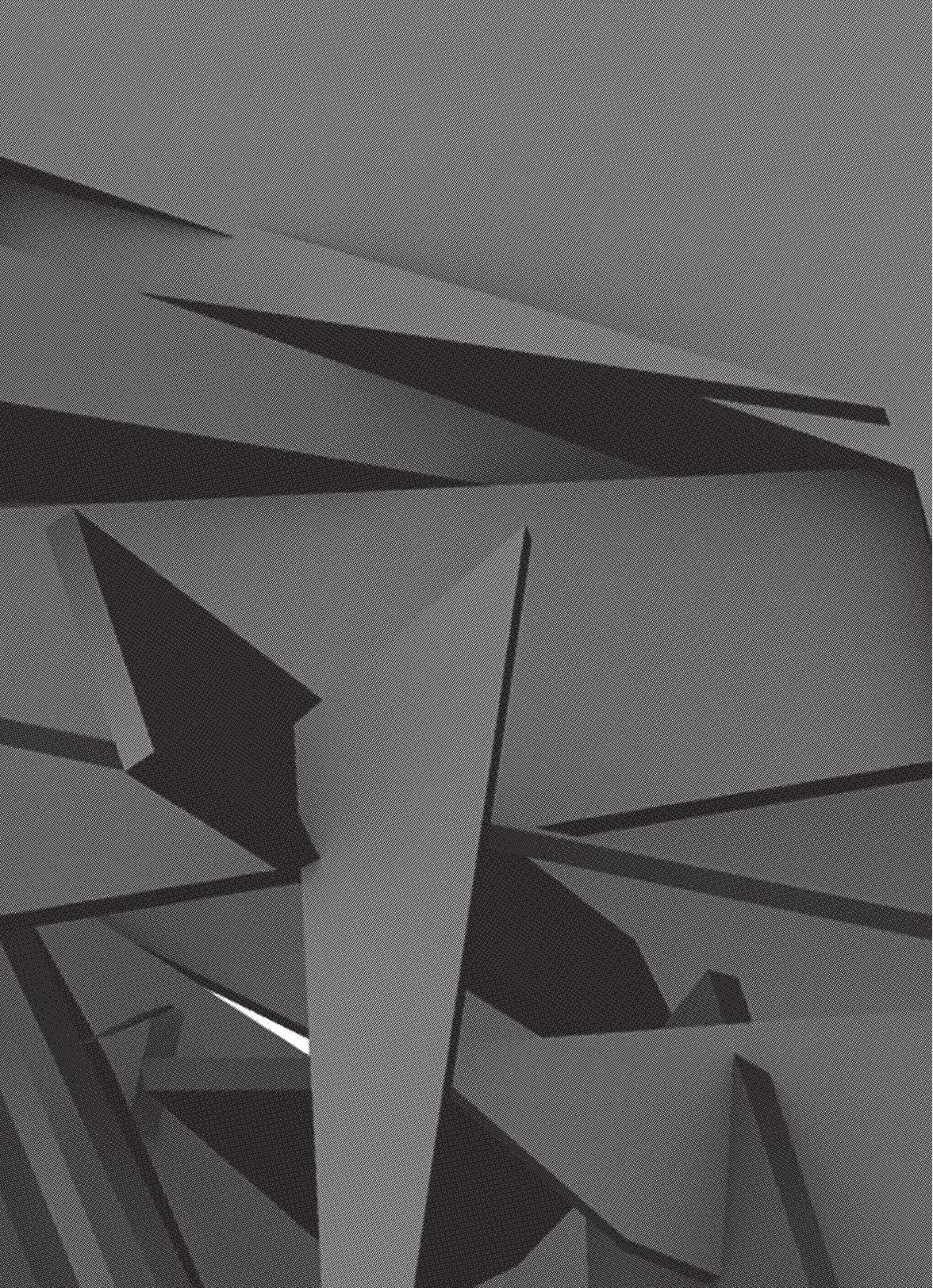
The first stage of UTPMP's interventions consists of replacing precariousness with emergency low-cost houses, built by volunteers in direct collaboration with families or individuals for whom they are designed. Through this strategy, UTPMP provides the beginning of a relationship that has two main objectives: the immediate improvement of living conditions of impoverished people and the possibility of training volunteers through practical and empirical knowledge on the field.

### References:

DAVIS, Mike (2006), *Planeta Favela*, Boitempo, São Paulo

SACHS, Jeffrey D. (2006) *O Fim da Pobreza. Como consegui-lo na nossa geração*. Casa das Letras, Lisboa

TIBAIJUKA, Anna Kajumulo (2009), *Building Prosperity: Housing and Economic Development*, Earthscan, London



# Oral Sessions VII



Oral Sessions VII > A1

# **Stress & Heart Physiology II**



# Monitoring Drivers' Vital Signs for characterisation of Stress and Fatigue in Urban Traffic

D. Carneiro<sup>1</sup>, F. Vieira<sup>2</sup>, J. Barros<sup>1</sup>

<sup>1</sup> Department of Electrical and Computer Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup> Instituto de Telecomunicações, University of Porto, Portugal.

This work is part of the MISC project “Massive Information Scavaging with Intelligent Transportation Systems”, and has as one of its aims the characterization of stress in urban traffic situations. Through the use of the VitalJacket (equipment designed by IEETA and developed as product by Biodevices), it is possible to make an assessment of physiological signals, such as stress and fatigue in drivers of the Public Transport Company of Porto (STCP) and in order to understand which factors have impact in the physical and mental state of drivers during the practice of their profession. Thereby, specific measures may be taken to support the drivers, both in terms of health care but also in terms of road safety [1].

The methodology applied for the resolution of this issue begins with drivers filling out questionnaires, both at the beginning as well as at the end of the work day, to enable a better understanding and analysis of the possible extra professional occurrences that might have an influence in the physiological analysis results. At the end of the day, the driver is also confronted with a map plotting the routes of the day where possible stress events are marked and the drive can rank them on a scale from 0 to 10.

Underlying these procedures is objective to analyse, through the heart rate variability (in the frequency domain), the behaviour of the autonomic nervous system, since it is a key factor in influencing the heart rate, as well as mediation and response to stressful events (Fig. 1).

In conclusion, it is shown that through the heart rate variability it is possible to infer stress events, based on the frequency domain analysis, as well as validation with complementary tools, such as questionnaires. The method is non-invasive, which allows holding unbiased evaluations and develop more accurate methods for the classification of events of stress and the characterization of the stress of public transport drivers in urban areas.

[1] J. Rodrigues, F. Vieira, J. Barros and J. P. Cunha, *A Non-Intrusive Multi-Sensor System for Characterizing Driver Behavior*, IEEE Conference on Intelligent Transportation Systems, Madeira, Portugal, September 2010.

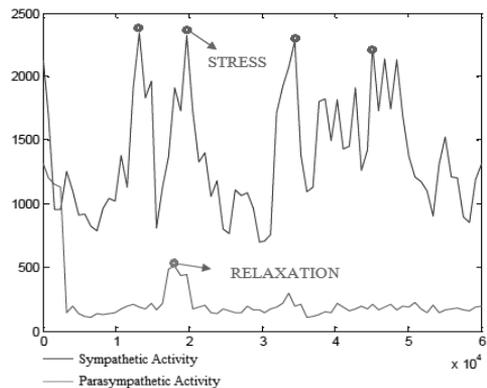


Figure 1-Heart rate variability along a trip.

## Angiotensinogen concentration in patients with chronic heart failure

**L. Morgado<sup>1</sup>, M. Morato<sup>1,2</sup>, M. Reina Couto<sup>1,3</sup>, T. Sousa<sup>1,2</sup>, J. Afonso<sup>1</sup>, M. Marques<sup>1</sup>, L. Belo<sup>4</sup>, P. Bettencourt<sup>3</sup>, A. Albino-Teixeira<sup>1</sup>**

<sup>1</sup>Department of Pharmacology and Therapeutics, Faculty of Medicine, and Neuropharmacology, Institute of Molecular and Cell Biology (IBMC), University of Porto, Portugal.

<sup>2</sup>Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal.

<sup>3</sup>Internal Medicine Department, São João Hospital, Portugal.

<sup>4</sup>Laboratory of Biochemistry, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Heart failure (HF) is a common chronic disabling disease responsible for high levels of morbidity and mortality as well as marked economic burden. Chronic renin-angiotensin system (RAS) activation leads to long-term deleterious effects in HF[1]. One of the cornerstones of chronic HF (CHF) treatment is the RAS blocking effect of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II (AngII) receptor blockers (ARB)[1]. AngII modulates angiotensinogen (AGT) synthesis, while AGT levels influence AngII production[2]. We aimed at characterizing AGT concentrations in CHF patients treated with RAS blocking drugs (either ACEI or ARBs).

The project was approved by the Health Ethics Commission of the Hospital S. João (HSJ). Sixty one patients with CHF were selected from the Heart Failure Clinic of HSJ, informed about the study and asked to participate giving their informed consent. On the day of the visit, data on age, gender, functional status (New York Heart Association classes I-IV) and chronic medication were taken and blood samples collected. AGT concentrations were determined using a commercial ELISA kit (IBL#27412). Renin, angiotensin-converting enzyme (ACE), brain natriuretic peptide (BNP) and cystatin C (CysC) concentrations were determined using commercial kits and an automated biochemical analyzer. Statistical analysis was performed using independent samples t-test or Kurskal-Wallis ANOVA as appropriate.

Patients were divided in four groups according to the use of RAS blocking drugs (ACEI-only, ARB-only, both or none) and ACEI-only and ARB-only were compared. AGT and ECA concentrations were significantly decreased in ACEI-only versus ARB-only patients (AGT: 23,71±8,79 vs 33,91±10,19 µg/ml, n=38/6, respectively, p<0,05; ECA: 437,14±750,85 vs 454,42±738,36 U/l, n=42/6, respectively, p<0,05). There were no significant differences in BNP, CysC, age, gender or functional class between ACEI-only and ARB-only treated CHF patients.

ARB-only treated CHF patients have higher concentration of AGT and ECA when compared to ACEI-only treated CHF patients. This might be due to the regulatory role that AngII has on AGT synthesis, since ARB-only patients are expected to have higher AngII concentrations than ACEI-only patients. Future experiments will evaluate AngII concentrations in this population in order to confirm this hypothesis.

References:

[1] Werner, C.M. and M. Bohm. (2008), *The therapeutic role of RAS blockade in chronic heart failure*. Ther Adv Cardiovasc Dis., 2(3): p. 167-77.

[2] Deschepper, C.F. (1994), *Angiotensinogen: hormonal regulation and relative importance in the generation of angiotensin II*. Kidney international., 46(6): p. 1561-3.

## Effect of fatty acids upon catecholamine handling by adrenal chromaffin cells

**A. Gomes<sup>1</sup>, G. Correia<sup>1</sup>, M. Moz<sup>1</sup>, M. Coelho<sup>1</sup>, A.L. Teixeira<sup>2</sup>, R. Medeiros<sup>2</sup>, F. Leite<sup>1,3</sup>, D. Torres<sup>4</sup> and L. Ribeiro<sup>1</sup>**

<sup>1</sup> Department of Biochemistry, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> Molecular Oncology GRP, IPO-Porto, Portugal

<sup>3</sup>Unidade Multidisciplinar de Investigação Biomédica - UMIB

<sup>4</sup>Faculty of Nutritional and Food Sciences, University of Porto (FCNAUP), Portugal

Adrenaline (AD) and noradrenaline (NA), the main mediators of the sympathoadrenomedullary system, play crucial roles in the regulation of metabolic and cardiovascular (CV) homeostasis. Interestingly enough, relatively to metabolic syndrome (MS), AD and NA seem to behave very differently: whereas NA levels positively correlate with obesity and CV risk, AD shows an inverse association with CV mortality [1]. Fatty acids (FA) overload or dysfunction of their metabolism could contribute to the development of obesity and/or type 2 diabetes [2]. The typical western diet is overloaded with omega-6 FA and contains insufficient omega-3 FA, and this dietary imbalance in FA is a fundamental underlying cause of many chronic diseases including CV disease [2]. In line with this, omega-3 FA are usually associated with cardioprotective benefits [2]. On the other hand, consumption of trans FA, even at low levels of intake, significantly increases the risk of coronary events [2]. Although our group has already described that saturated fatty acids affect CA content and release [3], very limited information is available concerning the effect of these molecules on CA handling by adrenal chromaffin cells.

The aim of this work was to investigate the effect of several fatty acids on catecholamine (CA) synthesis and secretion from chromaffin cells. Tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT) expression was also evaluated under the same conditions. For that purpose, the effect of acute (60 minutes) and chronic (24 hours) exposure to monounsaturated, trans and polyunsaturated fatty acids upon the above parameters was investigated, using bovine adrenal chromaffin cells. CA in cells and liquids were quantified by HPLC and mRNA levels of both enzymes were analyzed by relative quantification using quantitative real-time PCR with SYBR Green I detection.

With the exception of eicosapentaenoic acid, which was able to significantly reduce TH mRNA levels ( $p=0,043$ ), none of the fatty acids studied significantly affected the expression of this enzyme. PNMT mRNA levels were significantly diminished after cells incubation with elaidic acid ( $p=0,019$ ),  $\gamma$ -linolenic acid ( $p=0,015$ ), linoleic acid ( $p<0,001$ ),  $\alpha$ -linolenic ( $p=0,015$ ) and eicosapentaenoic acid ( $p=0,031$ ), comparatively to control conditions. In conclusion, fatty acids differently affect the expression of both CA synthesis enzymes, TH and PNMT, in chromaffin cells, suggesting distinct roles in AD and NA production. These findings, together with data from functional studies, currently under analysis, will help to clarify the role of monounsaturated, trans and polyunsaturated fatty acids upon CA handling by these cells.

This work was supported by U.Porto/Santander Totta" (IJUP) and FCT through a scholarship to A. L. Teixeira (SFRH/BD/47381/2008).

[1] Reims HM, Sevre K, Fossum E, Mellem H, Eide IK, Kjeldsen SE (2005) Adrenaline during mental stress in relation to fitness, metabolic risk factors and cardiovascular responses in young men. *Blood Press* 14: 217-26.

[2] Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC (2006) Trans fatty acids and cardiovascular disease. *N Engl J Med* 354: 1601-13.

[3] Ribeiro L, Martel F, Azevedo I (2008) Catecholamine (CA) content and release from adrenal chromaffin cells is differentially influenced by fatty acids *FASEB J* 22: 1195.

## Neuregulin attenuates right ventricular hypertrophy and dysfunction in an experimental model of pulmonary hypertension

**Maia-Rocha C<sup>1</sup>, Adão R<sup>1</sup>, Mendes-Ferreira P<sup>1</sup>, Mendes MJ<sup>1</sup>, Pinho S<sup>1</sup>, Lourenço AP<sup>1</sup>, De Keulenaer GW<sup>2</sup>, Leite-Moreira AF<sup>1</sup>, and Brás-Silva C<sup>1,3</sup>**

<sup>1</sup> Department of Physiology, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> Laboratory of Physiology, University of Antwerp, Belgium.

<sup>3</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

Neuregulin (NRG)-1 is implicated in the preservation of left ventricular (LV) performance in pathophysiological conditions [1]. Nevertheless, the role of NRG-1 in right ventricular (RV) failure secondary to pulmonary arterial hypertension (PAH) is still unknown. This study analysed the effects of NRG-1 chronic treatment in an animal model of PAH and HF.

Male Wistar rats (180-200g) randomly received monocrotaline (MCT, 60mg/Kg, sc) or vehicle. After 14 days, animals from these groups were randomly assigned to receive treatment with either NRG-1 (4ug/Kg/day, ip) or vehicle. The study resulted in 4 groups: control (n=10); control+NRG (n=10); MCT (n=10); MCT+NRG (n=10). Echocardiography, RV invasive hemodynamic evaluation and sample collection were performed 25 to 28 days after MCT administration.

MCT group developed PAH, as shown by the increase in RV maximum pressure (MCT vs control: 63±3 vs 34±3mmHg) and by the decrease in cardiac output (MCT vs control: 34.4±4.4 vs 64.6±3.4mL/min) which were both attenuated in the MCT+NRG group (53±3mmHg and 52.2±1.6mL/min). Ecocardiographic data confirmed these results and showed a marked dilatation of the RV, and a decrease in the pulmonary artery acceleration time in the MCT group, changes that were also reduced by NRG-1 treatment. Animals from the MCT group developed RV hypertrophy (RVweight/tibia length ratio MCT vs control: 0.08±0.002 vs 0.05±0.003 g/cm) and pulmonary congestion (lung weight/tibia length ratio MCT vs control: 0.7±0.03 vs 0.4±0.03g/cm), both changes were minimized by the NRG-1 treatment (0.06±0.002 g/cm and 0.6±0.03 g/cm, respectively). Histological analysis also revealed a decrease of RV cardiomyocyte hypertrophy and fibrosis in the MCT+NRG group in comparison with MCT group.

The RV of MCT group animals presented an increased expression of brain natriuretic peptide (BNP) and endothelin (ET)-1 (17.5 and 5.0 times vs control, respectively). These changes were attenuated or reversed in the MCT-NRG group.

NRG-1 chronic treatment significantly reduced the severity of PAH and RV hypertrophy, as well as the expression of genes associated with overload and ventricular hypertrophy. These findings suggest that the NRG-1 pathway has a relevant role on the pathophysiology of PAH and right ventricular HF, representing a potential therapeutically target.

[1] De Keulenaer GW, Doggen K, Lemmens K. (2010). *The vulnerability of the heart as a pluricellular paracrine organ: lessons from unexpected triggers of heart failure in targeted ErbB2 anticancer therapy.* Circ Res, 106:35-46.

# Differential expression of A<sub>1</sub> receptors between sinoatrial node and cardiomyocytes does not account for adenosine chronoselectivity in the spontaneously beating rat atria

**B. Bragança, N. Oliveira-Monteiro, F. Ferreirinha, A.P. Fontes-Sousa  
& P. Correia-de-Sá**

Laboratório de Farmacologia e Neurobiologia, UMIB, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto (UP), Portugal.

It is widely accepted that adenosine (ADO) and its analogues are able to reduce cardiac chronotropism, which makes this nucleoside clinically relevant to control supraventricular tachyarrhythmias. ADO produces its effects through activation of different receptors subtypes, being the A<sub>1</sub> receptor (A<sub>1</sub>R) subtype the most expressed in the heart. Like muscarinic M<sub>2</sub> receptors responsible for the negative chronotropic and inotropic effects of acetylcholine, A<sub>1</sub>R effects are mediated by hyperpolarization of sinoatrial (SA) node cells primarily by inducing potassium currents through  $\beta\gamma$  subunits of G protein-coupled inwardly rectifying K<sup>+</sup> channels (GIRK or K<sub>IR</sub> 3.1/3.4). Using the spontaneously beating rat atria, we have previously observed that the A<sub>1</sub>R agonist R-PIA exhibited significant “chronoselectivity”, since the negative chronotropic effect was evidenced at much lower concentrations than the negative inotropic action. This phenomenon was not observed with the M<sub>2</sub>R agonist, oxotremorine (Oxo) (Oliveira-Monteiro *et al.*, unpublished observations). To explore a rationale to support functional data, we aimed at investigating the distribution of A<sub>1</sub>R, M<sub>2</sub>R and GIRK1 throughout the rat atria, focusing our attention specifically in the sinoatrial (SA) node as compared with surrounding cardiomyocytes.

Experiments were performed on spontaneously beating isolated atria from Wistar rats, continuously superfused with gassed (95% O<sub>2</sub> + 5% CO<sub>2</sub>) Tyrode's solution, at 37°C. Isometric muscle tension was continuously monitored on a computer screen through a PowerLab data acquisition system (Chart 5, v.4.2 software; AD Instruments, USA). Reported parameters were the rate and force of contraction. The atrial distribution of A<sub>1</sub>R, M<sub>2</sub>R and GIRK1 was evaluated by immunofluorescence confocal microscopy. Oxo (0.01-3  $\mu$ M; n=30-46) and R-PIA (0.3 nM-1  $\mu$ M, n=40-47) concentration-dependently decreased the rate and the force of spontaneous contractions of the rat atria. As mentioned before, both agonists were about equipotent in decreasing the rate of contraction ( $IC_{50}$ ~0.03  $\mu$ M) (negative chronotropism), but Oxo was significantly ( $P<0.05$ ) more potent ( $IC_{50}$ ~0.03  $\mu$ M) than R-PIA ( $IC_{50}$ ~1  $\mu$ M) upon reducing the force of spontaneous contractions (negative inotropism). Selective blockade of A<sub>1</sub>Rs with DPCPX (100 nM) and M<sub>2</sub>Rs with AF-DX 116 (10  $\mu$ M) shifted significantly ( $P<0.05$ ) to the right the concentration-response curves of R-PIA (0.3 nM-1  $\mu$ M) and Oxo (0.01-3  $\mu$ M), respectively. As expected, inhibition of GIRK/KIR3.1/3.4-mediated potassium currents with tertiapin-Q (300 nM) prevented the negative chronotropic and inotropic actions of A<sub>1</sub>R and M<sub>2</sub>R agonists. In contrast with surrounding cardiomyocytes, the SA node is recognized by showing positive immunostaining with anti-NF160 and for being negative for anti-conexin 43 antibodies under the confocal microscope. In spite of these features, we found no differences in the expression of A<sub>1</sub>R and M<sub>2</sub>R between SA node and surrounding cardiomyocytes. Moreover, immunolabeling against K<sup>+</sup> channel Kir3.1 (GIRK1) was abundantly and homogeneously expressed throughout the right atria.

Data suggest that adenosine A<sub>1</sub>R-mediated chronoselectivity in the spontaneously beating rat atria is, apparently, not due to differential expression of A<sub>1</sub>R between the sinoatrial node and the surrounding cardiomyocytes.

*Work supported by FCT (FEDER funding, UMIB-215/94). B. Bragança was in receipt of a Young Investigator Fellowship from FCT (BII/UMIB-ICBAS/2009-2).*



Oral Sessions VII > A2

# Legal Studies & Criminology II



## The costs of law enforcement in the drivers' crimes: The case of the *Tribunal de Pequena Instância* of Oporto

T. Dias<sup>1</sup> & P. Sousa<sup>2</sup>,

<sup>1</sup> School of Criminology, Faculty of Law, University of Porto, Portugal.

<sup>2</sup> School of Criminology, Faculty of Law, University of Porto, Portugal.

Since the 1970s there has been a growing interest in knowing the costs of crime in countries like France [1], United States [2] and United Kingdom [3].

However, in Portugal, one does not find scientific studies oriented to estimate the costs of behaviours that are defined as crimes by the criminal law and that require a response by the justice system when detected.

This study is motivated by the acknowledgement of that gap, and it aims to contribute theoretically, methodologically and empirically to the scientific discussion of this theme in Portugal.

In particular, this study focuses on the costs of law enforcement, namely the costs generated by the *Tribunal de Pequena Instância Criminal* of Oporto. The analysis confines itself to the crime of illegal driving, i.e., driving without license (article 3.º of Decree Law 2/98) and to the crime of driving while intoxicated or under the influence of narcotic drugs or psychotropic substances (article 292.º of Criminal Code).

For this study, we have selected the geographic area of the Oporto city, the time period 2007-2010 and, at this initial stage, the court's operation costs. With a sample of 411 cases (judged = 250; suspended = 161), we measured the average number of days that each individual process is in active status at the court, per type of crime, and in each stage of the process path.

At the present stage, we still can not provide final conclusions about monetary values. Instead, we are able to present some inferential results about the average time that a typical judicial process has occupied the functioning of the court, for each of these types of crime, computations that until now were not made with scientific methodology as we do. The crime of illegal driving, when it is judged, requires, on average, about 33.10 days to be solved (SD = 12.457), while the crime of driving while intoxicated requires 28.87 days (SD = 10.739). When the processes are suspended, the average duration is 11.22 days in the crime of illegal driving (SD = 3.219) and 13.62 in the crime of driving while intoxicated (SD = 3.397).

### References:

- [1] Robert, P. and Godefroy, T. (1977), *Le cout du crime ou l' économie poursuivant le crime*, Masson – Médecine et Hygiène, Genève.
- [2] Anderson, D.A. (1999), *The Aggregate Burden of Crime*, Journal of Law and Economics, 42 (2), 1-57.
- [3] Brand, S. and Price, R. (2000), *The economic and social costs of crime*, Home Office Research Study, 217.

# **Civil Liability for contents uploaded and transmitted on the Internet: The user generated content and the web 2.0. paradigm**

**João Pedro Fachana Cardoso Moreira da Costa**

Faculty of Law, University of Porto, Portugal.

On the first decade of the current millennium, Internet and the World Wide Web have been the stage of a unique technical and social evolution in all of its aspects. Today, the most visible feature of that evolution is the proliferation of tendentially free on-line services that allow Internet users to upload and transmit contents created or generated by them.

Under the advent of web 2.0. Internet users are not mere consumers of on-line services but true active participants in the creation and development of those services, through the contents they provide.

As a counterpoint of this amazing development of the web, there is an increase of unlawful acts on the Internet, that produce damages on third parties, mainly through upload and transmission of unlawful contents.

With this paper, we propose to talk about some of the main issues investigated in our Master's dissertation, presented and discussed last December. We intend to present the main issues regarding the application of the civil liability traditional dogmatic system to the present Internet space, in order to appraise the way that the civil liability approach can be used to act upon unlawful contents uploaded and disseminated in the web, mainly when these are generated by the actual users of Internet-based services.

In a first stage we will focus on key questions concerning civil liability of the user that uploads or disseminates unlawful contents in the web, as well as the problem drawn by anonymity in the network.

In a second stage, we will explain how civil liability concerning intermediary service providers whose activities are capable of distributing unlawful contents in the web is being held by some legal systems, in particular the Portuguese legal system, the uniform European legal system and the North American legal system.

Finally, we will try to debate if, under the paradigm of web 2.0, the current system keeps its original strength, when facing the new tendencies that are emerging in several law orders.

We conclude for the maintenance of the core of the regime of exemption of liability for intermediary service providers notwithstanding, at least, that the new web 2.0 reality will require an inversion of the burden of proof of liability exemption.

# **Nationalism and Extreme Right: The Portuguese Reality in Post April 25, 1974**

**João Caseiro**<sup>1</sup>

<sup>1</sup> Faculty of Law, University of Porto, Portugal.

There is no doubt that nationalism and extreme right are generally a taboo subject in society or in criminology Portuguese in particular. Although many researchers have argued that nationalism and the extreme right are a taboo in Western modernity, is increasingly accepted that the recent explosion of academic and popular interest in the subject precludes such an argument. There is now a vast body of literature on nationalism and the extreme right that has been developing during the last decade and this trend seems predisposed to continue. In a sense, then, nationalism and extreme right have a strong presence in Western societies.

However, while nationalism and extreme right are not a forbidden subject, a subject remains hidden in the sense that is usually sequestered from public space. There is a danger that a job like this one, may result in the creation of an "ideological criminology", a specialty within criminology, reflecting generally this hidden character of nationalism and the extreme right. I suggest that this danger must turn our attention to the more widespread kidnapping of nationalism and the extreme right of the public domain, the absence of considerations of right-wing nationalism and social life. Therefore, a criminogenic consideration of nationalism and the extreme right has to reflect on, and try to explain the apparent contradiction between the absence and presence of nationalism and the extreme right in contemporary societies, especially the Portuguese.

## **Abstract**

This review article is a theoretical introduction to Nationalism and Extreme Right: The Portuguese Reality in the Post 25 April 1974, which aims to fill a gap in the criminological overview Portuguese. Useful to the interested public and students need in their courses, to begin addressing a phenomenon that has reached global proportions. The reflection developed seeks to emphasize the importance of understanding nationalism and extreme right as a mechanism to raise awareness of criminal liability of organizations and institutions in general, and groups involved in particular. It approaches a subject - inextricably linked to Criminology - than their importance warrants a comprehensive study of concepts, its aspects, the social impact it has in contemporary societies - especially in Portuguese. Moreover, because it concerns a real phenomenon whose expansion is likely to harm a sizable segment of future generations, this approach provides some contributions to the understanding of nationalism and extreme right.

# When The Watchmen Take Your Watch: Corruption of Public Officials and its Causes, Consequences and Solutions

**Arv Ferreira da Cunha**<sup>1</sup>

<sup>1</sup> Faculty of Law, University of Porto, Portugal.

<sup>2</sup> Center for Legal and Economic Research (CIJE), University of Porto, Portugal

In modern history, all democratic political systems have been based upon the idea of popular sovereignty; uphold by a concept of citizenship in which the individual is the ultimate source of political legitimacy, and power; sustained by the notion that governments and rulers exist only to protect individual freedom and personal well-being. Public officials have the crucial role of being the agents of society chosen to serve its best interests. Citizens trust them with their property and freedom, so they can run public business, in a very similar way shareholders trust managers their money, so they can run private businesses.

Evidence from agency theory show us why managers and politicians alike have an incentive not to run public business according to the stakeholder's best interests. This happen when these agents work less then they should (shirking) or when they try to convince us to possess qualities they do not (adverse selection), for example, but it occurs in the most pernicious way when self-interest leads to corruption, instrumentalizing public power for private gain.

With such a great delusion about the political process, it's important for democracy not only to restate its founding principles, but, above all, for it to find new and rediscover old ways to restore trust among this wrecked relationship with citizens. Making policy focused on curving corruption should be a priority to those who aspire to that goal.

In this communication we compile and briefly analyze the latest scientific findings regarding corruption of public officials: on its causes, consequences and solutions. We will also have a and we try to a series of mechanisms that might be used to align interests between agents -- that is politicians -- and principals -- that is citizens -- fighting corruption and reestablishing the center of power in the relationship where it belongs.

We conclude that there is no "one size fits all" solution, that we need strong democratic institutions, and that this can hardly be done without redoubled vigilance and regained interest from society in political issues, setting a new paradigm of participatory democracy.

Oral Sessions VII > A3

# History & Cultural Studies II



## H2A Project – Heritage, History and Art

**P. Silva<sup>1</sup>, J. Mateus<sup>1</sup>, and H. Barreira<sup>2</sup>**

<sup>1</sup> Mestrando em História da Arte Portuguesa, FLUP.

<sup>2</sup> Doutorando em História da Arte Portuguesa, FLUP.

The H2A Project was born from the cooperation between three students of Art History from FLUP. Although transcending the usual research project, it had its origin and *modus operandi* directly connected with investigation methods of Art History, searching to provide, with its action, the same results to different types of public and, at the same time, to emphasize the role of the Art History researcher.

Formed in the context of *Educação Patrimonial* class of the degree in Art History, the first practical implement of the theoretical formulation was a platform/business company that would allow to transform and showcase the products of the scientific research in Art History, converted in services to the community. For that, a blog was developed (<http://h2aproject.blogspot.com>) showcasing a first prototype product: two tours of the Playhouses' Architecture in Porto (Theatre and Cinema). The tours have their foundation on the study about the cultural phenomena and the architectures that embodied them in the city, enabling the learning of the specifics of these buildings, their image and memory and their integration on Porto's architecture, as well as the cultural and social dimension of the shows they housed. Along with the study that served as base for the creation of the tours, a research on Patrimonial Education, Cultural Mediation and other related areas was made, leading to a new goal: to articulate the developed research with Heritage's safe keep.

From the background investigation to the information systematization, turning it in divulgation goods, H2A always values the accuracy of the developed knowledge, opposing to excessive simplifications that denies quality cultural and patrimonial mediation. These kinds of simplification are so many times the main offer in this area, and the absence of scientific and professional foundation on the preparation of the displayed data adds to neglect that inflicts our heritage.

H2A adapts to diverse types of public (national, foreign, of different ages and backgrounds), on more or less didactic context, according to the needs. Having as a base the specific value that the research practice in a broad spectrum science as Art History confers, H2A develops cultural products where scientific knowledge, properly suited, allows the public establish a better and healthier connection with their Heritage.

Opposite to the individualism that investigation practice often incurs, H2A lives of the permanent sharing of knowledge and thought between three young researchers, seeking the safe keep and value of Heritage and Culture and the role of Art History in dialogue with various publics. On these foundations, against the harsh Present, we believe that dynamic and effective mediation of the signs of the Past will concur to edification of a better and solid Future.

## Casa Amarela; Collectivization and Resistance

C. do Couto Soares<sup>1</sup>

<sup>1</sup> Department of Communication Sciences, Faculty of Letras, University of Porto, Portugal.

Among all the forecasts for the twenty-first century which led to demonstrations, have confirmed a more painfully, the decollectivisation." Every moment the world ceases to be a little more collective to be a little more individual. The slogan "One for all and all for one" has given way to "save yourself if you can" show and mobilization, solidarity and collective works have become rare, especially when they relate to youth.

The same youth was, in other decades, more than faithful representation of the community, with its revolutionary student movements and "alternative societies" to reverse charge "adult", is today surrendered before the forms that move, but do not add the comfortable social networks and the like. However there is still timidly, numerous collective organizations that arrange themselves in order to stand out against this apocalyptic reality. This, for "personal and cultural differences survive despite the internationalization of capital and technical and scientific rationalism. And the mass industrial society can be perceived as totally hyper-differentiated classes, people and ethos, where each one is motivated by factors specific to their contexts to build a sense for their particular existences, through pipelines driven by normative standards (ethical) and recreational (aesthetic) collectively created and established " 1

This is an example of the Casa Amarela Project, based at the Galleries of Paris Street, 34, which concentrates more than painted walls and poetry written in chalk. The Casa Amarela brings the ideal of an independent youth, busy with art, motivated policies that go beyond the opposition and situation, and therefore, chosen as the bias of this investigation.

The survey went on two methodological forms, focus groups and in-depth interview. The first being defined as "a fast, easy and practical to put in touch with the people who want to investigate,"<sup>2</sup> or "an informal discussion group and size, with the purpose of obtaining qualitative information on depth "<sup>3</sup> and the second corresponding to the individual interview.

After a few months employees living in constant with the Collective, the investigation resulted in the realization of a video documentary about the project and his activism, and the theoretical explanation about the actual mechanisms of mobilization.

References:

[1] VIANNA, Leticia C. R. "The Media Age: A reflection on myth of youth in mass culture." Anthropology 121 Series. Brasília: UnB, 1992. Available at: <http://vsites.unb.br/ics/dan/Serie121empdf.pdf> .

[2-3] Neto, O, C. Moreira, M, R. Sucena, L, F, M. Focus Groups and Qualitative Social Research: the debate as technique-oriented research. 2002. Unicamp, Proceedings. Available at : [http://www.abep.nepo.unicamp.br/docs/anais/pdf/2002/Com\\_JUV\\_PO27\\_Neto\\_texto.pdf](http://www.abep.nepo.unicamp.br/docs/anais/pdf/2002/Com_JUV_PO27_Neto_texto.pdf).

## José Júlio de Souza Pinto na Bretanha

### A. Santos<sup>1</sup>

<sup>1</sup> Department of Heritage Studies, Faculty of Arts, University of Porto, Portugal.

José Júlio de SOUZA PINTO (1856-1939) was a Portuguese naturalist painter of the first generation, and developed much of the work studied in this dissertation in France, more specifically in Pont-Scorff at Bretagne.

His formation in Paris with Professor Cabanel has consolidated his formation in Academia Portuense in which allowed him to find his unique and unmistakable artistic personality.

His works produced in Brolles *Calções Rotos*, *Macieira Partida*, *Hóspede Inconsolável*, *Viandante* and *Volta do trabalho* were reflected in several awards at the *Salons* that he ran, achieving the *hors-concours*. He produced in Portugal *Molhado até aos ossos* and *Espera dos barcos* which transmits a refined lusitanian sense.

In Bretagne he developed a naturalistic thematic of its own, both at pastel and oil with verdant scenes highlighting the laundresses of Pont-Scorff, children playing along the river or tender fishing scenes. The excellence of those works led to be high demanded and even reproduced in engravings.

Especially during the First World War, the artist using the portuguese light knew how to develop and use the impressionism light materializing it through the form in his paintings of Francelos, Miramar, Valongo and Caldas da Rainha.

José Júlio de Souza Pinto was recognized among the best painters and is considered an essential pastel handler of his time. In his vast work of more than sixty years, he always remained faithful to the school that formed him, either in rigor of drawing as in composition, discovering the freedom and expression in the application of the technique of colour having strengthened his artistic individuality and persevering personality.

He developed in Bretagne most of his work and was recognized with respect and esteem among the population and the social and cultural communication following up his stated artistic value in the parisian society in which he was integrated and accepted as being one of them. In 1900 he accepted the invitation to join the jury of the painting exhibition at the Universal Exposition in Paris.

Sousa Pinto knew how to capture attention of his work enabling him to live in wealthy allowing him to make an artistic career in Europe, not forgetting his homeland.

# Inventory of Pictorial Portraits of Bishops of Porto

M. Conde<sup>1</sup>

<sup>1</sup>Department of Heritage Studies, Faculty of Arts, University of Porto, Portugal.

This work is essentially a pictorial survey of the portraits of the Bishops of Porto, to the realization of its inventory.

Due to the large number of sculptures and paintings found, we decided, to meet the deadline set for the project, to select the artistic genre of Painting, leaving the Sculpture work to a later stage.

The theme of this research - Inventory of Pictorial Portraits of Bishops of Porto - is based on a publication in 1992 on the collection of Portraits of Bishops of the Porto's Episcopal Palace.

We found appropriate to update this catalog and to search for pictures of the Bishops of this city located outside the Episcopal Palace.

After studying / biographical summary of each prelate, the places where he staged and exercised his teaching, we made maps of the places where there are representations, chips and tokens biographical inventory on each painting. We detected two painting types: some pictures with the artist's signature and others only with the Bishops "representation".

During the project we were able to add a significant collection of new works.

Indeed, to the 23 works originally known, we added 26 paintings, making a total of 49, which were located:

- 7 in the city of Porto.
- 16 in other five cities of Portugal.
- 3 abroad.

This geographic dispersion enables to evaluate the effort and time that were required to accomplish this task which result we understand, exceeded the initial expectations to available term.

It is noted that this project only was possible thanks to the reception received from the several entities visited and very particular thanks to the collaboration of Oporto Diocese, institution that from the beginning engaged like a partner in the project realization.

Keywords: Inventory, Portrait, Painting, Oporto's Bishops

## For the practice of Dance Criticism as a form of Legacyis

Vera Santos<sup>1</sup>

<sup>1</sup> Department of Art Sciences Group, Faculty of Fine Arts, University of Porto, Portugal.

Art criticism lives on artistic experience while fostering it simultaneously. Both artistic creation and artistic criticism are inscribed in art history, and art theory has the task of distinguishing them, so we can understand its tangible objects better. When we reflect on the practice of criticism as a mediation activity and consider its task of establishing reception contexts, it is important to highlight the facilitating role of art criticism (and theory) throughout history. In fact, it has dealt with issues such as the sense of beauty and technique, the relationship between art and nature, authorship and original creativity, representation and identity in confrontation with reality, death or freedom from history or from new technologies. If, on the one hand, the production of recurrent criticism is necessary, and it should follow artistic production while being external to it, on the other hand, documentation on that production is required for the archives of art history.

Dance remains a transient art form, even when it is related to other art forms and increasingly connected to different areas of knowledge. Presumably, it does not exist any other support and transaction system as complete and adequate to dance as the body itself, which substantiates dance. After experiencing fruition from choreographic works, it matters to acknowledge the permanence of the artistic production legacy and, also, of the ideas which communicate its historical, artistic and cultural context into the future. This legacy, free from the body, deserves particular interest as it is unrepeatable. A way to preserve it is by establishing inventories, connections, materializing its presence in a universe in which the legacy can once again be an object of fruition, with a sense of collective belonging.

This is the framework for the practice of dance criticism as a form of legacy. Arising from the memory and the repercussion of the choreographic work over time, since its disappearance, first in the eyes of the audience (when the audience believes that something is emerging in front of it) and, later, when the memory of the choreography is obliterated from the dancer's body.

For the purpose of this paper, I have isolated a case study which might provide an illustrative practical analysis of the possibilities of the proposal for interpretation and analysis that I have brought forward. The piece being analysed is “como rebolar alegremente sobre um vazio interior”, a creation by Vera Mantero for Ballet Gulbenkian in 2001. To this end, we analyzed the company and personal files, interviews and cross-memories (written, sound, graphic and oral) resulted in a critical analysis of the piece that, after 10 years would be difficult to recover from any one element alone.

The result is a historical (and distant) reading, an interpretation of the artistic process and the artistic issues under the watch and the cross memories (also distant). It's a critical that confronts a heritage detached from the body, resulting from the dance.

## Who is going to the movies in *Grande Porto*? A profile of the consumer

A. Matos<sup>1</sup>, R. Silva<sup>1</sup>

<sup>1</sup> Master's Degree's Student in Communication Sciences, Faculty of Letras, University of Porto, Portugal

In the absence of a cinema consumer profile in *Grande Porto*, it was established the need to explore this theme. Who is going to the movies in this area? Which are the most visited movie theatres? How often people are going to the movies? These are some of the questions that represented the starting point of this study, which includes a new subject: would people be interested in having a new cinema in Oporto downtown? How much they will be willing to pay?

For this purpose, we conducted an online survey in the Google Docs's platform, which was released large-scale on social networks, obtaining a total of 326 answers. Accepting the limitations that an online survey can bring - the representativeness of the sample - the authors consider that this article is a pilot study and can be a starting point for further investigation. We also used the SPSS software, in order to cross some variables, and we achieved very interesting results.

Contrary to what we expected, we found that the majority of the young people and students of our sample rarely go to the movies. On the other hand, we found that the unemployed and people over the age of 44 years go "twice a month" to the movies.

Regarding the possibility to create a movie theatre in Oporto downtown, we found that, even against a backdrop of crisis, people were willing to pay more for ticket in these conditions. Another finding of our study is that people don't want a different movie theatre, open air, for example, but a commercial one, just like in the shopping centers.

The main conclusions of this research are that young women, students and single, aged between 18 and 24 years, interested in North American comedies and dramas, shape the current profile of the cinema consumers of Grande Porto. These women go once a month to the movies. A vast majority of our sample said "yes" to a movie theatre in Oporto downtown.

## **A blogosfera como plataforma privilegiada para as notícias hiper-regionais: Associações desportivas de Vale de Sousa**

**Varandas, M.<sup>1</sup>, Duarte, A.<sup>1</sup>, Mário, A.<sup>1</sup>**

<sup>1</sup>Departamento de Jornalismo e Ciências da Comunicação, Faculdade de Letras, Universidade do Porto, Portugal

This paper is about the study of the blogosphere as a privileged platform to the hyper-regional news. In it are exposed some perspectives, concepts and reflections about the access and use of blogs to the publication of information. It is our aim to understand in what measure the regional newspapers use blogs as source, as it is to expose the influence and use that blogs have to the regional press. On this case, we intend to analyze the blog importance to the sports journalism in the region of Vale do Sousa. Thus, we propose to make a discourse analysis about the journalism made in four online regional newspapers, comparing it to six blogs, regarding to regional sports.

In a first step, it is studied the exponential growth of blogs and their definition of concept and utilization. Sometimes they serve as an expression of will that people have to be heard and understood, which results in new models of mass communication and in new information systems.

All of this to reach the goal that is to detect which is the blog application in the information services and what the professionals of communication can achieve with that advantage.



Oral Sessions VII > A4

# Public Health & Epidemiology



## Susceptibility profile of deep-seated yeasts isolates from a university hospital in the northern region of Portugal.

I. Faria-Ramos<sup>1</sup>, J. Maia<sup>1</sup>, P. Tavares<sup>1</sup>, S. Farinha<sup>1</sup>, J. Santos-Antunes<sup>1</sup>, A.G. Rodrigues<sup>1,2,3</sup>, C. Pina-Vaz<sup>1,2,4</sup>

<sup>1</sup> Department of Microbiology, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> Cardiovascular Research & Development Unit, Faculty of Medicine, University of Porto.

<sup>3</sup> Burn Unit, Department of Plastic and Reconstructive Surgery, Hospital S. João, Porto.

<sup>4</sup> Department of Microbiology, Hospital S. João, Porto.

A prospective, observational study was conducted at the biggest hospital in Portugal, aiming to evaluate the epidemiology of fungal infections.

Between September 2010 and 2011, all yeasts isolated from sterile body sites of the patients admitted at a university hospital of Porto were collected; urinary samples were excluded since they often represent colonization and not true infection. Clinical and demographic data were registered. Antifungal susceptibility testing was performed regarding 8 antifungals. Chi-square test and multivariable logistic regression were used to analyze data.

One hundred and sixty four isolates were identified from blood (61.5%), peritoneal liquid (29.5%), cerebrospinal fluid (6.4%), and pleural effusion (2.6%) cultures. Fifty-two percent were *Candida albicans*, followed by *C. glabrata* (15.2%), *C. parapsilosis* (10.9%), *C. lusitaniae* and *Cryptococcus neoformans* (5.4%), *C. krusei* (3.3%), *C. tropicalis* and *C. dubliniensis* (2.2%) and, finally, *C. kefyr* and *Trichosporon mucoides* (1.1%). Most isolates were collected from the Surgery department (38%) and Intensive Care Units (19.6%). Most patients were aged between 41 and 60 years (39%) and 67% were male. Regarding antifungal susceptibility testing, 16.3% of all strains showed resistance to fluconazole (FLU), mostly *C. albicans* (42.9%); 4.7% were resistant to voriconazole and posaconazole (all *C. albicans*) and 2.3% to 5-flucytosine (*C. krusei*) and caspofungin (*C. parapsilosis*); no resistance was found to anidulafungin, micafungin or amphotericin B.

Epidemiological studies concerning fungal infections are rare in Portugal, but extremely valuable. Overall, this study showed that *C. albicans*, *C. glabrata* and *C. parapsilosis* were the most common species and little resistance was observed being FLU the antifungal with the most associated resistance.

# Characterization of the oral fungal microbiota in smokers and non-smokers

**F. Monteiro-Silva<sup>1</sup>, R. Araujo<sup>2</sup>, B. Sampaio-Maia<sup>1</sup>**

<sup>1</sup> Department of Microbiology, Faculty of Dental Medicine, University of Porto, Portugal.

<sup>2</sup> Institute of Molecular Pathology and Immunology of the Porto University, Portugal.

The dynamic oral microbiome is an essential component of the on changing balance between health and disease. Although overlooked in the majority of published studies, fungi are also important components of the oral microbiome [1, 2]. Smoking has several deleterious effects on human health, including a variety of changes in the oral cavity. Its effects on oral bacterial have been well characterized, but its impact on fungal population is still largely unknown [3-5].

Thus, the purpose of the present investigation was to characterize the oral fungal microbiota – yeasts and moulds - of healthy young subjects and assess the effect of smoking in this mycobiome.

A group of 40 volunteer students from a Dental Medicine faculty, 20 smokers and 20 non-smokers, were selected. The final sample was composed by 26 females (65%) and 14 males (35%), with a mean age of 24.0±2.8 years old. Clinical examination was performed to obtain caries prevalence and plaque index. Oral rinse was collected and incubated in a fungi selective medium at 25°C and 37°C for 7 days. The final number of fungal colonies was obtained and the fungi identified, when possible, based on macro and microscopic morphology.

All subjects showed fungal growth, 38% yeasts and 62% moulds (at 25°C). The most prevalent mould genera identified were *Penicillium* and *Aspergillus*. Smokers' presented significantly higher levels of yeasts in samples incubated at 25°C and higher levels of moulds potentially pathogenic, in comparison to non-smokers.

In conclusion, the characterization of oral microbiome is still not completely clarified. Fungi's role on the oral microbiota equilibrium, including the overlooked moulds, is apparently more relevant than previously perceived. Tobacco smoking appears to alter the oral mycobiome, facilitating the colonization of oral cavity.

## References:

- [1] Kuramitsu HK, He X, Lux R, Anderson MH, Shi W. Interspecies interactions within oral microbial communities. *Microbiol Mol Biol Rev.* 2007 Dec;71(4):653-70.
- [2] Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, et al. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. *PLoS Pathog.* 2010 Jan;6(1):e1000713.
- [3] Heikkinen AM, Pitkaniemi J, Kari K, Pajukanta R, Elonheimo O, Koskenvuo M, et al. Effect of teenage smoking on the prevalence of periodontal bacteria. *Clin Oral Investig.* 2011 Feb 22.
- [4] Haffajee AD, Socransky SS. Relationship of cigarette smoking to the subgingival microbiota. *J Clin Periodontol.* 2001 May;28(5):377-88.
- [5] Soysa NS, Ellepola AN. The impact of cigarette/tobacco smoking on oral candidosis: an overview. *Oral Dis.* 2005 Sep;11(5):268-73.

## Biofilm-production ability of widespread *Escherichia coli* clones

C. Montenegro<sup>1</sup>, Â. Novais<sup>1</sup>, L. Peixe<sup>1</sup>

<sup>1</sup> REQUIMTE. Department of Microbiology, Faculty of Pharmacy, University of Porto, Portugal

**Introduction:** Particular widespread *Escherichia coli* clones, frequently associated with urinary tract infections, have been responsible for the expansion of antibiotic resistance determinants worldwide. The reasons motivating their successful spread and maintenance in diverse settings and environments remain to be elucidated. The aim of this study is to assess the ability of representative *E. coli* strains belonging to phylogenetic groups A, B1, B2 and D isolates from different settings, geographic origins and time-frames to adhere and form biofilm on abiotic surfaces.

**Methods:** One hundred and seven representative *E. coli* isolates from phylogroups B2 (n=20 ST131), D (n=13 ST69, n=12 ST393, n=11 ST405), A (n=22 ST10, n=14 ST23 complexes) and B1 (n=8 ST155 complex, n=7 ST359) were analysed. They were identified in 9 European (n=89) and 2 South American (n=5) countries, the USA (n=9), Korea (n=3) and Kuwait (n=1) from 1980-2010. Isolates were responsible for nosocomial (59.22%) and community (13.59%) outbreaks, or were identified among healthy volunteers (11.65%), animals (12.62%) and environmental samples (2.91%).

The ability to adhere and form biofilm in abiotic surfaces was evaluated by a modified quantitative biofilm production assay. Briefly, 20µl of an ON culture (adjusted to 0.5McF) was incubated in 180 µl LB broth supplemented with 1% glucose (m/m) for 24h at 37°C in 96-well flat-bottomed plastic tissue culture plates (5 wells/strain). The wells were washed with 1% PBS, dried and stained for 15min with crystal violet (2%), rinsed off with tap water and solubilized with 33% (v/v) glacial acetic acid. The optical density (O.D.) was measured at 570nm using an ELISA microplate reader. Each assay was repeated at least 3 times. *E. coli* CFT073 and LB (1% glucose) without bacteria were used as positive and negative controls, respectively.

**Results:** Most strains were classified as weakly adherent (n=86), although non-adherent (n=3), moderately (n=15) or strongly (n=3) adherent strains were also identified. Weakly adherent strains belonged to ST131 (n=20), ST10 (n=20), ST69 (n=9), ST393 (n=9), ST405 (n=8) and ST23 (n=8), ST155 (n=7) and ST359 (n=5) strains with O.D. ranging between 0.13 and 0.43. Moderately adherent strains belonged to ST10 (n=2; 0.48<O.D.<0.73) or ST23 complexes (n=5; 0.27<O.D.>0.48), ST155 (n=1; O.D.=0.52), ST69 (n=2; 0.26<O.D.>0.36), ST393 (n=3; 0.30<O.D.>0.49) and ST405 (n=2; 0.25<O.D.>0.29). Two ST69 isolates from ready-to-eat salads as well as one ST405 strain from an hospitalized patient were classified as strongly adherent (O.D.≈0.5). Isolates categorized as non-adherent included 2 ST359 (O.D.≈0.1) and 1 ST23 isolate (O.D.=0.14).

**Conclusion:** Most of the isolates demonstrated a weak ability to adhere to abiotic surfaces, at least in the conditions tested. Presumptive biofilm production was inferred only in some strains (moderate or strong adherent) from different clonal groups. Interestingly all isolates belonging to ST131, a predominant and widespread lineage in human infections, were weakly adherent. Consequently, factors other than biofilm formation might be influencing the epidemiological success of these *E. coli* clones.

# Molecular and phenotypic characteristics of the emerging *Salmonella enterica* serotype 4,5,12:i:- (monophasic variant of *S. Typhimurium*) from Portugal

J. Mourão<sup>1</sup>, L. Dias<sup>2</sup>, L. Fernandes<sup>2</sup>, T. Alves<sup>1</sup>, C. Novais<sup>1,3</sup>, L. Peixe<sup>1</sup> and P. Antunes<sup>1,2</sup>

<sup>1</sup> REQUIMTE, Department of Microbiology, Faculty of Pharmacy, University of Porto, Portugal

<sup>2</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal

<sup>3</sup> CEBIMED, Faculty of Health Sciences, Fernando Pessoa University, Porto, Portugal

**Background:** *Salmonella enterica* serotype 4,5,12:i:-, a monophasic variant of *S. Typhimurium* (4,5,12:i:1,2), rank among the most worldwide common serovars isolated from humans and transmitted through the food chain. In order to better understand the recent emergence of this variant we characterize Portuguese isolates belonging to the serotype *S. enterica* 4,5,12:i-, obtained from different sources, in relation to resistance to antimicrobial and/or biocides.

**Methods:** The study included 132 isolates (2002-2010): human (n=115), food (n=9), environment (n=4) and piggeries (n=4) of the serotype *S. 4,[5],12:i:-*. Serotype was confirmed by PCR (gene *fljB* and intergenic region *fliB-fliA*). Antibiotic susceptibility to 10 antibiotics was tested by disk diffusion method (CLSI). Characterization of antibiotic and biocide resistance genes, plasmid and integron backbones (PCR, PCR-RFLP and/or sequencing), transferability and genomic location (*I-CeuI/S1* nuclease hybridization) was performed. Clonality was established by PFGE (*XbaI*) and *multilocus sequence typing* (MLST).

**Results:** All isolates except two (99%) were resistant to at least one antibiotic, being 93% multidrug-resistant (2-8 antibiotics). Resistance to sulfamethoxazole (Su, 92%), tetracycline (T, 91%), streptomycin (S, 88%), ampicillin (A, 67%), chloramphenicol (C, 45%), trimethoprim (Tr, 35%; *dfxA12*), gentamicin (G, 27%), nalidixic acid (4%) and kanamycin (3%) was detected. We identified three major groups associated with three different genotypes: i) European clone (n=48; 4 PFGE-types and ST34-SLV of ST19), ASSuT profile (*blaTEM*, *strA-strB*, *sul2*, *tetB*), *pcoD*<sup>+</sup> and *merA*<sup>+</sup>, all chromosomally located; ii) MDR Spanish clone (n=45; 2 PFGE-types; ST19 and phage type DT104/U302) most of them ACGSSuTTr phenotype (n=27; *blaTEM*, *cmlA1*, *aac(3)-IV*, *aadA*, *sul1-sul2-sul3*, *tetA*, *dfxA12*), with *merA*, atypical (*estX-psp-aadA2-cmlA1-aadA1-IS440-qacH-sul3*) and conventional (*dfxA12-orfX-aadA2-qacEd1-sul1*) class 1 integron on large non-conjugative IncA/C plasmids; iii) CSSuTTr type (n=15; ST19; *cmlA1*, *aadA*, *sul3*, *tetB*, *dfxA12*), carrying an atypical class 1 integron (*dfxA12-orfF-aadA2-cmlA1-aadA1-IS440-qacH-sul3*) on large, non-conjugative IncR plasmids.

**Conclusion:** The spread of three MDR genotypes belonging to worldwide spread clonal lineages in association with biocide resistance determinants (*pcoD*, *merA*, *qacEΔ1* and/or *qacH*) might account for the recent emergence and success of this serotype.

**Acknowledgments:** This research was supported by Universidade do Porto/ Santander Totta “Projectos Pluridisciplinares 2010”.

## Opinions of Caregivers concerning school meals

Carvalho A.<sup>1</sup> e Rocha A.<sup>1</sup>

<sup>1</sup>Faculty of Nutrition and Food Science, University of Porto, Portugal.

Introduction: Childhood is a crucial step in acquiring a healthy lifestyle. It's central to focus the intervention in the two most important poles of nutritional education in preschool children: Caregivers and school. Objective: Evaluate the opinions of caregivers in relation to school meals. Methodology: A survey was developed and applied to caregivers of three types of kindergartens (public, semi-private and private). Demographic and socio-economic data and opinions on various parameters related to school meals were collected. Results: 85.5% of respondents usually check the menu, but 56.8% do not plan meals at home according to the school menu. The consumption of soup was the one referred to be encouraged. It was given little importance to egg and bread and it was privileged bottled water (85.7%) instead of public water. Salt reduction was the item which importance is prominent (84.4% on the soup and 86.8% on the plate). The elaboration of menus by a Nutritionist and the inclusion of the nutritional composition of meals were also valued parameters by caregivers. Conclusions: This study was a preliminary approach in the perception of the criteria valued by caregivers in relation to school meals. There is a need to demystify the "myths" in relation to egg and public water consumption. It is essential to give feedback to caregivers about the choices made by schools to encourage healthy eating habits so that food education can be effectively extended at home.

## Characterization of school food service of Portuguese Municipalities

**M. Barbosa<sup>1</sup>, H. Ávila<sup>2</sup>, A. Rocha<sup>3</sup>**

<sup>1,3</sup> Nutritionists, Faculty of Nutrition and Food Science, University of Porto, Portugal.

<sup>2</sup> Nutritionist; Portuguese Association of Nutritionists, Portugal

In Portugal, the responsibility of school meals was transferred from the Ministry of Education to the municipalities. This work aims to identify the measures that local authorities are taking to ensure healthy and safe meals, the food policies developed, as well as examine the framework of the nutritionist in the municipality. An on-line questionnaire was developed for collecting data. 109 municipalities responded to the questionnaire, corresponding to 39,2% of the total municipalities from mainland Portugal.

It was observed that the school food service is mostly leased and in large part to private catering companies. The number of schools receiving transported meals is higher than schools with cooking units. Most municipalities do not have a nutritionist in charge of the school food service, or it is involved in the development of the contract catering in 58,7% of local authorities. Nevertheless, in 48,5% of the municipalities inquired there is a nutritionist. It was observed that in the academic year of 2010/2011, 48,6% had a Nutritional Education Program in course and 37,6% of municipalities have already developed an assessment of nutritional status of the school population.

This work reveals that the majority of Portuguese municipality assume the responsibility of school food service without formal technical support, pointing out to the need for assistance and intervention of specialized technicians and skilled in this area.

This finding assumes even more importance given the growing demand of municipal interventions in meeting the needs of health and well-being of local populations, as well as the number of children using the school canteens.

Oral Sessions VII > A5

# Architecture II



## **A Casa do Arquitecto Agostinho Ricca, Porto: 1963 / 1974**

**Joana Pastor**<sup>1</sup>

<sup>1</sup> Faculty of Architecture, University of Porto, Portugal.

Key words: single family home, domestic architecture, 20th century Portuguese architecture, client.

Not only does the context appears as a physical place but also as a cultural and subjective surrounding environment of the work.

As my interest lies in single family's houses I've decided to focus on the architect's own house. Therefore my case study is Agostinho Ricca's own home, built in 1963 with whose architectural language I identify myself.

The deep look into the architect's architectural concept through its own house is, in this chapter, used to understand certain aspects that usually are not so perceptible at first sight, aspects that can be found are inserted in his work pieces. In contrast with the methods which explore a more expository character of it's architectural concept, graphics were added to allow understanding better the idea behind the creation as well as it's implementation.

In order to do so, a presentation with detailed documentary expressions of the instrumental and disciplinary procedures will be used. It uses the architect's sketches which allows the perception of "an imaginary constructed reality". Consequently, key sketches for the project evolution and implementation are also included. After a thorough examination of a wide number of working documents such as sketches, meticulous drawings, construction process details and photographs of the house are included in this project in order to establish the endurance of the architectonical materialization. It is important to notice that the analysis will take place in two distinct time periods, firstly the analysis in the construction year, 1963, and secondly in the year of the house change, 1974.

I will try to expatiate the meaning and the relevance that this workpiece had for Agostinho Ricca as an architect and also as a client. In order to do so, I'll look back to the mid 20th century to see this house for it's "stories of experiences".

# **José Marques da Silva: From the trip to the project**

## **I. Miranda<sup>1</sup>**

<sup>1</sup> Faculty of Architecture, University of Porto, Portugal.

The present dissertation is a reflection on the issue of travel in the act of projecting and its important contribution to the understanding of the project and the work, of the architect and his architecture.

Considering the present work also as a journey, the first chapter, “Departing”, presents the object of study, sets up the final objectives and displays the structure chosen for the development of all research.

The second chapter, “Travel”, outstands a theoretical approach to the proposed topic and it establishes the basic concepts for the understanding of the work that follows. This chapter presents travel as an important tool for the formation of the architect himself, which is further supported with case studies, and its influence upon him. It also contextualizes a trip throughout Portuguese architecture, which is also illustrated with examples.

The third chapter, “About The trip of Marques da Silva,” is defined by the approach to the object of study and is divided into two parts, the first of which leads us to get to know the architect, his identity and his formative path, whose description is indispensable to the necessary understanding of the second part. This second part conveys a research and an analysis on seven of the several trips made by the architect, each one structured according to trip motivations, destination and route and log analysis, this one providing vital information to the development of the fourth chapter.

The fourth chapter, “From the trip to the project”, is also divided into two parts: the first, consisting of the analysis to the case study, the Theatre of S. João, comprises one first moment where the whole process of design and construction of the project is described, and a second moment where the possible influences gathered by the architect while travelling are identified; in the second part of this chapter, it is reinforced this process of identification of common elements observed during his trips, but applied to other case studies.

The fifth and last chapter, “Arriving”, the initial objectives of the work become explicit.

# Photography, design process reading by images

I.Gomes

Department of Architecture, Faculty of Architecture, University of Porto, Portugal.

*Photography, design process reading by images*, develops an analysis on images of architecture, in their conception and production of their meaning. The development is aimed to be critical and theoretical, with respect to the potential that works produced by different contemporary photographers induce in a reflected design process practice.

Architecture and photography communicate in order to establish a dialogue in which the contagiousness of the photographic glance of architecture, and the influence of the architectonic work in Photography, promotes the discovery of the identity of the two areas, and in particular, it contributes, as we intend to defend in this essay, for an evolution of a project practice which is conscious and responsible.

The dissertation is divided into three broad chapters: artistic architectural photography, documentary architectural photography, and photography of architecture.

Through a study of the Authors Hiroshi Sugimoto and Andreas Gursky, the first chapter expressed an interpretative view and one intends to understand the way in which this aspect can benefit, at an initial stage, the concept of the project. The second chapter, with the study of documentary photography, intends to analyse the importance of the record of "landscapes" in the construction of a more conscious urban and sociological knowledge. In this sense, we seek a reading of the problematics of the city that the chosen authors, Michael Wesley and Gabriele Basilico, raise so well. The third chapter, concerning the photography of architecture, explores the advantages that photography offers as a tool in the project process: from its use as a database related to a record and the study of constructive details, of textures and of colours; to the simulation of a real relationship, spatial and volumetric of the models. Photography as an indispensable tool in communicating the project is explored, and one approaches the relation between the architectural piece and its aesthetic properties, within the scope of a visual culture. The limits between the shape and the contents of the image are questioned, emphasising an aesthetic structure. One debates with architecture photographers, Daniel Malhão and Miguel Coelho, the image as concrete and operational object, as well as fiction and achievement, as a result of what was thought, as matter and apprehension.

The conclusion presents a practice of image and expression of the architectonic thought, delator of critical potentialities and of a formal search. One consolidates with its apology alerting, however, for the incongruities that it might generate, evidencing a defense of such instrument, as a generator of a theoretical thought and reflected on the practice of architecture.

In the interdisciplinarity between analytic speech and poetic exploration, this image based reading of design process investigates a correspondence between different expressions of photography and the conception, development and communication of architecture.

## Archaeology of drawing – mapped Rome

**Paulo Azevedo<sup>1</sup>, M. Teresa Fonseca<sup>1</sup>**

<sup>1</sup> Faculty of Architecture, University of Porto, Portugal.

Roma's cartography is perhaps the most complete and former of the Western world that accompanied its history since the founding of the city. The discovery of the iconographic heritage associated with all this long history of a single city, and the direct exploitation of space in the present for almost two years, as a result of the first year developed under ERASMUS mobility and a second-year self-study for the purposes of Integrated Thesis, directed by Prof. Dra. Teresa Fonseca, constitute this work's subject.

In an ambitious thorough selection process of existing iconography from the beginning of the III century, accompanied by extensive bibliographic research, it gives a kind of archaeology of the space representation. That is to say, we want to write a text which creates an explanation of the city image from its representation mode.

Rome's image, in each of the eight phases chosen by obvious impacts in the city formation, presents itself as a current project, turning the story into space. Using different data, both bibliographic and iconographic, it was reconstructed a clear and simple picture of the city in digital form.

With this method, a memory strategy, it is proposed an urban recognition of cities with an applicable process to other contexts, which registers the urban evolution with current means.

## **Architectural Interactive Surfaces Modeled in Composed Materials: The Multi Sensorial Stimuli**

**M. Lucas<sup>1</sup>, Eng.º J. Lino<sup>2</sup>, Arq.º G. Furtado<sup>1</sup>, Eng.º N. Lopes<sup>3</sup>, Eng.º J. Lopes<sup>4</sup>**

<sup>1</sup>Faculdade de Arquitectura da Universidade do Porto; <sup>2</sup>Faculdade de Engenharia da Universidade do Porto; <sup>3</sup>Instituto Politécnico de Leiria; <sup>4</sup>Faculdade de Ciências e Tecnologia da Universidade de Coimbra

The project consists of a multidisciplinary investigation developed with the support of the Oporto University and Santander Bank. The investigated components are supposed to lead us to a multisensorial experience of space to a surface.

In a time when society is becoming increasingly affected by the technological and digital world, the connection to electronic engineering and to computers is inevitable. Bind so architecture cannot be indifferent before this scenario and the greatest challenge is to bind the existing materials to the technological elements in order to create new patterns of experience. Conceptually, the reasons for this cross can be varied: interactivity, communication, understanding of space and/or its “deconstruction”, “education of behaviors”, and so on.

The project pursued the idea of stimulating as many senses as possible, trying to assemble the greatest number of interactive moments in order to contribute to a better understanding of space or simulating other space’s possibilities. All the components have both the capacity of emphasizing reality and simulate/create “other realities”.

The proposal consists of a unit of modular nature – a parallelepiped of 0,60 x 0,60 x 0,10 meters, in polyester with fiberglass, and a flexible covering on its frontal surface. The choice of the materials for the sheath based itself on composed materials. In the inner side of the module operates the “heart” of interaction. It is in the inside of the module that the treatment of “Inputs” and “Outputs”, key-elements of all the wanted communication takes place.

The acoustic features, temperature, the human body position, the sounds and the touch validates all the interactive experiment. Microphones, sprays, RGB Leds and servomotors perform, in such way a rhythmical dynamism to the module and to the surface.

The module will be able to be assembled multiple times, according to the number of modules one intends to get, possessing its own autonomous power supply, turning it into something capable of boosting any given space in which it might be inserted.

Finally, and once again we emphasize that this presentation entitled “Architectural Interactive Surfaces Modeled in Composed Materials” only was possible due to a multidisciplinary investigation, with the contribution of different areas such as informatics, mechatronics, design, architecture, and materials science.

# An Investigation into the Use of Cement-based Materials as Alternative Printmaking Substrates

**C. Marques<sup>1</sup>, L. Esteves<sup>2</sup> and G. Machado<sup>1</sup>**

<sup>1</sup> Department of Drawing, Faculty of Fine Arts, University of Porto, Portugal

<sup>2</sup> Department of Civil Engineering, Porto Engineering Institute, Portugal

This work [1] aims to test the efficiency of using artistic printing techniques to directly imprint images into cement-based materials.

Fine art print studio methods such as etching, lithography and relief printing are tested on a new type of support: concrete. The concrete formulation is adjusted to meet specific artistic requirements, from which optimized final objects become part of an international exhibition held in France in 2011. All methods used are based on a direct transfer of matter (ink), opposite to what happens with other known methods for printing in concrete, which derive from an indirect transfer based on surface retarders.

This project allows selecting photopolymer plates for its outstanding capability to reproduce permanent and detailed photographic or drawn images into the cement-based system. (Fig. 1)

Therefore it is possible to use fine art print studio methods and materials to transfer images into cement-based slabs with remarkable quality, interchanging printmaking workshops with industrial sites. This multidisciplinary investigation involving Fine Arts, Engineering and Architecture defines a new creative potential for practical applications of printmaking into concrete, challenging with complexity and variety.

By means of a broader range of printing possibilities, the research contributes to the expansion of printmaking substrates as well as to define new expressions for concrete.

[1] IJUP multidisciplinary project number 136.

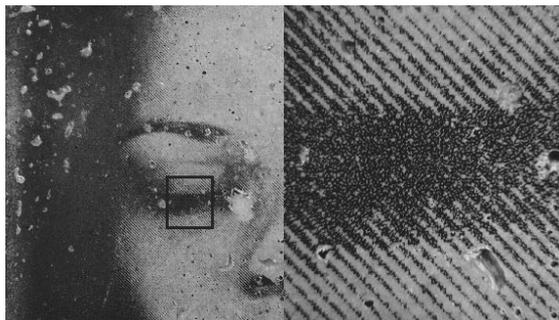
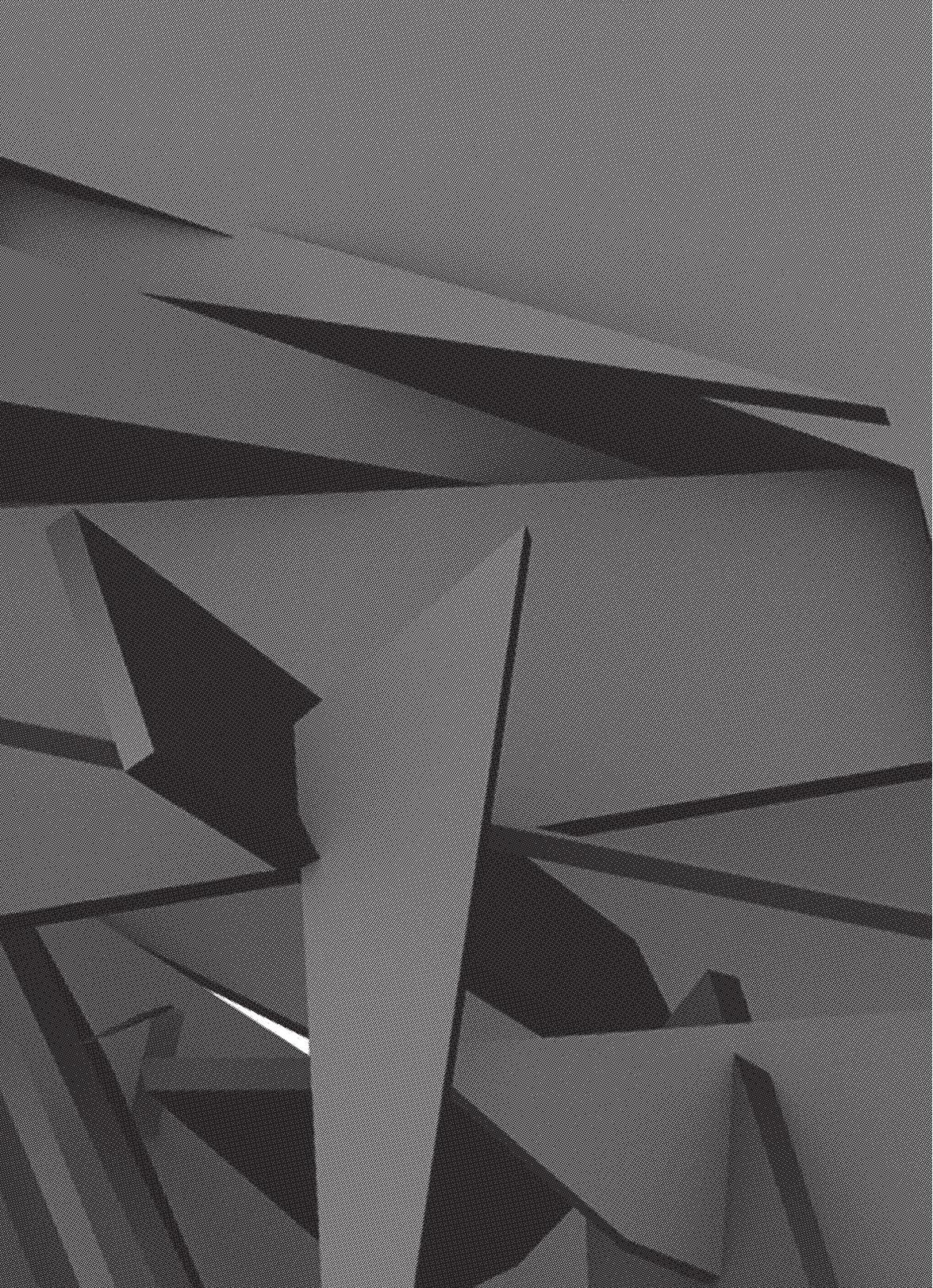


Fig. 1







# Oral Sessions VIII



Oral Sessions VIII > A1

# Molecular Medicine II



# Cortical Thymic Epithelial Cells are controlled by lympho-stromal interactions during thymic development and are regenerated upon genotoxic aggression in the adulthood.

**A.R. Ribeiro<sup>1,2,3</sup>, P. Rodrigues<sup>1,3</sup>, H. Schmid<sup>1</sup>, A. Moreira<sup>1</sup>, A. Carmo<sup>1,2</sup>, J. Di Santo<sup>4</sup> and NL Alves<sup>1</sup>**

<sup>1</sup> Instituto de Biologia Molecular e Celular (IBMC), Porto, Portugal.

<sup>2</sup> Instituto Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Portugal

<sup>3</sup> Faculdade de Ciências, University of Porto, Portugal

<sup>4</sup> Institut Pasteur, Paris, France

The thymus provides a specialized microenvironment for the development of T cells. Thymopoiesis is not a thymocyte-autonomous process and depends on unique signals provided by thymic epithelial cells (TECs). There are two known subsets of TECs: cortical (cTECs) and medullary (mTEC) that derive from a common precursor. Their differentiation relies on reciprocal signals provided by developing thymocytes. Still, little is known about the molecular and cellular mechanisms involved in TEC maturation.

Interleukin 7 (IL-7) is an essential thymopoietic cytokine predominantly expressed by TECs. Using IL-7 reporter mice, in which yellow fluorescent protein (YFP) expression identifies a primitive TEC subset that express high levels of *Il7* ( $Il7^{hi/YFP+}$  TECs), we studied the lineage relationship between  $Il7^{hi/YFP+}$  TECs and the two classic epithelial subsets throughout thymic organogenesis. We found that  $Il7^{hi/YFP+}$  TECs (1) emerge early during thymic development, (2) progressively acquire, and retain, a cortical phenotype ( $CD205^+BP1^+CD40^{dim}$ ), (3) co-express cortical-associated thymopoietic factors (*Dll4*, *Ccl25*, *Psmb11*) (Fig. 1) and continuously segregate from  $CD80^+CD40^{high}Aire^+$  mTECs. The establishment of mature mTECs depends on RANK ligand, belonging to the TNF receptor superfamily, provided by developing thymocytes. Using fetal thymic organ cultures, we showed that the RANK-induced formation of  $CD80^+Aire^+$  mTECs and the thymocyte-mediated decline in  $Il7^{hi/YFP+}$  TECs are two independent events. Together, our results indicate that  $Il7^{hi/YFP+}$  TECs are a determinant of a cortical epithelial lineage and that distinct thymocyte-derived signals controlled the establishment of the cortical and medullary compartment.

In addition, we show that  $Il7^{hi/YFP+}$  TECs, which comprise a minor subset in the adult thymus, remerge upon thymic atrophy induced by  $\gamma$ -irradiation, but not glucocorticoid treatment. This reappearance is accompanied by a decrease in mTEC frequency and a regeneration of cTEC population, recapitulating earlier stages of thymic development. Our findings suggest that there is a subset of TECs that retain the functional plasticity to re-establish the cortical epithelium upon genotoxic aggression. The timely extension of this stress-induced response may be taken in consideration in therapeutic strategies aimed in fasting thymic recovery.

## Adenosine mediates a positive feedback mechanism facilitating ACh release from rat myenteric motoneurons through the cross-talk between A<sub>2A</sub>, M<sub>3</sub> and NK<sub>1</sub> receptors

**P. Marques, I. Silva, C. Vieira, M. Duarte-Araújo, F. Ferreirinha, T. Magalhães-Cardoso & P. Correia-de-Sá**

Laboratório de Farmacologia e Neurobiologia / UMIB, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS) - Universidade do Porto (UP), Portugal.

At the rat myenteric plexus, extracellular adenosine (ADO) is released as such through equilibrative nucleoside transporters existing in both neuronal and non-neuronal cell types [1]. ADO may also originate from the catabolism of ATP released together with acetylcholine (ACh) during long periods of nerve stimulation through the ectonucleotidase pathway. Endogenous ADO contributes significantly to maintain cholinergic neurotransmission at the myenteric synapse through the activation of facilitatory A<sub>2A</sub> receptors on cholinergic nerve terminals [2]. Recently, we demonstrated that muscarinic M<sub>3</sub> receptors activation facilitates ACh release via a mechanism that depends on ADO outflow leading to activation of excitatory A<sub>2A</sub> receptors [3]. As tachykinins (*e.g.* substance P) have also been implicated in the facilitation of cholinergic neurotransmission, we decided to investigate the interplay between A<sub>2A</sub>, M<sub>3</sub> and NK<sub>1</sub> receptors on evoked [<sup>3</sup>H]-ACh release in longitudinal muscle-myenteric plexus (LM-MP) preparations of the rat ileum. Stimulation-evoked (5 Hz, 200-3000 pulses) release of [<sup>3</sup>H]-ACh and adenine nucleosides (INO+ADO) were measured by liquid scintillation spectrometry and HPLC, respectively [3].

Using confocal microscopy, we showed that intramuscular interstitial cells of Cajal (ICC-IM) of the rat ileum are endowed with M<sub>3</sub> and NK<sub>1</sub> receptors, whereas A<sub>2A</sub> receptors are predominantly localized in VACHT-positive cholinergic nerve terminals. Activation of A<sub>2A</sub> receptors with CGS21680C (3 nM) facilitated [<sup>3</sup>H]-ACh release by 53±10% (n=4). The facilitatory effect of CGS21680C (3 nM) was abolished in the presence of (1) adenosine deaminase (ADA, 0.5 U/ml, n=8), (2) the selective A<sub>2A</sub> receptor antagonist, ZM241385 (50 nM, n=4), and (3) the selective M<sub>3</sub> receptor antagonist, J104129 (6 nM, n=5). Blockade of NK<sub>1</sub> receptors with L732138 (20 nM) only partially attenuated CGS21680C-induced facilitation of [<sup>3</sup>H]-ACh release. The muscarinic M<sub>3</sub> receptor agonist, oxotremorine (Oxo, 300 μM), also facilitated the evoked [<sup>3</sup>H]-ACh release (34±4%, n=3). Oxo-induced facilitation was prevented upon blocking both M<sub>3</sub> and A<sub>2A</sub> receptors with J104129 (6 nM) and ZM241385 (50 nM), respectively, but it was kept unaltered in the presence of the NK<sub>1</sub> receptor antagonist, L732138 (20 nM). When used alone the selective NK<sub>1</sub> agonist, s,m-Substance P (s,m-SP, 300 nM), increased [<sup>3</sup>H]-ACh release by only 16±4% (n=4). The effect of s,m-SP (300 nM) was significantly (*P*<0.05) enhanced after pretreating the preparations with (1) CGS21680C (3 nM, 48±8%, n=4), (2) EHNA (50 μM, 27±6%, n=5), which increases ADO accumulation by inhibiting ADA activity, and (3) forskolin (3 μM, 31±6%, n=4), an activator of adenylate cyclase activity. All receptor agonists, CGS21680C (3 nM), Oxo (300 μM) and s,m-SP (300 nM), increased ADO outflow from the stimulated myenteric plexus.

Data indicate that facilitation of evoked [<sup>3</sup>H]-ACh release by A<sub>2A</sub>, M<sub>3</sub> and NK<sub>1</sub> receptor agonists depends on ADO outflow (possibly from ICC-IM) leading to retrograde amplification of transmitter release via pre-junctional A<sub>2A</sub> receptors. Our results are consistent with ADO being a modulator of the modulators playing a role to control cholinergic neurotransmission at the rat myenteric synapse.

[1] Correia-de-Sá *et al.* (2006), *Auton. Neurosci.*, **127**, 211-24; [2] Duarte-Araújo *et al.* (2004), *Br J Pharmacol.*, **141**, 925-934; [3] Vieira *et al.* (2009), *Neurogastroenterol. Motil.* **21**, 1118-1128.

Work supported by FCT (FEDER funding, PTDC/CVT/74462/2006 and UMIB-215/94) and Univ. Porto / Santander Totta.

## Diclofenac interactions with lipid membranes - insight into the NSAIDs-induced gastrointestinal toxicity

**J. X. Soares<sup>2</sup>, J. Brittes<sup>1</sup>, M. Lúcio<sup>1</sup>, C. Nunes<sup>1</sup>, J.L.F.C. Lima<sup>1</sup>, C. Afonso<sup>2</sup> and S. Reis<sup>1</sup>**

<sup>1</sup> REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Portugal.

<sup>2</sup> Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto. \*jxaviersoares@gmail.com

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) well-known for its therapeutic value. Nevertheless, its use is frequently limited by gastrointestinal (GI) toxicity. Regarding this toxicity effects, several evidences point towards a pivotal role in assumed by non-prostaglandin-mediated effects, known as “topical” effects.[1,2]

The “topical” effects of NSAIDs are assumed to be a consequence of the ability of these drugs to interact with phosphatidylcholine (PC) in the gastric protective layer[1,2]. So, the diclofenac-membrane interactions were studied using a liposome model composed of DPPC, which is the most abundant phospholipid in the gastric mucus gel. This study was conducted at different pH values, in order to mimic the physiological pH (pH 7.4) and the acidic pH (pH 5.0) at the gastric mucus layer. Furthermore, diclofenac showed to act as uncoupler and as inhibitor of mitochondrial oxidative phosphorylation [3]. In view of this, another study of diclofenac-membrane interaction was also performed using a liposome model which mimics the mitochondrial inner membrane (DOPE, DOPC, CL (cardiolipin)). In this work, several biophysical techniques were employed: derivative spectrophotometry and fluorescence quenching studies to determine drug partition and location within the membrane; differential scanning calorimetry measurements for the thermoanalysis of the phase behavior; and synchrotron small-angle and wide angle X-ray scattering to evaluate the structural modifications.

The results obtained give new highlights into the mechanism of NSAIDs-toxicity since they indicate that diclofenac can modulate the membrane structure properties being capable of disturbing the membrane functional properties in both used models.

### References:

[1] Lichtenberger, L.M., et al. (1995), *Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: Insight into the mechanism and reversal of NSAID-induced gastrointestinal injury*, *Nature Medicine*, 1(2), 154-158.

[2] Lúcio, M. et al. (2010), *Drug-membrane interactions: molecular mechanisms underlying therapeutic and toxic effects of drugs* in *Ideas in chemistry and molecular sciences: where chemistry meets life*, Bruno Pignataro, Editor, Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany. p. 191-203.

[3] Krause, M.M., et al. (2003), *Nonsteroidal antiinflammatory drugs and a selective cyclooxygenase 2 inhibitor uncouple mitochondria in intact cells*, *Arthritis & Rheumatism*, 48(5), 1438-1444.

Acknowledgements: The authors thank HASYLAB at DESY, Hamburg, Germany, for beam time and support through the project II-20100139 EC.

# Changes in the purinergic modulation of high-affinity GABA and glutamate uptake in human epileptic brain

**A. Barros-Barbosa, S. Guerra-Gomes, F. Ferreira, M. T. Magalhães-Cardoso, M.G. Lobo, P. Correia-de-Sá and J.M. Cordeiro**

Laboratório de Farmacologia e Neurobiologia, UMIB, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Portugal

Excitability in the central nervous system (CNS) depends on a balance between GABAergic and glutamatergic inputs. Since both transmitters rely on high affinity transporters to terminate signaling, we sought to determine if their regulation by widely distributed endogenous neuromodulators, such as purines (e.g. ATP and adenosine), could have an impact on neuronal excitability in normal and epileptic human brain tissues. In this study, we tested the effects of stable adenosine and ATP analogues, which are specific ligands respectively for A<sub>1</sub> and P2X<sub>7</sub> purinoceptors, on high-affinity GABA and glutamate uptake by synaptic plasma membrane vesicles (SPMs) isolated from synaptosomes originated from cadaveric human brain neocortex (obtained from forensic autopsies). Radiolabeled neurotransmitter accumulation was evaluated by liquid scintillation spectrometry. The expression of A<sub>1</sub> and P2X<sub>7</sub> purinoceptors was evaluated by Western Blot analysis in cadaveric (control) specimens and in samples collected from patients with drug-resistant meso-temporal lobe epilepsy (MTLE) submitted to surgery for epileptic focus removal. All procedures were approved by the Ethics Committee of CHP-HGSA, IML-DN and ICBAS.

In cadaveric (control) samples, the uptake of [<sup>3</sup>H]-GABA and [<sup>14</sup>C]-glutamate decreased respectively by 60% and 46% in SPMs loaded with ATP (7 mM) as compared with unloaded SPMs. The extracellular reaction medium of SPMs loaded with ATP contained approximately 66 μM ATP, 65 μM ADP, 13 μM AMP, 1 μM adenosine and 2 μM inosine measured by HPLC. Extracellular ATP inactivation with apyrase (2 U/mL) increased [<sup>3</sup>H]-GABA and [<sup>14</sup>C]-glutamate uptake by 18% and 28%, respectively. The adenosine A<sub>1</sub> receptor agonist, R-PIA (100 nM), decreased (16%) [<sup>3</sup>H]-GABA uptake, without significantly affecting [<sup>14</sup>C]-glutamate transport. The inhibitory effect of R-PIA on [<sup>3</sup>H]-GABA uptake was prevented by the A<sub>1</sub> receptor antagonist, DPCPX (10 nM). ATPγS (30-300 μM) and the preferential P2X<sub>7</sub> agonist, BzATP (3-100 μM), reduced [<sup>3</sup>H]-GABA, but not [<sup>14</sup>C]-glutamate, uptake in a concentration-dependent manner; the inhibitory effect of ATPγS (100 μM; 20%) was prevented by the non-selective P2 receptor antagonist, PPADS (30 μM). Concurrently, the expression levels of the A<sub>1</sub> receptor in isolated nerve terminals of the neocortex of MTLE patients was half of those observed in cadaveric (control) samples, but the opposite was detected for the P2X<sub>7</sub> receptor whose expression almost doubled the control value.

Data suggest that purines control negatively GABA, but not glutamate, uptake through A<sub>1</sub> and P2X<sub>7</sub> receptors, thus favoring GABAergic inhibition of neuronal firing. Adenosine and ATP acting respectively on high-affinity A<sub>1</sub> and low affinity P2X<sub>7</sub> receptors broadens the dynamic range of the purinergic synaptic control, *i.e.* under mild stimulation conditions adenosine A<sub>1</sub> receptor activation may prevail, a situation that might change towards activation of P2X<sub>7</sub> receptors by high ATP amounts. Decreases in the expression of the adenosine A<sub>1</sub> receptor found in MTLE patients may hasten GABAergic transients rendering neuronal networks more prone to firing. Conversely, increased expression of P2X<sub>7</sub> receptors might endure the GABAergic neurotransmission even more than in control tissue. This may paradoxically induce GABAergic rundown found in epileptic tissues, thus promoting excitability as well.

*Work supported by FCT (FEDER funding, PIC/IC/83297/2007; UMIB-215/94 and FSE-POPH-QREN) and by Univ. Porto / Santander Totta. ABB is in receipt of a PhD Scholarship from FCT (SFRH/BD/79259/2011).*

## Antioxidant activity mediated by non-steroidal anti-inflammatory drugs of the anthranilic acid chemical group

A. Alves<sup>1</sup>, D. Ribeiro<sup>1</sup>, D. Couto<sup>1</sup>, M. Freitas<sup>1</sup> and E. Fernandes<sup>1</sup>

<sup>1</sup>REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal.

Nonsteroidal anti-inflammatory Drugs (NSAIDs) are widely used for the treatment of inflammatory disorders and painful conditions such as rheumatoid arthritis, gout, bursitis, painful menstruation and headache. Their main mechanisms of action is the inhibition of cyclooxygenases (COX-1 and COX-2), though it has long been postulated that the therapeutic effects of NSAIDs on a variety of human diseases may be also due to their ability to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as to inhibit the oxidative burst of neutrophils triggered by various activating agents. Although this has been shown for NSAIDs derived from groups with defined chemical structures, namely carboxylic acids, acetic acids, propionic acids, pyrazolones and indole analogs, the anthranilic acids (mefenamic acid, meclofenamic acid, and tolfenamic acid) (Fig. 1), have not been tested before.

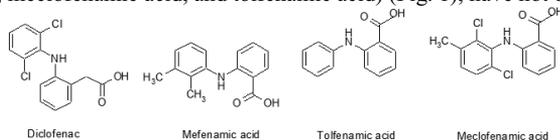


Figure 1. Chemical structures of the studied NSAIDs

The aim of the present work was to evaluate and compare the potential scavenging activity the above mentioned anthranilic acids as well as of the structurally related NSAID diclofenac, for an array of ROS ( $O_2^-$ ,  $^1O_2$ ,  $H_2O_2$ ,  $ROO^\cdot$  and  $HOCl$ ) and RNS ( $NO$  and  $ONOO^\cdot$ ) using *in vitro* non-cellular screening systems as well as a cellular model (human neutrophil oxidative burst). The results obtained in the present work demonstrate that, under the present experimental conditions, many of the studied NSAIDs showed  $O_2^-$  scavenging activity (meclofenamic acid with higher potency than mefenamic acid, but no effect for diclofenac),  $H_2O_2$  (similar activity for mefenamic and tolfenamic acids, but no effect for meclofenamic acid or diclofenac),  $HOCl$  (Higher activity for mefenamic acid, compared to tolfenamic acid, but no effect for meclofenamic acid or diclofenac),  $^1O_2$  (only mefenamic acid revealed activity),  $NO$  (again, only mefenamic acid revealed activity),  $ONOO^\cdot$  (all tested NSAIDs revealed activity, but mefenamic and tolfenamic acids were especially potent). In spite of these results, neutrophil oxidative burst was also not affected by the studied compounds up to 500  $\mu M$ . The observed effects, if confirmed *in vivo*, may strongly contribute to the anti-inflammatory therapeutic activity observed for these NSAIDs.

### Acknowledgements

Authors greatly acknowledge the financial support given by Reitoria da Universidade do Porto for "Projectos Pluridisciplinares para estímulo à Iniciação à Investigação na U. Porto' 2010". Marisa Freitas acknowledges FCT for the post-doctoral grant (SFRH/BPD/76909/2011). Diana Couto and Daniela Ribeiro acknowledge FCT financial support for the PhD grants (SFRH/BD/72856/2010 and SFRH/BD/72966/2010, respectively).

## ***In vitro* assessment of NSAIDs-membrane interaction: the role of biophysics on drugs pharmacological and toxic effects**

**C. Pereira-Leite<sup>1\*</sup>, C. Nunes<sup>1</sup>, J. L. F. C. Lima<sup>1</sup>, S. Reis<sup>1</sup> and M. Lúcio<sup>1</sup>**

<sup>1</sup> REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Portugal, \*  
catarinapl12@gmail.com

Non-steroidal anti-inflammatory drugs (NSAIDs) are extensively used in clinical practice due to their analgesic, anti-inflammatory and antipyretic effects. The mechanism of action of NSAIDs can explain their pharmacologic and adverse effects. NSAIDs decrease the endogenous production of inflammation mediators, prostaglandins, by inhibiting the integral membrane enzyme, cyclooxygenase (COX). The inhibition of constitutive COX-1 isoenzyme is related to gastrointestinal (GI) injuries and alteration of platelet function. In contrast, the interaction of these drugs with inducible COX-2 explains their anti-inflammatory effect. Both non-selective NSAIDs and COX-2 selective NSAID, developed to avoid the GI damage, are used in clinical practice. Some factors that contribute to the acid-resistant properties of stomach are the line of surfactant-like phospholipids on the stomach surface and the mucus gel layer also formed by the same type of phospholipids. Thus, the toxic effects of NSAIDs can be also explained by the interaction of these drugs with the phospholipids of the protector gastric layer. Because of this fact and since COX-1 is an integral membrane enzyme, it is important to study the interaction between NSAIDs and membranes.

The aim of this work is to obtain the biophysical characterization of the interaction of meloxicam and celecoxib with membranes by using liposomes, as membrane model systems. Liposomes were made of DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) or DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), since phosphatidylcholines are major components of biomembranes. In order to mimic the physiologic and inflammation conditions, all the experiments were performed at pH 7.4 and 5.0, respectively. The partition coefficient of drugs between aqueous media and vesicles was determined by derivative spectrophotometry. In order to study the drug location within the membrane, fluorescence quenching studies were performed by using probes with a well-characterized location in the membrane. The influence of drugs on membrane fluidity was predicted by studying their effect on membrane microviscosity and structure. The drugs effect on membrane microviscosity was evaluated by determining the main transition temperature by fluorescence anisotropy or dynamic light scattering (DLS). To analyse the membrane structure in presence and absence of drug in what concerns to bilayer thickness and lipid packing, experiments of small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) were performed.

The biophysical characterization of NSAIDs interaction with biomembranes obtained in this work was important to interpret the pharmacological and toxic effects of these worldwide prescribed drugs.

Acknowledgements: The authors thank HASYLAB at DESY, Hamburg, Germany, for beam time and support through the project II-20100139 EC.

Oral Sessions VIII > A2

# Chemistry



## Quantification of cocaine and morphine in hair samples by a validated gas chromatographic – mass spectrometry method

**Carla Alexandra Gouveia<sup>1</sup>, Ana Oliveira<sup>2,3,4</sup>, Sandra Pinho<sup>5</sup>, Roxana Falcão Moreira<sup>2</sup>, Ricardo Jorge Dinis-Oliveira<sup>2,3,6,7</sup>**

<sup>1</sup>Faculty of Medicine, University of Porto, Porto, Portugal, <sup>2</sup>Department of Sciences, Advanced Institute of Health Sciences – North, CESPU, CRL, Gandra, Portugal, <sup>3</sup>REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal, <sup>4</sup>Molecular Oncology GRP and Virology LB, Instituto Português de Oncologia-Porto, Porto, Portugal, <sup>5</sup>CESPU, CRL, Gandra, Portugal, <sup>6</sup>Department of Legal Medicine and Forensic Sciences, Faculty of Medicine, University of Porto, Porto, Portugal, <sup>7</sup>Department of Clinical Analysis and Public Health, Center of Research in Health Technologies (CITS)-IPSN-CESPU, CRL, Vila Nova de Famalicão, Portugal.

The developments of analytical techniques that enable the use of hair as alternative matrix for the analysis of drugs of abuse are useful. Generally, drugs analyses are usually based on blood or urine, sometimes on liver or kidney tissue in post-mortem cases. Nevertheless, blood and urine specimens only reflect dosages of several hours and several <sup>[1, 2]</sup>.

The present work reveals a qualitative and quantitative developed and validated method for the simultaneous determination of cocaine and morphine in human hair. Hair samples (20 mg), after decontamination, were incubated with a mixture of methanol/hydrochloric acid (2:1) at 65°C overnight (≈16h) in order to extract the drugs of the matrix. The purification of standards was performed in mixed-mode extraction cartridges. After derivatization with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), quantitative GC/EI/MS analyses were performed and then analyzed by gas chromatography/electron impact/mass spectrometry (GC/EI/MS).

The developed method proved to be specific, accurate and precise within the calibration range (0.25–10 ng/mg), where good linearity was observed for both the analytes with correlation coefficients ranging 0.9989 and 0.9991. The coefficients of variation oscillated between 0.83% and 14.6%. The limits of quantification (LOQ) for cocaine and morphine were 0.03 and 0.06 ng/mg, 0.01 and 0.02 ng/mg for the limits of detection (LOD), respectively. Accordingly the Society of Hair Testing rules, these results are acceptable.

The proposed GC/EI/MS method can be successfully applied in the screening and quantification of these compounds in real hair samples, and is appropriate for application in forensic toxicology analysis.

### References:

- [1] R. J. Dinis-Oliveira, F. Carvalho, J. A. Duarte, F. Remião, A. Marques, A. Santos, and T. Magalhães, *Toxicology Mechanisms and Methods*, 2010,7, 363-414.
- [2] Kintz, P., *Analytical and practical aspects of drug testing in hair*, 2007, CRC Press, Boca Ranton, Florida

## On the voltammetry of chalcones

**E.M. Tavares<sup>1</sup>, L.M. Gonçalves<sup>1</sup>, J.A. Rodrigues<sup>1</sup> and A.A. Barros<sup>1</sup>**

<sup>1</sup> Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Chalcones (1,3-diphenyl-2-propene-1-one, whereas 2 aromatic rings are connected by a 3-carbon link, Fig. 1) are a particular subclass of flavonoids. Chalcones are an example of compounds present in many plants with a high therapeutic and preventive potential of many diseases. Chalcones are particularly interesting for their chemopreventing properties, one should keep in mind that cancer is one of the major death causes worldwide and such even tends to increase. Moreover, these compounds could be easily introduced in human's diet or in pharmaceutical formulations with great added value considering that they are not synthesized by the human body [1].

In this study, electrochemical analysis was applied to three chalcones (xanthohumol, cardamonin and trans-chalcone) in order to obtain qualitative and quantitative information about them. This was performed by cyclic voltammetry (CV) on a hanging mercury drop electrode (HMDE) [2,3].

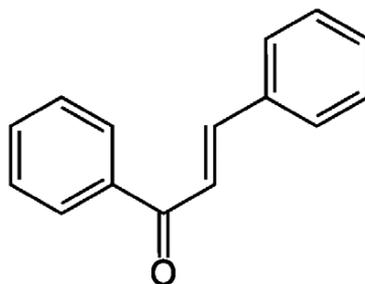


Fig. 1 – Basic chalcone structure.

### References:

- [1] Magalhães, P.J., Carvalho, D.O., Cruz, J.M., Guido, L.F. and Barros, A.A. (2009), *Fundamentals and health benefits of xanthohumol, a natural product derived from hops and beer*; Natural Product Communications, 4, pp. 591-610
- [2] Moreira, M.M., Carvalho, A.M., Valente, I.M., Gonçalves, L.M., Rodrigues, J.A., Barros, A.A. and Guido, L.F. (2011), *Novel application of square-wave adsorptive-stripping voltammetry for the determination of xanthohumol in spent hops*, Journal of Agricultural and Food Chemistry, 59, pp. 7654-7658
- [3] Carvalho, A.M., Gonçalves, L.M., Valente, I.M., Rodrigues, J.A. and Barros, A.A., *Analysis of cardamonin by square wave voltammetry*; Phytochemical Analysis, in press, doi: 10.1002/pca.1370

## New Biomolecular Force Field: lipid components

**J. T. S. Coimbra**<sup>1</sup>, **S. F. Sousa**<sup>2</sup>, **P. A. Fernandes**<sup>1</sup>, **M. C. Rangel**<sup>2</sup>, **M. J. Ramos**<sup>1</sup>

<sup>1</sup> REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007, Porto, Portugal.

<sup>2</sup> REQUIMTE, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Largo Prof. Abel Salazar, 2, 4099-003, Porto, Portugal.

Computational biochemistry has been growing in the last decades and is now an important tool for the atomistic and mechanistic understanding of diverse chemical, physical and biological processes. Our work aims to develop molecular mechanics parameters for lipid components, namely glycerophospholipids, to be applied in the assembly and study of lipid bilayer structures. This will grant us with biological membrane computational models, which are of high pharmacological interest.

We have modeled up to 12 different glycerophospholipid bilayers. The molecular dynamics simulations were carried out for systems containing 200 glycerophospholipids and TIP3P water model, in an NPT ensemble. The simulations were performed applying the General Amber Force Field (GAFF) and up to 80 ns dynamics were conducted for each of the bilayer systems created.

The structural and dynamic properties of the bilayer systems were evaluated and they include: the area per lipid, the volume per lipid, the bilayer thickness, electron density profiles, and the analysis of the water/lipid interface and the glycerophospholipid's lateral diffusion coefficients. The results were compared with available literature data in order to validate the obtained membrane models. We have found great stability of the bilayer systems throughout the computational simulations and a great similarity of both the volume per lipid values and the diffusion coefficients regarding experimental available data. We have also observed that the applied methodology tends to overestimate the bilayer thickness and underestimate the area per lipid.

All things considered we have achieved a set of consistent parameters for different lipid models that allowed for the atomistic simulation of this type of systems, without imposing any constraints (often used in this type of simulations). We are currently applying these models in a study of drug/membrane interaction in order to rationalize the experimental data obtained by the group managed by Professor Maria Rangel. [1,2]

We thank the financial support provided by FCT (projects PTDC/QUI/67915/2006, PTDC/QUI-QUI/103118/2008 and grant no. Pest-C/EQB/LA0006/2011).

### References:

- [1] Fernandes, S. S., Nunes, A., Gomes, A. R., de Castro, B., Hider, R. C., Rangel, M., Appelberg, R. and Gomes, M. S. (2010), *Identification of a new hexadentate iron chelator capable of restricting the intramacrophagic growth of Mycobacterium avium*, *Microbes and Infection*, 12, 287-294.
- [2] Nunes, A., Podinovskaia, M., Leite, A., Gameiro, P., Zhou, T., Ma, Y., Kong, X., Schaible, U. E., Hider, R. C., and Rangel, M. (2010), *Fluorescent 3-hydroxy-4-pyridinone hexadentate iron chelators: intracellular distribution and the relevance to antimycobacterial properties*, *Journal of Biological Inorganic Chemistry*, 15, 861-877

## Coffee Silverskin nutritional profile: agro agricultural by-products valorisation

**SE. Pereira<sup>1</sup>, J. Santos<sup>1</sup>, A. Costa<sup>2</sup>, RC. Alves<sup>1,3</sup>, MBPP. Oliveira<sup>1\*</sup>**

<sup>1</sup> REQUIMTE, Depart. of Chemical Sciences, Faculty of Pharmacy, Univ. of Porto, Portugal

<sup>2</sup> Faculty of Sciences, University of Porto, Portugal, <sup>3</sup> REQUIMTE, Porto Superior Institute of Engineering, Polytechnic Institute of Porto, Portugal \*beatoliv@ff.up.pt

The growing of population and the difficulty on food supplying is one of the most disturbing issues of the global society. Thus, we must focus on the food industry when treating the sustainability matter. One of the problems of this industry is the by-products management. The industrial technology can, nowadays, give effective answers and tools to re-use and valorize the by-products, transforming these residues in new raw material and new products.

Currently, there are many cases of use and valorization of materials that are rejected by the food industries and this should be an example for all food producing processes. The coffee industry could be one case of success due to the large amount of residues produced and discarded. An example is spent coffee grounds, which are used nowadays to produce adsorbents <sup>[1]</sup>.

The aim of this work was to determine the nutritional profile of silverskin, a by-product of roasted coffee beans. When roasted, the silverskin can, and usually does, crack off the coffee bean. The silverskin cracks off because it does not expand like the inner coffee bean when roasted. This brings two problems: first, it looks like some kind of chaff which is undesirable and must be removed from the batch of roasted coffee beans for cosmetic reasons; and second, it can easily catch fire.

Soxhlet method (using petroleum ether as solvent) was used to quantify total fat. Protein content was determined using Kjeldahl method. Moisture content was verified with electronic moisture analyser Scaltec model SMO01 (Scaltec Instruments, Heiligenstadt, Germany). Ashes were determined recurring to incineration at 450-500°C. Dietary fiber content was determined according to the protocol referred by SIGMA for their Total Dietary Fibre assay kit that was based on the method by the Association of Official Analytical Chemists (AOAC).

The samples were provided by a local roasting industry and their composition fluctuated with the percentage of Arabica and Robusta coffee in the blend. Nutritional analysis revealed a high fiber content for all samples (about 60g/100g). The protein content varied between 19 and 21%, ashes presented results between 7 and 9%, moisture was around 4-7%, total fat varied between 1 and 3% and (calculated by exclusion) carbohydrates content resulted between 4 and 6%.

Low fat and carbohydrates content as well as high fiber level are evident. This nutritional profile is a starting point to evaluate the benefits of new applications of coffee silverskin in the future.

### References:

[1] Franca A S, Oliveira L S. Chemistry of Defective Coffee Beans. In: Ernst N. Koeffler. (Org.). Food Chemistry Research Developments. New York: Nova Publishers, 2008, Cap. 4, p. 105-138

### Acknowledgments:

R. C. Alves is thankful to Fundação para a Ciência e a Tecnologia (FCT) for a post-doctoral grant (SFRH/BPD/68883/2010) and a doutoral Grant (SFRH/BD/66476/2009) financed by POPH - QREN - Tipologia 4.1 - Formação Avançada, subsidized by Fundo Social Europeu and Ministério da Ciência, Tecnologia e Ensino Superior. This work has been supported by FCT through grant no. PEst-C/EQB/LA0006/2011. We thank BICAFÉ for providing the coffee silverskin samples.

## Development of ibuprofen selective electrode for pharmaceutical formulations and surface waters

**Tiago Sousa, Célia Amorim, Alberto Araújo, Maria da Conceição Montenegro**

REQUIMTE, Laboratório de Química Analítica e Físico-Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal.

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties usually used in the treatment of rheumatoid arthritis, degenerative joint disease, ankylosing spondylitis and acute gouty arthritis. Ibuprofen is administered orally and 1% excreted in urine unmetabolized. Due to the fact that this drug does not require medical prescription its high consumption contributes to a high level of environmental contamination. As in the case of many other pharmaceutical, chemical control of ibuprofen is based on different methods such as HPLC, capillary electrophoresis, spectrophotometry, infrared spectrometry, conductometry and potentiometry. In this work a simple, low cost and sensitive potentiometric procedure for ibuprofen determination is presented. The method is based on the use of potentiometric sensor developed for this purpose. The plastic membrane electrode is based on cyclodextrin as ionophore. In order to optimize the membrane characteristics several cyclodextrins and a molecular imprinted polymer (specific to NSAID), plasticisers and lipophilic ionic species were evaluated. The polymeric membrane with better characteristics incorporates  $\alpha$ -cyclodextrin (1.2% w/w) as ionophore, 2-nitrophenyloctyl ether (65.6% w/w) as plasticizer and tetradecylammonium bromide (5.39 mmol/Kg) as cationic additive. The electrode presented low response times with an analytical linear range between  $3.87 \times 10^{-6}$  –  $1.00 \times 10^{-2}$  mol L<sup>-1</sup>, slopes of  $-60.5 \pm 2.9$  mV/dec and PDL of about  $2.78 \times 10^{-5}$  mol L<sup>-1</sup> when tested in a buffer solution of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>/NH<sub>3</sub> 0.01M with the pH adjusted to 9.9. The response of the electrode is highly selective concerning to the representative species, usually present in pharmaceutical samples. The electrode was directly used for ibuprofen determination in medicines and also in contaminated surface waters after being subject to a solid phase extraction (LiChrolut EN/RP-18). The ibuprofen recoveries for surface waters varied between  $89 \pm 4$  and  $96 \pm 5\%$ . Comparison of obtained results for pharmaceuticals with those provides by the reference method and recovery assays, revealed adequate accuracy for control assays.

### Acknowledgments:

A gratefully acknowledge to Renato Lopes Gil, Ruben Filipe Monteiro Calaia, Pedro Miguel Ferreira Maurício and Elisabete Cardoso Mendes Moura for some laboratory supporting

# TD-DFT/MM Study of the Firefly Multicolor Bioluminescence

**L. Pinto da Silva, J.C.G. Esteves da Silva**

Centro de Investigação em Química (CIQ-UP), Department of Chemistry and Biochemistry, Faculty of Sciences of University of Porto, Portugal. Email: luist311@hotmail.com.

Firefly luciferase (Luc) catalyzes a two-step light-emitting reaction: first, luciferin reacts with ATP-Mg<sup>2+</sup> to form an adenylyl intermediate; the second step consists in the oxidation of this intermediate with release of AMP, CO<sub>2</sub> and the light emitter anionic keto-form oxyluciferin (Keto(-1)) [1]. The most relevant characteristics of this system are its dependence on ATP-Mg<sup>2+</sup>, high quantum yield and its multicolor bioluminescence (562-620 nm) [2]. Currently, this system has gained numerous bioanalytical, biomedical and pharmaceutical applications, among others [1,2].

The mechanism that controls the multicolor bioluminescence is still far from elucidated, despite decades of research [2]. Crystallographic studies have indicated that green emission (562 nm) is achieved in a closed and hydrophobic conformation, while a more open and polar conformation is responsible for red emission (620 nm) [3].

This work aims to clarify the origin of the multicolor bioluminescence, by studying the interaction between the first excited state of Keto(-1) and active site molecules in both conformations of Luc. To this end, we docked AMP and Keto(-1) to the active site by means of protein-ligand docking. The resulting complexes were minimized by using molecular mechanics (MM), and the emitter and some active site molecules were withdrawn from the resulting structures. Time dependent density functional (TD-DFT) calculations on these active-site models allowed us to determine the effect of different amino-acids, AMP and water molecules on the excited state of the bioluminophore [4,5].

In conclusion, our study demonstrated that the red-shift in the emission results mainly from decreased interaction of Keto(-1) with AMP and increased interaction of the bioluminophore with a water molecule and Phe249. Breaking of a hydrogen bond between the benzothiazole oxygen atom with formation of a similar bond to the thiazolone oxygen atom is also fundamental [4,5].

References:

- [1] Marques, S.M., Esteves da Silva, J.C.G. (2009), *Firefly bioluminescence: a mechanistic approach of luciferase catalyzed reactions*, IUBMB Life, 61 (1), 6-17.
- [2] Pinto da Silva, L., Esteves da Silva, J.C.G. (2011), *Computational Studies of the Luciferase Light-Emitting Product: Oxyluciferin*, Journal of Chemical Theory and Computation, 7 (4), 809-817.
- [3] Nakatsu, T., Ichiyama, S., Hiratake, J., Saldanha, A., Kobashi, N., Sakata, K., Kato, H. (2006), *Structural basis for the spectral difference in luciferase bioluminescence*, Nature, 440 (7082), 372-376.
- [4] Pinto da Silva, L., Esteves da Silva, J.C.G. (2011), *Theoretical modulation of the color of light emitted by firefly oxyluciferin*, Journal of Computational Chemistry, 32 (12), 2654-2663.
- [5] Pinto da Silva, L., Esteves da Silva, J.C.G. (2011), *Study on the effects of intermolecular interactions on firefly multicolor bioluminescence*, ChemPhysChem, 12 (16), 3002-3008.

Oral Sessions VIII > A3

# Sport Sciences



## Effect of a program of hidrogymnastic on body composition and strength. Study performed with and without drag material.

**E. Louro<sup>1</sup>, J. Ribeiro<sup>2</sup> and S. Soares<sup>3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal.

<sup>2</sup> Faculty of Sport, University of Porto, CIAFEL, Portugal.

<sup>3</sup> Faculty of Sport, University of Porto, CIFI2D, Portugal.

The health benefits of the practice of hidrogymnastic are unquestionable <sup>[1,2,3]</sup>. However, the scientific literature seems to be scarce in what concern effects of aquatic exercise programs which includes the effects of the utilization of drag material in classes. Therefore, the purpose of this study was to determine the influence of an aquatic program in the alteration of body composition and muscular strength and observe the effects of the utilization of drag material.

A sample of 22 female subjects was divided in 2 groups, one with 13 individuals (group1) and another with 9 (group 2). Both groups participated in the same exercise protocol, but the group 2 performed it using drag material (gloves and ankle cuffs). Along 13 weeks, the subjects participated in 25 sessions of 45 min, twice a week. In each session, the warm up and the aerobic segment had the duration of 16 min and were compounded by aerobic exercises of moderate to high intensity. During the local segment, subjects performed a randomized sequence of 8 upper body and 8 lower body movements. The fat mass, lean mass and bone mineral content were determined by Dual-energy X-ray absorptiometry. Muscular strength was measured with 30-second Chair Stand and 5-repetitions Chair Stand. All measurements took place before and after the exercise protocol.

After the application of the aquatic program, group 1 decreased fat mass (2.02% and 3.31%), fat mass percentage (3.5% and 6.87%) and total mass (1.67% and 3.45%) in appendicular and trunk region, respectively, and showed no fluctuation on bone mineral content scores. Group 2 observed a reduction of fat mass percentage at the appendicular region (1.34%). Muscular strength increased after de exercise protocol in both groups. The heterogeneity of the responses of the subjects to exercise protocol, for all variables, was very high.

### References:

- [1] Barbosa, T.; Marinho, D.; Reis, V.; Silva, A. and Bragada, J. (2009), *Physiological assessment of head-out aquatic exercises in healthy subjects: A qualitative review*, Journal of Sports Science & Medicine, 8, 179-189.
- [2] Colado Sánchez, J. and Moreno Murcia, J. (2001), *Fitness acuático*, Inde, Barcelona.
- [3] Fomina, O. (2009), *Active exercises in water for advanced age women as the way to improve their physical workability and to correct body composition*, Adv Gerontol, 22 (2), 343-347.

## **Motor performance and body composition of children aged seven and eight of the participating centers Municipal Sports and Recreation in Manaus.**

**W. Domingues<sup>1</sup>, D. Leão<sup>2</sup>, I. Ferreira<sup>2</sup> and E. Bezerra<sup>2,3</sup>**

<sup>1</sup> Faculty of Sports University of Porto, Portugal.

<sup>2</sup> Faculty of Physical Education and Physiotherapy, Federal University of Amazonas, Brazil

<sup>3</sup>Laboratory Study of Human Performance, Brazil.

Studies on motor performance and body composition has received enormous attention and value to researchers in the field of health, that they are recognized as important indicators of health status of an individual, as can be seen in studies by Ferreira and Böhme (1998), Guedes (1994) also highlighted the study in schoolchildren in the city of Manaus, Leo (2005). The objective of this study was to investigate the behavior of variables of motor performance and body composition of children participating in the Municipal Centers Sporting Goods Manaus in the 2007 academic year. The study included 213 children, 106 boys (49.7%) and 107 girls (50.3%) of seven and eight years of age selected randomly. Was performed by a transversal approach a battery of tests of motor performance and anthropometric measures AAHPERD (1980). Statistically significant differences were found in favor of girls in the variable triceps skinfold (10.7 mm) in only seven years old. In children of eight years was statistically significant variable in the triceps skinfold (11.9 mm) and subscapular (5.38 mm) also in favor of girls. In tests of motor performance were statistically significant differences in favor of boys in the variables, abdominal strength at the ages of seven (18.0 cm) and eight (21.0 cm) and endurance at the age of eight years (1,361 m), but in the flexibility test, the result was in favor of girls in the age of seven (23.3 cm) ( $p < 0.05$ ). Based on engine testing, the analysis revealed differences with high performance levels of boys over girls, particularly in the abdominal modified tests, run / walk for nine minutes and nearly all ages in the sit and reach test. With respect to body composition when compared with average values of references, it was observed that the girls proved to be more sensitive to be a larger accumulation of body fat than boys, as they have been found in other populations studied.

### References:

- [1] Ferreira, M., & Bohme, M. T. S. (1998). Diferenças sexuais no desempenho motor de crianças: Influência da adiposidade corporal *Revista paulista de educação física*, 2, 92-181.
- [2] Guedes, D. P. (1994a). *Crescimento, composição corporal e performance motora em crianças e adolescentes do município de Londrina*. Tese de Doutorado, Universidade de São Paulo, Escola de educação física e esporte.
- [3] Leão, D. P. (2005). *Crescimento, Desenvolvimento e Aptidão Física do Escolar de Manaus*. Dissertação Mestrado Dissertação, Universidade de São Paulo, São Paulo.
- [4] AAHPERD. (1980). *Health related physical fitness test manual*. Reston: American Alliance for Health, Physical Education, Recreation and Dance.

## **Dynamic balance of surfers: The effect of sex and foot base position on board**

**A. Gomes<sup>1</sup>, L. Oliveira<sup>1</sup>, M. Botelho<sup>1</sup>, O. Vasconcelos<sup>1,2</sup>**

<sup>1</sup> Faculdade de Desporto, Universidade do Porto, Portugal.

<sup>2</sup> Responsável pela UC Aprendizagem Motora, Faculdade de Desporto, Universidade do Porto, Portugal

The practice of surf has been growing exponentially and therefore it urges to understand and analyze it through a scientific perspective. The issue regarding balance has remained largely disregarded despite its importance to the practice of this sport.

It's following this perspective that emerges the need of this study. We aim to investigate dynamic balance according to sex and to the foot base position on board: Goofies and Regulars (with the right or left foot in the front of the board, respectively). We also intend to verify the functional foot asymmetry differences with respect to the variables study (sex and foot position).

The sample comprises 18 subjects, 8 females (4 Goofies and 4 Regulars) and 10 males (5 Goofies and 6 Regulars). The ages range from 14 to 21 years old ( $16,72 \pm 1,708$ ). It was applied an experimental approach based on the Flamingo Balance Test [1], but in this study we used the adapted instrument as an instable platform to assess the dynamic balance. Statistical procedures included descriptive and inferential tests (Mann-Whitney Test). The significance level was set at  $p \leq 0.05$ .

The results show (i) In the entire sample, an absence of the effect of sex in dynamic balance; (ii) When comparing both positions, Goofies performed better with both feet; (iii) The balance was significantly improved in Goofies males both in preferred and non-preferred foot. The same happened for female's Goofies but just in non-preferred foot; (iv) According to the functional foot asymmetry, we conclude that females are more asymmetric than males even though significant differences were not observed.

[1] EUROFIT (1983) - *Physical Fitness Test Battery* (Council of Europe, 1983) Flamingo Balance Test

## EFFECT OF AN ALL-OUT ROWING ERGOMETER TEST IN THE PROPRIOCEPTIVE SENSIBILITY AND MANUAL DEXTERITY IN YOUNG ROWERS

DAVID CARDOSO<sup>1</sup>, JOÃO PEREIRA<sup>1</sup>, JOSÉ MOREIRA<sup>1</sup>, DOUTOR MANUEL BOTELHO<sup>1</sup>,  
DOUTORA OLGA VASCONCELOS<sup>1,2</sup>

<sup>1</sup>FACULDADE DE DESPORTO DA UNIVERSIDADE DO PORTO  
<sup>2</sup>RESPONSÁVEL PELA UC APRENDIZAGEM MOTORA

MANUAL DEXTERITY (MD) AND MANUAL PROPRIOCEPTIVE SENSIBILITY (MPS) ARE BOTH ESSENTIAL FOR THE ACCURATE CONTROL IN THE MANIPULATION OF SMALL OBJECTS AND FOR THE NEEDS OF MANY DAILY ACTIVITIES [1]. THE STUDY OF THESE MOTOR ABILITIES IN ROWING IS OF CRUCIAL IMPORTANCE FOR THE FACT THAT THE HANDS ARE THE LINK BETWEEN THE BODY AND THE MAIN LEVER OF THE BOAT IN WHICH THE POSITION OF THE HANDS IN THE STROKE CYCLE PLAY AN IMPORTANT ROLE. THE AIM OF THIS STUDY IS TO VERIFY THE CHANGES OF THESE TWO VARIABLES (MD AND MPS) BEFORE AND AFTER AN ALL-OUT 2000 METERS TEST PERFORMED IN ROWING ERGOMETER CONCERNING PREFERRED HAND, NON-PREFERRED HAND AND ITS FUNCTIONAL ASYMMETRY. THE SAMPLE COMPRISES 15 MALE ROWERS, WITH AGES BETWEEN 14 AND 16 YEARS OLD. THE PLATING TAPPING TEST OF EUROFIT TESTING BATTERY (1983) WAS APPLIED TO ACCESS MD. THE DISCRIMINATING WEIGHTS TEST WAS APPLIED TO EVALUATE THE MPS. THE STATISTICAL PROCEDURES USING SPSS VERSION 19 INCLUDED: DESCRIPTIVE STATISTIC (MEAN AND STANDARD DEVIATION) AND INFERENTIAL STATISTICS (WILCOXON TEST). SIGNIFICANCE LEVEL WAS FIXED ON  $p \leq 0,05$ . THE MAIN CONCLUSIONS WERE: (i) IN MD ROWERS SIGNIFICANTLY IMPROVED THEIR PERFORMANCE FROM THE BEGINNING TO THE END OF THE TEST ON BOTH HANDS BUT NOT IN THEIR DIFERENTIAL; (ii) IN MPS NO SIGNIFICANT DIFFERENCES OCCUR FROM THE BEGINNING TO THE END OF THE TEST ON BOTH HANDS AND ITS DIFFERENTIAL. IN FUTURE STUDIES IT WOULD BE INTERESTING TO INVESTIGATE THESE TWO VARIABLES IN ROWING TECHNIQUE, AND THE EFFECT OF PREFERRED HAND IN THE ROWERS FUNCTIONAL ASYMMETRY, AS WELL AS AND THE PERFORMANCE OF EACH ARM.

### REFERENCES:

[1] SILVA, J., VASCONCELOS, O., & CARVALHO, J. (2008). *EFFECTS OF A PHYSICAL ACTIVITY PROGRAM IN MANUAL DEXTERITY AND MANUAL PROPRIOCEPTIVE SENSIBILITY IN TWO DIFFERENT INSTITUTIONALIZED AGE GROUPS OF ELDERLY PEOPLE*. II CONGRESSO INTERNACIONAL DE CIÊNCIAS DEL DEPORTE. PONTEVEDRA: UNIVERSIDADE DE VIGO

## Anaerobic Threshold assessment in young swimmers

**M. Sousa<sup>1</sup>, J. P. Vilas-Boas<sup>1</sup> and R. J. Fernandes<sup>1</sup>**

<sup>1</sup> CIFI2D, Faculty of Sport of University of Porto, Portugal.

The assessment of blood lactate concentrations has been considered one of the essential procedures in diagnosis of swimming performance [1]. However, for more complete analysis of the swimmers performance, it is also important to examine the technical characteristics concurrently with physiological responses [2]. The purpose of this study was to assess individual anaerobic threshold velocity and corresponding blood lactate concentrations in young swimmers, and to compare these results with the traditionally used value of 4 mmol/l proposed by Mader et al [3]. Complementary, it was also aimed to analyze the kinetics of stroke rate, stroke length, stroke index throughout the incremental protocol used for anaerobic threshold assessment.

Fifteen young swimmers ( $10.73 \pm 0.70$  years old) performed a 5x200m intermittent and incremental protocol. Swimming velocity was controlled by an acoustic signal each 50 m. The blood samples were collected from the ear lobe (Lactate Pro, Arkay, Inc), at rest and after each step. Stroke rate was registered by a chronofrequencemeter base 3, and stroke length, stroke index and velocity, were calculated at the end of each 200 m.

The anaerobic threshold occurs at a mean  $[La^-]$  of  $2.29 \pm 0.59$  mmol/l, much lower than the traditionally used 4 mmol/l value (or even of 3.5 mmol/l), and corresponding velocity was  $1.03 \pm 0.05$  m/s. Velocity corresponding to 4 and 3.5 mmol/l occurred at  $1.08 \pm 0.06$  m/s and  $1.07 \pm 0.06$  m/s, respectively. Stroke rate increased and stroke length decreased throughout the 5x200 m protocol, i.e., accordingly with the velocity increments. The stroke index presented a significant increase from the first to the second steps, and stabilized in the last three stages.

The velocity corresponding to 4 mmol (and 3.5 mmol) does not represent the individualized anaerobic threshold in young trained swimmers and age group swimmers, as the older swimmers [4] prefer to increase their velocity through the increase of stroke rate. Given the importance of developing swimming technique in age-group swimmers, coaches should implement the lengthening of their stroke cycles in their training practice routines, so that their swimmers are able to resist to the degradation of the SL when velocity increases.

### References:

- [1] Bonifazi, M., Martelli, G., Marugo, L., Sardella, F., Carli, G. (1993). Blood lactate accumulation in top level swimmers following competition. *Journal of Sports Medicine and Physical Fitness*, 33(1), 13-18.
- [2] Pyne, D., Maw, G., Goldsmith, W. (2000). Protocols for the Physiological Assessment of Swimmers. In C. Gore (Ed.), *Physiological Tests for Elite Athletes*, Australia, 2000, pp. 372-382.
- [3] Madder, A.; Liesen, H.; Heck, H.; Philippi, H.; Rost, R.; Schurch, P.; Hollmann, W. (1976) – Evaluation of lactic acid anaerobic energy contribution by determination of post-exercise lactic concentration of ear capillary blood in middle-distance runners and swimmers. In: *Proceedings of an International Congress of Physical Activity Science* (edited by F. Landry and WHK Orban), pp. 187-199. Miami, FL: Symposium Specialists.
- [4] Pyne, D., Maw, G., Goldsmith, W. (2000). Protocols for the Physiological Assessment of Swimmers. In C. Gore (Ed.), *Physiological Tests for Elite Athletes*, Australia, 2000, pp. 372-382.

## Manual Dexterity and Functional Motor Asymmetry. Study in boys and girls without sports practice.

Filipe Silva<sup>1</sup>, Juan Molina<sup>1</sup>, Ricardo Santos<sup>1</sup>, Rita Monteiro<sup>1</sup>, Doutor Manuel Botelho<sup>1</sup>, Doutora Olga Vasconcelos<sup>1,2</sup>

<sup>1</sup> Faculdade de Desporto da Universidade do Porto

<sup>2</sup> Responsável pela UC Aprendizagem Motora

Most of the behaviors of the children are seized and they are the result of an enormous ability to adapt when they are confronted with new experiences. However, even nowadays, cultural affordances are different for boys and girls concerning the opportunities to play and to learn new skills. Previous studies in this domain have been done (e.g. Barroso, 2008) [1] but it lacks more investigation about this question. With this study we intend to evaluate manual dexterity in children from 7 to 9 years old, who don't practice sports, and to compare their performance with respect to sex and hand of execution. The sample consisted of 42 children attending the 3rd and 4th grade, 1st Cycle of Basic Education, College Camões, located in the district of Porto. To assess manual dexterity we used the *Plate Tapping Test* (Eurofit, 1983) [2] and we assessed preferred hand, non-preferred hand and the correspondent functional hand asymmetry. Statistical procedures included descriptive statistics (mean and standard deviation) and inferential statistics (independent sample t-test to compare sexes and paired sample t test to compare hands.). The significance level was set at  $p \leq 0.05$ .

The findings of this study were: **i)** According to sex, there were no statistically significant differences; **ii)** With respect to the comparison between hands, in each sex, we observed either for boys and girls a statistically significant better performance with the preferred hand; **(iii)** boys and girls did not differ in the functional hand asymmetry.

[1] Barroso, Jorge (2008): Preferência Lateral e Assimetria Motora Funcional em Crianças do 1º Ciclo. Mestrado em Ciência do Desporto, na área de especialização de Desenvolvimento Motor, pela Faculdade de Desporto da Universidade do Porto.

[2] EUROFIT (1983) - *Physical Fitness Test Battery* (Council of Europe, 1983). Plate Tapping.

## Videogame practice and reaction time. Study in first and second grade school children

A. Oliveira<sup>1</sup>, R. Santos<sup>1</sup>, T. Neves<sup>1</sup>, M. Botelho<sup>1,2</sup>, O. Vasconcelos<sup>1,2</sup>

<sup>1</sup> Faculdade de Desporto, Universidade do Porto, Portugal

<sup>2</sup> Responsável pela UC Aprendizagem Motora, Faculdade de Desporto, Universidade do Porto, Portugal

Within the latest technological advances, video games occupy a considerable sum of children's free time. This reality brings several costs, both physical and psychological – in one hand, the knowledge acquisition and motor skills development are stated as the most benefic consequences; in the other hand, sedentarism and isolation are found as the most malign effects.

This study aims to appraise the effect of videogame practice in the hand reaction speed of 6 and 7 years old children – videogame players and non-players – applying *The Nelson Hand Reaction Test* and, for data assessment, the descriptive statistics and the Mann-Whitney test, with the significance level established for  $p \leq 0,05$ .

The sample comprises 20 right-handed children from the school Escola Básica de Corveiros – 12 on the first grade and 8 on the second grade, who didn't practice any after-school sportive activity. To evaluate their hand preference and videogame practice, it was conducted a semi directed interview to the children, in which ten affirmed that use to play videogames more than 1 hour per day and the remaining children didn't play at all.

The main results show no difference between videogame players and non-players as for the hand reaction speed of both hands. When comparing both sexes, it was realized that male children show a lower Functional Hand Asymmetry mean value than female subjects, although not being statistically significant. Otherwise, relatively to the subject's age, it was verified that 7 years old children are more "symmetrical" than their pairs, classifying this difference as "marginally" significant.



Oral Sessions VIII > A4

# **Biological Sciences IV**



# Phytoremediation of a metalaxyl-photo-Fenton-treated solution by *Solanum nigrum* L. plants

**José Tiago Moreira<sup>1,2</sup>, F. Fidalgo<sup>1,2</sup>, Adrián M.T. Silva<sup>3</sup> and J. Teixeira<sup>1,2</sup>**

<sup>1</sup>Center for Biodiversity, Functional & Integrative Genomics (Bio FIG)

<sup>2</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

<sup>3</sup>Laboratório de Catalise e Materiais (LCM), Laboratório Associado LSRE/LCM, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Portugal

The increasing contamination of waste waters streams by recalcitrant organic constituents from industrials and municipal waste waters [1] has prompted the development of newer technologies able to degrade these contaminants, such as the advanced oxidation processes (AOPs). Photocatalysis is an AOP that consists on the use of a photocatalyst that is activated by light, creating a strong oxidation agent that mineralizes the organic matter. In this work, an artificial waste water initially composed of 151 ppm of metalaxyl, a commonly used pesticide, was treated by the photo-Fenton reaction, in which ferrous iron ( $\text{FeSO}_4$ ) decomposes hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) involving the production  $\text{HO}\cdot$  [2], the latter oxidizing metalaxyl to a concentration lower than 25 ppm [3]. However, this procedure produces secondary products such as small organic molecules,  $\text{Fe}^{3+}$  and  $\text{SO}_4^{2-}$ , making the resulting solution less but still harmful to the environment. The objective of this work was to access the potential of *Solanum nigrum* L. plants to phytoremediate the resulting photo-Fenton-treated solution by determining its effects on two enzymes related to the detoxification of synthetic compounds in plants: the guaiacol peroxidase (GPX) and Glutathione-S-Transferase (GST), that participate in their activation and conjugation, respectively [4]. *S. nigrum* seedlings were grown hydroponically in a mixture of vermiculite:perlite (2:1) watered with a metalaxyl photo-Fenton-treated solution supplemented with a commercial liquid fertilizer, and maintained in a plant growth chamber, at optimal growth conditions. After one month of treatment, at least 4 plants from each condition (control and treated) were collected, frozen and grinded under liquid  $\text{N}_2$  and stored at  $-80\text{ }^\circ\text{C}$  until used. The obtained results indicate that both enzymes activities augmented with the exposure to the resulting solution. It is suggested that GPX activity is enhanced because of Fe, which is a cofactor of this enzyme, and of some reminiscent  $\text{H}_2\text{O}_2$  that is the enzyme's substrate. As for GST, its activity augmented in the aboveground tissues due to the transport of the photocatalysis side-products to these organs, where they were conjugated to glutathione in order to become less toxic to the plant.

## References:

- [1] Stasinakis, A.S. (2008), Global NEST Journal.
- [2] Augugliario V. et al., (2006), Journal of Photochemistry and Photobiology C: Photochemistry Reviews.
- [3] Silva, A.M.T. et al. (2012), Journal of Chemical Engineering (in press).
- [4] Edwards, R et al., (2011), In Organic Xenobiotics and Plants. Pp: 125-148. Ed. Schröder & Collins. Springer, New York

The financial support from the University of Porto (projeto pluridisciplinar PhotoWeed), with the contribution of Santander Totta, is gratefully acknowledged. Syngenta Agro S.A. Portugal is acknowledged for providing Ridomil®. AMTS acknowledges financial support from POCL/N010/2006.

# Susceptibility of European eel, *Anguilla anguilla*, and this parasite, *Anguillicoloides crassus*, to heavy metals concentrations in the International river Minho

**A.C. Braga<sup>1\*</sup>, M.J. Araujo<sup>1</sup> and C. Antunes<sup>12</sup>**

<sup>1</sup> CIIMAR Centro Interdisciplinar de Investigação Marinha e Ambiental. Rua dos Bragas, 289. 4050-123 PORTO.

<sup>2</sup> *Aquamuseu do Rio Minho. Parque do Castelinho. 4920-290 VILA NOVA DE CERVEIRA*

\* Correio electrónico: catarinatrabalho@gmail.com

The European eel, *Anguilla anguilla* a migratory species of high ecological and commercial importance is nowadays classified as "Endangered". This species although, having raised great interest in recent decades in science level remains an enigma and presents a very long and complex catadromous life cycle. Their stocks have been decreased very quickly, although the causes of this decline are not well defined. Among them are, infection with a parasite exotic *Anguillicoloides crassus* and contamination with anthropogenic substances, such as heavy metals, therefore these two factors were chosen to be study subjects.

During this work, we then perform a progress report regarding the population structure of eels in the River Minho, during which it is found that a population composed mainly of young and sexually undifferentiated.

It was analyzed the presence of the parasite *Anguillicoloides crassus* in this population and its dispersion and infection parameters. We conclude that, the dispersion of this parasite in the Minho increased significantly, when we compare our results with previous data for this area covering new areas of the river and in some of these sites its prevalence reaches 100%.

Ultimately we evaluated the amount of metals present in the sediment of the river Minho, in eels and in the parasite *A. crassus*, getting at relatively low metals concentrations that lead us to considered the river Minho as a clean stream. In the same way, we found that metals concentrations in eels, differ throughout their life cycle, but in the two phases analyzed, glass eels and yellow eels, the amounts are below the legal limit regarding the human consumption. Concerning the values obtained in the parasites they are not related with eels values therefore *A. crassus* has no impact on metals concentrations in its host.

# Ecological characterization of surface water bodies from the Hydrographic Regions 1 and 2

**P. Ribeiro<sup>1</sup>, N. Formigo<sup>1</sup>**

<sup>1</sup> Department of Biology, Sciences Faculty, University of Porto, Portugal.

On the 23rd October 2000 the European Parliament approved the Directive 2000/60/EC, known as the Water Framework Directive (WFD), which changed the way the water resources were managed in all member states. The main objective of the WFD is to reach at least the rank of good ecological and chemical status of all water bodies by the year 2015 [2, 3].

The characterization of the ecological status of surface water bodies from hydrographic regions 1 (Minho and Lima rivers) and 2 (Cávado, Ave and Leça rivers), was based on physicochemical, biological and hydromorphological elements, with 5 possible results: Bad Poor, Fair, Good and Excellent, as required by the WFD [2, 3].

The data were collected in sampling campaigns sponsored by INAG during the years 2004 and 2005. Before beginning any statistical analysis, a validation of the data was carried out, followed by the categorization of values in the variables. Five categories were used, to match the categories established by the WFD [2, 3].

For the statistical analysis we used the procedure of multiple correspondence analysis (MCA) present in the SPSS software. The MCA is a statistical process of data reduction, which allows us to understand the affinity between two or more dimensions, studying the relationships established between the various variables [1].

In the graphs obtained, the occurrence of a geographical gradient of the sampling points (Source – Mouth) was evidenced. Another evidence was that the most degraded water bodies corresponded to those closer to the mouth. The water bodies in a better ecological status are associated with the sampling stations near the source.

The watersheds that have a better ecological quality for their water bodies were those of the Minho and Lima, being the most degraded watersheds those of rivers Ave and Leça.

Finally, it is possible to conclude that the use of semi-quantitative metrics generates perfectly acceptable results, that can be used to assess water quality and the status of water bodies. This is important, because the use of this kind of approach allows for significant cost savings in data collection and processing, that could be used to increase the amount of information that is gathered, namely in terms of spatial and temporal scales.

[1] Carvalho, H. (2008), *Análise multivariada de dados qualitativos - Utilização da ACM com o SPSS*. 1ª edição, Edições Sílabo, Lda.

[2] INAG, I. P. (2005), *Relatório síntese sobre a caracterização das regiões hidrográficas prevista na directiva-quadro da água*. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I.P..

[3] INAG, I. P. (2006), *Implementação da Directiva quadro da água*. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I.P..

## Growth of common gobies *Pomatoschistus microps*: is growth similar for populations distant apart?

**P. Costa<sup>1</sup>, R. Guillot<sup>2</sup>, and J. Campos<sup>3</sup>**

<sup>1</sup>Department of Biology, Faculty of Sciences University of Porto, Portugal.

<sup>2</sup>Department of Biology, University of Bergen

<sup>3</sup>CIMAR/ CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Portugal.

In this work we used the common goby, *Pomatoschistus microps* (Krøyer, 1838) as our subject of study. It is an epibenthic and euryhaline fish which inhabits fjords, estuaries and shallow near shore waters from Northeastern Atlantic. These organisms play a relevant role in the ecosystem functioning due to their high abundance, high fecundity, and trophic role as a predator on meio- and macro fauna and as prey for larger fish species and seabirds.

Since the species has a large geographic range inhabiting areas of different temperature patterns, we intended to investigate if the growth rate is differs between populations very distant apart. The growth rate was compared between gobies from three different locations: River Minho and Ria Formosa, Algarve, both in Portugal, and Bergen, in Norway. For this experiment it was used four aquaria at different temperatures: 10, 15, 20 and 25°C. The salinity was maintained constant and similar in all aquaria. Water was changed weekly and the temperature and salinity were measured every day. Food was given ad libitum on a daily basis. About 30 gobies per population and temperature level were used. Each fish was placed individually in the aquarium in order to enable to identify them and follow the individual growth.

Every two weeks the gobies' total length was measured. The dead ones were bagged and tagged, and replaced by another fish from the same population randomly chosen from the maintenance aquarium.

Length data were treated to determine daily growth rates in each aquarium. Growth rates were compared between sexes, size classes, temperatures levels and between origin populations. Results were further compared with previous results obtained with gobies from Minho estuary. For that multivariate ANOVA was applied using the software Systat 13.

### References:

[1] Christiansen P.D, Brozek M, Hansen B.W (1998) *Energetic and behavioral responses by the common goby, Pomatoschistus microps (Krøyer), exposed to linear alkylbenzene sulfonate*, Environmental Toxicology and Chemistry, pp 2051–2057.

# A GIS predictive performance of habitat suitability model for Iberian red deer (*Cervus elaphus hispanicus* Hilzheimer, 1909)

**J. Carvalho<sup>1,2</sup>, L. Martins<sup>1,3</sup>, J. Silva<sup>1,3</sup>, J. Santos<sup>2</sup>, R. Torres<sup>2</sup>, C. Bateira<sup>1,3</sup>, C. Fonseca<sup>2</sup>**

<sup>1</sup> Department of Geography, Faculty of Arts, University of Oporto, Portugal.

<sup>2</sup> Department of Biology & CESAM, University of Aveiro, Portugal.

<sup>3</sup> DyNat/CEGOT-UP - Natural Processes Assessment, Modelling and Mapping/Centro de Estudos em Geografia e Ordenamento do Território

The development of habitat suitability models is an important tool in conservation planning and game management [1]. Detailed knowledge about species distribution is a key aspect to examine the effects of biotic, abiotic and anthropogenic factors on species occurrence [2]. Such predictive models have become particularly important against the backdrop of climate and land use changes.

The present study was conducted in the northeast of Portugal, in *Lombada* National Hunting Area, Bragança, which is one of the national largest Iberian red deer free-ranging population habitat. Red deer has increased in number and distribution over the last decades in Portugal, fact that has urged to define a prospective approach considering their ecological characteristics as well as their sustainable exploitation as a game resource.

A multi-criteria model using the Analytical Hierarchic Process (AHP) and GIS (Geographical Information Systems) weighted spatial analysis with matrix algebra techniques were applied. The main goal was to evaluate the local capacity and effectiveness of the model in order to widen the scope of its applicability defining the greatest potential areas for colonization. This method was developed in three distinct phases: i) setting the objectives, ii) analyzing pairs of factors through the application of a comparison matrix and iii) its synthesis with the weight assignment that followed a predetermined numeric range according to the ecological requirements of the study species. The variables used in the habitat suitability model included the hydrographic network, asphalted roads, relief aspects and land use (divided in forest, agricultural and urban areas), all variables known to affect red deer occurrence and distribution.

Through GPS points and trigonometric operations it was achieved the exact position of the animals observed. A total of 129 observations were used in model validation. Using the AUC (Area Under the Curve) method a success rate of 81.5% was obtained.

Our results suggest that the methodology can be confidently applied on a larger scale. This multi-criteria model provides a rapid response to the defined objectives and allows the effect of change's assessment in the weights assigned to the variables under analysis.

## References:

- [1] Pearce, J. and Ferrier, S. (2000), *Evaluating the predictive performance of habitat models using logistic regression*, *Ecological Modelling*, 133, 225-245.
- [2] Guisan, A. and Zimmermann, N. E. (2000), *Predictive habitat distribution models in ecology*, *Ecological Modelling*, 135, 147-186.

## **Biogeography and conservation of the common snipe *Gallinago gallinago*: application of ecological niche models to different spatial scales during the breeding season**

**M. Rodrigues<sup>1</sup>, J.C. Brito<sup>2</sup>, T. Rodrigues<sup>2</sup>, J. Domínguez<sup>3</sup>, D. Gonçalves<sup>1,2</sup>**

<sup>1</sup>Departament of Biology, Faculty of Science, University of Porto, Portugal

<sup>2</sup>CIBIO - Research Center in Biodiversity and Genetic Resources, University of Porto, Portugal

<sup>3</sup>Department of Zoology and Physical Anthropology, Faculty of Biology, University of Santiago de Compostela, Spain

The common snipe *Gallinago gallinago* is a migrant wading bird with a wide range, frequent in Iberian Peninsula during winter, where also occurs in small breeding populations. The Iberian breeding populations represent the southwestern distribution limit of the species during the breeding season and are located in the Northwest part of the Peninsula and in the Iberian Mountain Central System. Despite the Iberian restricted range and the observed sharp population decline, ecological niche differences of breeding sites between Iberian populations and the remaining Europe have never been assessed. In this work, ecological niche-based models (ENMs) were developed for the common snipe distribution during the breeding season in the Iberian Peninsula and in the complete European range. Species occurrence data and environmental factors (topographic, climatic and habitats) from each study area were combined with GIS and ENM software (Maxent). Environmental factors most related to species occurrence were identified and potential breeding areas were predicted. Models developed for Iberia were projected into Europe and vice-versa, in order to check if the regional breeding niche (Iberia) is different from the global (Europe). Species occurrence is explained by different combination of factors at each scale. The most important factors for the occurrence of the common snipe at European scale are maximum temperature of warmest month, temperature seasonality, and shrub cover, closed-open and deciduous habitat type. In the Iberian Peninsula, besides the maximum temperature of the warmest month, as in Europe, precipitation seasonality and the tree cover, broadleaved, deciduous, and closed habitat type, are the most important factors. These differences suggest that the ecological niche of the Iberian populations may be related to different environmental pressures from those that occur, on average, in European ones and are reflected in the habitat suitability models, resulting in the inability of the European model to identify Iberian breeding areas and vice-versa, which highlights particular requirements concerning the selection of breeding habitat in Iberia. Results of this work increased the current knowledge about common snipe distribution and breeding habitat selection in the peripheral and threatened Iberian populations, which constitute framework tools for the development of optimised conservation strategies.

Oral Sessions VIII > A5

# Engineering IV



# Elasto-static analysis using a meshless method

S. Moreira<sup>1</sup>, L.M.J.S. Dinis<sup>2</sup>, R.M. Natal Jorge<sup>2</sup> and J. Belinha<sup>3</sup>

<sup>1</sup> Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup> Associate Professor, Department of Mechanical Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>3</sup> Mechanical Engineering Institute (IDMEC), Faculty of Engineering, University of Porto, Portugal.

In structural engineering, numerical methods are an important tool in the modulation and analysis of complex structures. For many years the Finite Element Method (FEM) was the most widespread numerical method used [1]. However in the last fifteen years meshless methods [2] enlarge their application field, and are today a competitive and alternative numerical method in structural analysis. In this work a meshless method [3] is used to study several solid mechanics benchmark examples considering an elasto-static analysis.

Generally, in meshless methods the nodes discretizing the problem domain can be randomly distributed, since the field functions are approximated within a flexible influence domain rather than an element. In meshless methods the influence domains may and must overlap each other, in opposition to the no-overlap rule between elements in the FEM. In this work a radial interpolator meshless method is used [3], where the nodal connectivity and the background integration mesh, totally dependent on the nodal mesh, are achieved using mathematic concepts, such as Voronoï Diagrams and the Delaunay tessellation [3]. The obtained interpolation functions, used in the Galerkin weak form, are constructed with the Radial Point Interpolators [3] and possess the delta Kronecker property. Due to the organic procedure employed to impose the nodal connectivity the displacement and the stress field are smooth and accurate, which can be observed in figure 1, for the solid mechanics classic example: the infinite plate with a central hole.

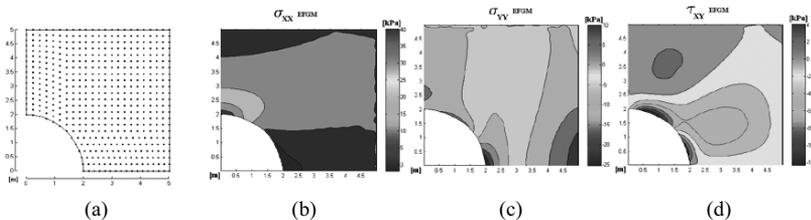


Figure 1 – (a) Domain discretization. Obtain stress field: (b)  $\sigma_{xx}$ , (c)  $\sigma_{yy}$  and (d)  $\tau_{xy}$ .

## References:

- [1] Zienkiewicz, O.C., (1989), *The Finite Element Method*. 4th ed., McGraw-Hill.
- [2] Nguyen, V.P., Rabczuk, T., Bordas, S., and Duflot, M. (2008), *Meshless methods: A review and computer implementation aspects*. Mathematics and Computers in Simulation, 79, 763–813.
- [3] Dinis, L., Jorge, R.N., and Belinha, J., (2008), *Analysis of 3D solids using the natural neighbour radial point interpolation method*. Computer Methods in Applied Mechanics and Engineering, 196, 2009–2028.

## Residual stress determination using FBG sensors

R. Barbosa<sup>1</sup>, J. E. P. C. Ribeiro<sup>2</sup>, P. M. G. P. Moreira<sup>2</sup>

<sup>1</sup> Department of Mechanical Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup> Institute of Mechanical Engineering and Industrial Management, Portugal

Residual stresses are locked-in stresses which exist in a structural part without the application of any service or other external loads. The effects of residual stress may be either beneficial or detrimental, depending upon the magnitude, sign, and distribution of the stress with respect to the in service applied loads. In most cases, residual stresses arise from the production process.

Today, due to weight and cost reduction policies there is an increasing pressure to better understand the effect of residual stresses on the mechanical properties of a component/structure. Its failure can be due to external loading conjugated with locked-in residual stresses. In most cases, residual stresses are created due to mechanically induced plasticity or by thermal effects [1]. Quantitative estimation of residual stresses is important for a safe performance of structural components [2].

In order to measure residual stress mechanically, the locked-in stress must be relieved so that a sensor can register the change in strain caused by the relaxation of the stress. One of the most common methods to measure residual stresses is the hole drilling method, which can be considered almost non-destructive due to the hole small diameter [3]. This method, technique widely accepted for measuring residual stresses, involves drilling a small hole into the surface of a component at the centre of a special strain gage rosette and measuring the relieved strains. This type of sensor can be problematic when used on industrial environments, mainly due to electrical noise and magnetic fields. In order to eliminate this drawback, this study is focused on the development of a new methodology to replace the electrical sensors with optic fiber sensors, fiber Bragg grating (FBG) sensors. After installation and residual stress measurements, this new type of sensors can also be used to monitor the strain loading of the component during service.

A MIG welded plate was used as a component containing residual stresses. Two optic fiber rosettes, with 1.6 and 3.2mm gratings, were developed and tested. For comparison, two similar electric strain gage rosettes were also used. The results obtained, particularly with the 3.2mm length grating sensors, were encouraging. The residual stress profile is in agreement with the one obtained for the electrical sensors.

R. Barbosa acknowledges the support of Porto University Pluridisciplinary projects program and FiberSensing for kindly supplying the necessary FBG sensors.

[1] Withers P. and Bhadeshia H., Overview - Residual stress part 2 - Nature and origins. *Materials Science and Technology*, 2001. 17(4): p. 366-375.

[2] Venkitakrishnan P., Philip J., and Krishnamurthy R., An assessment of stresses in thin walled welded tubes through hole drilling and sectioning methods. *Journal of Materials Processing Technology*, 2007. 185(1-3): p. 228-232.

[3] Mathar J., Determination of Initial Stresses by Measuring the Deformation Around Drilled Holes. *ASME Transactions*, 1934. 56(4): p. 249-254.

# Application of *Lean Production Management* to manufacturing modules of automotive industry parts

**Diogo A. Regalo**<sup>1,2</sup>, Manuel B. Silva<sup>2</sup>, Abel D. Santos<sup>1</sup>

<sup>1</sup> Department of Mechanical Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup> Simoldes Plásticos, Oliveira de Azeméis, Portugal.

Principles of *lean production management* involve the identification of customer value, reducing to zero all forms of waste in the production system. That is made through the management of the value stream, developing the capability to flow production, the use of *pull systems* to support flow of materials at constrained operations [1]. Lean production has actually challenged the accepted mass production practices established for decades, first in the automotive industry, now in all sectors of the industry. This challenge to mass production traditional management made by the so called *lean thinking* has been carried out through an important change in the relation between productivity and quality. The core idea of maximizing customer value while minimizing waste led to a rethinking of a wide range of manufacturing operations beyond the industrial repetitive production environment [2, 3].

This work presents the application of *lean production* approaches and methodologies in the industry of injection of plastic automotive products. Value stream mapping (VSM) methodology is applied in several production process analyses seeking to identify and eliminate waste in the value stream. The application of other methodologies, such as 5S, SMED and the use error-proof systems (also known as *Poka-yoke* systems) is described in this work, where results of actual improvement are shown. Additionally, it is shown the importance of involving all the workers in the process of continuous improvement, as well as the significance of *lean production management* methodologies by creating a strong positive impact in the value stream, thus improving the shop floor visual management and increasing the production flexibility and efficiency.

## References:

- [1] Peter Hines, Matthias Holweg, Nick Rich, (2004) "Learning to evolve: A review of contemporary lean thinking", *International Journal of Operations & Production Management*, Vol. 24 Iss: 10, pp.994 - 1011
- [2] Matthias Holweg (2007). "The genealogy of lean production". *Journal of Operations Management* **25** (2): 420–437. doi:10.1016/j.jom.2006.04.001.
- [3] J. Womack, D.T. Jones (1996), *Lean Thinking: Banish Waste and Create Wealth for Your Corporation*, Simon and Schuster, New York, NY.

# Land use/land cover changes and flooding surface estimation in Alqueva (Portugal) using 18 years of Landsat data

Dário Ferreira<sup>2</sup>, Sofia Rios<sup>2</sup>, Ana Teodoro<sup>1,2</sup>

<sup>1</sup> Centro de Investigação em Ciências Geo-Espaciais, Faculdade de Ciências da Universidade do Porto

<sup>2</sup> Dep. de Geociências, Ambiente e Ordenamento do Território, Faculdade de Ciências da Universidade do Porto

Land use/cover classification tops natural resources management priorities world-wide and especially in complex and heterogeneous landscapes such as the dry and semi-dry ecosystems. Landsat imagery can be used for detecting terrestrial land cover conditions, and tracking land vegetation, agricultural activity, urban growth, and surface hydrology and proved to be valuable in qualitative and quantitative terrestrial land cover changes.

All the Landsat images, which cover a period between 1992 and 2009, were submitted into pre-processing tasks. Firstly, all the images were cut into a Region Of Interest (ROI) correspondent to the study area (Alqueva area). Then, radiometric normalization was applied in order to remove radiometric distortions and make the images comparable. After the pre-processing tasks, the images were classified. The image classification procedure was divided as a pixel- and region-based approach.

The pixel-based classification presents the best results of two approaches. All the images showed an overall accuracy higher than 90%, and all the classes identified (according to CORIN land cover nomenclature) were well separated, considering the Bhattacharyya distance ( $>1.95$ ). The surface flooding surface increased from 14,000 ha in 1992 to approximately 23,000 ha in 2009. In the 1992-2009 period, the variation of pixels number results are given in Figure 1.

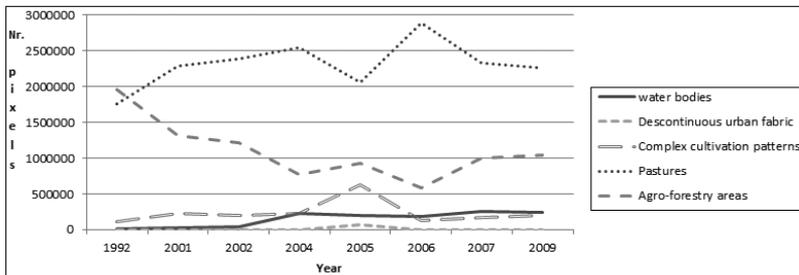


Figure 1: Classes evolution (in terms of number of pixels) between 1992 and 2009 considering the minimum distance classifier.

In this work, was proved that the pastures class present the higher land cover occupation class; the flooding surface (water bodies class) until floodgates were closed (February of 2002) is practically null; the increase and decrease on pastures class and agro-forestry areas are inversely proportional; the discontinuous urban fabric class has a very low representation (practically null); the agro-forestry areas class and the complex cultivate patterns had approximately the same behaviour after 2002. Therefore, as expected, the Alqueva dam construction changes the land cover of the surrounding areas.

# Expansion of IFC Data Model to Kinematic Sensors

**B. Ferreira<sup>1</sup>, J. Rio<sup>2</sup> and J. Poças Martins<sup>3</sup>**

<sup>1</sup> Department of Civil Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup> LABEST, Department of Civil Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>3</sup> Gequaltec, Department of Civil Engineering, Faculty of Engineering, University of Porto, Portugal.

The instrumentation and structural health monitoring of buildings has gained a growing importance in the construction industry. However, the resulting data is not always properly organized, stored and used. [1] For this reason, the aim of this work was to test the integration of the data collected in an information management system such as BIM (Building Information Model). BIM is based on the idea of integrating all information related to a building or project in a single digital model. This information can be associated prior, or during, the construction of the building or even during its lifetime. These tools are being developed rapidly, increasing their chances of information management.

The purpose of this study is to manage information from the instrumentation and structural health monitoring. As to achieve this goal, it was studied a standard construction model, with the use of a common language that serves as the industry standard, known as IFC (Industry Foundation Classes). In this work is performed an assessment of the applicability of the IFC standard, as a format for information exchange between sensors and BIM. It was also proposed the extension of the model based on kinematic sensors, since it presently only includes environmental sensors. [2]

Based on the model above, and using BIM programs, a real case study was done concerning the “Nave do INEGI” building using data from actual measurements. It was conducted a three-dimensional model of the building, studied the interoperability between various BIM tools and compatible properties were created within the IFC model capable of delivering the information recorded by the sensors.[3]

Thus, it was studied the issue of the instrumentation and structural health monitoring framed on the BIM software on a scale closer to the real, where the adversities and the problems substantially differ from those presented theoretically. The results suggest that the management of information from the BIM with the data obtained by the sensors is achievable.

## References:

[1] Akinci, B. (2003). Using sensor systems and standard project models to capture and model project history for building commissioning and facility management Burcu Akinci 2 . Problem Statement embedded sensor systems and integrated project models.

[2] Froese, T. (2003). Future directions for IFC-based interoperability. *Exchange Organizational Behavior Teaching Journal*, 8(April), 231-246.

[3] Ferreira, B. F. V. (2011). *Aplicação de conceitos BIM à instrumentação de estruturas*. Master's Thesis. FEUP.

# Strain sensitivity enhancement in suspended core fiber tapers

Ricardo M. André<sup>1,2</sup>, S. O. Silva<sup>1,2</sup>, M. B. Marques<sup>1,2</sup> and O. Frazão<sup>2</sup>

<sup>1</sup> Departamento de Física e Astronomia, Faculdade de Ciências, Universidade do Porto, Portugal.

<sup>2</sup> INESC Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal.

Optical fibers based on silica have become an irreplaceable asset in telecommunications, data transport and sensing. Fiber tapering of conventional fibers to reduce the waveguide dimensions has been thoroughly investigated. Low-loss tapers with subwavelength diameters offer empowering optical and mechanical properties such as strong confinement and consequent non-linear interactions [1], large evanescent fields, and high mechanical strength [2]. In the last 15 years, microstructured optical fibers have attracted a lot of attention due to their excellent properties. Over the last decade, tapering of microstructured optical fibers has been perfected [3] and it was shown that microstructured optical fibers can be tapered while retaining their cross-sectional profile with little or no distortion [4]. The cross-sectional profile can thus be scaled down proportionally producing structures with very small core diameters.

Suspended core fiber tapers with different cross sections (from 70  $\mu\text{m}$  to 120  $\mu\text{m}$  diameter) were produced by filament heating. They were deemed insensitive to temperature and external refractive index but extremely sensitive to strain. Sensitivities of  $-1.73$  and  $-3.12$   $\text{pm}/\mu\epsilon$  for 120 and 80  $\mu\text{m}$  taper structures, respectively, were obtained (see Figure 1). An increase of strain sensitivity with decreasing taper diameter was observed. It was also verified that even when the suspended-core fiber was tapered down to 40  $\mu\text{m}$  the holes did not collapsed.

Acknowledgements:

The authors would like to thank Fundação Calouste Gulbenkian for the grant provided under the award “Prémio de Estímulo à Investigação” attributed to Ricardo Melo André.

References:

- [1] S. G. Leon-Saval, T. A. Birks, W. J. Wadsworth, P. S. J. Russell, and M. W. Mason, *Supercontinuum generation in submicron fibre waveguides*, Opt Express 12, 2864-2869 (2004).
- [2] G. Brambilla, V. Finazzi, and D. J. Richardson, *Ultra-low-loss optical fiber nanotapers*, Opt Express 12, 2258-2263 (2004).
- [3] J. K. Chandalia, B. J. Eggleton, R. S. Windeler, S. G. Kosinski, X. Liu, and C. Xu, *Adiabatic coupling in tapered air-silica microstructured optical fiber*, IEEE Photonics Technol. Lett. 13, 52-54 (2001).
- [4] E. C. Magi, P. Steinvurzel, and B. J. Eggleton, *Tapered photonic crystal fibers*, Opt Express 12, 776-784 (2004).

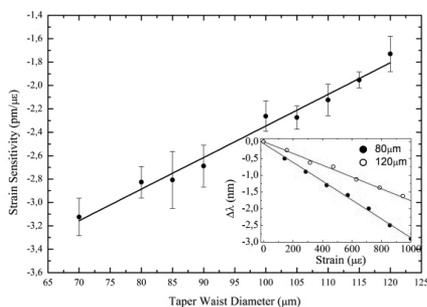


Figure 2 - Relationship between the taper waist and the strain sensitivity. Inset: wavelength change with applied strain for 80  $\mu\text{m}$  and 120  $\mu\text{m}$  SCF tapers.

# On the Viability of Cooling Integrated Circuits using Magnetocaloric Materials

**T. Lima<sup>1,2</sup>, D. Lopes<sup>1,2</sup>, B. Bordalo<sup>1,2</sup>, D. Silva<sup>1,2</sup>, A. Pereira<sup>1,2</sup>, J.O. Ventura<sup>1,2</sup> and J.P. Araújo<sup>1,2</sup>**

<sup>1</sup> Department of Physics and Astronomy, Faculty of Sciences, University of Porto, rua do Campo Alegre s/n, 4169-007, Portugal

<sup>2</sup> IFIMUP/IN – Material Physics Institute of the University of Porto – Nanoscience and Nanotechnology Institute, rua do Campo Alegre s/n, 4169-007, Portugal

In this work we propose novel approaches to refrigerate integrated micro-circuits and perform numerical simulations to attest their viability. These systems are based on magnetocaloric materials – materials that have the ability to absorb heat upon the application of an external magnetic field – the so-called *Giant Magnetocaloric Effect*. Such materials could prove useful in cooling integrated circuits (IC's) such as computer processors or sensors [1].

We designed a model [2] which uses a specific magnetocaloric material,  $Gd_5Si_2Ge_2$  (GSG), two microchannels where water flows and a dissipating power acting as our working IC (fig. 1).

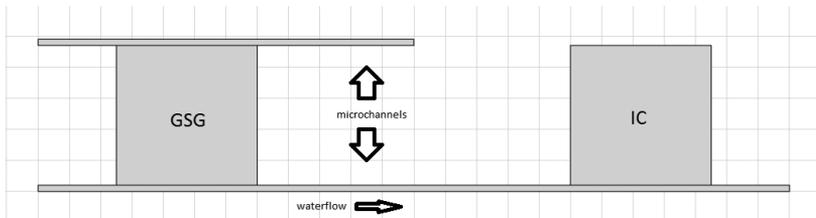


Figure 1: The microcooling system

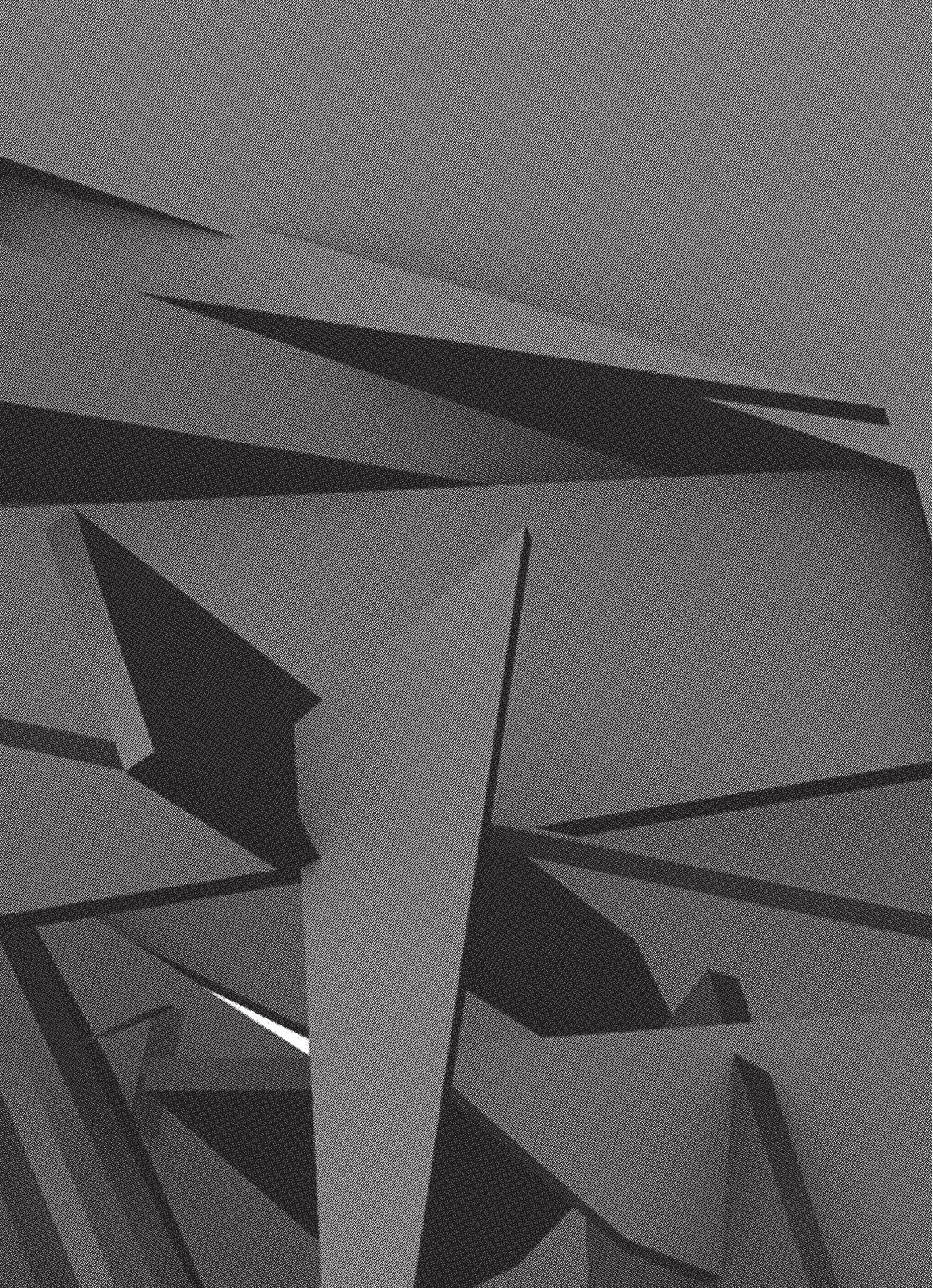
The system operates cyclically, i.e. we apply the magnetic field periodically on the GSG material, alternating the heat flow between the top and bottom microchannels. One of the microchannels is in contact with the IC, cooling it, while the other is used to exchange with the exterior the generated heat.

The main objective of this project was to simulate how the system behaves with different operation frequencies and different fluids. To do so we used the *COMSOL Multiphysics®* software. This project was developed in an extracurricular internship program (PEEC) of the Faculty of Sciences of University of Porto in cooperation with IFIMUP/IN.

References:

[1] Silva, D. (2010), *Simulation and Optimization of Magnetic Micro-Refrigeration Systems*, master's dissertation, FCUP

[2] Pereira, A.M., Oliveira, J.C.R.E., Soares, J.C., Ventura, J., Sousa, J.B., Araújo, J.P., *Simulations of refrigeration on integrated circuits using micro-channels*, Journal of Non-Crystalline Solids, 2008



# Posters



Posters

# Posters from Wednesday, 22<sup>nd</sup>



## Assessing service quality in public transportation: Metro do Porto

A.Bizarro<sup>1</sup>, A. Oliveira<sup>1</sup>, M. Ameixeira<sup>1</sup> and A. Brochado<sup>1</sup>

<sup>1</sup> Faculty of Economy, University of Porto, Portugal.

“Metro do Porto” has been performing a very important role in terms of transportation and mobility in the city since the time it was created. For this reason, the main objective of this work is to evaluate the performance of the service that “Metro do Porto” provides, according to its users and study the factors that are relevant.

Based on previous studies [1-3], a qualitative research was developed, using a modified SERVPERF scale and an online survey addressed to 738 users. The distribution of this questionnaire was through the online platform of the Faculty of Economy and Faculty of Engineering from Oporto’s University and social networks (convenience and snowball sampling).

Most of the respondents use a monthly pass to travel (55%) and most of the movements take place in the rush hour (70%). The frequency of use is mostly daily (40,6%) and occasionally (29,3%). The Line D Hospital de S. João – Santo Ovídio (this line crosses the university campus) has the most users (72,6%), which can be justified by the fact that the most common reason for travel is the study (56,9%) and most of our respondents have between 18 and 25 years (71,1%). The variable gender of Metro do Porto users in our sample is equally divided (female – 43,2% and male – 42,6%).

Factor analysis results revealed the existence of eight dimensions that influence the service quality of Metro do Porto. These factors were named as follows: tangibles, empathy, accuracy, reliability, social image, assurance, net coverage and price. All of these factors present a mean higher than 2,5 (in Likert scale with 5 points), with social image (4,02) and assurance (3,98) having the highest perceived service quality and price (2,87) and net coverage (3,09) the lowest. Cronbach’s alpha test shows that all factors are reliable (Cronbach’s alpha is greater than 0,7). The global quality of the service is 3,93, of which 39,2% is explained by the eight factors. Managerial implications of this study were also addressed.

References:

- [1] Eboli, L. and Mazzula, G. (2009), *A New Customer Satisfaction Index for Evaluating Transit Service Quality*. Journal of Public Transportation, Vol. 12 (3), pp. 21-37.
- [2] Maruvada, D. Prasad and Bellamkonda, R. Shekhar (2010), *Analyzing the Passenger Service Quality of the Indian Railways using Railqual: Examining the Applicability of Fuzzy Logic*. International Journal of Innovation, Management and Technology, Vol. 1 (5), pp. 478-482.
- [3] Randheer, K., Al-Motawa, A. and Vijay, J. P. (2011), *Measuring Commuters’ Perception on Service Quality Using SERVQUAL in Public Transportation*. International Journal of Marketing Studies, Vol. 3 (1), pp. 21-34.

# CHARISMATIC LEADERSHIP: THE CASE OF JORGE NUNO PINTO DA COSTA

André Sousa Machado<sup>1</sup>

<sup>1</sup> Master in Business Management, Faculty of Economy, University of Porto, Portugal.

Power is a phenomenon which the concept has been widely discussed in organizational studies, as well as in various areas of social sciences. With the growth of organizations shall be no more structures, tasks and skills fragmented and specialized positions functionally differentiated and codified knowledge, stored, evaluated and divided, requiring the creation of strategies for the joint management of the organization and to obfuscate issues and conflicts, arising from the division of labor. Emerges in this context (the present case), which is the analysis of the power of relations in organizations, in an attempt to establish a bridge between the work of *Michel Foucault* and the case in question, the charismatic leadership of Jorge Nuno Pinto da Costa at the Futebol Clube do Porto. Its longevity and how it has led the club over several decades allows us to extrapolate and relate their practices to the content studied, presented and disclosed. Do not exist at present time better examples of the emblematic figure cited here. The President (chairman) of FC Porto, Jorge Nuno Pinto da Costa, is within this framework, a living legend. The richness and complexity of the thought of Michel Foucault may raise some concern by the scientific community, especially related to the field of applied sciences, such as Management; use it for analysis of its objects of study. Foucault always advocated a theory: is that it is only useful if you allow this condition to achieve the objectives. As a toolbox:

[...] It must be useful, it must function and not only for itself. If no one uses it, beginning with the very theory that ceases to be theoretical, it is worthless or that the moment has not arrived yet. Do not revise a theory, others are made; there are others to be made (*Foucault, 1981*).

In this sense, the aim with the analysis of this case was to use Foucault's theory of power, in order to correlate structural and thought this theme with a real example of our days. This is a true example of successful leadership. The charismatic leadership of Jorge Nuno Pinto da Costa is now considered one of those responsible for national and international success that FC Porto won the last decades.

FC Porto is the club with the most titles won in the XXI century, having won in this period, four international trophies. The great European look for FC Porto recognizing its qualities and recognition by Jorge Nuno Pinto da Costa with the Career Award in Soccer Globe Awards 2011 is an example of this. Pinto da Costa, who led FC Porto since 1982, is the club president with more titles worldwide, bringing 55 trophies. Without doubt, an example to follow, an inspiration to us all!

References:

[1] Foucault, M. (1981), *Microfísica do Poder*, Edições Graal, Rio de Janeiro.

## Efficiency Analysis of Portuguese Hospitals

A. Silva<sup>1</sup>, L. Fonseca<sup>1</sup>, N. Monteiro<sup>1</sup>, A. Brochado<sup>1</sup>

<sup>1</sup> Faculty of Economics, University of Porto, Portugal

The National Portuguese Healthcare System (SNS-Serviço Nacional de Saúde) has suffered significant changes in the past few years. Indeed, since 2002, several hospitals have been transformed into public corporations (EPE Hospitals) and afterwards into public enterprises. Moreover, new business management models have been adopted by hospital health care units and considerable attention has been focused on hospital efficiency [1].

This study aims at analyzing efficiency levels of Portuguese Hospitals using a non-parametric method – data envelopment analysis (DEA). Data for 50 hospitals (20 SPA and 30 EPE) was collected for the year 2008.

Two different efficiency measures were obtained, each related to different sets of inputs: One relates to the costs of providing medical care, while the other includes the human resources available in each unit. Both models used as outputs variables that best reflect hospital services' results (such as patients discharged or emergency services).

Average efficiency levels are high, only having one or two units deviating to levels under 90%. Using both models we can see that EPE hospitals are, on average, more efficient than SPA ones, but the difference is small. From our sample of 50 hospitals, 30 operated with efficiency scores equal to 100%.

Comparing our two approaches, when costs of production are used as inputs, the average efficiency levels are higher and less volatile than when human resources are considered. The performance measures obtained are also used in a second stage Tobit regression in which environmental and structural variables are included (case mix index, legal status and average length of stay).

Comparisons with previous studies and managerial implications were also addressed.

### References:

[1] Rego, G., Nunes, R. and Costa, J. (2010). *The challenge of corporatisation: the experience of Portuguese public hospitals*. European Journal of Health Economics, Vol. 11, pp. 367–381

# Keynes e o Brasil de 1929

S. Leandro

<sup>1</sup>Department of Economic, Faculty of Economic, University of Porto, Portugal.

This article aims to show the vision of Celso Furtado on the 1929 crisis, which began in the United States and that is reflected rapidly in Europe and arrived in Brazil with a crisis in the Balance of Payments. In Brazil, one of the earliest exponents of Keynesian thought, despite its few explicit references to Keynes, was Celso Furtado. In this direction, perhaps his most influential work was the formation of Brazil Economic immediately recognized as an application of the approach proposed by Keynes to macroeconomic economic history. Anyway, the school ECLAC, while remaining active, represented an important stimulus not only to knowledge of the ideas of Keynes, but its use in contexts other than those in which the author developed theme preference for the presentation, ORAL or POSTER.

## References:

FURTADO, Celso. "Formação Econômica Brasileira",  
KEYNES, John. "A TEORIA GERAL DO EMPREGO, DO E DA MOEDA Juro"

## Portuguese investor profile

**S. Marques<sup>1</sup> and A. Oliveira Brochado<sup>2</sup>**

<sup>1</sup>Faculty of Economics, University of Porto, Portugal.

<sup>2</sup>UNIDE, ISCTE, University Institute of Lisbon, Portugal.

The search for investments decisions in financial market is an in-depth discipline, where the growing amount of empirical data is continuously advancing the understanding of investor behaviors. Being conscious of the empirical limitations of the *homo economicus* model for exploring the behavior of private individuals, behavioral finance broadens the view by combining knowledge from psychology and economics [1].

This study aim at identifying and profiling segments of individual investors based on their shared investing attitudes and behavior, by demographics and cognitive factors. A questionnaire was answered by 776 respondents in Portugal, during December 2011. The sample of this study is relatively heterogeneous, which allows us to have an overview of the financial attitudes and behavior tendencies of a variety of demographic groups.

Factor analysis revealed the existence of five almost independent dimensions on investing styles, named as: investment horizon, confidence, control, risk attitude, and personalization of loss. Moreover, a logistic regression model was used to study the impact of sociodemographic variables on investing decisions and behavioral tendencies – how to invest and to invest in saving modes or risk products. We found that variables as gender, age, income, marital status, residence, family members, occupation and education have a significant influence on investing decisions.

### References:

[1] Camerer, C.F. and Loewenstein, G. (2004), “Behavioral economics: past, present, future” in Camerer, C.F., Loewenstein, G. and Rabin, M. (Eds.), *Advances in Behavioral Economics*, Russell Sage Foundation, New York, pp. 3-51.

## HELENA ALMEIDA - DRAWING AS AN APPROPRIATION ACT FOR A PERFORMATIVE

Ana Margarida Sousa<sup>1</sup>

<sup>1</sup> History of Art, Faculty of Letters, University of Porto, Portugal.

All the work of Helena Almeida deals with the draw as a "**model**", a "**method**" as a "**line of thought**," but mainly as a **restored behavior** that **reorganizes** and **reconstructs** a performativity act.

The drawings are the real principle of their action, in which you can determine the development of such actions

This type of work that we call the **process of representation** involves the "**transfer-of-use**."

"**Transfer**" in the substitution of acts does not necessarily imply the physical presence of the author [1].

"**Use**" in the documents that may be diverted to accommodate other content [2].

These **actions** present in the work of Helena Almeida, we can consider '**performance**', are thought to **support** a single **photo**, as such, raises the question: To what extent can we consider 'performance'?

Since we did not see action in his time here, the performance is the starting point, is used as a resource for the construction of the concrete image of the photograph, not a thought to public space. It is often performed in a private space, the studio of the artist, and has as 'public' her husband, Artur Rosa, who shoots and sometimes shooting their actions.

Although the picture is the final support, turns out to be 'proof' documentation of actions, of what 'happened', resulting in an image, what we see.

The image-figure, but also the senses: hearing, feeling, seeing. And "minimal gestures: a hand that draws a line, a mouth that opens to blue, a black body that pours"[3].

References:

[1] ALMEIDA, Paulo Luís, *Desenho e Performatividade [3.9]. Transferências de uso: apropriação, citação e migração de actos entre campos performativos. Desenho e contágio motor. Fundamentos cognitivos e semióticos da conduta gestual no desenho*, in Web: [arquivoerepatorio.yolasite.com](http://arquivoerepatorio.yolasite.com), Faculdade de Belas-Artes da Universidade do Porto\_ Mestrado de Desenho e Técnicas de Impressão, 9 de Março de 2010, p.14.

[2] *Ibidem*.

[3] CARLOS, Isabel, *Helena Almeida*, Editorial Caminho, SA, p.20.

# Organizational metaphors in movies: an analysis of the archetypes in films that focus on interpersonal relationships present within corporations

T. Silva<sup>1</sup>, L. Filho<sup>2</sup>

<sup>1</sup> Department of Ciências da Comunicação, Faculty of Letras, University of Porto, Portugal. Student of mobility by the Federal University of Juiz de Fora, Brazil.

<sup>2</sup> Journalist and university professor. Ph.D. in Communication from the Federal University of Rio de Janeiro. Book author, professor, department head and coordinator of the Specialization Course in Corporate Communications from the Federal University of Juiz de Fora and professor of the Masters Program in Communication and Society from the same institution.

People around the world have managed to build stories from the metaphorical language. Instructions, advice and beliefs were passed on through new generations of fantastic stories, analogies and symbols with specific people.

For the reports and felt they could achieve in the minds of those features were needed to allow the understanding of metaphorical discourse present. The proximity of the report and the context experienced by listeners, and an identification between the past and present partners are analogies that make sense, which is essential because this is a symbolic system dependent on the cooperation of the individual to take effect.

According to Thomas Wood [1], studies on the use and interpretation of the symbols were left in the background until the early nineteenth century because they were linked to both religious tradition, with symbols to represent the Church's power and legitimize their acts of God ground and the power of rulers, both of which were intended to subdue the mind.

With the artistic expressions that mark the nineteenth century, as Dadaism and Surrealism, there is a symbolic resumption of studies, but this time with the freedom of creation and interpretation. The use of symbols beginning with the arts and from it there is the development of post-modern society and promoting the use of metaphor in the texts.

References:

[1]Wood, JR. Thomaz. *Organizations spectacular*. RJ: Editora FGV, 2001.

## Ausubel meaningful learning among Biology and Geology high-school students

A. S. Abreu<sup>1</sup>, A. P. Fonseca<sup>2</sup>, A. Valente<sup>3</sup>, C. Vasconcelos<sup>4</sup>

<sup>1</sup>Faculty of Sciences, University of Oporto, Portugal

<sup>2</sup>EB3/ Almeida Garrett Secondary School, Vila Nova de Gaia, Portugal

<sup>3</sup>Department of Biology, Faculty of Sciences, University of Oporto, Portugal

<sup>4</sup>Department of Geosciences, Environment and Spatial Planning, Faculty of Sciences, University of Oporto, Portugal

Epistemology helps teachers to improve their own nature of science concepts and their pedagogic action [1]. Aiming at meaningful learning for their students, teacher must use diverse strategies that make easier the comprehension of scientific knowledge. Pre-service and in-service teachers, they both know that it isn't enough to transmit and memorise. Students must understand the meaning of facts and evidences to be able to use it whenever it is required in everyday life. In fact, memorization does not influence comprehension [2] of the concepts under study. Meaningful learning only occurs when new information is assimilated and integrated in the student cognitive structure, acquiring meaning from its interaction with previous knowledge [1]. Concept maps are a graphic instrument that allows the representation of the scientific knowledge, through the use of concepts and links between them.

In our study concept maps were used with 24 students from a convenience sample (one 11<sup>o</sup> grade class from an EB3/ Almeida Garrett Secondary School, Vila Nova de Gaia) before and after teaching Biology and a Geology thematic within the scholar program (Sexual reproduction and Sedimentary rocks, historical earth archive). With the data collected a subject analysis will be done taking in account the scores proposed by Novak and Gowin [3], with some adaptations. The analysis of the obtained results shown a concept organization better scored in the final concept map, reflecting an increase in the number of concepts used and an increase in the meaningful links between them. All these improvements point out to a meaningful learning process in students.

We have no doubts that the use of concepts maps can be used to improve meaningful learning, although teachers and students aren't familiarised with this instrument. The initial difficulties, both to teachers and students, should not discourage their use. In the future a generalized application, and since the early ages, will undoubtedly help students in the process of learning how to build concept maps

### References

[1] Cachapuz, A., Praia, J & Jorge, M. (2002), *Ciência, Educação em Ciência e Ensino da Ciências*, Lisboa: Ministério da Educação (IIE).

[2] Novak, J.D. (2000), *Aprender, criar e utilizar o conhecimento*, Lisboa: Plátano Edições Técnicas, Coleção Plátano Universitária.

[3] Novak, J.D. & Gowin, D. B., (1999), *Aprender a aprender*, Lisboa: Plátano Edições Técnicas, Coleção Plátano Universitária.

## **Concept maps and meaningful learning: a study with Biology and Geology contents**

**A. Fernandes<sup>1</sup>, A. P. Fonseca<sup>2</sup>, C. Vasconcelos<sup>3</sup> & A. Valente<sup>4</sup>**

<sup>1</sup>Faculty of Sciences, University of Oporto, Portugal

<sup>2</sup>EB3/ Almeida Garrett Secondary School, Vila Nova de Gaia, Portugal

<sup>3</sup>Department of Geosciences, Environment and Spatial Planning, Faculty of Sciences, University of Oporto, Portugal

<sup>4</sup>Department of Biology, Faculty of Sciences, University of Oporto, Portugal

No longer is expected that students just memorize the information given by teachers. On the contrary, it is expected that they will be curious enough to pursue knowledge and that this interest in learn how to learn will increase their knowledge. The teacher role is to stimulate meaningful learning, developing and implementing strategies and materials to make it possible. Students should learn from daily-life problems, becoming active, informed, responsible and intervenient citizens, capable of thinking over their attitudes face to the world. Meaningful learning means that new knowledge is assimilated by the students, acquiring a meaning through the relationships that are established with existing concepts in the cognitive structure of the subject. Several tools are available to evaluate meaningful learning and the use of concept maps is one such tool. With the concept maps meaningful relationships are established between concepts in the form of propositions. Concept maps present a hierarchical structure of the concepts involved.

In this study, concept maps were used in a convenience sample (one 11<sup>o</sup> grade class of the EB3/ Almeida Garrett Secondary School of Vila Nova de Gaia with 24 students) before and after teaching Biology and a Geology thematic within the scholar program (Life cycles and Sedimentary rocks, first formation steps). With the data collected a subject analysis will be done taking in account the scores proposed by Novak and Gowin, with some adaptations.

The analysis of the concept maps made by the students (before and after the intervention) allowed us to detect an improvement of their concept structure and an increase in the number of both concepts used and the number of links established. The evidence let us conclude that a more meaningful learning occurred due to the process of teaching through concept maps.

Accordingly to our positive experience we advise the use of the concept maps to promote meaningful learning, in spite of the difficulties with its application mainly due to the fact that students aren't familiarized with that tool. Other difficulty results of the lack of knowledge or of the existence of alternative concepts that may unable the students to build a concept map with a proper mean. Due to that fact students showed difficulties in verbalizing the recent knowledge and incorporating it in the map. It is also highly recommended that concepts maps should be introduced to the students at early ages because they learn to produce better maps more quickly than older students, who have been learning mechanically.

## **Initial Training Teachers in Science Education at the UFPR - Coast - Brazil: Possibilities of Renovation**

**J. Santos<sup>1</sup>, D. A. Lima<sup>2</sup>, L.E. Silva<sup>3</sup>**

<sup>1,2</sup> Acadêmicos do Curso de Licenciatura em Ciências da UFPR Litoral;

<sup>3</sup> Professor Coordenador do Curso de Licenciatura em Ciências da UFPR Litoral

<sup>1</sup> Faculdade de Psicologia e Ciências da Educação da UP; <sup>1,2,3</sup> Universidade Federal do Paraná, Brazil.

This paper aims to present the possibilities of educational reform in the initial training of science teachers in UFPR Coast - Brazil. The implementation of this campus in the coast of Paraná is critical to foster the training of students to act in this reality. The Bachelor's Degree in Science education is aimed at training of science teachers to work in elementary school (6th to 9th grade) in the discipline of science. In the face of local and global science education the teachers have many difficulties to build a relationship between science and daily life due to the characteristic fragmented teaching. This approach is observed when a current global issue of human life needs to be better articulate to propose solutions in many different contexts.

From this point, the teacher training in Science Education at UFPR Coast has the interdisciplinary and multidisciplinary training that support the political, philosophical, human and professional approaches. The science teacher in this perspective acts in schools with the goal of training and citizenship. The science knowledge is developed in a manner that enables the understanding of nature with the human being with a member of this complex reality, and where it acts modifies the environment, and science as a human construction, set the time and place with intentions that underlie the political, social, economic and cultural aspects.

The formation of this teacher guided in three areas: theoretical and practical approaches (FTP), humanistic and cultural interactions (ICH) and based-learning projects (PA). In FTP students have contact with the knowledge about the reality of learning, the foundations of education, beyond the specific content of sciences. The contents of the local reality ever go away because it is based on the concrete that provides a meaningful learning. The ICH is learning-space that fosters student leadership. It's space that integrated students from different courses and classes with the local community. The activities are organized through workshops. The PA is the learning-space that possibility the autonomy of students. In this context, the research is structured from student's interest. The focus is to allow to students define issues. The teacher acts as a mediator. The initial training is structured from the first year, allowing students an approach to reality and school context. In this process the individual knows, understands and acts on a feeling he was trying dialectical criticality in practice, reflections before and after its shares, which require observation, dialogue, research, action and advanced theory about the nature of knowledge necessary for the whole process. Understanding their role and commitment in the relationship of teaching and learning of his career.

### References:

FREIRE, P. *Pedagogia da Autonomia: Saberes Necessários à Prática Educativa*. 17ª Ed. São Paulo: Paz e Terra, 2001.

PROJETO POLÍTICO DE CURSO: Licenciatura em Ciências, UFPR Litoral 2010.

## Perceptions of Physical Education pre-service teacher concerning the learning process during the practicum context

**F. Santos<sup>1</sup>, P. Batista<sup>2</sup>, M. Cunha<sup>3</sup>**

<sup>1</sup> Faculty of Sports, University of Porto, Portugal.

<sup>2</sup> Department of Sports Pedagogy, Faculty of Sports, University of Porto, Portugal.

<sup>3</sup> Department of Sports Pedagogy, Faculty of Sports, University of Porto, Portugal.

The teaching practice emerges as one of the most decisive moments of the Teaching Training Programs, usually characterized by the experiencing of dilemmas and mixed feelings in the mind of the pre-service teacher, inherent to the contradiction certainty / doubt (McIntyre et al. 1996). Thus, the structural and structuring character of the practicum refers to the premise advocated by Tardif M. (2000), one learns to do by doing. From this point of view, the practicum enables an approach to professional practice, because it promotes acquisition of knowledge, know-how and judging ability of the pedagogical-didactic activities developed in the workplace. On the other hand, the impact that the pre-service teacher experiences when starting his contact with the profession is defined in the literature as a “reality shock” (Veenman, S., 1984). Taking this framework as a starting point, this is the written presentation of a research project whose purpose is to examine how the pre-service teacher experiences the formative process during the practicum context, and what is the practicum's contribution to the construction of his/hers professional identity. The data collection instrument to be used is a questionnaire developed by Gilat, Kupferberg and Sagee (2006) and adapted by Ezer, Gilat and Sagee (2010). The questionnaire is structured in two sections: one involving closed questions and another open-ended questions. The closed section is composed of five categories relating to the teaching profession and to teachers' education: (1) motivation for teaching, (2) conceptualisations of teaching and learning, (3) roles of the teachers, (4) contents of teachers' training, and (5) training agents. The open-ended section of the questionnaire includes a question in which participants are asked to describe freely “the most significant change they experienced during their teacher's training”. The sample will consist of pre-service teachers of the school year 2011/2012 attending the Masters in Teaching Physical Education in Basic and Secondary Education of the Faculty of Sports, University of Porto.

### References

- [1] McIntyre, D. J., Byrd, F., and Fox, S. M. (1996), *Field and Laboratory Experiences*, in J. Sikula et al. (Ed.) *Handbook of Research on Teaching and Teacher Education*, (2<sup>nd</sup> Ed.), New York, Macmillan, 1996.
- [2] Tardif, M. (2000), *Saberes Profissionais dos Professores e Conhecimentos Universitários*, Revista Brasileira de Educação, 13, 5 - 24.
- [3] Veenman, S. (1984), *Perceived Problems of Beginning Teachers*, in: Review of Educational Research Summer, vol.54, no.2, pp.143-178.
- [4] Ezer, H., Gilat, I., Sagee, R., (2010), *Perception of Teacher Education and Professional Identity among Novice Teachers*, European Journal of Teacher Education, vol. 33, no. 4, pp. 391–404.

## **Adoption, identity and intimacy: An exploratory study of the adoptees' discourse.**

**E. Nicolas<sup>1</sup>, M. Barbosa-Ducharne<sup>1</sup>**

<sup>1</sup> Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

The present exploratory study focuses on the impact of the adoption experience in the development of the adopted emerging adults. More precisely, it focuses on the development of their identity and the construction of an intimate relationship outside the context of the adoptive family.

As stated by the attachment theory, it is known that the integration that is made of the early attachment experiences widely influences the way the individual will face the tasks that its development path puts him through. Further, it is also known that the early experiences are often difficult for the adopted people and that they face increased psychosocial challenges and specific tasks through their life-span.

Thus, by using a qualitative methodology, the purpose of this study was to collect the statements of four emerging adults, aged 19 to 23 years-old and that had been adopted during their first year of life. Through deep semi-structured interviews, several topics that were related to the identity construction and the development of intimate relationships during their life course were explored.

The participants' discourse was analyzed through a content analysis of categorical type. This analysis suggested that, for emerging adults that had been adopted at an early stage and to whom the adopted status was revealed in the pre-school years, for those who considered that the issue of adoption is discussed, within the adoptive family, in a natural, open, constructive and non-defensive way, and who built close and secure relationships with their parents in childhood, the experience of adoption is integrated in a positive way. Adoption is considered as a peculiarity that characterizes them, but that doesn't define them. In addition, the specific features of their developmental path seem to have more to do with the past and present experiences that are lived and observed in their relational contexts, rather than with the experience of adoption itself.

The data provides clues that inspire new lines of investigation in the psychology of adoption, namely in the expression of these experiences in the intimate relationships. Moreover, the results regarding the integration of the adoption experience in the life story of the adopted emerging adult suggests new possibilities of professional practice in order to optimize the post-adoption process.

# Interactive maternal behaviours and intellectual ability of children at school age

**M. Sousa<sup>1</sup>, O. Cruz<sup>2</sup>, and Lopes, S.<sup>3</sup>**

<sup>1</sup> Integrated Master in Psychology, Specialization in Psychological Intervention, Education and Human Development, Faculty of Psychology and Educational Science, University of Porto, Portugal.

<sup>2</sup> Associated Teacher, Department of Developmental and Educational Child Psychology, Faculty of Psychology and Educational Science, University of Porto, Portugal.

<sup>3</sup> Master in Psychology Themes, Specialization in Psychology of Child Development and Education, Faculty of Psychology and Educational Science, University of Porto, Portugal.

The present study aimed to study the influence of maternal interactive behaviours on the intellectual ability of children at school age, as observed in three distinct moments. Starting from a larger longitudinal project, we observed a subsample of 36 dyads when children were 1 to 3 years, 4 to 6 years and 8 to 10 years old.

Two dimensions of interactive maternal behaviour were observed – Responsiveness and Active Teaching – using the Teaching Styles Rating Scale (TSRS) [1], which was applied to mothers of school-age children for the first time through this research. The intellectual ability of children aged 8 to 10 years old was assessed using the Raven Colored Progressive Matrices.

From the methodological point of view, the TSRS proved to be an appropriate instrument for assessing the interactive behaviours of mothers of school-age children. The results indicated that the relationship between maternal interactive behaviours and the intellectual ability of children is only partially explained by maternal education. The hierarchical multiple regression model confirmed the influence of maternal interactive behaviours on the intellectual ability of children of school age, stressing the importance of responsiveness in childhood and the importance of active teaching in school-age children.

The findings of this research highlight the importance of promoting responsive and stimulating maternal behaviours in the intellectual development of children, and suggests clues for future research.

## References:

[1] McWilliam, R., Scarborough, A., Bagby, J. & Sweeney, A. (1998). *Teaching Styles Rating Scale (TSRS)*. Chapel Hill, NC: Frank Porter Graham Child Development Center, University of North Carolina.

# Development and validation of the Inventory “Articulation Person-Organization” (APO)

<sup>1</sup>T. Morais, <sup>2</sup>V. Ramos, <sup>3</sup>F. Jordão

<sup>1,2,3</sup>Psychology Center, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

A fundamental and enduring aspect of both organizations and people is their values [1]. Person-organization fit (P-O fit) has been defined as the congruence between the norms and values of organizations and the values of persons. In order to determine the effects that the organizational membership will have on an individual's values and behaviors and the effects that an individual will have on an organization's norms and values, we must assess the extent of agreement between the person's values and the organization's values [1]. The measurement of the fit between the organization and its workers has been made by taking into account three different approaches: considering organizational values from a specific context; considering the perception of the general culture; or considering the general perception of the fit. Usually, the measurement of fit is related with the lack of value conflict, but recently authors have described these two elements as independent [2]. As there wasn't any instrument available in Portugal that could assess the relationship between individual and organizational values, with this study we intended to create and validate this new inventory named “Articulation Person-Organization” (APO).

We've created and validated a 19 items scale for the Portuguese population to measure the articulation between the person and its organization according to their values through the measure of three constructs: acknowledgement of the organizational values; P-O fit and conflict between individual and organizational values. We displayed the instrument on-line and our sample was defined by 102 people (39 men and 63 women, working in both private and public organizations).

Using an exploratory factor analysis, we found a distribution in three main components. Based on this analysis, three items were removed. In the end, we got an inventory of 16 items with a total variance of 71,9%. This allows us to measure three different components: person-organization fit ( $\alpha = .91$ ), value conflict ( $\alpha = .89$ ) and value recognition ( $\alpha = .90$ ). These results demonstrate the reliability of the instrument and also show that the concepts of “person-organization fit” and “conflict between individual and organizational values” are really different. This study makes available a new instrument that allows us to evaluate, in the Portuguese context, the articulation that exists between individual and organizational values.

## References:

- [1] Chatman, J. A. (1989). Improving Interactional Organizational Research: A Model of Person-Organization Fit. *Academy of Management Review*, 14(3), 333-349.
- [2] Talbot, D., & Billsberry, J. (2011). *Similarities & Differences between Fit & Misfit*. Paper presented at the 15th conference of the European Association of Work and Organizational Psychology, Maastricht.

## **Intergenerational perspective of the adoption process: grandparents, parents and grandchildren**

**R. Barroso<sup>1</sup> and M. Barbosa-Ducharne<sup>1</sup>**

<sup>1</sup> Faculty of Psychology and Educational Sciences, University of Porto, Portugal

Considering that an adoptive family is above all a family, that the grandparents are an essential part of the family and that currently the various generations of a family have more opportunities for interaction for increasingly extended periods of time, it seems appropriate to introduce an intergenerational approach in family processes, such the adoption. Thus, the major objective of this study is to approach the process of adoption in an intergenerational perspective that involves grandparents, parents and adopted grandchildren, and in particular the exploration of intergenerational communication about adoption as a specific task of the adoptive family throughout the life cycle.

In this study the participants were 61 grandparents, 58 parents and 20 grandchildren. The instruments used in the collection of data were the EAPA - Interview with the Grandparents about the Adoption Process, the EPA - Interview about the Adoption Process and the ECAA - Interview with the Children and Adolescents about the Adoption.

The results characterize the experience of the adoption process by the grandparents in a very positive way, and through a clusters analysis led to the identification of two groups of grandparents with regard to the communication about adoption: the group with an attitude of openness communication and the group with an attitude of closed communication. Another clusters analysis made with the results obtained with the parents showed also the existence of two different groups with regard to the process of communication about adoption: the group with an attitude of openness communication and the group with an attitude of closed communication. Similarly, the grandchildren are distinguished by belonging to one of two groups, namely, a group with an open and timely communication and a group with a closed and delayed communication. Through the analysis of these groups in the study of the three generations of the family, we found five types of intergenerational chains of communication in the family, which are distinguished by the continuity or discontinuity of intergenerational communication openness about the adoption.

The results of this study are highlighted by the importance that they can assume to all the people that are directly related to the area of adoption, including researchers and professionals, as they provide information on how the grandparents live the adoption process, on their conceptions of adoptive family and of adoption and also about their role in the process of communication about adoption. Noteworthy are the results on how the process of communication about adoption is lived within the families, in the three generations that make them up: grandparents, parents and grandchildren.

The results evoke the need to devise new ways of professional practice in adoption, including the grand-parental system in the training of prospective and adoptive parents, and they suggest innovative research clues, particularly in terms of the family communication about adoption.

# Relationship between grandparents and adopted grandchildren

**A. Monteiro<sup>1</sup> and M. Barbosa-Ducharme<sup>1</sup>**

<sup>1</sup> Faculty of Psychology and Educational Sciences University of Porto, Portugal.

At the present the relationship between grandparents and grandchildren is recognized as an important relational context, not only for the direct participants, but also for the rest of the family. With regard to adoptive families, this relationship hasn't been object of systematic investigation. The only existing studies highlight the important role played by grandparents in the context of adoption. The way they accept the adopted child as their grandchild contributes for a more easily integration of the child into his/her new family.

This study was developed in the context of the theory of intergenerational family solidarity. The main goal of this investigation refers to the study of the relationship between grandparents and adopted grandchildren, and to the confrontation of that relationship with the one between grandparents-grandchildren in conventional families. In addition, we intend to visit the main experiences of the grandparents on adoption, understand their representations of it and explore the impact of the meaning they attach to the adoption on the relationship they establish with the adopted grandchild.

Sixty-one grandparents with an average age of 69 years-old ( $M=69.21$ ;  $SD=6.76$ ) participated in this study, 63.9% of them are female and 36.1% are male. In terms of lineage, 44.3% of grandparents are of maternal lineage and 55.7% are of paternal lineage. The adopted grandchildren are 8.57 years-old, on average ( $SD=3.31$ ), and have been living with their adoptive families for 5.33 years, on average ( $SD=3.08$ ). Only the grandparents were interviewed through the Interview to Grandparents about Adoption (Barbosa-Ducharme, Monteiro & Barroso, 2011), which covers several topics, one of them being the attitudes of grandparents towards adoption, their experience of adoption, and the nature of the relationship between grandparents and the adopted grandchild.

From the grandparents' perspective, the relationship between grandparents and adopted grandchildren is characterized as very positive, highlighting the affective dimension. The factors that determine variability in this relationship are: the lineage and years of schooling of the grandparent, the sex of the grandchild, the total number of grandchildren and the position that the grandchild occupies in the total set of grandchildren. Comparing this relationship with the relationship between grandparents-grandchildren in conventional families, it appears that with the adopted grandchildren the relationship is described with higher values in all dimensions for both of the age groups comparisons – middle childhood and adolescence. This study also shows that the experiences of grandparents regarding adoption are very positive and have a stronger impact in the development of the concept of adoption for grandparents than the information transmitted by the media. Moreover, it appears that the meaning attributed to the adoption by the grandparents has a differential impact on the dimensions of the relationship between grandparents and adopted grandchildren.

These results give clues for further investigation, but also constitute an important basis for reflections on the relevance of integrating the grandparents in the development of a project of adoption that is intended to be building a family through adoption.

# Socio-cognitive development of adopted preschoolers: Exploratory study

**J. Costa<sup>1</sup>, M. Barbosa-Ducharne<sup>1</sup>**

<sup>1</sup> Faculty of Psychology and Educational sciences, University of Porto, Portugal.

Skills such as emotional understanding and Theory of Mind (ToM) have remarkable progress in preschool age and play a leading role in the quality of children's social interactions. Research has revealed the impact of early adversity on these socio-cognitive skills. On the other hand, adoption has been shown to be an essential transition in the lives of children who experienced early deprivation, with significant potential of development recovery.

This research seeks to explore social cognitive development, such as the emotional knowledge and the ToM in adopted preschoolers.

In this study participated 30 adopted children, with a mean age of 5.07 years (SD = .75), with an average time of adoption of 3.35 years (SD = .98), after an average period of institutionalization of 1.60 years (SD = .82) and respective mothers with an average of 40.30 years (SD = 3.50) and 11.97 years of education (SD = 4.93).

The data on children were collected through the *Emotional Knowledge Tasks* for preschool children; the *Conflicting Emotion Task* of Baron-Cohen; the *Unexpected Content false belief Task*; *Unexpected transfer Task* and the *vocabulary subscale* of the WPPSI-R. Mothers completed the *Strengths and Difficulties Questionnaire* (SDQ) as well as the *Interview about Adoption Process* (EPA – Portuguese version).

The results of this study suggest that the adopted children show a development process in regard to emotional understanding, focusing initially on the facial and situational cues and moving progressively to integrate mental states, based on experienced emotions. In what concerns the ToM, children show an understanding of other's actions, based mainly in the desire than the belief. The mothers' perceptions of child behavior as well as the child's level of vocabulary present normative values. However, it was found that the characteristics of the biological family are related to pro-social behavior of children and that time of institutionalization had a negative impact in emotion recognition and vocabulary. The emotional knowledge and ToM are strongly correlated, although they constitute distinct dimensions. In general, a more sophisticated social cognitive development is related to positive behaviors towards peers.

The results of this study are read as evidence of the recovery character of the adoptive placement of children with early experiences of adversity. Indeed the transition of these children into a responsive, stable and protector context seems to have contributed positively to the current achievement level of emotional knowledge, ToM, vocabulary and behavior.

## **“The transition to adulthood: The quality of parental relationship, career exploration and family-work perspectives on young people with different academic trajectories.”**

**D. Couto<sup>1</sup> and P. M. Matos<sup>1</sup>**

<sup>1</sup> Faculdade de Psicologia e de Ciências da Educação da Universidade do Porto.

The present study focuses on the transition to adulthood considering the fact that it has suffered over the past decades, major changes regarding the characteristics that traditionally marked in a clearly defined way the integration with the adult's role. The theoretical background on which this work was done corresponds to the model of emerging adulthood proposed by Arnett (e.g., 1996[1], 2000[2], 2002[3], 2004[4], 2006[5]). According to model, this distinct period has specific characteristics that mark adolescence and adulthood, being guided by the high level of exploratory behaviors of identity, without an assumption of responsibility that characterizes the adult role. With the present work, we intend to address the model above, through a detailed analysis of its characteristics, with special attention to the Portuguese reality, and factors such as education level, gender and age, which may shape the way this period is experienced by young people. The main objective of this empirical study aims to understand the perception that emerging adults have of the quality of the relationship with both parents, verifying the link between this behavior and dimensions of career exploration, the importance attached at present to various aspects of life (e.g., having a romantic stable relationship, professional success), and the importance in considering a job (current or future). In particular, it is intended to compare a sample of emerging university adults and a sample of emerging adults who have engaged on other paths, as well as analyze the role of gender and age. In total, the sample consists of 294 participants of both genders, aged between 18 and 30 years old. It was found that gender, age and education levels exert an effect on emerging adults' expectations for the variables studied. There were also associations between quality of parental relationship, the dimensions of career exploration behavior, and the importance attached to work and family.

**Keywords:** emerging adulthood; quality of parental relationship; career exploration behavior; balance work and family.

References:

- [1] Arnett, J. (1996). Learning to stand alone: The contemporary American transition to adulthood in cultural and historical context. *Human Development*, 41, 295-315.
- [2] Arnett, J. (2000). Emerging adulthood: A theory of development from the late teens through the twenties. *American Psychologist*, 55, 469-480.
- [3] Arnett, J. (2002). The psychology of globalization. *American Psychologist*, 57, 774-783.
- [4] Arnett, J. (2004). *Emerging adulthood: The winding road from the late teens through the twenties*. New York: Oxford University Press.

## Agar as a film forming agent for food packaging and edible coating

**J. Santos<sup>2</sup>, A.T.S.C. Brandão<sup>1</sup>, J.F.F.V. Baldaia<sup>1</sup>, A.M.M. Sousa<sup>1</sup>, R. Alves<sup>2</sup>, A. Costa<sup>2</sup>, M.B.P.P. Oliveira<sup>2</sup>, M. P. Gonçalves<sup>1</sup> and C.M.R. Rocha<sup>1</sup>**

<sup>1</sup> REQUIMTE, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Portugal.

<sup>2</sup> REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal.

Currently, mainly non-biodegradable petroleum-based synthetic polymers are used as packaging materials for foods, because of their availability, low cost and functionality. However, biodegradable/edible films can be made from polysaccharides, proteins, and lipids without the environmental issues of petroleum-based polymers. On the other hand, the use of edible coatings can work as an additional barrier to food spoilage and can help reducing the amount of packaging material needed.

The main objective of this work was to evaluate the potential use of hot-water extracted agar from the red algae *Gracilaria vermiculophylla*, after cultivation in an integrated multitrophic aquaculture (IMTA) system, as a film forming agent for food packaging and edible coating. For comparison purposes commercial agar was also used. The behaviour of agar coatings when applied to two different food systems (sliced apples and strawberries) was also evaluated.

Films were made using a knife-coating system. Glycerol (20 %; dry basis) was used as plasticizer. All films were conditioned for 48 hours at 53 % relative humidity before further tests. Films were tested for visual appearance, thickness, water sorption, mechanical, barrier and thermal properties. Strawberries were coated by dipping in an agar solution of 1.5% (w/w) and 0.1% Tween80 at 50°C for 5s. Sliced apples were dipped in a 1.20% (w/w) agar solution at 41°C and a dipping time of 20 seconds (with or without citric acid). After drying, they were stored at 10°C and 80% of relative humidity. Coated apples and coated strawberries were periodically tested for visual appearance, loss weight, texture, soluble solids and nutritional changes.

Flexible, transparent films were obtained with both sources of agar (commercial and IMTA). Elongation at break and gas barrier properties were similar for films from both agar sources. The GAB model adjusted well to the water sorption data and no significant differences were observed in the hygroscopic behaviour of both films. Although films from commercial agar were slightly stronger (higher tensile strength) films of IMTA agar showed appropriate properties for food packaging applications. Moreover, the exploitation of this low-value biomass can be of great economic interest.

The presence of an edible coating was benefic to the improvement of shelf life, when compared with no coating, minimizing the weight loss and the oxidation caused by the contact of the fruit with air. Exception to this was the coating of sliced apples with IMTA agar without citric acid. In this case, oxidation of the sliced apples was relevant.

Acknowledgments:

Thanks are due to U.Porto/Santander Totta “Projectos Pluridisciplinares 2010 and to FCT (grants SFRH/BD/64315/2009 to A.M.M. Sousa, SFRH/BD/66476/2009 to J. Santos and SFRH/BPD/68883/2010 to R. Alves) for financial support. Rui Pereira, Helena Abreu from CIIMAR and A. Coelho & Castro, Lda are acknowledged for kindly supplying the biomass.

## Determination of selenium in food supplements by HR-CS-ETAAS

S. Moreira<sup>1</sup>, I.M.C. Almeida<sup>1</sup>, R.C. Alves<sup>2</sup>, M.T. Oliva-Teles<sup>2</sup>, C. Delerue-Matos<sup>2</sup>, M.B.P.P. Oliveira<sup>1</sup>

<sup>1</sup> REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> REQUIMTE, School of Engineering, Porto Polytechnic Institute, Portugal.

Selenium (Se) is an essential micronutrient that, through its incorporation into selenoproteins, plays a vital part in many metabolic functions including protection against oxidative stress, the immune response and thyroid metabolism [1]. The recommended daily allowance (RDA) for total Se is 55 µg/day and the acceptable upper limit ranges between 300–400 µg/day, in the European Union. It has been reported that Se has a cancer-protective potential, when supplemented above the recommended dietary requirements. This has led to a great interest in selenium as a food supplement by manufacturers and consumers. Nevertheless, the intake of Se in excessive amounts can be toxic, with the gap between essentiality and toxicity being very narrow.

The aim of this work was to quantify total Se in dietary supplements, obtained from local stores in Porto city, and assess compliance with the stated label level. Six different brands of dietary supplements containing Se, as sodium selenite, selenomethionine and Se-enriched yeast, were evaluated.

For the analysis the samples (capsules and tablets) it was developed a procedure that includes an acid digestion (with nitric acid) in a microwave oven and quantification by high resolution continuum source atomic absorption spectrometry with electrothermal atomization (HR-CS-ETAAS). The heating program conditions, the absorbance signal profiles and the influence of different chemical modifiers were the parameters studied for detection. A mixture solution of Mg(NO<sub>3</sub>)<sub>2</sub> and Pd(NO<sub>3</sub>)<sub>2</sub> was selected as matrix modifier. Detection was made at 196.0267 nm (primary line). The standard addition method was used. The accuracy and precision of the method were evaluated.

The results of the samples analyzed showed that total Se for 4 of the 6 supplements were in concordance with the ones presented by the manufacturers, within an error below 18%. For the two other samples, the differences were <26%.

Acknowledgments: This research was supported by U.Porto /Santander Totta “Projectos Pluridisciplinares 2010”. I.M.C. Almeida and R.C. Alves are grateful to FCT for a PhD grant (SFRH/BD/66032/2009) and a PostDoc grant (SFRH/BPD/68883/2010), respectively, financed by POPH-QREN and subsidized by ESF and MCTES. This work has also been supported by FCT through grant n°. PEst-C/EQB/LA0006/2011 and project REEQ/724/QUI/2006. C. Delerue-Matos and M.T. Oliva-Teles are grateful to Instituto Politécnico do Porto (PAPRE - Programa de Apoio a Publicações em Revistas Científicas de Elevada Qualidade).

### References:

[1] Papp, L.V., Lu, J., Holmgren, A., and Khanna, K.K. (2007), *From selenium to selenoproteins: synthesis, identity, and their role in human health*, Antioxidants and Redox Signaling, 9, 775-806.

# Exploratory evaluation of nutritional information displayed on breakfast cereals package labels

**M. A. Costa<sup>1</sup> and L. M. Cunha<sup>2,\*</sup>**

<sup>1</sup>MSc Consumer Science and Nutrition, Faculty of Sciences and Faculty of Food and Nutrition Sciences, University of Porto, Portugal.

<sup>2</sup>REQUIMTE/DGAOT, Faculty of Sciences, University of Porto, Portugal

\*Corresponding author: lmcunha@fc.up.pt

Nutritional labelling is intended to emphasize essential information about the nutritional value and composition of the products. Although it is currently voluntary within the EU, the majority of the pre-packaged products on the European market and in the national space contemplate this kind of information on their packaging<sup>1</sup>. Breakfast is considered the most important meal of the day. There is evidence that people who eat cereal on breakfast have shown a better overall nutritional profile, show improvements in cognitive functioning and are less likely to have overweight<sup>2</sup>. The main objective of this work was to evaluate the nutritional information displayed on ready-to-eat breakfast cereals package labels. Collected information contained brand and product description, existence of nutritional claims and nutritional content. Reported work is based on the analysis of 81 packages collected at different locations. From these, 58 % yield manufactures' brands with 7 brands, while 42 % display among 7 private label brands. Breakfast cereals were also classified as children's breakfast cereal (43.2 %) or non-children cereals. Data was compared using non-parametric Mann-Whitney test at a 95 % confidence level. Results, on a 100 g of cereal base, shown that energy level ranged from 257 to 462 kcal, sugar content from 0.0 to 43.0 g, fibre content from 1.0 to 32.0 g, saturated fats from 0.1 to 10.8 g and sodium from 0.01 to 0.84 g. When comparing manufacturers' with private label brands, significant differences were found for vitamins B1, B12 and E and Iron content. Contrarily, when comparing children's breakfast cereals with others, significant differences were found in energy value, carbohydrates and sugar content, all higher in children's cereals, and in protein, fibre, sodium and vitamins B6 and B12 content, all smaller in children's cereals. Concluding, when comparing the nutritional content, children's ready-to-eat breakfast cereals are generally less healthy than regular cereals. Future work is under development, involving the collection and analysis of a larger variety of breakfast cereals, thus allowing for comparisons between brands and between cereals subcategories.

## References:

[1] Storcksdieck Genannt Bonsmann, S., Celemin, L. F., Larranaga, A., Egger, S., Wills, J. M., Hodgkins, C. & Raats, M. M. (2010) *Penetration of nutrition information on food labels across the EU-27 plus Turkey*. Eur J Clin Nutr.

[2] Schwartz, M., Vartanian, L., Wharton, C., Brownell, K. (2008). *Examining the Nutritional Quality of Breakfast Cereals Marketed to Children*, Journal of the American Dietetic Association, 108(4), pp. 702-705.

## Identification of *Hypericum* species for herbal tea authentication

**B.R. Campos<sup>1</sup>, J. Costa<sup>1</sup>, M.E. Nunes<sup>2</sup>, M.B.P.P. Oliveira<sup>1</sup>, I. Mafra<sup>1\*</sup>**

<sup>1</sup> REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto

<sup>2</sup> CIBIO, Laboratory of Vegetal Genetics, Faculty of Sciences, University of Porto

(\*E-mail: [isabel.mafra@ff.up.pt](mailto:isabel.mafra@ff.up.pt))

With the increasing consumption of medicinal plants in developed countries a more frequently monitorization of adulteration of these based products for medicinal use is needed. Since the adulteration represents a commercial fraud and can lead to health issues, it becomes important to establish easy, quick and efficient detection methods.

In Portugal, two *Hypericum* species are widely consumed for their medicinal properties namely, *H. perforatum*, the most commonly used with proved antidepressant properties [1], and *H. androsaemum*, used mainly for its hepatoprotective qualities [2]. The latter, sold at prices 3 to 4 times higher than *H. perforatum*, may become an adulteration target. Therefore, the detection of this particular adulteration is of extreme importance, especially for patients taking prescribed medicine, in order to avoid risk interactions of *H. perforatum* with a large number of prescribed drugs [3].

In this study, we searched for potential adulteration of commercial teas containing *H. perforatum* and *H. androsaemum* using a DNA barcode candidate (ITS1), which represents a highly variable intergenic locus between two conserved regions, 18S and 5.8S. Specific PCR primers were designed targeting 298 bp fragments of ITS1 sequences of both species.

The results showed the potential use of ITS1 region for the discrimination of these two species, and consequently the detection of incorrect labeling of commercial teas allegedly containing *H. perforatum* or *H. androsaemum*. After sequencing, the next step will be to exploit the development of a simple approach based on real-time PCR with High Resolution Melting analysis.

Acknowledgments: Joana Costa is grateful to FCT PhD grant (SFRH/BD/64523/2009) financed by POPH-QREN (subsidised by FSE and MCTES).

### References:

- [1] Greeson, J.M., Sanford, B. and Monti, D.A. (2001), *St. John's Wort (Hypericum perforatum): A review of the current pharmacological, toxicological, and clinical literature*, Psychopharmacology, 153 (4), 402-414.
- [2] Valentão, P., Dias, A., Ferreira, M., et al. (2003), *Variability in phenolic composition of Hypericum androsaemum*, Natural Product Research, 17 (2), 135-140.
- [3] Izzo, A.A. and Ernst, E. (2009), *Interactions between Herbal Medicines and Prescribed Drugs: An updated systematic review*, Drugs, 69 (13), 1777-1798.

## Identification of the mycotoxin system patulin/producing mold in different fruits

V. Pereira<sup>1</sup>, T.M. Oliveira<sup>1</sup>, M.A. Faria<sup>1</sup>, M.E. Pinto<sup>2</sup>, M.E. Nunes<sup>3</sup> and S.C. Cunha<sup>1</sup>

<sup>1</sup> REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto.

<sup>2</sup> CEQUIMED, Departamento de Ciências Biológicas, Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto.

<sup>3</sup> CIBIO, Laboratório de Genética Vegetal, Faculdade de Ciências, Universidade do Porto.

Patulin is a mycotoxin produced by several species of moulds belong to the genera *Penicillium* and *Byssoschlamys* [1]. It can be responsible for severe acute (convulsions, nausea, ulceration) and chronic (carcinogenic, genotoxic and immunotoxic) hazardous effects in humans and has been found mainly in apples as well as products derived from these fruits [2].

The main goals of these work were: i) to extend the screening of patulin levels to fruits in which the presence of this mycotoxin is probable, namely apples, tomato and persimmon and; ii) to isolate and identify the species of molds present in the same fruits, by classical morphological techniques and by using a DNA barcode system.

Patulin levels were determined by gas chromatography coupled to mass spectrometry (GC-MS) using <sup>13</sup>C<sub>5-7</sub> patulin labeled as internal standard. Patulin levels in raw fruits ranged from 18 to 64 µg/kg in tomato and from 3 to 1209 µg/kg in apples. The levels of patulin in process food is lower than those found in raw materials, with levels ranging from 0.15 to 47 µg/kg in processed tomato and from 0.25 to 70 µg/kg processed apples foodstuff.

In what respects mold species identification, a previous evaluation of the best *loci* (BenA, ITS, CO1, nrSSU, and nrLSU) was performed in known standard species [3]. The *locus* ITS showed to be the most efficient in terms of percentage of amplification and identification certainty due to the number of sequences available in databases. As concerns the identification of molds in the above listed fruits about 40 have been isolated, amplified, and sequenced for ITS and additional selected *loci* accordingly to genera information from the morphological identification. Results are presented which allowed the comprehension of system patulin/producing organism.

### Acknowledgments:

This research was supported by grant from by U.Porto /Santander Totta “Projectos Pluridisciplinares 2010”. S.C.C. is grateful to “POPH-QREN- Tipologia 4.2, Fundo Social Europeu e Fundo Nacional MCTES”.

### References:

- [1] Richard, J.L and Payen G. A. (2003), *Mycotoxins: Risks in Plant, Animal and Human Systems*, Koog, Edir Council for Agricultural Science and Technology.
- [2] Moake, M.M., Padilla-Zakour, O.I., Worobo, R. W. (2005). *Comprehensive Reviews in Food Science and Food Safety*, 1, 8–21.
- [3]. Begerow, D., Nilsson, H., Unterseher, M., Maier, W. (2010) *Appl Microbiol Biotechnol*, 87, 99–108.

# Keratin-based biodegradable films from bovine hair, human hair and chicken feathers

F. Coelho<sup>1</sup>, J. Bessa<sup>1</sup>, L. Barbosa<sup>1</sup>, S. Neto<sup>2</sup>, C. Sousa<sup>2</sup>, J. Costa<sup>2,3</sup>, C.M.R. Rocha<sup>1</sup>, O.M. Freitas<sup>2</sup>, A. Crispim<sup>2</sup>, C. Delerue-Matos<sup>2</sup>, M.P.Gonçalves<sup>1</sup>

<sup>1</sup> REQUIMTE, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Portugal

<sup>2</sup> REQUIMTE, Departamento de Química, Instituto Superior de Engenharia do Porto, Portugal

<sup>3</sup> CIETI, Instituto Superior de Engenharia do Porto, Portugal

Feathers and bovine hair are waste-products that in Portugal are disposed of through incinerators or landfills. An alternative use for these waste-products is highly desirable. One way to do so is to use them in the production of biodegradable films.

The goal of this work was to evaluate the viability of using solid wastes containing keratin – chicken feathers, human hair, and bovine hair – as a sustainable source for the production of biodegradable films by thermo-compression.

The preparation of materials was carried out according to their type and source. The chicken feathers were washed with hot water containing detergent, then thoroughly rinsed with water and dried at room temperature. The human and bovine hair were washed with water at room temperature and dried at 60 °C. Feathers and hair were milled and sieved to a final particle size of < 100 µm and < 50 µm, respectively. The samples were characterized concerning humidity, fat content, Kjeldahl nitrogen, mineral matter amount and total organic matter. The analytical determinations followed standard procedures.

Films were made by thermo-compression in a Carver Press Autofour/15P (model 25-12HC, Carver, Inc., Wabash, USA), using the sieved keratinous material mixed with 23% of glycerol. The process parameters (temperature, pressure and time) were optimized.

The resulting films were tested in terms of optical, thermal, mechanical and barrier properties. Films behaviour towards the presence of water was evaluated by determining water solubility and sorption isotherms, at 25 °C, by a gravimetric method.

With these conditions, human hair was not able to produce films. All bovine hair-based films were dark and opaque. Feather-based films were light and opaque. Solubility was higher for films processed at higher temperatures. The Guggenheim-Anderson- de Boer (GAB) model gave a good fit of the experimental results for the moisture sorption isotherms.

Results for feathers and bovine hair were very promising and proved that it is possible to produce biodegradable films from keratin wastes that can be used as an alternative to synthetic films, for instance in agriculture.

## Acknowledgments

Thanks are due to U.Porto /Santander Totta “Projectos Pluridisciplinares 2010” and to AdI for financial support to FILMEQUE project: Development of keratin films from waste poultry feathers and bovine hair (SI IDT – 5551/2009 AdI)



## Packaging contaminants: bisphenol A and B in mussels

**A. R. Ferreira, J.O. Fernandes, and S.C. Cunha**

REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal. Email:sara.cunha@ff.up.pt

Bisphenol A (BPA) is used in the manufacture of polycarbonate plastic found in such items as reusable drinking bottles, infant feeding bottles and storage containers, and in the lining of some food and drinks cans. Due to the possible association of BPA with negative health effects, the endocrine active substance has been the subject of considerable attention worldwide.

The tolerable daily intake (TDI) level established by the U.S. Environmental Protection Agency as well as that recent recommend by the European Food Safety Authority (EFSA) is 0.05 mg/kg of bodyweight. The amount of BPA legally permitted to migrate from packaging into food, known as the specific migration limit, is based on TDI and it was set at 0.6 mg/kg by EU commission [1].

The most common sample preparation procedures applied to the determination of BPA and bisphenol B (BPB) on foodstuffs are based in liquid-liquid extraction and solid-phase extraction (SPE) followed by purification of extracts and concentration by evaporation with nitrogen. The quantification is usually carried out by liquid chromatography combined with either UV or fluorescence detection, or by gas chromatography coupled to mass spectrometry (GC-MS).

The main aim of this work was to simplify the sample preparation required for the determination of BPA and BPB in raw and canned mussels samples. For this purpose, the method used previously for the determination of BPA in soft drinks and liquid infant formula products [2] was adapted and further validated attending the improvements achieved. The method is based on a dispersive liquid-liquid microextraction (DLLME) with derivatization *in situ* of the BPA and BPB followed by GC-MS analysis. Particular attention was paid to set up a pre-extraction procedure compatible with DLLME. In order to avoid complex pre-extraction procedures, with consequent possible poor recoveries, a previous partitioning with acetonitrile was selected. Several samples of canned mussels and raw mussels collected in the marine coast of Portugal were evaluated for their levels in BPA and BPB.

### Acknowledgments:

This research was supported by grant from the FCT project “PTDC/AGR-ALI/101583/2008” and COMPETE FSE/FEDER/OE and “PEst-C/EQB/LA0006/2011”. S.C.C. is grateful to “POPH-QREN- Tipologia 4.2, Fundo Social Europeu e Fundo Nacional MCTES”.

### References:

[1] Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intending to come into contact with foodstuffs.

[2] Cunha, S.C., Almeida, C., Mendes, E. and Fernandes, J.O. (2011), *Simultaneous determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid-liquid micro-extraction and heart-cutting multidimensional gas chromatography-mass spectrometry*, Food Add. Cont. A, 28, 513-526.

# Polyphenolic content of grapevine leaves and stems: optimization of extraction parameters

**A. S. Reis<sup>1</sup>, B. Matos<sup>1</sup>, M. F. Barroso<sup>1</sup>, T. Oliveira<sup>2</sup>, M. Correia<sup>1</sup> and C. Delerue-Matos<sup>1</sup>**

<sup>1</sup> REQUIMTE/Instituto Superior de Engenharia do Porto, Politécnico do Porto, Portugal.

<sup>2</sup> WeDoTech, Porto, Portugal.

Polyphenols represent a biologically relevant group of natural compounds, which have generated growing interest from consumers, food manufacturers, cosmetics and pharmaceutical industries [1]. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects [2]. Consequently, the interest in obtaining these active compounds in a cheap, easy and rapid way is increasing. One source of polyphenols is the agricultural by-products, such as the by-products of wine/grape juice processing. Leaves, stems and pomace obtained after the wine making process have been underutilized despite representing a good source of antioxidants and bioactive compounds.

Portugal is one of the most important wine producing countries, where every year, several tons of wine by-products are generated. The extraction, characterization, quantification and evaluation of the antioxidant activity of these by-products are important tasks, in order to evaluate the potential in using these products as new sources of antioxidant compounds.

The aim of this work was to assess the polyphenolic content of extracts prepared from grapevine leaves and stems. The experimental extraction parameters were optimized, including ratio of extraction solvent (water/ethanol), temperature and time of extraction. Polyphenols (such as phenols and flavonoids) quantification was determined spectrophotometrically.

The polyphenolic content of a set of samples of grapevine leaves and stems (fresh or previously dried) will be presented and compared.

## Acknowledgements

This work has been supported by Fundação para a Ciência e a Tecnologia (FCT) through grant no. PEst-C/EQB/LA0006/2011. M. Fátima Barroso is grateful to FCT for the Ph.D. grant SFRH/BD/29440/2006.

## References:

[1] Chamorro, S., Goñi, I., Viveros, A., Hervert-Hernández, D., Brenes, A. (2012), *Changes in polyphenolic content and antioxidant activity after thermal treatments of grape seed extract and grape pomace*, European Food Research and Technology, 234, 147–155.

[2] Balasundram, N., Sundram, K., Samman, S. (2006), *Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses*, Food Chemistry, 99, 191-203.

# **A morphometric analysis of the Cholinergic fiber varicosities in the Retrosplenial Cortex of the Epileptic rat**

**J. Ferreira**

Department of Anatomy, Faculty of Medicine, University of Porto, Portugal.

It has been previously hypothesized that changes in cholinergic neurotransmission can play an important role in epileptogenesis, the neuronal reorganization process that leads to the establishment of abnormal cerebral circuits responsible for Epilepsy. The main purpose of this study was to address the issue whether prolonged seizures (status epilepticus, SE) and/or brief repeated seizures are associated with changes in the cholinergic neurotransmission in the rat retrosplenial granular cortex (Rgc), a cerebral cortical area strongly interconnected with other epilepsy-related limbic structures, including the hippocampal formation.

SE was induced by treating rats with pilocarpine (350 mg/kg). Brief seizures were induced by electroconvulsive shock (ECS). In this model, rats were given six ECS seizures, the first five of which were spaced by 24-h intervals, whilst the last two were 2h apart. Two months later, the brains of the animals were processed for immunostaining with a marker of vesicular acetylcholine transporter protein (VAcHT) and the densities of fiber varicosities immunoreactive to VAcHT were estimated.

SE produced a statistically significant increase in the densities of cholinergic varicosities in Rgc layers I, II/III, IV and VI, but not in layer V. In ECS group there was a slight and nonsignificant increase in the densities of VAcHT positive varicosities in all layers of the Rgc cortex.

To the best of our knowledge the present study is the first to demonstrate that SE induces long-term dysfunction of the cholinergic neurotransmission system in the Rgc cortex.

According to the obtained results, prolonged seizures are associated with an increase in the cholinergic pre-synaptic activity in most layers of Rgc. This points to an involvement of acetylcholine in the epileptogenic process observed in the SE model, in which the animals develop spontaneous motor seizures after the treatment what doesn't happen in the ECS model. Attending to the neurotrophic effects of acetylcholine and its activity in neuronal plasticity in the normal brain, the increase of acetylcholine synaptic release in cerebral cortical areas might be one of the elements leading to the reorganization of neural networks which eventually lead to the establishment of Epilepsy. Furthermore, the cholinergic neurotransmission dysfunction may possibly be a future prophylactic or therapeutic target for some forms of Epilepsy.

Thus, results of the present study are consistent with the emerging theory that changes in the activity of the cholinergic system in specific cortical areas of the limbic system can be involved in epileptogenesis.

**Acknowledgments:** This investigation was supported by Fundação para a Ciência e a Tecnologia, Grant PTDC/SAL-NEU/68141/2006, Grant SFRH/BD/21596/2005, and Centro de Morfologia Experimental. The author thanks to Ph.D N. Lukoyanov and Ph.D A. Cardoso for their technical assistance and valuable comments.

## Accelerated release pharmaceutical dosage forms: solid dispersions using the solvent evaporation method

C. Valada, P. C. Costa, J. M. Sousa Lobo

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal.

The objective of this study is to improve the solubility of a model drug, belonging to Biopharmaceutics Classification System (BCS) Class II [1], using solid dispersions. From several BCS Class II drugs, which show to have low water solubility and high permeability, phenytoin (diphenylhydantoin, DPH) was chosen. DPH is an antiepileptic agent extensively used in the treatment of generalized tonic-clonic epileptic seizures. Solid dispersions (SD) refers to the dispersion of one or more drugs in a hydrophilic matrix in solid state, that allows a particle size reduction, so when the system is exposed to an aqueous solution, the carrier is dissolved and the drug is released in small particles that are quickly dissolved.

Solid dispersions were obtained by two methods: fusion method and solvent evaporation method [2, 3]. Because of the high melting point of phenytoin, the fusion method was abandoned and it was decided to use the solvent evaporation method. Using data from an earlier phase of this study PVP K30 was chosen as matrix and SD were produced at 30:70 (DPH:PVP K30) ratio. In the solvent evaporation method the drug was dissolved in the same solvent as the matrix - ethanol - which was evaporated under vacuum producing SD.

The SD was analyzed using Differential Scanning Calorimetry (DSC) and compared with the respective physical mixture and with the matrix and the drug itself. Thermal analysis was conducted from 0°C to 350°C, under a nitrogen flow rate of 40 mL.min<sup>-1</sup> at a heating rate of 10 K.min<sup>-1</sup> (200 F3 Maia® - NETZSCH, Germany).

The tablets with phenytoin will be submitted to a dissolution assay using three different pH buffer solutions to simulate the gastrointestinal tract. For the analysis of the SD spectrophotometry UV at 200-400 nm was used, however this method was abandoned because phenytoin and PVP K30 absorb in the same wavelength and so it was decided to use a HPLC method. For method validation eight different concentrations of the active compound in ethanol were made. The DPH concentrations of saturation were determined using three different pH buffer solutions (1.2, 4.5, 6.8).

The results obtained so far are an important guidance, however to prove the efficacy of SD dissolution assays are required.

References:

- [1] Amidon, G.L., et al (1995), *A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability*, Pharm Res, 12(3): pp. 413-20.
- [2] Chiou, W.L. and Riegelma, S. (1971) *Pharmaceutical Applications of Solid Dispersion Systems*, Journal of Pharmaceutical Sciences, 60(9): pp. 1281-&.
- [3] Leuner, C. and Dressman, J. (2000) *Improving drug solubility for oral delivery using solid dispersions*, Eur J Pharm Biopharm, 50(1): pp. 47-60.

## Aged female diet anti-oxidant supplementation: effect on reproductive outcome

A.I. Soares<sup>1</sup>, H. Almeida<sup>1,2</sup> and E. Silva<sup>1,2</sup>

<sup>1</sup> Department of Experimental Biology, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> IBMC – Instituto de Biologia Molecular e Celular, University of Porto, Portugal.

**Introduction:** In mammals advanced maternal age is known to be a risk factor for loss of reproductive capacity. Although age-related changes in the ovary account for most of the loss, aging also affects the function of the uterus. As a consequence, both maternal and progeny outcome is affected by maternal age [1]. Since an imbalance in the redox status with enhanced reactive oxygen species production or reduced scavenging has been suggested to play a role in the development of pregnancy complications, in the present work it was aimed to determine whether diet anti-oxidant supplementation of aged female mice before and during pregnancy might improve reproductive ability.

**Experimental description:** Non-parous female C57BL/6J mice aged 8–10 weeks or 42-45 weeks were mated with male C57BL/6J mice aged 3–4 months. Aged females were treated with a SOD mimetic (TEMPOL, 1 mM) or a NOX inhibitor (apocynin, 5 mM) in drinking water prior to and during pregnancy. Following natural mating, fetal mice, placenta and uterine samples were collected at day 21 of pregnancy after inhalation anesthesia in adequate surgical environment. Fetal number and weight were quantified. Results are presented as mean  $\pm$  standard error mean.

**Results:** In controls, the number of fetuses from aged females decreased significantly [young females  $6.0 \pm 1.2$  (n=9) and aged females  $2.0 \pm 0.4$  (n=6),  $P=0.03$  (Student's t test)]. This decrease was accompanied by early and late fetal loss and reabsorption. No differences were observed in water intake between aged control and aged anti-oxidant treated female mice. Anti-oxidant treatment increased the number of fetuses from aged female mice [control  $2.0 \pm 0.4$  (n=9), TEMPOL  $3.5 \pm 0.9$  (n=4) and apocynin  $4.2 \pm 0.6$  (n=5),  $P=0.05$  (ANOVA)].

**Conclusions:** Aged female mice showed an age-related impairment in reproduction. Anti-oxidant treatment ameliorated aged female reproduction outcome.

### References:

[1] Lopes, F.L., Fortier A.L., *et al.* (2009), *Reproductive and epigenetic outcomes associated with aging mouse oocytes*, Human Molecular Genetics, 18(11), 2032-2044.

# **An auscultation system using electronic stethoscope and Smartphone's**

**F. Almeida<sup>1</sup>, M. Coimbra<sup>2</sup> and R. Correia<sup>3</sup>**

<sup>1</sup> Faculty of Medicine and Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Faculty of Sciences, University of Porto, Portugal.

<sup>3</sup> Faculty of Medicine, University of Porto, Portugal.

Cardiac auscultation was first referenced by Hippocrates<sup>[1]</sup> and is a wide used technique to detect heart problems. The instrument used to perform auscultations, the stethoscope, became a very well know symbol of medical practice and identifies clinicians as physicians.

The evolution and constantly evolving of technological tools opened doors that could not be opened before on medicine. Features like recording hearts sounds or re-listen auscultations, now can be made through stethoscopes linked with computers throw Bluetooth technology. Currently there are stethoscopes in the market that allows the transition of auscultation sound to be saved in computers as data.

The aim of this study is to develop a prototype application for mobile devices based on Android operating system, allowing collecting data through an electronic stethoscope that has enabled Bluetooth communication with paired devices. The recorded data sounds of auscultations will be sent to a unique repository to help the development of a decision support system. The decision support system will be later integrated into the same application that does the auscultation reads.

General practitioners (GP) and internal students will be able to compare the decision obtained with the prototype and the decision that they made about auscultations. The prototype should help internals and GPs to improve their qualities of listen and detecting heart problems.

References:

[1] Am Heart J. (2001), *Hippocrates and Cardiology*, 141 (2), 173-83.

## An expert view on Perinatal Indicators

**J.V. Santos<sup>1</sup>, C. Correia<sup>1</sup>, F. Cabral<sup>1</sup>, J. Freitas<sup>1</sup>**

<sup>1</sup> Department of Health Information and Decision Science, Faculty of Medicine, University of Porto, Portugal.

Perinatal indicators are one of the tools that can help European countries to evaluate perinatal health problems and their eventual causes<sup>[1]</sup>. Routine reporting on a wide range of perinatal health indicators is possible in Europe<sup>[2]</sup>. However, problems persist, and a joint effort is needed, which motivated us to pursue this project. Our main goal is to investigate, according to experts who have publications related to Perinatology, which indicators are more relevant, in order to develop a standardized list with the prospect of unifying new born child's care.

This is an observational and cross-sectional study. We created a questionnaire on the relevance of selected perinatal indicators that was sent, by e-mail, to the authors (n=860) of the articles previously selected (January '2010 to February '2011) on a restrict number of journals (n=19), elected based on their relation with our theme and ISI impact factor. The data collected from the e-mail replies was carefully organized and analyzed statistically in IBM SPSS Statistics 19<sup>TM</sup>.

When the specialists are asked to select which indicator is more relevant in each of the four groups, the indicators that seem to have more relevance are the following: *Multiple Birth Rate by Number of Fetuses*, *Distribution of Maternal Age* and *Distribution of Mother's Education* (Fetal, neonatal and child care); *Percentage of All Pregnancies Following Fertility Treatments and Mode of Delivery* (Health care services); *Prevalence of Severe Maternal Morbidity* (Population characteristics/Risk factors); *Fetal Mortality Rate*, *Neonatal Mortality Rate* and *Severe Neonatal Morbidity Among Babies at High Risk* (Maternal Health). When the specialists are asked to evaluate the relevance of each indicator included in the survey, the indicators that seem to have more relevance are *Neonatal Mortality Rate*, *Prevalence of Severe Maternal Morbidity*, *Percentage of Highly Preterm Babies Delivered in Units Without a NICU* and *Severe Neonatal Morbidity Among Babies at High Risk*.

There are agreements on what are the most important indicators between specialists on Perinatology. So the effort to develop a standardised list can be conclusive. However, taking into account the low answer number (n=52) and the unequal distribution of answers per country that we have obtained, a further study is necessary to develop a good-based standardised list of indicators.

Acknowledgements: Prof. Altamiro Pereira, Eng. Jorge Gomes, Dr. Ricardo Santos.

### References:

- [1] Wildman K, Blondel B, Nijhuis J, Defoort P, Bakoula C. European indicators of health care during pregnancy, delivery and the postpartum period. Eur J Obstet Gynecol Reprod Biol. 2003 Nov 28;111 Suppl 1:S53-65.
- [2] Zeithlin J, Mohangoo A. European Perinatal Health Report. [Internet]. Paris. EURO-PERISTAT ; 2008 [updated 2008 ; cited 2011]. Available from: <http://www.europeristat.com/publications/european-perinatal-health-report.shtml>.

## Antimicrobial resistant bacteria in shrimp

**I.Oliveira<sup>1</sup> and H. Ferreira<sup>1,2</sup>**

<sup>1</sup> Department of Biological Sciences - Microbiology, Faculty of Pharmacy, University of Porto, Portugal.  
<sup>2</sup> REQUIMTE

In the last years we have assisted to a great diffusion of aquaculture raised seafood consumption, once this means a cheaper way to assess these products, associated to new feeding habits. Some raising practices involve antimicrobial agents use, to prevent or treat shrimp illnesses, of unknown impact in human health. The occurrence of antibiotic-resistant bacteria in foods of animal origin is a potential health threat.

The aim of our study was the detection of antimicrobial resistant bacteria in shrimp used for human consumption.

Shrimp samples were purchased in supermarkets or specialized frozen food stores. We studied crude deep frozen and previously boiled, ready to heat shrimp, of different aquaculture sources.

Shrimp samples were homogenized in sterile saline and suspended in Trypticase soy broth. Isolates were selected by sample spreading on Mac Conkey agar with ampicillin, oxiiiminobeta-lactams, meropenem, tetracycline and ciprofloxacin. Colonies in different selective culture media were randomly selected for antimicrobial susceptibility study and those from oxiiiminobeta-lactams media were screened for ESBL production, by the double disc synergy test and clavulanic acid addiction, according to the Clinical Laboratory Standards Institute (CLSI) guidelines. Identification of the selected strains was achieved by ID 32 GN. Susceptibility to antimicrobial agents was determined by the agar diffusion method, according to the CLSI.

Multidrug resistant *Acinetobacter baumannii* was detected in samples of crude frozen shrimp.

This is a relevant finding once this is a health-care associated pathogen responsible for reported nosocomial outbreaks of difficult eradication, able of colonization for long-term periods with consequent impact in infection installation in debilitated people. This is a therapeutically challenging opportunistic pathogen due to the multi-resistant behavior as highlighted by recent news of an abnormal number of deadly cases reported in a hospital in Azores.

## Antioxidant capacity and related parameters of commercial fruit smoothies

**M.Nunes<sup>1,2</sup>, A. Costa<sup>1,2</sup>, R. C. Alves<sup>2,3</sup>, A. Rocha<sup>4</sup>, MBPP. Oliveira<sup>2</sup>**

<sup>1</sup> Faculty of Sciences, University of Porto, Portugal

<sup>2</sup>REQUIMTE, Depart. of Chemical Sciences, Faculty of Pharmacy, Univ. of Porto, Portugal

<sup>3</sup>REQUIMTE, Superior *Institute* of Engineering, Polytechnic *Institute* of Porto, Portugal

<sup>4</sup>REQUIMTE, Faculty of Food Science and Nutrition, University of Porto, Portugal

Smoothies are blends of fruits, fruit purées, pulps and/or fruit juices without additives. Nutritional and organoleptic characteristics of fruits are preserved through a minor thermal processing. The result is a minimally processed fresh-like product with a short storage life. Smoothies are, therefore, beverages with a high concentration of nutrients, namely antioxidants, and low energy content [1].

The complex composition of smoothies (mixture of different fruits and therefore, antioxidants with complementary mechanisms of action), seems to provide a greater protection against free radical injury than other commercial fruit juices.

Antioxidant capacity is related to compounds able to protect the biological systems against deleterious effects caused by free radicals reactions or processes [2].

The aim of this work was to analyze antioxidant activity (by DPPH assay) and related determinations as total phenolics content (by Folin-Ciocalteu method), flavonoids and vitamin C (by colorimetric assays). Some representative commercial smoothies of the Portuguese market (n=8) were sampled.

In what concerns to the analyzed parameters, a great variability was found, probably due to the different fruits used to prepare each smoothie.

### References:

[1] Derek, F.K., Brunton, N.B., Gormley, R. and Butler, F. (2011), *Effects of thermal and high hydrostatic pressure processing and storage on the content of polyphenols and some quality attributes of fruit smoothies*, J. Agric. Food Chem., 59, 601–607.

[2] Kwon, Y., Choi, K. H., Kim, S. J., Choi, D. W., Kim, Y. S. and Kim, Y. C. (2009), *Comparison of peroxy radical scavenging capacity of commonly consumed beverages*, Arch. Pharm. Res., 32, 283-287.

### Acknowledgments:

R. C. Alves is thankful to Fundação para a Ciência e a Tecnologia (FCT) for a post-doctoral grant (SFRH/BPD/68883/2010) financed by POPH - QREN - Tipologia 4.1 - Formação Avançada, subsidized by Fundo Social Europeu and Ministério da Ciência, Tecnologia e Ensino Superior. This work has been supported by FCT through grant no. PEst-C/EQB/LA0006/2011.

# Antioxidant potential of binary mixtures of phenolic compounds: Synergistic or antagonistic effects

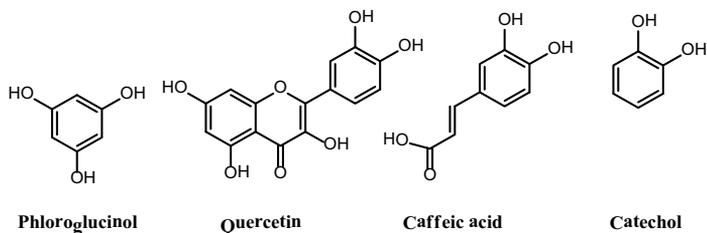
**R. Pereira, G. Silva, P. Valentão, P. B. Andrade and C. Sousa**

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal

Polyphenols have the ability to trap free radicals, which contributes for their known antioxidant activity [1]. In plant extracts, these secondary metabolites may act in concert, in such a way that their combined activities will be superior to their individual effects (synergistic interaction).

1,1-Diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) is one of the most popular radicals used to study the antioxidant potential of isolated compounds and natural extracts because it is stable, is commercially available, and possesses a strong absorption band in the visible region of the electromagnetic spectrum.

In this work the DPPH<sup>•</sup> scavenging activity of four different polyphenols (Fig. 1) was evaluated: the simple phenols catechol and phloroglucinol, caffeic acid representing the hydroxycinnamic acids family, and quercetin as representative of one of the most important classes of plant polyphenols, the flavonoids. The antioxidant potential of binary mixtures containing quercetin was also evaluated in order to check for synergistic or antagonistic interactions between the compounds [2].



As expected, the most effective compound was the flavonol quercetin, followed by catechol and caffeic acid. Phloroglucinol, the only studied phenol without a catechol group in its structure, was also able to scavenge DPPH<sup>•</sup>, although it was considerably less effective than the other compounds. The DPPH<sup>•</sup> scavenging ability of mixtures containing quercetin and another polyphenol were not additive, though they were higher than that of quercetin alone.

## References:

[1] Ferreres, F., Sousa, C., Vrchovska, V., Valentão, P., Pereira, J.A., Seabra, R. M. and Andrade, P.B. (2006), *Chemical composition and antioxidant activity of tronchuda cabbage internal leaves*. European Food Research and Technology, 222, 88–98

[2] Alonso-Amelot, M.E. and Calcagno, M.P. (2000), *Modeling Synergistic and Antagonistic Interactions in Xenobiotic Compounds*. Journal of Chemical Ecology, 26, 513-531.

## ***Aplysia fasciata* Poiret: the potential of a sea slug**

**J. Vinholes, A. Alves, A. M. Vaz, A. Ramos, A. Sá, I. P. Duque, J. G. Neves, J. Magalhães and S. Cortal**

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Till recently terrestrial vegetal species have been the main source of bioactive molecules. Considering that about 70% of the Earth is covered by the oceans and that 80% of the living organisms of the planet are found in aquatic ecosystems, it is surprising that the great majority of the natural compounds with interesting pharmacological properties arise from terrestrial organisms and not from marine ones.

*Aplysia* (Phylum Mollusca, Class Gastropoda) is a genus of sea slugs with a tropical and subtropical distribution, feeding on algae. In this work an ethanol extract of *Aplysia fasciata* Poiret was analysed by HPLC/DAD and GC/MS in order to characterize its chemical composition. In addition, its antioxidant and antibacterial capacity was assessed by microassays.

HPLC/DAD analysis allowed the detection of a red pigment derivative of phycoerythrobilin. By GC/MS several compounds were determined, including amino acids, fatty acids and sterols (Fig. 1).

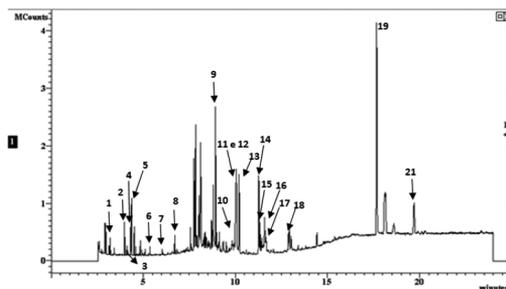


Fig. 1. GC/MS chromatogram of the ethanol extract of *A. fasciata*. (1) alanine; (2) valine; (3) urea; (4) glycerol; (5) leucine; (6) threonine; (7) methionine; (8) phenylalanine; (9) palmitic acid; (10) linolenic acid; (11) linoleic acid; (12) oleic acid; (13) stearic acid; (14) arachidonic acid; (15) eicosapentaenoic acid; (16) eicosadienoic acid; (17) *cis*-11-eicosanoic acid; (18) docosahexaenoic acid; (19) 5- $\alpha$ -colestan-3- $\beta$ -ol; (20) unidentified sterol; (21) stigmasterol.

The ethanolic extract revealed a reduced activity against DPPH and nitric oxide. *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and *Escherichia coli* were susceptible to this extract, the last being the most resistant bacteria species.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the 5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy, University of Porto, under the responsibility of Paula Andrade and Patrícia Valentão.

## Assesement of *Cynara cardunculus* L. ethanol extracts biological potential

**V. Machová<sup>1,2</sup>, P. Valentão<sup>1</sup>, P. B. Andrade<sup>1</sup>, R. F. Gonçalves<sup>1,2</sup> and C. Sousa<sup>1</sup>**

<sup>1</sup> REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> Department of Pharmacognosy, Faculty of Pharmacy, Charles University, Czech Republic.

Cardoon (*Cynara Cardunculus* L.) is an ancient herbaceous perennial plant, originating from the southern Mediterranean area, with a long traditional use in Southwest Europe. Cardoon leaves are traditionally used for their diuretic, choleric and hepatoprotective effect, being also recognized for their protective effect against reactive oxygen species. This species is known to be a rich source of polyphenolic compounds, with mono- and dicaffeoylquinic acids and flavones as the major polyphenolic components [1].

In this work, the phenolic composition of the ethanol extract of *C. cardunculus* leaves was determined using HPLC-DAD. The profile was similar to the infusion's one previously described [1]. It mainly contained caffeic acid, caffeoylquinic acids derivatives and luteolin-7-*O*-glucoside, the last being the major compound. The total amount of phenolic compounds was 883 mg/kg of leaves (dry weight).

The knowledge on the biological potential of the species was extended by evaluating its effect against nitric oxide radical (\*NO) through spectrophotometric *in vitro* microassay. Also its acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities were assessed. In order to establish some relationship with the extract's phenolic composition two standard compounds, 5-*O*-caffeoylquinic acid and luteolin-7-*O*-glucoside, were assayed under the same conditions of the extract. The ethanol extract showed ability to scavenge \*NO in a concentration-dependent manner, the standard compounds being even more effective. In what concerns to cholinesterases inhibitory activity, the extract performed better regarding AChE, although it also exhibited some inhibitory effect on BuChE. In the same manner luteolin-7-*O*-glucoside was more effective at inhibiting AChE. 5-*O*-Caffeoylquinic acid didn't show any BuChE inhibitory activity.

The results provide evidence that the phenolic compounds contribute to the potential demonstrated by the extract of *C. cardunculus* to scavenge nitric oxide and to inhibit cholinesterases.

### References

[1] Valentão, P., Fernandes, F., Carvalho, F., Andrade, P.B., Seabra, R.M. and Bastos, M.L. (2002), *Antioxidative properties of cardoon (Cynara cardunculus L.) infusion against superoxide radical, hydroxyl radical, and hypochlorous acid*. Journal of Agricultural and Food Chemistry, 50, 4989-4993.

## Bacterial resistance to copper and antibiotics: two sides of the same problem in the animal production setting?

E. Silveira<sup>1</sup>, L. Dias<sup>1,2</sup>, P. Antunes<sup>1,2</sup>, A. R. Freitas<sup>1</sup>, T. M. Coque<sup>4</sup>, L. Peixe<sup>1</sup>, C. Novais<sup>1,3</sup>

<sup>1</sup> REQUIMTE, Microbiology Department, Pharmacy Faculty, Porto University, Portugal.

<sup>2</sup> Faculty of Nutrition and Food Sciences, Porto University, Portugal.

<sup>3</sup> CEBIMED, Health Sciences Faculty, Fernando Pessoa University, Porto, Portugal.

<sup>4</sup> Microbiology Service, Ramón Cajal Hospital, Madrid, Spain

**Background:** Copper (Cu) is highly used in the animal production setting as feed supplement or in antiseptic formulations. We hypothesized that the use of Cu might contribute to select antibiotic resistant (AB<sup>R</sup>) pathogenic and non-pathogenic bacteria of animal origin, which might reach human by food chain.

**Methods:** *Enterococcus* spp (180 *E. faecalis*, 171 *E. faecium*, 199 from other species) and *Listeria* spp (13 *L. monocytogenes*, 13 *L. innocua*, 11 *Listeria* spp) were included in this study. They were collected (1999-2011) from swine feces and piggeries environmental samples, trout aquacultures (*Enterococcus* spp), poultry carcasses for human consumption (*Enterococcus* spp, *Listeria* spp), raw turkey meat and other raw/cooked food types (*Listeria* spp). Susceptibility to AB was studied by agar dilution/disk diffusion method (CLSI guidelines) and the search of AB/Cu<sup>R</sup> genes by PCR and sequencing. Their ability of transfer was evaluated by conjugation assays and plasmid location by S1-PFGE hybridization. Clonality of representative isolates of different species and sources that carried Cu<sup>R</sup> genes was studied by PFGE/MLST.

**Results:** Only a *L. innocua* (3%) from raw turkey meat and resistant to clindamycin showed the multicopper oxidase gene *mco*<sub>P<sub>LMS578</sub></sub> while the incidence of Cu<sup>R</sup> genes among *Enterococcus* spp was 21%. The genes *tcuB* (coding for copper export ATPase) and *cueO* (multicopper oxidase) were detected in 17% and 16% of all *Enterococcus* spp, respectively. A higher incidence of Cu<sup>R</sup> genes was detected in piggeries isolates (31% vs 15%- aquacultures, 10%-poultry) and in *E. faecium* (35% vs 13% *E. faecalis*, 15% other species). Isolates carrying Cu<sup>R</sup> genes belonged to a polyclonal population: i) 55 *E. faecium*-37 PFGE clones (ST185, ST150/CC5; ST132, ST393/CC17; ST432; ST434; STnew); ii) 14 *E. faecalis*-10 PFGE clones (ST224/CC21; ST16; ST445; ST260; ST288; ST49; ST53). Nevertheless, *E. faecium* PFGE type A (ST185/CC5) was detected in different pig farms. *Enterococcus* (n=29) carried plasmids of different sizes (150-320kb) with *tcuB* and/or *cueO* plus genes coding for resistance to tetracycline (*tetM*-18, *tetL*-23), erythromycin (*ermB*-24) and/or vancomycin (*vanA*-4). These plasmids were successfully transferred in all cases studied (n=17), both alone or with other smaller plasmids (25-70kb) also carrying AB<sup>R</sup> genes (n=4; *tetM*, *tetL*, *ermB*, *aac6-aph2*).

**Conclusion:** *Enterococcus* spp seem to be better reservoirs of the Cu<sup>R</sup> genes studied than *Listeria* spp. Cu<sup>R</sup> and AB<sup>R</sup> genes were often co-located within large mobile plasmids or were co-transferred by different plasmids in the same genetic event. The intensive use of Cu might contribute to the maintenance of AB<sup>R</sup> bacteria/genes in the animal setting, which can reach human by food chain.

**Acknowledgments:** This work was supported by Universidade do Porto/Santander Totta "Projectos Pluridisciplinares 2010"

## Biological activities of *Echium plantagineum* L. pollen hydro-alcoholic extract

**E. Moita, L. R. Silva, P. Valentão, C. Sousa and P. B. Andrade**

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

*Echium plantagineum* L. (Paterson's curse) is native of the western European Basin and the bee pollen is a source of potential benefits to human health, being applied for centuries in traditional medicine among other honey bee-derived products.

*E. plantagineum* pollen is rich in phenolic compounds [1]. It has been shown that these compounds have antioxidant activity, being considered as possible protective agents for reducing oxidative damage of important biomolecules, including lipoproteins and DNA [2]. Therefore, they have attracted a great deal of attention mainly concentrated on their role in preventing several diseases, like cancer, inflammation and cardiovascular disorders and also aging [3].

In the present study interest was focused on the determination of antibacterial, antioxidant and anti-inflammatory activities of a hydro-alcoholic extract from *E. plantagineum* bee pollen for the first time.

The results obtained by HPLC/DAD showed that flavonoids, namely quercetin and kaempferol derivatives, are abundant. A high antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus* and *Micrococcus luteus*) was found, but no protective effect was observed against Gram-negative ones (*Salmonella typhimurium*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa*).

The extract also displayed interesting results against superoxide and nitric oxide radicals. Additionally, it showed an evident anti-inflammatory activity, with concentration-dependent decrease of nitric oxide production in murine macrophage RAW264.7 cells.

These results contribute to the valorization of this bee-product, which deserves to be further studied concerning other pharmacological properties.

### References:

- [1] Ferreres, F., Pereira, D.M., Valentão, P., Andrade, P.B. (2010). *First report of non-coloured flavonoids in Echium plantagineum bee pollen: differentiation of isomers by liquid chromatography/ion trap mass spectrometry*, Rapid Communications in Mass Spectrometry, 24, 801-806.
- [2] Marghitas, L.A., Stanciu, O.G., Dezmirean, D.S., Bobis, O., Popescu, O., Bogdanov, S., et al. (2009), *In vitro antioxidant capacity of honeybee collected pollen of selected floral origin harvested from Romania*, Journal of Food Chemistry, 15, 878-883.
- [3] Niciforovic, N., Mihailovic, V., Maskovi, P., Solujic, S., Stojkovic, A. and Muratspahic, D.P. (2010), *Antioxidant activity of selected plant species: potential new sources of natural antioxidants*, Journal of Food and Chemical Toxicology, 48, 3125-3130.

## Biological effects of exemestane metabolites in an estrogen-receptor-positive aromatase-overexpressing breast cancer cell line

A. Lopes<sup>1</sup>, C. Amaral<sup>1,2</sup>, G. Correia da Silva<sup>1,2</sup> and N. Teixeira<sup>1,2</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Pharmacy, University of Porto, Portugal

<sup>2</sup> Institute for Molecular and Cell Biology (IBMC), University of Porto, Portugal

Approximately one third of all breast cancers is estrogen dependent and will respond to estrogen deprivation. Aromatase inhibitors (AIs) inhibit the conversion of androgens to estrogens, increasing in that way the disease-free survival in patients with estrogen-receptor-positive breast cancer. Exemestane is a third-generation AI currently prescribed for postmenopausal hormone-dependent breast cancer, but the mechanism of action in cancer cells is not completely elucidated. We studied the effects of principals metabolites of exemestane, 17-beta-hydroxyexemestane (**17-BHE**) [1], 6 $\beta$ -spirooxiranandrosta-1,4-diene-3-17-dione (**32**) [2] and 1 $\alpha$ ,2 $\alpha$ -epoxy-6-methylenandrosta-4-ene-3,17-dione (**33**), *in vitro*, in an ER-positive aromatase-overexpressing breast cancer cell line (MCF-7aro). Metabolite **33** was synthesized by our group and was obtained by chemical modifications of exemestane.

MCF-7aro cells were cultured in steroid-free medium with testosterone and treated with different concentrations (1-15  $\mu$ M) of each compound during different time-points (24-144 hours). Cell proliferation and cell viability was evaluated by <sup>3</sup>[H] thymidine incorporation assays, MTT and LDH assays. Morphological alterations were evaluated by Giemsa and Hoechst staining. The results showed that exemestane metabolites induce a decrease in cell viability in a time dependent manner, and that, compound **32** is the less potent metabolite. The proliferation is also suppressed but in a dose-dependent manner and compound **33** is the most potent metabolite. The LDH release in the culture medium indicates that these metabolites are not cytotoxic. By the analysis of cell morphology we observed a decrease in cell density and condensation of chromatin for the highest doses of compound **17-BHE**.

These observations are important for the elucidation of the cell death mechanisms in cancer cells that occur following exemestane treatment, which is key to better understand the discovery of effective targets to breast cancer treatment.

The authors thank Fundação para a Ciência e Tecnologia (FCT) for grants of C. Amaral (SFRH/BD/48190/2008).

### References:

- [1] Buzzetti, F., Di Salle, E., Longo, A., & Briatico, G. (1993). *Synthesis and aromatase inhibition by potential metabolites of exemestane (6-methylenandrosta-1,4-diene-3,17-dione)*. Steroids, 58(11), 527-532.
- [2] Kamdem, L. K., Flockhart, D. A., & Desta, Z. (2011). *In vitro cytochrome P450-mediated metabolism of exemestane*. Drug Metab Dispos, 39(1), 98-105.

# Brown macroalgae from Portuguese coast: a source of antioxidants

**M. Barbosa, R. Matos, J. Vinholes, P. B. Andrade and P. Valentão**

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Antioxidants can be used in different fields, such as medicine (for cancer, cardiovascular disorders, and chronic inflammations), pharmaceuticals (anti-ageing) or food (food preservative), among others [1]. In the last years, the search for new antioxidant compounds from natural sources has increased. This is due to health concerns regarding the potential toxic and side effects caused by synthetic antioxidants, as well as changes in consumer preferences for natural products [2]. In this sense, ethanol extracts of five brown macroalgae species from the Portuguese coast were prepared and their biological potential was assessed towards different radicals, namely, nitric oxide ( $\cdot\text{NO}$ ), superoxide ( $\text{O}_2\cdot^-$ ) and hydroxyl ( $\cdot\text{OH}$ ). In order to relate the biological activity with their chemical composition, extracts were analyzed by means of gas chromatography coupled to mass spectrometry (GC–MS).

A concentration-dependent effect was observed for all extracts (Fig. 1), which can be partly explained by their composition in terms of amino acids, phloroglucinol, sugars, fatty acids and sterols.

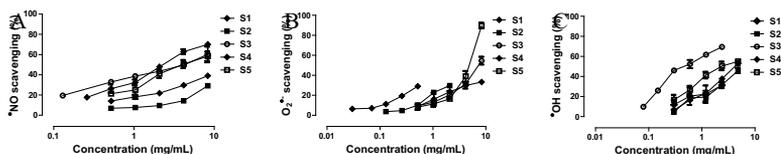


Fig. 1. Radical scavenging activity of brown macroalgae against nitric oxide (A), superoxide (B) and hydroxyl (C) radicals. Results show mean  $\pm$  standard error of three assays, performed in triplicate. S1, *Padina pavonica*; S2, *Sargassum vulgare*; S3, *Cystoseira tamariscifolia*; S4, *Cystoseira nodicaulis*; S5, *Fucus spiralis*.

Our preliminary results indicate that the five brown algae ethanolic extracts studied might be good sources of antioxidants that can be used in different food and pharmaceutical products.

## References:

- [1] Cornish, M. L., and Garbary, D. J. (2010). *Antioxidants from macroalgae: potential applications in human health and nutrition, Review*. *Algae*, 25 (4), 155-171.
- [2] Safer, A. M., and al-Nughamish, A. J. (1999). *Hepatotoxicity induced by the anti-oxidant food additive, butylated hydroxytoluene (BHT), in rats: an electron microscopical study*. *Histology and Histopathology*, 14 (2), 391-406.

# Modeling users for interactive decision support systems for gastroenterology

C. Gomes 1,2, A. Sousa 1,2, M. Coimbra2

<sup>1</sup> Department of Medicine, Faculty of Medicine, University of Porto, Portugal

<sup>2</sup> Department of Computer Science, Faculty of Science, University of Porto, Portugal

The rapid and dizzying technological change in recent years allowed digestive endoscopy to extend beyond the mere diagnosis and have a more interventionist role and definitive treatment in clinical situations that are difficult to resolve and often avoiding the use of alternative more penalizing therapies.

The CAGE project, funded by FCT, aims to research and develops a CAD system that can be deployed in current gastroenterology rooms, which can support the decision regarding the physician's clinical diagnosis of the patient. Thus, the challenge posed is to explore new ways to assist the physician in assessing the patient's diagnosis. The doctor will be able to hold the endoscope tube in one hand and its affiliates, and another joystick while looking at a big screen HD? Or vocally ask the computer to show similar tests? Should we display all the tests in the picture or just one at a time? Is the introduction / application of new technologies in endoscopy it feasible and easily accepted by physicians?

This study aims to study and model the various users of such a system using Human-Computer Interaction methodologies, paving the way for answering the difficult questions posed by the design of interactive systems for gastroenterology. Users include patients undergoing elective exams, doctors and nurses. Contextual studies are underway in two different hospitals and current results show that although the techniques of each doctor are very similar, there are important differences in the functionalities of the gastroenterology room equipment and its environment.

## References:

- [1] Isabelle Cremers(2009), EndoNews, news global information for doctor, Sydney.
- [2] Papavramidis ST, Theocharidis AJ, Zaraboukas TG, Christoforidou BP, Kessissoglou II, Aidonopoulos AP. Upper gastrointestinal endoscopic and histologic findings before and after vertical banded gastroplasty. *Surg Endosc* 1996; 10(8):825-30.
- [3] Schirmer B, Erenoglu C, Miller A. Flexible endoscopy in the management of patients undergoing Roux-en-Y gastric bypass. *Obes Surg* 2002; 12(5):634-8.
- [4] Stellato TA, Crouse C, Hallowell PT. Bariatric surgery: creating new challenges for the endoscopist. *Gastrointest Endosc* 2003; 57(1):86-94.

# Can fungicide application in vineyards induce azole resistance in *Aspergillus* spp.?

**M. Lago<sup>1</sup>, A. Natário<sup>1</sup>, C. Fernandes<sup>1</sup>, A. Marques<sup>2</sup> and E. Pinto<sup>1</sup>**

<sup>1</sup> CEQUIMED, Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal

<sup>2</sup> REQUIMTE/GRESA, Departamento de Geociências, Ambiente e Ordenamento do Território, Faculdade de Ciências, Universidade do Porto, Portugal

The emergence of antifungal drug cross-resistance among clinical pathogens, like *Aspergillus* spp., represents a considerable human health risk. In agriculture, fungi are responsible for important losses of crop yields and for production of mycotoxins. The widespread use of fungicides is essential to protect a wide variety of crops from fungi, like in the vineyards. Considering the 7500 million hectares of vineyard in the world, it can be estimated that a big amount of fungicides is systematically spread in the air to prevent mildew. Azoles, differing in structure but exhibiting the same mode of action, have been extensively used in agriculture and medicine which led to the development of resistances that are becoming a problem nowadays (1). The aim of this work is to understand the role that the massive use of farm fungicides may have in the development of resistance to drugs used in human treatment.

Two vineyards from North Portugal were selected and monitored during vegetative period from spring to autumn of the same year. The vineyards were divided in plots and different number of sprays was done in each plot. One vineyard was in “Vinhos Verdes” region and was divided in three plots: in “P2Bx” no azoles were sprayed, in “CIMA” penconazole was sprayed once and in “P5STOPAZE” five sprays with penconazole were done. The other vineyard was in “Douro” region and was divided in four plots: in “BIO” no synthetic fungicides were sprayed; in “TF0” no azoles were sprayed; in “TF1” penconazole was sprayed once, and in “TN5” penconazole was sprayed three times. In each vineyard, air, soil and plant samples were collected in three moments: before any fungicide application, just after all fungicide applications, and before grape harvest. The total number of fungi was evaluated in MEA and DG18 medium and the strains of *Aspergillus* species were isolated and identified by morphological and molecular techniques. Seventy three strains of *Aspergillus* were isolated, being seventy from air and soil samples. *Aspergillus* strains were evaluated for their susceptibility to azoles used in human therapeutic (itraconazole, ketoconazole, posaconazole and voriconazole) and used to prevent/treat plant diseases (fenarimol and penconazole). Susceptibility testing was based on the Clinical and Laboratory Standards Institute recommendations (M38-A2 document).

Most of the isolates (96%) were obtained from air and soil, being *A. niger* the specie most frequently isolated. Eleven strains showed resistance to voriconazole, used in the treatment of aspergillosis, with a minimal inhibitory concentration (MIC)  $\geq 4$  mg/L. In addition, 25 strains showed high values of MIC ( $\geq 16$  mg/L) for penconazole, used in treatment of the vineyards.

References:

(1) Serfling, A., Wohlrab, J and Deising H.B. (2007). *Treatment of a clinically relevant plant-pathogenic fungus with and agricultural azole causes cross-resistance to medical azoles and potentiates caspofungin efficacy*. Antimicrobial Agents and Chemotherapy, 51 (10), 3672-3676.

The authors acknowledge U. Porto/Santander Totta and CEQUIMED-UP (I&D 4040/2007) for financial support.

## Cardiovascular and structural abnormalities induced by histone deacetylase inhibitors on zebrafish embryonic development

B. R. Pinho<sup>1</sup>, J. S. O. Magalhães<sup>1</sup>, A. Fonseca-Silva<sup>1</sup>, M. M. Santos<sup>2</sup> and J. M. A. Oliveira<sup>1</sup>

<sup>1</sup> REQUIMTE, Dep. Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> CIIMAR, Environmental Toxicology Lab, University of Porto, Portugal

Histone deacetylases (HDACs) and acetyltransferases modulate histone acetylation levels and, consequently, gene transcription. This acetylation homeostasis is reportedly disrupted in neurological disorders, conditions in which HDAC inhibitors may have therapeutic application [1]. Zebrafish (*Danio rerio*) is increasingly used to model disease pathogenesis and drug testing. Genetic knockout of some classical HDACs was shown to induce developmental abnormalities in zebrafish [2]. Currently, the effects of knocking out other HDACs, namely sirtuins, and of selective pharmacological inhibition remain unexplored in zebrafish. Here we chronically exposed fertilized eggs to HDAC inhibitors with different selectivity profiles and assessed their effects on zebrafish development, focusing on structural and cardiovascular abnormalities. Selective (HDAC) inhibitors were: AGK2 (SIRT2), EX527 (SIRT1) and MS275 (HDAC1); Non-selective were: trichostatin-TSA and valproate-VPA. Fertilized eggs were randomly distributed in 12-well plates (10/well) and exposed to HDAC inhibitors, from 4 to 80 hours post fertilization (hpf). Readings were made at 8, 32, 56 and 80hpf and embryos classified as normal, abnormal or dead, and visually screened for abnormalities. The heart rate was measured at 80hpf. AGK2 (0.5-10 $\mu$ M) was neither lethal nor teratogenic; higher AGK2 concentrations presented water solubility problems. All other compounds evoked developmental abnormalities (LC<sub>50</sub>/NC<sub>50</sub>>1). For each drug, the concentration causing the highest proportion of abnormalities at 80hpf was: TSA (1  $\mu$ M) < EX527 (50  $\mu$ M) < MS275 (100  $\mu$ M) < VPA (1 mM). At these concentrations, HDAC inhibitors induced mainly cardiovascular abnormalities, such as oedema. The following compounds induced cardiac oedema (% total embryos): MS275 (80 $\pm$ 9%, n=2, P<0.05), VPA (64 $\pm$ 8%; n=8, P<0.05), EX527 (61 $\pm$ 11%, n=4, P<0.05) and TSA (27 $\pm$ 8%; n=7). Also, bradycardia (maximal % decrease in heart rate vs. control) was detected for VPA (0.3-1mM; 61 $\pm$ 5%, n=8), TSA (0.25-1 $\mu$ M; 40 $\pm$ 6% n=4) and EX527 (50 $\mu$ M; 36 $\pm$ 6%, n=3). At 80hpf, the order of lethality (LC<sub>50</sub>  $\mu$ M) was: TSA (1.29 $\pm$ 0.18, n=5) > EX527 (60.3 $\pm$ 16.8, n=4) > ENT (300, n=1) > VPA (3.1E3 $\pm$ 1.1E3, n=3). The co-exposure AGK2 and EX527 showed no synergic toxicity. In summary, with the exception of AGK2, all HDAC inhibitors induced abnormalities, mainly cardiovascular, within the range of concentrations tested. At their reported isoform concentration concentrations, however, none of the compounds exhibited detectable toxicity. In ongoing studies we are assessing their protective potential in disease models.

Acknowledgments: Fundação para a Ciência e a Tecnologia (SFRH/BD/63852/2009, Pinho BR); Universidade do Porto & Santander Totta, IJUP2010 #195.

References: [1] Haberland, M.R., Montgomery, R. L. and Olson, E. N. (2009), *The many roles of histones deacetylases in development and physiology: implications for disease and therapy*, Nature Reviews Genetics, 10, 32-42. [2] Pillai, R., Coverdale, L. E., Dubey, G. and Martin, C. C. (2004), *Histone deacetylase 1 (HDAC-1) required for the normal formation of craniofacial cartilage and pectoral fins of the zebrafish*, Developmental Dynamics, 231, 647-654.

## Characterization of Macrogols phase transitions

A.R. Lucas, P.C. Costa, J.M. Sousa Lobo

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology,  
Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

The Macrogols (PEGs) are a series of water soluble synthetic polymers, widely used as pharmaceutical excipients [1]. Some authors reported that, using DSC, the shape of the curves of enthalpies of same samples of Macrogols depended on the heating and the cooling conditions. For fast heating rates, the endothermic peaks were not clearly defined, while for slow heating rates (ie,  $+1\text{ }^{\circ}\text{C}/\text{min}$ ) endothermic curve presented three distinct peaks [2].

For these authors, who used PEG 6000, report that the three peaks correspond, respectively: fusion of the PEG 6000 spherulitic phase, the devitrification of the vitreous interspherulitic phase and, finally, the melting of the new crystalline phase [2]. Using DSC, this phenomenon could only be observed for slow heating rates. Thus was demonstrated the presence of folded chain crystals in the sample at room temperature. Due to the increase in the proportion of double chain with the sample cooling rate, care should be taken in conservation and preparation of the sample. To confirm the previous results Macrogols 1500, 4000 and 6000 were used [2].

The samples were analyzed using Differential Scanning Calorimetry (DSC). Thermal analysis was conducted from  $0^{\circ}\text{C}$  to  $100^{\circ}\text{C}$ , under a nitrogen flow rate of  $40\text{ mL}\cdot\text{min}^{-1}$  at a heating rate of  $5\text{ K}\cdot\text{min}^{-1}$  (200 F3 Maia® - NETZSCH, Germany). In our experience it was observed that the results of previous authors occurred [2]. For low molecular weight Macrogol (1500) only the first two peaks appeared. For medium molecular weight Macrogol (4000 and 6000) the three peaks were visible in the DSC thermogram (Figure 1).

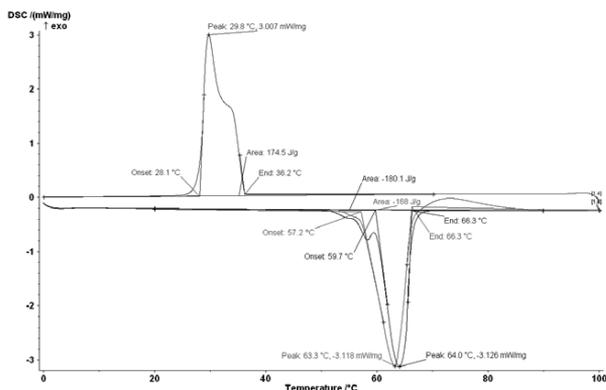


Figure 1 - DSC thermogram of Macrogol 6000.

### References:

- [1] Pharmaceutical Society of Great Britain and American Pharmaceutical Association (2006), *Handbook of Pharmaceutical Excipients*, 5th edn. Pharmaceutical Press, London.
- [2] Delahaye N; Duclos R; Saiter JM (1997), *Characterization of PEG6000 phase transitions Influence of kinetic conditions*, Int. J. Pharm. 157: 27 – 34.

## Characterization of the immune response to experimental calicivirus infection

**S. Cunha**<sup>1,2</sup>, **R. M. Marques**<sup>1,2</sup>, **A. P. Águas**<sup>1,2</sup>, **P. G. Ferreira**<sup>1,2</sup> and **L. Teixeira**<sup>1,2</sup>

<sup>1</sup> Departamento de Anatomia, ICBAS – Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Portugal.

<sup>2</sup> UMIB – Unidade Multidisciplinar de Investigação Biomédica, Porto, Portugal

Rabbit Haemorrhagic Disease (RHD) is an important cause of the worldwide decline in the number of rabbits that has been observed since 1980s. It is an infectious disease caused by a calicivirus that kills more than 90% of adult European Rabbits (*Oryctolagus cuniculus*), between 24-72 hours of infection. This calicivirus replicates in the liver, causing a necrotizing hepatitis and haemorrhagic diathesis. An interesting phenomenon is the resistance of the young rabbits (less than 8 weeks old) to this infection.

In this work we compare the immune response of young and adult rabbits to Rabbit Haemorrhagic Disease Virus (RHDV), focusing on the study of naturally occurring CD4<sup>+</sup> Foxp3<sup>+</sup> regulatory T cells (Treg cells) in several organs affected by RHD. European rabbits (*Oryctolagus cuniculus*), 5 and 11 weeks old, were infected with RHDV and sacrificed 24 hours after inoculation. The organs of interest were collected and later processed and analyzed by flow cytometry, in which Treg cells were stained with anti-rabbit CD4-FITC antibody and anti-human Foxp3-PE antibody. Portions of liver and appendix were also analyzed by immunohistochemistry (IHC) for Treg cell detection.

A decrease in the frequency Treg cells in the spleen and mesenteric lymph nodes of infected adult rabbits was observed when compared to non-infected controls and to infected young rabbits. In the spleen, there was also a significant decrease in the number of Treg cells in infected adult rabbits. No alteration in the frequency of this cellular population was observed in the liver of infected young and adult rabbits comparatively to non-infected controls.

These results suggest that Treg cells may play a role in the susceptibility of adult rabbits to calicivirus infection, since there was a significant decrease in the frequency of this cellular population in the spleen and mesenteric lymph nodes of adult rabbits infected with RHDV.

This work was funded by grants from FCT (TDC/CVT/66656/2006), Portugal. Luzia Teixeira was supported by Fundo Social Europeu and MCTES through POPH-QREN – Tipologia 4.2.

## Clinically relevant bacteria in bovine meat

**J. Machado**<sup>1</sup> and **H. Ferreira**<sup>1,2</sup>

<sup>1</sup> Department of Biological Sciences - Microbiology, Faculty of Pharmacy, University of Porto, Portugal.  
<sup>2</sup> REQUIMTE

We have been observing a rise in vacuum preserved bovine meat consumption, in the last few years, mainly imported from different origins. Some bovine raising practices involve antimicrobial agents use, to prevent and treat animal illnesses in big scale production, of unknown impact in human health.

Meat samples of different origins, were studied, some were imported vacuum preserved and other national fresh meat.

Meat samples were homogenized in sterile saline and suspended in Trypticase Soy Broth. Isolates were selected by sample spreading on Mac Conkey agar with ampicillin, oximinobeta-lactams, meropenem, tetracycline and ciprofloxacin. Colonies were randomly selected and susceptibility to antimicrobial agents was determined by the agar diffusion method, according to the Clinical Laboratory Standards Institute (CLSI) and screened for extended-spectrum beta-lactamases (ESBL) production, by the double disc synergy test and clavulanic acid addition, according to the CLSI guidelines. Identification of the selected strains was achieved by ID 32 GN.

*Acinetobacter baumannii* was detected in samples of imported vacuum preserved crude bovine meat. This finding is of relevance once this is a health care associated pathogen able to easily develop multidrug resistance, relevant in opportunistic infection of colonized people.

In a national meat sample we detected an *Enterobacter sakazakii* resistant to third generation cephalosporins and a tetracycline resistant *Escherichia coli*. In another imported sample a *Morganella morganii* was detected.

These results show the importance of detection of opportunistic pathogens and antimicrobial resistant bacteria in national and imported meat, to ensure that consumers are not exposed to hazards of clinically relevant and antimicrobial-resistant bacteria, in food. This might represent a threat in terms of population colonization with consequences in infection installation and spread.

## Comparative study of the sun protection factor and water resistance of two silicone emulsions containing ethyl hexyl methoxycinnamate

**A. Pereira, M. H. Amaral, I. F. Almeida, P. A. L. Lobão, P. C. Costa, J. P. Silva, J. M. Sousa Lobo**

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Sun protection has a prominent role in health programs for the prevention of skin cancer<sup>[1]</sup>. High water resistance is a key feature regarding sunscreen efficacy. Following this underlying principle, this study aimed to formulate water resistant silicon based sunscreens-water/silicone (W/S) and water/silicone/water (W/S/W) emulsions containing 3 % (w/w) of a chemical sunscreen, 2-ethylhexyl *p*-methoxycinnamate (EHMC) and evaluate the sun protection factor (SPF) and the water resistance. Physical stability of silicone formulations was also studied by the analysis of texture (firmness and adhesiveness), viscosity, pH and accelerated stability by centrifugation. A comparative study of these emulsions with a reference sun protection water-in-oil emulsion indicated by COLIPA (The European Cosmetics Association) was also performed<sup>[2]</sup>.

The *in vitro* evaluation of SPF was performed by spreading 1 mg/cm<sup>2</sup> of each product in roughened polymethylmethacrylate (PMMA) plates. Then, UV-spectra from 290 nm to 400 nm were obtained using a spectrophotometer<sup>[2]</sup>. For water resistance studies, PMMA plates were immersed in 1000 mL of purified water at 37.0 ± 0.5°C, and submitted to an agitation speed of 150 rpm for 1 hour. The percentage of water resistance retention (WRR) is the ratio between the SPF value measured before and after the immersion<sup>[3]</sup>. Several measurements were made to evaluate the variation of SPF and WRR values after storage of formulations at room temperature during 4 weeks.

The results showed that the SPF obtained for the reference formulation (15.2) was in agreement with the specifications of COLIPA guidelines. The mean SPF values obtained for W/S/W and W/S emulsions were 2.3 and 6.0, respectively. However, W/S/W emulsion showed higher WRR (99.66%) than W/S (75.18%) and reference formulation (80.28%). Both silicone bases incorporating EHMC showed a shear thinning behavior and a decrease of firmness and pH after 4 weeks of storage at room temperature. However, all formulations were stable after being subjected to accelerated stability studies by centrifugation at 3000 rpm, during 30 min.

Despite presenting a greater resistance to water, multiple W/S/W emulsion containing EHMC showed lower SPF values and lower physical stability than W/S emulsion or the reference formulation.

### References:

- [1]- Bahia MFG. Protecção solar-Actualização, Editora Universidade do Porto, Porto; 2003.
- [2]- COLIPA. Method for *in vitro* Determination of UVA protection. COLIPA; 2009.
- [3]- Ahn S, Yang H, Lee H, Moon S, Chang I. Alternative evaluation method *in vitro* for the water-resistant effect of sunscreen products. *Skin Research and Technology*, 2008;14(2):187-91

# COMPARISON OF L-DOPA UPTAKE BETWEEN PROLIFERATIVE AND DOPAMINERGIC NEURONS LIKE SHSY5Y CELLS AS A CELLULAR MODEL FOR PARKINSON DISEASE STUDIES

**E. Magalhães-Ribeiro<sup>1</sup>, M.P. Serrão<sup>1</sup>, E. Moura<sup>1,2</sup>, M.A. Vieira-Coelho<sup>1,2</sup>**

<sup>1</sup>Departamento de Farmacologia e Terapêutica, Faculdade de Medicina, Universidade do Porto, Porto, Portugal; <sup>2</sup>Departamento de Neurofarmacologia, Instituto de Biologia Molecular e Celular, Porto, Portugal.

**Introduction** The molecular mechanisms underlying Parkinson's disease (PD) are not completely understood, but the treatment with L-DOPA remains to date the most effective treatment. The human neuroblastoma cell line SH-SY5Y has been widely used as a cellular model of dopaminergic neurons for PD research [1]. In this study, we attempted to determine the differences in L-DOPA uptake between proliferative and retinoic acid (RA), 12-O-Tetradecanoylphorbol 13-acetate (TPA) and TPA/RA differentiated cells (dopaminergic neurons like SHSY5Y cells).

**Methods and Statistics** Proliferative SHSY5Y cells were maintained with DMEM/F12 medium plus 5% of fetal bovine serum (FBS). Differentiation was triggered by lowering FBS to 1% and by 6 day treatment with 10  $\mu$ M RA or 80 nM TPA or by 3 days treatment with 10  $\mu$ M RA and 3 days with 80 nM TPA (TPA/RA). L-DOPA levels in SH-SY5Y cells were evaluated by high performance liquid chromatography with electrochemical detection. Results are presented as mean  $\pm$  standard error mean.

**Results** L-DOPA transport was concentration (2.5-2500  $\mu$ M) dependent for undifferentiated (CT) [ $K_M$  ( $\mu$ M) =  $292 \pm 37$  and a  $V_{max}$  (nmol/mg protein/6 min) =  $60.7 \pm 2.2$ , n = 6] and TPA [ $K_M$  ( $\mu$ M) =  $279 \pm 77$  and a  $V_{max}$  (nmol/mg protein/6 min) =  $60.4 \pm 4.8$ ] and TPA/RA [ $K_M$  ( $\mu$ M) =  $280 \pm 78$  and a  $V_{max}$  (nmol/mg protein/6 min) =  $2412 \pm 194$ ] differentiated cells. The L-DOPA uptake in TPA/RA differentiated cells was significantly different from CT and TPA differentiated cells. RA differentiated cells transport L-DOPA in a linear way [ $K_M$  ( $\mu$ M) =  $5227 \pm 6237$  and a  $V_{max}$  (nmol/mg protein/6 min) =  $3785 \pm 3238$ ]. The uptake of L-DOPA (2.5  $\mu$ M) was reduced (~80% inhibition) by the inhibitor of the L-type amino acid transporters 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH, 1 mM) in the CT, TPA/RA and TPA differentiated cells but not by the inhibitor of the A-type amino acid transporters *N*-(methylamino)-isobutyric acid (MeAIB, 1mM). L-DOPA (2.5  $\mu$ M) uptake remained unaltered by either lowering the pH from 7.4 to 6.2 or by removal of extracellular sodium  $Na^+$  in both differentiated or CT cells. The effect of the L-Amino-acids (1 mM) on the accumulation of L-DOPA in TPA/RA and TPA differentiated cells did not differ in general from what happens in proliferative cells, but in RA-differentiated cells L-DOPA uptake in the presence of several aminoacids was significantly different from the other differentiated and proliferative cells.

**Conclusions** L-DOPA transport in SH-SY5Y was similar between proliferative cells and TPA and TPA/RA differentiated cells, although the latter presented increased ability to transport L-DOPA (increased  $V_{max}$ ). However in RA differentiated cells L-DOPA transport differed significantly from the other differentiated cells.

## References

[1] Xie HR, Hu LS, Li GY, *SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease*, Chin Med J (Engl) 2010 Apr 20;123(8), pp. 1086-92.

## **Concept Analysis: Healthy Lifestyles, according to the traditional method of Walker and Avant (2005)**

**F. Pires<sup>1</sup> M. Barbieri<sup>2</sup>**

<sup>1</sup> Department of Master of Science in Nursing, Faculty of ICBAS, University of Porto, Portugal.

<sup>2</sup> Department of Nurse, Faculty of ESEP, University of Porto, Portugal

Nowadays, the healthy lifestyles concept is increasing among human population, seeking for more and better health. However, in the scientific community, this concept is applied in different contexts with different meanings, so it is important to clarify it in the nursing field.

The aim of this work is to perform a concept analysis, according to the traditional model of Walker and Avant (2005) [1], which comprises eight steps: select the concept, define the purpose of analysis, identify the use of the concept, determine the defining attributes, develop model cases and other cases, identify antecedents, consequents, and set empirical referents.

The results obtained allowed the identification of some critical attributes of the Healthy Lifestyles concept, highlighted antecedents, subsequent and empirical referents, which provided support in the illustration of the use of the concept through the model case, borderline case, related case, opposite case and invented case.

This analysis provides the conceptual basis for the development of an operational definition. Moreover, we noticed that sometimes the Healthy Lifestyles concept is not fully applied since it does not take into account the target parameters evaluated in the context of nursing, thereby emerging new directions of scientific research under the concept under study.

[1] Walker, L. O., Avant K. C. (2005), *Strategies for Theory construction in nursing*. Person Prentice Hall, upper Saddle River, New Jersey. 4.<sup>a</sup> edition.

# Contribution to the study of the relationship between occlusal contacts, head posture and center of gravity of the body, in patients with parafunctional and temporomandibular disorders

Rui Duarte<sup>1</sup>, Vanessa Silva<sup>1</sup>, César Ferreira<sup>2</sup>, Marcelo Castro<sup>3,4</sup>,  
Miguel P. Clemente<sup>1</sup>, Joaquim Gabriel<sup>2,4</sup>, J. Paulo Vilas-Boas<sup>3,4</sup>, João C. Pinho<sup>1</sup>

<sup>1</sup>Faculdade de Medicina Dentária UP (FMDUP), Porto

<sup>2</sup>IDMEC, Faculdade de Engenharia UP (FEUP), Porto

<sup>3</sup>Faculdade de Desporto (FADEUP), Universidade do Porto, Porto

<sup>4</sup>LABIOMEPE, Universidade do Porto, Porto

email: jcpinho@fmd.up.pt

## INTRODUCTION

Temporomandibular disorders (TMD) due its high prevalence, has been considered to be a public health condition having become a subject of interest among professionals and researchers. The association between TMD and head, neck and mandibular, and full body postures has been studied giving rise to some controversies [1-3]. When a person stands quietly, the center of mass of the body and the point of application of the ground reaction force (center of pressure - CoP) shows a natural migration referred to as postural sway, were this quantification can be achieved by the use of a force platform [4]. The aim of this study was to relate TMD and orthostatic posture stability in humans, measured through the CoP sway on a force plate.

## METHODS

Twenty randomly individuals were submitted to a clinical examination, in the Unity of Occlusion, TMJ and Orofacial Pain of FMDUP, which was complemented using the RDC/TMD by a trained examiner. The individuals were divided into two groups: Group 1, with diagnosis of TMD and Group 2 (Control), without TMD. Intra-oral appliances like occlusal splints (Fig. 1a), thermoplastic appliances and anterior jig were made for each individual, according to the principles advocated by the Unity of Occlusion, TMJ and Orofacial Pain of FMDUP. Measurements of the head position was made with a special pair of glasses that incorporated a 3D accelerometer (Fig. 1b), connected wirelessly with a personal computer. A Bertec (4060 15) force platform was used to determine the body's CoP (Fig. 1c). Each participant underwent two measurements, interspersed by ten minutes, with and without the intra-oral appliances.

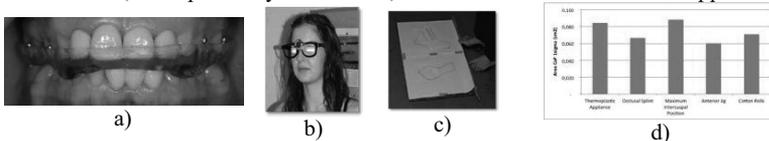


Fig. 1 Intra-oral appliance (a), Accelerometer (b), Force platform (c), Area of CoP (d)

## RESULTS

These preliminary results show that there are differences on the body posture stability (CoP sway) regarding the use of different intra-oral appliances, when evaluating the surface area of the stabiligram in the platform force (Fig. 1d) and the 3D accelerometer. Our results suggest that occlusal changes can have an influence on postural stability of an individual.

**References:** [1] Perinetti G. Dental occlusion and body posture: no detectable correlation. *Gait & posture*. 2006, pp. 165-8. [2] Gangloff P, Louis JP, Perrin PP. Dental occlusion modifies gaze and posture stabilization in human subjects. *Neuroscience letters*. 2000, pp. 203-6. [3] Saito ET, Akashi PMH, Sacco ICN. Global body posture evaluation in patients with temporomandibular joint disorder. *Clinics*. 2009, pp. 35-9. [4] Zatsiorsky V, Duarte M. Rambling and trembling in Quiet Standing. *Motor control*. 2000. pp. 185-200.

## Cultural competence: an analysis of the concept

**F. Rodrigues<sup>1</sup>, J. Martins<sup>2</sup>, M. Pereira<sup>3</sup>**

<sup>1</sup> Nurse, student of Master of Science in Nursing at the Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

<sup>2</sup> Nurse, Phd in sociology of education, teacher at the Portuguese Catholic University, Portugal.

<sup>3</sup> Nurse, post-doctorate in social psychology, teacher at the Master in Clinical Care at the University of Ceará, Brazil.

The cultural competence is an important phenomenon experienced in nursing practice<sup>[1,2]</sup>, it includes strategies to improve the quality of health care, reduce disparities and reduce the imposition of care formatted as a guide to care practices. This study aimed to: explore the meaning of the concept of cultural competence in order to clarify it in nursing practice. We used the method proposed by Walker and Avant for analysis of concepts. To identify the various uses of the concept of cultural competence were selected 15 articles in English and Portuguese, available on the Virtual Health Library, Scielo-Brazil and through EBSCOhost databases of CINAHL, MEDLINE, Academic Search Complete, to present the terms "cultural competence\*" in the title and "transcultural care" in the text, and were classified in the "transcultural nursing". It was used as an exclusion criterion for the time frame: 2005 to April 2011. We identified critical attributes, antecedents, consequent and empirical references to the existence of this concept. It is observed that the association between the ethical and humanitarian features personal and professional, cultural skills with a focus on communication and cultural knowledge were identified as the main empirical references to the existence of cultural competence. We can only observe in the intersubjective aspect regarding the concept translated into the existence of influences that shape the world views of individuals and the degree to which they identify as their own culture. It is concluded that this circularity between some of the related concept of cultural competence, may contribute little to the operationalization of the concept. Assuming at this point, cultural competence so simple, the concept takes the following definition applies also in nursing: the face of increasing diversity in society and understanding of a field cross product of globalization, the new education proposal and explore the political organization cultural information, cultural skill and cultural attitudes in the professionals and services. This allows for a reorientation of nursing practice through the systematic understanding of cultural needs to be met, as an integrator and facilitator of holistic care matching the characteristics of each person and expansion of harm reduction.

### References:

[1] Meleis, A.I. (2007), *Theoretical Nursing: development and progress*. 4th ed. Philadelphia: Lippincott Williams & Wilkins.

[2] Parker, V.A. (2010 - 2011), *The importance of cultural competence in caring for and working in a diverse America*, Journal of the American Society on Aging, 34(4), 97-102.

## ***Cystoseira tamariscifolia* (Hudson) Papenfuss (Phaeophyta): an alternative to plants?**

**G. Lopes, A. Leitão-Rocha, A. Santos, C. Almeida, D. Costa, D. P. Costa, M. Magalhães and S. V. Barros**

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Algae and their derivatives are used in diets and folk medicine, especially in Asian countries, where they are credited with several health benefits. In recent years they have been studied as a promising source of novel drug substances, cosmetic ingredients, food additives and, also, to produce biodiesel.

Herein we report the chemical composition, antioxidant and antibacterial potential of an ethanol extract of *Cystoseira tamariscifolia* (Hudson) Papenfuss, a brown seaweed whose habitat is limited to the intertidal shores of the north Atlantic ocean, Mediterranean and Black Sea.

By HPLC/DAD three carotenoides were detected and using GC/MS we identified sixteen compounds (fatty acids, amino acids, alcohols, phenolics and sterols).

The antioxidant capacity of the extract was assessed against DPPH and nitric oxide radicals and a potent dose-dependent effect was observed (Fig. 1).

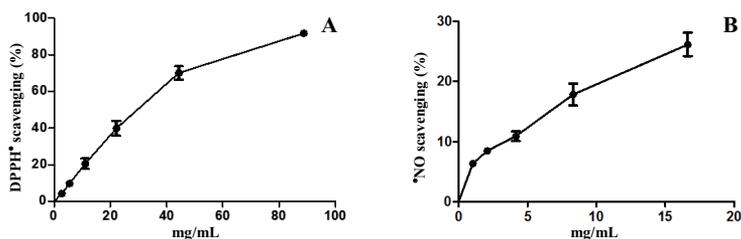


Fig. 1. Scavenging activity of *C. tamariscifolia* ethanol extract against DPPH (A) and nitric oxide (B) radicals. Results correspond to mean  $\pm$  standard error of three assays, performed in triplicate.

Also, it showed antibacterial activity against the Gram positive bacteria *Staphylococcus aureus* and *Bacillus cereus* (concentration  $\geq 15.63$  mg extract/mL).

In conclusion, the ethanol extract of *C. tamariscifolia* was found to have interesting features, which might be used in the prevention of oxidative stress and in antibiotic therapy.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade and Patrícia Valentão.

# Dental Polishing in Composite Resin Restorations: State of the Art Searching for Efficiency

**C. Almeida<sup>1</sup>, A. Moreira<sup>2</sup>, A. Portela<sup>3</sup>, T. Oliveira<sup>4</sup>, M. Vasconcelos<sup>3</sup>**

<sup>1</sup> Integrate Clinic Specialization Student, Faculty of Dental Medicine, University of Porto

<sup>2</sup> PhD student, Faculty of Dental Medicine, University of Porto, Portugal.

<sup>3</sup> Department of Biomaterial's Science and Technology, Faculty of Dental Medicine, University of Porto, Portugal.

<sup>4</sup> Department of Operative Dentistry, Faculty of Dental Medicine, University of Porto, Portugal.

Nowadays, there is a growing quest for esthetics and the perfect smile. This is the genesis for a large proportion of patients to search a dentist. Polishing involves the use, sequenced, abrasives finer grain size in order to reduce surface irregularities. The surface roughness is directly related to bacterial adhesion, which is the first step for of plaque's accumulation and gingival inflammation, and excessive pigmentation [1,2].

The purpose of this study is to evaluate which type of polishing's protocol for dental restorations with composite resin are performed by Portuguese dentists, and how important this procedure is for them. We also want to evaluate which protocol is more effective for polishing dental composite resins.

Questionnaires were conducted to dentists, according to the polishing method used and its importance. In the laboratory there were made 35 disks of two type of composite resin. A fine grain sandpaper was applied to discs and they were divided randomly into six groups (control, A to E). The first group was not polished (control); in group A, polish was performed with diamond burs; in group B, it was performed with carbamide burs; group C was subjected to polishing with abrasive rubbers (Astropol ®); group D, with polishing pastes (Enhance ®); in group E, polishing discs with aluminum oxide (Swissflex™) were used. The reading of roughness was conducted with the roughnessmeter. Discs were placed in an aqueous medium and at a temperature of 37 ° C for 48 hours to mimic the conditions of the oral cavity. Then they were subjected to the same type of polishing, as described above. There were new readings of roughness, using the same roughness.

Through the questionnaires analysis, it was verified that the majority of dentists perform tooth polishing and recognizes their importance to aesthetics and durability of a restoration. The most effective polishing system are the aluminum oxide discs (SwissFlex™) system followed by polishing paste (Enhance ®), rubber abrasive system (Astropolis ®), carbamide tungsten multilayered drill and finally the diamonds extra fine and fine drills. The surface roughness of composite resin restorations in aqueous and 37 ° C, does not present any significant change. The polish is effective and necessary to reduce surface roughness.

## References:

- [1] Barbosa SH, *et al.*(2005) Effect of Different Finishing and Polishing Techniques on the Surgace Roughness of Microfilled, Hybrid and Packable Composite Resins. *Braz Dent J.* 16(1):339-44;
- [2] Heintze SD, Forjanic M, Rousson V. (2006) Surface roughness and gloss of dental materials as a function of force and polishing time in vitro. *Dent Mater.* Feb;22(2):146-65;

# Development and optimisation of targeted drug delivery systems towards visceral leishmaniasis

**D. Barros<sup>1</sup>, A. Cordeiro da Silva<sup>1,2</sup> and S. A. Costa Lima<sup>1</sup>**

<sup>1</sup> IBMC-INEB Infection and Immunity- Parasite Disease Group. Rua do Campo Alegre, 823, 4150-180 Porto, Portugal

<sup>2</sup> Laboratório de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal

Current therapies against visceral leishmaniasis, a neglected tropical disease caused by obligate intra-macrophage protozoa, are associated with emergence of resistances and elevated toxicity. In that way is important to develop alternative treatments, like nanotechnology-based drug delivery systems which may reduce toxicity and side effects of the drug, and enhance their efficacy.

The purpose of this work was to prepare mannose coated nanoparticles by different techniques and do their biophysical characterization in order to see which of those techniques is more advantageous in the preparation of these nanoparticles. The nanoformulations cytotoxicity towards macrophages and their activity against the *Leishmania infantum* (*L.infantum*) parasite were also evaluated.

Empty poly( lactic-co-glycolic acid) nanoparticles (PLGA-NPs) were prepared by the nanoprecipitation method [1] and mannose was attached to the PLGA-NPs by three different techniques: physical adsorption, one-step chemical reaction and two-steps chemical reaction. Physicochemical characterization of the nanoformulations included size, shape, polydispersity index and zeta potential determined by transmission electron microscopy and dynamic light scattering. We also use Fourier transform infrared spectroscopy to confirm the structure of M-PLGA NPs and the lectin binding assay to assess the surface orientation and availability of mannose ligand after formation of the NPs. Mannose was indirectly quantified by phenol-sulfuric acid reaction. The biological effect was evaluated on THP1 differentiated macrophages, *L.infantum* promastigotes and intracellular *L.infantum* amastigotes.

The mean diameter of M-PLGA NPs prepared by physical adsorption, one-step and two-step chemical reaction were  $222 \pm 2.6$ ;  $208 \pm 12.6$  and  $200 \pm 9$  nm, respectively. The NPs presented low polydispersity ( $< 0.1$ ) and anionic surface charge ( $-15$  to  $-20$  mV). TEM photographs suggested that M-PLGA NPs prepared by physical adsorption and one-step chemical reaction were spherical in shape, whereas the ones that were prepared by two-step chemical reaction present an “oval” form. Mannose in M-PLGA NPs is capable of interacting, in a gretar extent, with the lectin receptors. The nanoformulations don't present cytotoxicity against THP1 differentiated macrophages and do not have any effect on intracellular *L.infantum* amastigotes up to 2 mg/ml in polymer.

From these results we conclude that one-step chemical reaction is more advantageous in the preparation of M-PLGA NPs.

## References

[1] Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule Formation by Interfacial Polymer Deposition Following Solvent Displacement. Int J Pharm 1989; 55: R1-R4.

# Differential distribution of ecto-NTPDases 1, 2 and 3 at the rat urinary bladder: Functional implications and drug targeting

**C.R. Moreira<sup>1</sup>, M.A. Timóteo<sup>1</sup>, I. Carneiro<sup>1</sup>, C. Vieira<sup>1</sup>, M.T. Magalhães-Cardoso, F. Ferreirinha<sup>1</sup>, J. Sévigny<sup>2</sup> & P. Correia-de-Sá<sup>1</sup>**

<sup>1</sup>Lab. Farmacologia e Neurobiologia / UMB, ICBAS – Univ. Porto, Portugal, and <sup>2</sup>Centre de Recherche en Rhumatologie et Immunologie, Univ. Laval, Québec, Canada

Bladder filling, inflammation and chemical irritation stimulate the release of nucleotides (*e.g.* ATP) from the bladder epithelium, which are thought to play important roles in bladder function via the activation of ionotropic P2X and metabotropic P2Y receptors. ATP-sensitive P2X<sub>1</sub> receptors are found on the detrusor smooth muscle, whereas receptors containing the P2X<sub>3</sub> subunit are found on pelvic nerve afferent nerves implicated in the micturition reflex. The role of ATP may, however, be counteracted by compartmentalization of purinoceptors together with ecto-NTPDases at cell surface microdomains. Impairment of the catabolism of ATP into its active metabolites, namely ADP and adenosine, by ecto-NTPDases contributes to hyperexcitability of the urinary bladder by preventing the activation of co-localized inhibitory P2Y<sub>1</sub> and P1 receptors, respectively (Correia-de-Sá *et al.*, unpublished observations).

In this study, we investigated the kinetics of the catabolism of ATP, ADP and AMP and the formation of metabolites in the isolated urinary bladder of male Wistar rats (300–450 g) by HPLC with UV detection. The differential distribution of ecto-NTPDases 1, 2 and 3 at the rat urinary bladder was evaluated by histoenzymatic analysis and immunofluorescence confocal microscopy. Effects of subtype-selective ecto-NTPDase inhibitors on the micturition reflex triggered by bladder distension (intravesical saline infusion 2.4 ml/h) were investigated in urethane-anaesthetized rats.

The half-degradation time of 30 µM ATP, ADP and AMP in the rat isolated urinary bladder was 40±5 min (*n*=4), 44±5 min (*n*=4) and 38±5 min (*n*=4), respectively. Histoenzymatic assays revealed that ATP and ADP were metabolized preferentially in the urothelium, as compared with lamina propria and detrusor smooth muscle layer. Immunolocalization studies confirmed that rat urothelial cells are highly enriched in ecto-NTPDase 2 and 3 enzymes. Nerve fibers at the lamina propria are immunoreactive against ecto-NTPDase 1 and 2, whereas detrusor smooth muscle layer exhibits faint labeling for these enzymes. Regarding AMP dephosphorylation into adenosine by ecto-5'-nucleotidase, both histoenzymatic assay and immunofluorescence labeling indicate that it takes place predominantly at the detrusor smooth muscle and at a thin layer corresponding to the most basal (progenitor) urothelial cells. The specificity of ecto-5'-nucleotidase activity was confirmed with α,β-methylene ADP (AOPCP, 200 µM), which is a selective inhibitor of this enzyme. Intravesical administration of the preferential ecto-NTPDase 1 inhibitor, ARL67156 (200 µM), increased the voiding frequency (VF) in anaesthetized rats by 41±8% (*n*=3), without significantly (*P*>0.05) affecting the amplitude (A) and the duration (Δt) of bladder contractions. Likewise, inhibition of ecto-NTPDases 1 and 2 with POM-1 (200 µM) also increased the VF in the rat *in vivo*, but this effect was smaller (29±1%, *n*=4) than that obtained with ARL67156 (200 µM). Surprisingly, the selective inhibitor of ecto-NTPDase 3, PSB06126 (10 µM), decreased bladder activity, *i.e.* the VF was reduced by 26±3% (*n*=5) as compared with the control situation. Exogenous application of NTPDase 1 (also called CD39, ATPDase or apyrase, 2 U/ml), the enzyme that dephosphorylates ATP directly to AMP with almost no release of ADP, significantly reduced (60±16%, *n*=4) the micturition reflex.

Considering (1) the geographical distribution of P2 purinoceptors and ecto-NTPDases in the urinary bladder wall and (2) the fact that ecto-NTPDase 2 is a preferential nucleoside triphosphatase which hydrolyses ADP 10 to 15 times less efficiently than ATP, whereas ecto-NTPDase 3 is described as a functional intermediate between NTPDase 1 and -2, our results are consistent with the hypothesis that ATP and its metabolite ADP exert opposite neuromodulatory actions to control micturition in the rat urinary bladder. Bladder hyperexcitability was observed upon increasing ATP accumulation by inhibiting ecto-NTPDase 1 with ARL67156, as this enzyme co-localizes with P2X<sub>3</sub> receptors on suburothelial sensory nerve fibers. ATP-induced bladder excitability was partially counteracted (through MRS2179-sensitive inhibitory P2Y<sub>1</sub> receptors activation) by ADP formation via ecto-NTPDase 2 localized in the urothelium and lamina propria. Because of their involvement in multiple physiological processes, namely the bladder micturition reflex, ecto-NTPDases are now considered as potential new drug targets.

*Work supported by FCT (FEDER funding, PTDC/SAU-OSM/104369/2008 and UMB-215/94), Assoc. Port. Urologia and Univ. Porto / Caixa Geral de Depósitos.*

## Disturbance of fine-tuning modulation of excitatory nicotinic $\alpha 3\beta 2$ and muscarinic $M_1$ autoreceptors by ADA overactivity in rats with toxin-induced *Myasthenia gravis*

D. Trigo, T. Morais, C. Costa, D. Meireles, M.T. Magalhães-Cardoso, M.A. Timóteo, L. Oliveira & P. Correia-de-Sá

Laboratório de Farmacologia e Neurobiologia, UMIB / Instituto de Ciências Biomédicas de Abel Salazar (ICBAS) – Universidade do Porto, Portugal.

Adenosine (ADO)  $A_{2A}$  receptors control neuromuscular transmission through a sophisticated interplay with cholinergic autoreceptors, which are either excitatory, like nicotinic  $\alpha 3\beta 2$  and muscarinic  $M_1$  receptors, or inhibitory, like the  $M_2$  receptor [1]. Retrograde signalling mediated by ADO released from skeletal muscle fibres is significantly impaired in  $\alpha$ -bungarotoxin-induced *Myasthenia gravis* (TIMG) rats, thus causing profound changes in the control of neuromuscular transmission particularly during high frequency nerve stimulation (50 Hz-bursts) [2]. Most attempts to improve muscle weakness in myasthenic patients involve blockade of acetylcholine breakdown with cholinesterase inhibitors. The therapeutic benefit of cholinesterase inhibitors (e.g. neostigmine) may be partially mediated by the activation of excitatory cholinergic autoreceptors. This prompted us to evaluate the neuromodulatory role of ADO in terms of its interaction with excitatory nicotinic  $\alpha 3\beta 2$  and muscarinic  $M_1$  autoreceptors in TIMG rats.

Rats were chronically injected with the muscle-type nicotinic antagonist,  $\alpha$ -bungarotoxin, as previously described [3]. [ $^3$ H]ACh release was evoked by phrenic nerve stimulation with 50 Hz-bursts (5 trains of 150 pulses each delivered with a 20-s interburst interval). The total amount of endogenous ADO release was determined by HPLC with UV detection [4].

Inhibition of [ $^3$ H]-ACh release induced by the nicotinic  $\alpha 3\beta 2$  receptor antagonist, DH- $\beta$ -E (3  $\mu$ M), was more intense in TIMG (25 $\pm$ 6%,  $n=9$ ) than in control (9 $\pm$ 4%,  $n=5$ ) animals. Likewise, the selective muscarinic  $M_1$  receptors antagonist, MTx-7 (1 nM), inhibited by 47 $\pm$ 6% ( $n=9$ ) the release of evoked [ $^3$ H]-ACh in TIMG rats, but it was without effect in control animals. There is remarkable parallelism between tonic enhancement of transmitter exocytosis via nicotinic  $\alpha 3\beta 2$  and muscarinic  $M_1$  and the reduction of ADO  $A_{2A}$  activation (revealed by ZM241385-induced inhibition) in myasthenic animals. Attenuation of the ADO  $A_{2A}$ -mediated tonus in myasthenic rats may be due to a faster kinetics of extracellular deamination of the nucleoside; inactivation of exogenously added ADO (30  $\mu$ M) into inosine by adenosine deaminase (ADA) was faster ( $P<0.05$ ) in TIMG rats (32 $\pm$ 11 min,  $n=5$ ) as compared to control littermates (59 $\pm$ 13 min,  $n=3$ ). Lower levels of endogenous ADO were also detected following phrenic nerve stimulation in TIMG animals as compared to control preparations.

Data indicate that increased activity of ADA (a hallmark of *Myasthenia gravis*) impairs synaptic ADO accumulation to levels required to activate  $A_{2A}$  receptors. Neuromuscular tetanic failure due to prevention of  $A_{2A}$ -receptor-mediated facilitation may be partially compensated through disinhibition of ACh exocytosis via nicotinic  $\alpha 3\beta 2$  and muscarinic  $M_1$  autoreceptors.

[1] Oliveira *et al.* (2002) *Eur. J. Neurosci.* **11**, 1728-736; [2] Noronha-Matos *et al.* (2011) *J. Neurochem.* **117**, 797-811; [3] Plomp *et al.* (1992) *J. Physiol. (London)* **458**, 487-99; [4] Magalhães-Cardoso *et al.* (2003) *J. Physiol. (London)*, **549**, 399-408.

Work supported by FCT (FEDER funding, PTDC/SAU-FCF/108462/2008 and UMIB-215/94) and by Univ. Porto / Santander Totta.

# Diversity and virulence profiles of *Escherichia coli* ST69, ST393 and ST405 clones

Pires J.<sup>1</sup>, Novais A.<sup>1</sup> and Peixe L.<sup>1</sup>

<sup>1</sup> REQUIMTE-Department of Microbiology, Faculty of Pharmacy, University of Porto, Portugal

**Background:** The worldwide spread of extended spectrum  $\beta$ -lactamases (ESBLs) has been linked to the expansion of particular extra-intestinal pathogenic *Escherichia coli* (ExPEC) clones. [1,2] Studies analysing their diversity and virulence profile include mostly temporally and geographically confined isolates. [3] In this work, we characterized a representative collection of phylogenetic group D (ST69, ST393 and ST405) *E. coli* isolates from different origins, geographic regions and temporal frames.

**Methods:** Thirty-six representative ESBL and non-ESBL producing isolates (13 ST69, 12 ST393 and 11 ST405) were collected from nine countries/three continents and sources (83% human infections, 8% healthy volunteers and 8% food-products) isolated between 1980 and 2010. *E. coli* phyloroups and O antigens were identified by PCR, and clonal relatedness was established by PFGE and MLST. The screening for 38 ExPEC virulence factors (VFs) was performed by PCR. Antibiotic resistance (AbR) profiles to non- $\beta$ -lactams (aminoglycosides, tetracycline, quinolones, sulphonamides, trimethoprim, chloramphenicol and nitrofurantoin) were assessed by disk diffusion tests. [4]

**Results:** Twenty-seven (73%, mostly from ST69 and ST393 clonal groups) isolates were classified as ExPEC (75% from UTI/bacteremia, 25% from healthy volunteers/food products), whereas 22% of non-ExPEC isolates (all identified as ST405) also caused extra-intestinal infections. A high virulence score was observed among ST69 (median 14; range 9-15) and ST393 (median 13; range 3-15) isolates, and lower for ST405 clonal group (median 6; range 2-14), all of them exhibiting high AbR rates. The most common VFs among all STs were *fimH* (82-100%), *fyuA* (62-100%), *iutA* (55-92%), and in a lesser extent *sat* (55-77%). ST69 and ST393 were enriched in *pap* alleles (69%-92%), *iha* (77%-83%), *kpsMTII-K5* (92%-100%) and *ompT* (50%-92%), whereas ST405 exhibited more frequently *kpsMT III* (46%), PAI (64%) or *fyuA* (100%). Despite the high clonal diversity observed, particular lineages sharing common PFGE-patterns and virulence profiles were identified in different niches and/or geographic regions for several years.

**Conclusion:** The absence of correlation between non-ExPEC and extraintestinal disease suggests the contribution of other non-explored VFs. Although with variable relative contribution, both virulence and AbR profiles seem to be influencing the widespread of ST69, ST393 and ST405 clonal groups. We further demonstrate the spread of particular lineages within different niches and geographic regions throughout time.

## References:

- [1] Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiology Reviews. 2011
- [2] Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG. Epidemic Clonal Groups of *Escherichia coli* as a Cause of Antimicrobial-Resistant Urinary Tract Infections in Canada, 2002 to 2004. Antimicrob Agents Chemother. 2009;53(7):2733-9.
- [3] Coelho A, Mora A, Mamani R, López C, González-López JJ, Larrosa MN, et al. Spread of *Escherichia coli* O25b:H4-B2-ST131 producing CTX-M-15 and SHV-12 with high virulence gene content in Barcelona (Spain). Journal of Antimicrobial Chemotherapy. 2011;66(3):517-26.
- [4] Coque T, Novais A, ACarattoli, Poirel L, Pitout J, Peixe L, et al. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum  $\beta$ -lactamase CTX-M-15. Emerg Infect Dis. 2008.

# Drug screening of bisnaphthalimidopropyl derivatives on *Trypanosoma brucei*

**P. Varela**<sup>1,2</sup>, **P.K.T.Lin**<sup>3</sup>, **A.Cordeiro-da-Silva**<sup>1,4</sup> and **S.A.C. Lima**<sup>1</sup>

<sup>1</sup> IBMC-INEB Infection and Immunity- Parasite Disease Group. Porto, Portugal

<sup>2</sup> Faculty of Sciences, University of Lisbon, Portugal

<sup>3</sup> School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, UK.

<sup>4</sup> Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal

*Trypanosoma brucei* found in many countries of Africa is a haemoflagellate protozoan parasite, transmitted by the bite of the tsetse fly (*Glossina* sp.). The subspecies *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* cause the debilitating disease Human African trypanosomiasis also known as 'sleeping sickness' [1]. The urgent need to develop new drugs against this neglected disease is supported by inadequate standard therapies, usually associated with high toxicity and inefficacy.

The anti-leishmanial properties of bisnaphthalimidopropyl (BNIP) derivatives have been described [2,3]. To further evaluate their application in the treatment of infections caused by trypanosomatids as *Trypanosoma brucei*, this study describes a cell-based drug screening towards human African trypanosomiasis disease.

The *T.brucei brucei* bloodstream form strain Lister 427, known to affect domestic and laboratory animals, was used to optimize an *in vitro* 96-well plate assay for drug screening. The two viability assays chosen were the resazurin-based cell viability (Alamar blue) and the tetrazole 3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay. The signal linearity was determined after 2, 3, 4 and 5h of incubation at 37°C. Due to the sensitivity, simplicity and cost benefit of the resazurin-based assay, it was the selected method to perform the screening of a series of BNIP derivative compounds towards *T.brucei*. As control drugs pentamidine and suramine were used.

As accordingly with the literature pentamidine exhibited an IC<sub>50</sub> value against *T.brucei* around 4 nM [4]. Two of the BNIP derivatives had IC<sub>50</sub> values in the same range of pentamidine, while four of the ten tested compounds had an IC<sub>50</sub> value above 1 μM.

A 96-well plate resazurin based assay was successfully optimized for drug screening on *T.brucei* allowing the identification of active compounds from the BNIP derivative compounds family.

## References:

- [1] Jacobs,R.T.,Nare,B. and Philips,M.A.(2011)*State of the Art in Trypanosome drug discovery*. Curr Top Med Chem. 11(10), 1255-1274. [2] Tavares,J.,Ouaissi,A.,Thoo Lin,P.K.,Tomas,A. and Cordeiro-da-Silva, A.(2005)*Differential effects of polyamine derivative compounds against Leishmania infantum promastigotes and axenic amastigotes*.Int. J. Parasitol.35, 637-646.[3] Oliveira,J.,Ralton,L.,Tavares,J.,Codeiro-da-Silva,A.,Bestwick,C.S.,McPherson,A.and Thoo Lin,P.K.(2007) *The synthesis and the in vitro cytotoxicity studies of bisnaphthalimidopropyl polyamine derivatives against colon cancer cells and parasite Leishmania infantum*. Bioorg. Med. Chem. 15, 541-555. [4] Jones,D.C.,Hallyburton,I.,Stojanovski,L.,Read,K.,Frearson,J., Fairlamb, A. (2010) *Identification of a k-opioid agonist as a potent and selective lead for drug development against human African Trypanosomiasis*. Biochem Pharm. 80, 1478-1486.

## **Effect of doxycycline on the proliferation and differentiation of rat bone marrow-derived osteoblastic cells**

**J. Silva<sup>1,2</sup>, B. Colaço<sup>3</sup>, M.H. Fernandes<sup>2</sup>, P.S. Gomes<sup>2</sup>**

<sup>1</sup> Master's Degree in Biomedical Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup> Laboratory of Pharmacology and Cellular Biocompatibility, Faculty of Dental Medicine, University of Porto, Portugal.

<sup>3</sup> Department of Zootechny, University of Trás-os-Montes and Alto Douro

Tetracyclines are broad-spectrum antibiotics that are able to modulate several biological effects in cellular mechanisms in processes unrelated to the inhibition of ribosomal protein synthesis – their principal antibacterial mechanism of action [1] Previous reports have shown that therapeutic doses of tetracyclines are beneficial in the management of bone tissue-related diseases such as rheumatoid arthritis and periodontal disease. [2] Although the established effects, little is known about the direct or indirect action of these drugs on the osteoblastic differentiation process.

This work aims to evaluate the proliferation and differentiation events of osteoblastic-induced rat bone marrow-derived cells, in the presence of therapeutic concentrations of doxycycline (a semi-synthetic tetracycline).

Cell cultures were established from bone marrow femoral and tibial aspirates of 4 months old females Wistar rats. First subcultured cells were seeded at  $2 \times 10^4$  cells.cm<sup>-2</sup> in standard culture conditions, for 12 days, in the presence of representative therapeutic concentrations of doxycycline (0.5 and 1 µg.ml<sup>-1</sup>). Cell morphology was addressed by confocal laser scanning microscopy following staining of cytoskeleton and nucleus. Cultures were further characterized for cell viability/proliferation and expression of phenotypic markers (alkaline phosphatase and collagen). Doxycycline, at assayed concentrations, did not alter cell morphology – as observed by confocal microscopy – or influence cell proliferation throughout the culture period. Both concentrations induced the synthesis of collagen and alkaline phosphatase, as assessed by biochemical and histochemical methodologies, at late culture time points.

Overall, the results suggest that low concentrations of tetracyclines induce the differentiation of rat bone marrow-derived osteoblastic cells, without influencing cell morphology and proliferation. Tetracycline application may combine an expected local antibacterial activity with a potential anabolic effect regarding osteoblastic differentiation, with prospective application in bone tissue engineering applications.

### References

[1] Klein, N.C. and Cunha, B.A. (1995), Tetracyclines. *The Medical Clinics of North America*, 79(4), 789-801.

[2] Sapadin, A.N. and Fleischmajer, R. (2006), Tetracyclines: Nonantibiotic properties and their clinical implications. *Journal of the American Academy of Dermatology*, 54, 258-65.

## EFFECT OF MEDITERRANEAN LIFESTYLE IN REDUCING THE WEIGHT AND BLOOD PRESSURE

**M. Neves<sup>1</sup>, R. Santos<sup>2,3</sup>, E. Tejera<sup>3,4</sup>, I. Rebelo<sup>3,4</sup>**

<sup>2</sup> Departamento de Medicina Interna, H S João-Porto

<sup>3</sup> Serviço de Bioquímica -Faculdade Farmácia-Porto

<sup>4</sup> Instituto Biologia Molecular e Celular- Porto

**Introduction:** Despite the uncertainty of the relationship between obesity and hypertension, it is known that obesity is an independent risk factor for hypertension and other cardiovascular risk factors. This should raise awareness among clinicians about the importance of obesity and encourage a more active approach in its prevention and treatment

**Objective:** Prospective study to evaluate the effect of decreasing the weight on blood pressure, waist circumference, type 2 diabetes mellitus and dyslipidemia

**Methods:** We evaluated 777 consecutive patients in outpatient nutrition / internal medicine, with different levels of obesity (BMI > 30 Kg/m<sup>2</sup>), underlining the age, waist circumference, BMI and years of hypertension as variables to adjust, given its association to blood pressure, 199 (25.6%) males and 578 (74.4%) females. Of the 534 patients studied 68.7% had Hypertension, 243 (31.3%) had no hypertension, 219 (28.2%) with DM type 2, 558 (71.8%) without DM type 2, 231 (29.7 %) with Dyslipidaemia (DL) and 546 (70.3%) with no Dyslipidaemia. In this sample, several evaluations were performed: i) anthropometric: weight, height, waist circumference; ii) assessment of blood pressure (BP). Patients were advised to have a lifestyle Mediterranean (Mediterranean hypocaloric diet and 40 minutes of aerobic activity three times per week) which was adjusted to age, gender, physical activity, social and/or professional lifestyle. All patients, over 17 years old, were divided into five age groups of dichotomy between them. The period of follow-up was 10 years, having been carried out observations for monitoring and dietary adjustments every 3 months.

**Results:** SBP increases significantly with age, weight, BMI and waist circumference, both for hypertensive or normotensive. The SBP is higher in women than in men, either in hypertensive or in normotensive. The DBP increases with age, weight, and circumference of waist and BMI, although not significantly in group 1 and 2, while in groups 3 and 4 were significantly increased.

In the age group older than 70, there is an inflection in relation to the previous groups. With regard to diastolic blood pressure, the highest value was at 50 years of age. In relation to follow-up, time (10 years) showed that the SBP had a significant reduction of 0.91 mmHg (p <0.001) for each year of follow-up of patients, this value was adjusted to an increase of 0.25 mmHg per year of age (p <0.001), an increase of 0.21 mmHg for every cm of waist circumference and an increase of 0.53 mmHg per kg/m<sup>2</sup> of body mass index. In relation to DBP, this study showed that DBP had a significant reduction of 0.56 mmHg (p <0.001) for each year of

Follow-up of patients, this value was adjusted to an increase of 0.30 mmHg for each year of hypertension (p <0.001), an increase of 0.18 mmHg for every cm of waist circumference and an increase of 0.31 mmHg per kg/m<sup>2</sup> of body mass index. The weight reduction to get the maximum benefit is 8.5 kg.

**Conclusion:** weight loss and consequent waist circumference decrease, reduces blood pressure and causes a significant improvement of co-morbidities associated with overweight or obesity. Hypertensive patients are those with better adherence to dietary and regular physical activity and for this reason hypertensive patients lose more weight and reduce WC. Patients with co-morbidities associated with hypertension and obesity present increased benefits (greater BP decrease) over time.

## Effects of uridine nucleotides on the proliferation and type I collagen synthesis in fibroblasts of the rat subcutaneous tissue: interplay between P2Y<sub>2</sub> and P2Y<sub>6</sub> receptors

A.R. Pinheiro<sup>1</sup>, C. Reis<sup>1</sup>, M.A. Costa<sup>1,2</sup> & P. Correia-de-Sá<sup>1</sup>

<sup>1</sup>Lab. Farmacologia e Neurobiologia and <sup>2</sup>Dept. Química, UMIB, Instituto de Ciências Biomédicas Abel Salazar - Universidade do Porto (ICBAS-UP), Portugal.

Connective tissue may be involved in the pathogenesis of a wide variety of disease conditions. Increased connective tissue stiffness due to fibrosis may be an important link to the pathogenic mechanism leading to drug-resistant chronic pain (Langevin & Sherman, 2007). In addition, extracellular adenine and uridine nucleotides seem to be involved in the pathophysiology of chronic pain (Burnstock, 2001). Therefore, we aimed at investigating the effect of uridine nucleotides on the proliferation and synthesis of type I collagen by rat fibroblasts of subcutaneous connective tissue.

Experiments were performed in the first subculture of fibroblasts isolated from the subcutaneous connective tissue of Wistar rat. Cultures were characterized for cell proliferation (MTT assay) and type I collagen production (Sirius Red F3BA assay) during 28 days. Uridine nucleotides-sensitive P2 purinoceptors expression was confirmed by immunofluorescence confocal microscopy.

Continuous incubation of UTP (0.3-100  $\mu$ M), concentration-dependently increased fibroblasts proliferation, as also increased the synthesis of type I collagen above the control levels. Conversely, the selective P2Y<sub>2</sub> agonist, MRS 2768 (10  $\mu$ M,  $n=3$ ), was devoid of effect in what concerns proliferation, but significantly ( $P<0.05$ ) decreased type I collagen synthesis. Since the increase in type I collagen synthesis induced by UTP (100  $\mu$ M) was proportional to the increase in the amount of cells in the culture (fibroblasts proliferation), we hypothesized that such an increase could be related to the increase in the cell number rather than a higher synthetic activity. Thus, we performed a more detailed data analysis, in which we normalized type I collagen production taking into consideration the MTT values obtained at the same time points, and we observed no longer significant differences between control and UTP-exposed cells. Discounting the contribution of MRS 2768-sensitive P2Y<sub>2</sub> receptors, UTP (100  $\mu$ M)-induced increase in cells proliferation could be due to P2Y<sub>4</sub> and/or P2Y<sub>6</sub> receptor activation. Since RB-2 (10  $\mu$ M,  $n=5$ ), a non-selective antagonist that acts preferentially on the P2Y<sub>4</sub> subtype, did not modify the effect of UTP (100  $\mu$ M), P2Y<sub>4</sub> does not seem to be involved. In turn, MRS 2578 (100 nM), which is a selective P2Y<sub>6</sub> antagonist, significantly attenuated the proliferative action of UTP (100  $\mu$ M). Corroborating this interpretation, fibroblasts of the rat subcutaneous tissue exhibited positive immunoreactivity against P2Y<sub>2</sub> and P2Y<sub>6</sub> receptors showing a cytoplasmic/membrane labeling pattern, which is typical for those receptors in many different cells. This contrasts with the nuclear labeling pattern exhibited with the antibody against the P2Y<sub>4</sub>.

Data suggest P2Y<sub>2</sub> and P2Y<sub>6</sub> receptors play opposing roles in fibroblasts of the rat subcutaneous tissue, in which P2Y<sub>2</sub> might have a preventive activity on the fibrotic response by reducing type I collagen synthesis, whereas P2Y<sub>6</sub> may favor fibroblasts proliferation. This outcome led us to propose that targeting the pathways regulating uridine nucleotide signals may represent an opportunity for designing novel therapeutic strategies to overcome the pathogenesis of superficial fascia remodeling and, thus, musculoskeletal chronic pain.

*Work supported by FCT (FEDER funding, PTDC/SAU-FCF/108263/2008 and UMIB-215/94). AR Pinheiro is in receipt of a FCT PhD Studentship (SRFH/BD/47373/2008).*

## Endocannabinoids and programmed cell death: Anandamide mediates p38 MAPK activation in rat decidual cells

**Almada M<sup>1</sup>, BM Fonseca<sup>1,2</sup>, G. Correia-da-Silva<sup>1,2</sup> and NA Teixeira<sup>1,2</sup>**

<sup>1</sup> Laboratory of Biochemistry, Biological Sciences Department, Faculty of Pharmacy, University of Porto  
<sup>2</sup> Institute for Molecular and Cell Biology (IBMC), University of Porto, Portugal

Recently a new “clan” of lipid mediators, the endocannabinoids, have emerged and at the moment there is increasing evidence of their role as regulators of key cell-signalling pathways in female reproduction. In rodents, the implanting blastocyst triggers a synchronized process of proliferation and differentiation of stromal cells forming a new tissue, the decidua. Later, decidual cells undergo a cycle of regression, which occurs mainly by apoptosis, in order to accomplish placenta development. We have previously shown that ECs machinery operates on decidual cells [1] and found that anandamide (AEA), the main endocannabinoid, induced apoptosis in decidual cells through cannabinoid receptor 1 (CB1) [2]. We found that AEA induces an increase in ceramide levels, effect dependent of CB1 activation. CB1 is a member of the superfamily of G-protein-coupled receptors (GPCRs), which inhibit adenylyl cyclases, regulate ionic channels and modulate the activation of mitogen-activated protein kinases (MAPK). MAPK cascades are a highly conserved signal transduction pathway involved in a vast array of biological processes, including gene expression, cell proliferation, differentiation and apoptosis. In the present study we intend to investigate the involvement of ceramide in MAPK signal transduction pathway in decidual cells induced by anandamide via CB1. Therefore we analysed the expression of protein kinases, in particular p38, by immunoblotting after AEA treatment (10  $\mu$ M, 25  $\mu$ M, and with pre-treatment with CB1 receptor antagonist), using primary decidual cell cultures. We found that AEA induced phosphorylation of p38 MAPK, throughout the observation of protein expression levels on AEA treated cells. In addition, the effect revealed to be dependent on CB1 receptor activation. Collectively, in primary decidual cells, anandamide induced an increase in ceramide levels which may evoke p38 MAPK phosphorylation. Although, more studies are required to understand the exact biochemical mechanisms underlying the decidual regression process, and the involvement of anandamide in activation of p38 pathway mediated by ceramide, which subsequently may result in apoptosis.

[1] Fonseca BM, Correia-da-Silva G, Taylor AH, Lam PM, Marczylo TH, Konje JC, et al. 2010 N-acyl ethanolamine levels and expression of their metabolizing enzymes during pregnancy. *Endocrinology* 151 3965-74.

[2] Fonseca BM, Correia-da-Silva G, Teixeira NA 2009 Anandamide-induced cell death: dual effects in primary rat decidual cell cultures. *Placenta* 30 686-92.

[3] Sarker KP, Biswas KK, Yamakuchi M, Lee KY, Hahiguchi T, Kracht M, Kitajima I, Maruyama Morris, 2003 ASK1-p38 MAPK/JNK signaling cascade mediates anandamide-induced PC12 cell death. *J Neurochem.* 85(1):50-61.

Acknowledgements: The project was partially supported by “Projectos Pluridisciplinares” Universidade do Porto/Santander Totta. BM Fonseca thanks Fundação para a Ciência e Tecnologia (FCT) for the post-doctoral grant (SFRH/BPD/72958/2010)

# Ethics in clinical studies: a systematic review on patients' consent for publication of case reports

**M.J. Bernardes<sup>1</sup> and R. Nunes<sup>2</sup>**

<sup>1</sup> Final year medical student. Department of Bioethics and Medical Ethics, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> Professor and Director. Department of Bioethics and Medical Ethics, Faculty of Medicine, University of Porto, Portugal.

**Introduction:** Although the compliance with the participants' right to autonomy and privacy in research involving humans is notorious in medical journals, closely following international guidelines [1], the same does not seem to occur in case reports. The objective of this paper was the development of a systematic review on patient's consent for the publication of case reports.

**Material and Methods:** A search, in the PubMed and ISI Web of Knowledge, was performed, using the query "case and consent and (report or reports)", with the limits "English, Portuguese and humans", complemented by a hand search. Relevant papers were selected with the scrutiny of two assessors.

**Results:** Thirty five papers were included in the final analysis: 3 guidelines, 9 surveys of editors, Internet or medical journals, 11 letters to the editors, 8 commentaries and 4 editorials, most of them coming from General/Internal Medicine and Anaesthesiology. In the analysed papers, patient's consent for the publication of case reports has only been mentioned in 0 to 23% of the cases. There was a consensus on the patient's right to privacy, but the obligation of written consent for all case reports was controversial.

**Conclusions:** Compliance with the patients' right to autonomy and privacy may be lacking in the publication of case reports. More research is needed to elucidate which is the best way to deal with the patient's right to autonomy and privacy *versus* the doctors' duty of publishing on behalf of the progress of medicine and of the public interest.

**Acknowledgments:** Professors Linda Ohler, Madison Powers, Marjan Kljakovic and Sara Douglas are kindly acknowledged for sending us the papers we have requested them.

## References:

[1] International Committee of Medical Journal Editors (ICMJE). *Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Privacy and Confidentiality*.  
[http://www.icmje.org/ethical\\_5privacy.html](http://www.icmje.org/ethical_5privacy.html) (accessed on 1 November 2011)

## Evaluation of compressibility indicators of technological excipients of tablets

**J. Conceição, M. Estanqueiro, M.H. Amaral, J.P. Silva, J.M. Sousa Lobo**

Department of Drug Sciences, Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Porto, Portugal

A medicine is a physical system whose properties depend on the individual contributions of drug(s) and excipients [1]. Technological or primary excipients have an essential role on the success of any formulation, as they play a major role on the final characteristics of the drug product [1]. The evaluation of technological characteristics of primary excipients is extremely important in any formulation study [1]. The knowledge of the compressibility characteristics of powders or granules is very important and is a very important phase of the pharmaceutical development of solid dosage forms [1, 2]. However, these tests are usually performed with pure excipients and there are only a few published studies concerning mixtures of excipients [1].

The aim of this work is to evaluate the compressibility indicators (angle of repose, flow time, Compressibility Index (CI), Carr Index (CrI) and Hausner Ratio (HR)) of primary excipients of tablets that behave differently during compression, either pure or in binary mixtures whose composition varies between 20%(w/w) and 80%(w/w) at intervals of 20% (w/w). The excipients studied were Pharmatose 200 mesh, Elcema P100, Avicel PH-200, Tablettose 80 and Emcompress [2, 3]. The mixtures were performed at WAB Turbula mixer for 15 minutes. For each mixture or pure excipient (150 g samples were used), the apparent volumes were evaluated using a compaction apparatus Tap Density Tester (USP) ETD-1020 (USPII variant) [2, 4]. The apparent volume values were used to evaluate compaction capacity (CC), CI, CrI and HR [2, 4]. The angle of repose and the flow time were determined with an apparatus Erweka GT [2, 4]. All determinations were done in triplicate for each sample.

Pharmatose 200 mesh, Elcema P100 and their mixtures did not flow and had poor compressibility characteristics. Mixtures of Avicel PH-200 with Tablettose 80 and Avicel PH-200 with Emcompress showed better compressibility characteristics. Finally, the powders were directly compressed in an instrumented alternative machine DOTT.Bonapace model CPR-6. It was not possible to obtain tablets with the mixtures of Pharmatose 200 mesh with Elcema P100. From the four mixtures of Avicel PH-200 with Tablettose 80, it was possible to obtain tablets, and the tablets obtained from the 80:20 mixture presented the better aspect. With the four mixtures of Avicel PH-200 with Emcompress and Avicel PH-200 it was possible to obtain tablets with good aspect. The compressibility indicators values obtained with the binary mixtures are not very different from those obtained with the pure excipients, indicating that ability to compression does not change. With this work we conclude that, by studying the compressibility indicators, it is possible to predict the suitability of materials to obtain tablets.

### References:

- [1] Prista LN, Alves AC, Morgado R. Tecnologia Farmacêutica. III Volume. 6.ª ed: Fundação Calouste Gulbenkian; 2009.
- [2] Prista LN, Alves AC, Morgado R, Lobo JS. Tecnologia Farmacêutica. I Volume. 6.ª ed: Fundação Calouste Gulbenkian; 2003.
- [3] Rowe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical Excipients. Fifth ed: The Pharmaceutical Press. American Pharmaceutical Association; 2006.
- [4] Farmacopeia Portuguesa 9: INFARMED; 2008.

## Evaluation of oxidative stress markers in Chronic Heart Failure

Marta Couto<sup>1,2</sup>, Teresa Sousa<sup>1,3</sup>, Manuela Morato<sup>3</sup>, Jorge Carvalho<sup>1</sup>, Marco Marques<sup>1</sup>, Luís Morgado<sup>1</sup>, Joana Afonso<sup>1</sup>, Maria João Valente<sup>1</sup>, Félix Carvalho<sup>4</sup>, Paulo Bettencourt<sup>2</sup>, António Albino Teixeira<sup>1,5</sup>

(1) Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, Portugal (2) Internal Medicine Department, São João Hospital, Porto; Portugal; (3) Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal (4) REQUIMTE-Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto (5) Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal.

**Introduction:** Increased oxidative stress has been recognized as an important contributory mechanism for the development and progression of cardiovascular diseases. The excessive generation of reactive oxygen species overwhelming the endogenous antioxidant defenses may constitute potential targets of therapy. However, knowledge of redox mechanisms is still insufficient, which might explain the disappointing results obtained with antioxidant supplementation used in the latest clinical trials. The aim of our work was to evaluate the redox status of ambulatory outpatients with Chronic Heart Failure (CHF).

**Methods:** Fifty four patients with CHF (New York Heart Association (NYHA) functional class I to IV) were selected from the Heart Failure Clinic of Hospital S. João. On the day of the visit, patients were examined and blood and urine samples were collected. Plasma total antioxidant status (TAS) was evaluated using a Cayman Chemical Antioxidant Assay Kit. H<sub>2</sub>O<sub>2</sub> and isoprostanes were evaluated in 24-hour urine using commercially available kits (Amplex Red Hydrogen Peroxide Kit, Molecular Probes; Urinary Isoprostane ELISA kit, Oxford Biomedical Research). Plasma myeloperoxidase (MPO) concentration was evaluated using an ELISA assay kit (Bioxytech MPO-EIA, Oxis Research). Plasma MPO activity was evaluated by monitoring the oxidation of tetramethylbenzidine at 655 nm. Serum uric acid (UA), urinary creatinine and plasma B-type natriuretic peptide (BNP) were also determined using commercial kits and an automated biochemical analyzer.

**Results:** Patients were stratified into mild (NYHA classes I and II) and severe (NYHA classes III and IV) CHF. Patients with severe CHF had significantly increased levels of plasma TAS (3.90±0.12 vs 3.19±0.16 mM Trolox, n=9-21, p=0.011), serum uric acid (91.11±10.13 vs 67.37±2.86 mg/l, n=8-19, p=0.0069) and plasma MPO activity (41.87±11.69 vs 16.46±3.04 micromol/min/mg, p=0.034, n=16-18) but not MPO concentration (28.22±8.00 vs 36.55±7.29 ng/ml, p=0.45). Urinary isoprostanes and urinary H<sub>2</sub>O<sub>2</sub> levels were also significantly increased in severe CHF patients (isoprostanes: 2.58±0.38 vs 1.62±0.11 ng/mg creatinine, n=19-27, p=0.008; H<sub>2</sub>O<sub>2</sub>: 0.027±0.009 vs 0.013±0.002 nmol/mg creatinine, p=0.047, n=8-18). BNP analysis confirmed our clinical evaluation of the patients as described in literature (1296.0±328.2 vs 456.8±105.6, p=0.009, n=23-31) but we didn't find any correlation between BNP levels and redox markers.

**Conclusions:** Severe CHF patients have higher levels of redox status markers, namely plasma TAS, plasma MPO activity, urinary excretion of isoprostanes and urinary H<sub>2</sub>O<sub>2</sub>, compared to mild CHF patients. However, these measures do not correlate with already validated and routinely used biomarkers like BNP.

## Exploring Argentine honey and propolis

**F. Rios<sup>1</sup>, P. B. Andrade<sup>2</sup>, P. Valentão<sup>2</sup>, D. Torres<sup>3,4</sup>, N. Samman<sup>1</sup> and L. R. Silva<sup>2</sup>**

<sup>1</sup> Faculty of Engineering, National University of Jujuy, Argentina.

<sup>2</sup> REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

<sup>3</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

<sup>4</sup> Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Portugal.

In recent years there has been a renewed interest in the study of honey and propolis composition and biological properties. The use of these matrices in the treatment and prevention of numerous diseases has been documented [1]. This beneficial role is partially attributed to their antibacterial activity. In addition, part of the therapeutic properties of honeybee products is due to their antioxidant capacity. This also justifies honey effectiveness against deteriorative oxidation reaction of food [2].

In the present study interest was focused on the determination of the botanical origin and phenolic composition and on the evaluation of the antioxidant and antibacterial capacity of honey and propolis collected from Jujuy province in Argentina. Pollen spectrum was determined following melissopalynological methods. Phenolic compounds were analyzed by HPLC-DAD. Antioxidant activity was assessed against DPPH, nitric oxide and superoxide radicals by scavenging microassays.

The results obtained revealed that *Baccharis* sp. was predominant in both honey and propolis samples, representing 76 and 23% of the pollen spectrum, respectively.

Regarding phenolic compounds, hydroxycinnamic acids and flavonoids were found, propolis showing highest diversity and content comparatively to honey.

Concentration-dependent antioxidant effect was noticed: propolis proved to be the most active against DPPH, while honey revealed higher capacity against nitric oxide and superoxide radicals.

Both extracts showed better activity against Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Enterococcus faecalis* and *Micrococcus luteus*) than Gram-negative (*Salmonella typhimurium*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa*) bacteria.

### References:

[1] Molan, P. C. (1999), *Why honey is effective as a medicine. 1. Its use in modern medicine*. Bee World, 80(2), 80-92.

[2] McKibben, J, Engeseth, N. J. (2002), *Honey as a protective agent against lipid oxidation in ground turkey*. Journal of Agricultural and Food Chemistry, 50(3), 592-595.

## Activation of IL-27p28 gene transcription on antigen presenting cells infected with *Leishmania infantum*

**A. Robalo<sup>1,2</sup>, J. Pereira<sup>1,3</sup>, M. Resende<sup>1</sup>, D. Moreira, J. Cunha<sup>1,4</sup>, S. Costa-Lima<sup>1</sup>, A. Cordeiro-da-Silva<sup>1,5</sup> and R. Silvestre<sup>1</sup>**

<sup>1</sup> Parasite Disease Group, IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal

<sup>2</sup> Escola Superior de Biotecnologia – Universidade Católica do Porto, Portugal

<sup>3</sup> Faculdade de Ciências, Universidade do Porto, Portugal

<sup>4</sup> Instituto de Ciências Biomédicas Abel Salazar e Faculdade de Medicina da Universidade do Porto, Portugal

<sup>5</sup> Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal.

*Leishmania* parasites are obligate intracellular eukaryotic protozoan pathogens that thrive on the mononuclear phagocytic system. The virulence related to the pathology seems to be linked to an induced lack of immune response control that begins with the manipulation of innate immune cells. In this context, cytokines are critical coordinators of the immune response necessary for resolving the infection. In particular, the IL-12 family of cytokines is key player in the initiation and regulation of cell-mediated immunity. The bioactive IL-12p70 is critical for the induction of Th1 responses. Other two members of this family, IL-23 and IL-27 have been demonstrated to induce or antagonize, respectively, the development of inflammatory Th17 cells. Bioactive IL-27 composed by p28 and Epstein-Barr-Virus-induced gene 3 (EBI3) has been similarly associated with the initiation of Th1-type immune responses and the attenuation of immune/inflammatory responses in various experimental settings. In this study, we have evaluated the kinetics of mRNA production of each member of the IL-12 family (p19, p28, p35, p40, EBI3) in bone marrow macrophages (BMM) and dendritic cells (BMDC) infected *in vitro* with *L. infantum* promastigotes. A general downregulation of all these members was observed. Nevertheless, increased levels of p28 secretion but not bioactive IL-27 were found in the supernatant of infected BMM and BMDC. Moreover the expression of IL-27p28, in opposition to IL-12p35 and IL-12p40, was found to be independent of IL-10. More importantly, macrophage and dendritic cells recovered from the spleen of acutely infected mice correlate the *in vitro* results demonstrating a specific increase of IL-27p28 subunit. Taken together, these results suggest a potential role for IL-27p28 subunit, which can exert biological activities independently of EBI3, in the modulation of early events of infection that will warrant further studies.

## Behaviour of a group of lions (*Panthera leo*) in captivity

Joana Sousa<sup>1</sup>, Nuno Alvura<sup>2</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal

<sup>2</sup> Maia's Zoo Veterinarian

In captivity, lions behaviour isn't well documented, so my work, at a primary stage consists into study their behaviour.

In Maia's Zoo there is a group of lions, consisted by two females and one male. According to the records of zoo they are about 12 to 14 years old, so it's presumptive that their reproductive performance is starting to decline.

To study the behaviour of this group, it was necessary to learn how to perform *Ad libitum* and focal sampling. *Ad libitum* sampling consists into take note of everything it is seen, without any restrictions. Focal sampling consists into record the behaviour of a specific animal and his interactions with others, during a period of time, previous specified. This sampling could also be direct to a determinate group of animals, e.g. In this case, it was used focal sampling to the three lions behavioural study.

These observations started in August of 2011 and after a month of *Ad libitum* sampling, it was already understandable some behaviour of this particularly group. Therefore it was elaborated a table with some of those behaviours to also be able to do focal sampling in a better way. Since October 2011, focal sampling it is done for about 3 times per week, during all day. It was also done some environmental enrichment once, using recorded roars of others lions to see how the group would react. Anything happened.

At this moment, it was realised that there is not anything new in their behaviour, due maybe to the act of their long permanence at the zoo. However it is necessary to keep doing sampling because in this current year, this group of lions will have a new place to be lodged, due to the increasing of zoo's facilities. Then it will be compared the behaviour of the group with the one's previously done.

## Brow shrimp *Crangon crangon* feeding preferences: do multiple choice experiments confirm field opportunistic feeding?

I. Jorge<sup>1</sup>, C. Moreira<sup>1,2</sup> and J. Campos<sup>2</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental – University of Porto, Ruas dos Bragas 289, 4050-123 Porto, Portugal

Brow shrimp *Crangon crangon* is a common species in the Minho River estuary, described as an omnivorous. But, despite the large range of preys (about 60 different species), there is hardly no knowledge of its feeding strategy.

In this study, we tried to determine the feeding preferences of *C. crangon* within a small group of food items belonging to different resource categories of its habitat and food regime. The study was performed in the absence of intra and interspecific competitive interactions, in order to identify if the species has a preferred specific type of food, or if it's an opportunistic benthonic species that feeds upon the most available of food items in its ecosystem.

In each observation trial it was prepared an aquarium with a set of 12 different items (Table 1), previously weighed (wet weight), randomly disposed and equally distant from the center of the aquarium and from each other. The disposition of the food items was registered on an aquarium map for each observation. About 100 observations were made with shrimps randomly selected from a maintenance aquarium. The shrimps were kept fastening for 48 hours and placed in a Petri dish in the center of the experience's aquarium (one for each replicate). Food items eaten by each shrimp were registered - as well as the food item that was in front of them when they got out of the Petri dish, to determine if the choice was made by proximity or preference - and timed (for 10 minutes observations). In the end, all food items were weighed again and dried (48h at 60°C) to determine the dry weight. Chesson's selectivity index was calculated for males and females separately as well as for size classes.

**Table 1:** List of food items used in the feeding trials

Food specie/type	Food category
<i>Nereis sp.</i>	Polychaeta
<i>Arenicola marina</i>	Polychaeta
<i>Crangon crangon</i>	Crustacean
<i>Gammarus sp.</i>	Crustacean
Mysidaceans	Crustacean
<i>Carcinus maenas</i>	Crustacean
<i>Scrobicularia plana</i>	Bivalve mollusk
<i>Corbicula fluminea</i>	Bivalve mollusk
<i>Pomatoschistus microps</i>	Fish
<i>Platichthys flesus</i>	Fish
<i>Enteromorpha sp.</i>	Green algae
Sand	Sediment

## Communication between *Planctomycetes*

**R. Costa<sup>1</sup>, K. Tait<sup>2</sup>, and O. M. Lage<sup>1,3</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Plymouth Marine Laboratory, United Kingdom.

<sup>3</sup> CIMAR/CIIMAR – Interdisciplinary Centre for Marine and Environmental Research– University of Porto

Quorum sensing enables bacteria to communicate between them using specific chemical compounds to modulate gene expression in accordance with changing population density. There are many known signaling molecules but the most intensively investigated are the N-acylhomoserine lactone (AHL) family in Gram-negative bacteria and the peptide autoinducers of Gram-positive bacteria. AHL are produced at a constant low rate when there is low cell density, until it reaches a critical threshold. Then, it modulates the gene expression, by regulatory circuits. Once this threshold is reached, AHLs activate LuxR family proteins which drive the expression of multiple target genes. Among these genes, it is included the responsible for AHL synthesis, but also other genes involved in secondary metabolism, virulence and biofilm development in a variety of bacteria associated with plants, animals, soils and marine and freshwaters environments.

A large community of *Planctomycetes* was isolated from the biofilm of macroalgae [1]. This peculiar phylum has ecological (annamox process), taxonomical (part of a superphylum with *Verrucomicrobia* and *Chlamydiae*) and structural (peptidoglycan-less cell walls) interest to Science. In this work OJF collection of *Planctomycetes* were screened for AHL production and quorum quenching, the ability to affect other species quorum sensing.

*Planctomycetes* were grown in media M13 and M14 and the growth media searched for signal molecules, extracted with an appropriated solvent. A luminescence assay was performed to measure the quantity of AHLs produced using a quorum sensing bio reporter. For the detection of quorum quenching, cell pellets were collected and resuspended in phosphate buffered saline (PBS) and left in contact with AHLs. After this incubation, the quantity of AHLs was accessed by a luminescence assay using a quorum sensing bio reporter.

Some *Planctomycetes* strains had shown positive results in AHL screening but not in TLC analysis which may indicate that *Planctomycetes* produce AHLs mimics. Quorum quenching activity was not detected. For some strains quorum sensing seems to be nutritionally dependent.

### References:

[1] Lage, O.M. and Bondoso, J. (2011), *Planctomycetes diversity associated with macroalgae*, FEMS Microbiology Ecology, 78(2):366-75.

## Development of an inhibition bioassay for the prediction of ionic liquid's human toxicity

**José P.S. Carvalho, E. Cunha, Paula C.A.G. Pinto, José L.F.C Lima, M. Lúcia M.F.S. Saraiva**

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

Ionic liquids (ILs) are organic salts, composed solely by ions, with unique properties such as negligible vapour pressure, high thermostability and reduced inflammability. Due to these characteristics, ILs have been considered green solvents and are being increasingly used as alternatives to conventional organic solvents in a variety of fields such as organic synthesis, catalysis, biocatalysis and bioreactor technology, analytical chemistry and electrochemistry, among others [1]. This, associated with extensive academic research on ILs field is leading to a constant release of these compounds to the environment with unknown long-term consequences. Indeed, even though ILs may reduce air pollution, their release into the environment may cause water contamination, affecting living organisms and ultimately human life. It is then mandatory to evaluate the toxicity of these compounds before their release into the natural environment in order to predict their impact, avoiding irreparable damages [2]. This evaluation shall be implemented in the early stage of development of new compounds and must be integrated in a multidisciplinary work, incorporating distinct classes of researchers, aiming the assessment of the environmental risk of ILs by combining quantitative structure-activity relationships (QSAR) and (eco)toxicological tests. Thus, to facilitate this evaluation it is important to keep on investing on simple and robust methodologies that can be easily implemented as screening tools and can help to predict the impact of these compounds on human health.

In, this context we developed a sequential injection analysis (SIA) [3] methodology for evaluating the activity of human carboxylesterase, a widely accepted toxicity biomarker. The assay was based on the degradation of 4-methylumbelliferone acetate (MUA) with release of the fluorescent 4-methylumbelliferone ( $\lambda_{exc}=365$  nm;  $\lambda_{em}=460$  nm). The developed methodology was applied to the evaluation of ILs toxicity through the extent of enzyme inhibition in the presence of the test compounds, with calculation of  $EC_{50}$  values. *O*-nitrophenyl acetate was used as a positive control in the inhibition assays.

References:

[1] Wasserscheid, P., Welton, T. (2008) *Ionic liquids in synthesis*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

[2] Romero, A. et al. (2008) *Toxicity and biodegradability of imidazolium ionic liquids*, J. Hazard. Mater., 151, 268-273.

[3] Pinto, P.C.A.G., Saraiva, M.L.M.F., Lima, J.L.F.C. (2008). *Sequential injection analysis as a tool for implementation of enzymatic assays in ionic liquids*, Talanta, 77, 479-483.

This work has been supported by Fundação para a Ciência e a Tecnologia through grant no. PEst-C/EQB/LA0006/2011 and Reitoria da Universidade do Porto.

# Ectoparasitic fauna of Atlantic mackerel, *Scomber scombrus*, from Matosinhos and Figueira da Foz

**R. Castro, M.J. Santos**

Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal.  
CIMAR Laboratório Associado / CHIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental,  
Universidade do Porto, Portugal.

Atlantic mackerel, *Scomber scombrus* Linnaeus, 1758 (Scombridae) is a fish species occurring all over the Portuguese coast. It is an economically important species due to large fishing activities. Moreover, the knowledge about its parasitological fauna is still scarce and outdated, thus, this study aims to make the current characterization of Atlantic mackerel's ectoparasitic fauna in two localities off Portuguese coast (Matosinhos and Figueira da Foz), in July 2011.

Five parasitic species were collected from Matosinhos fishes (n=40; total length =  $35.4 \pm 3.1$  [42.0 – 28.5]cm): *Grubea cochlear*, *Kuhnia scombri* (Monogenea), *Nematobothrium scombri* (Digenea), *Caligus pelamydis* (Copepoda) and an unidentified *Isopoda*. The highest prevalence was recorded for *K. scombri* (72.5%) and the highest mean abundance for *C. pelamydis* (3.7). From Figueira da Foz fish (n=39; total length =  $35.4 \pm 1.3$  [37.8 – 33.1]cm) were collected six ectoparasitic species: *G. cochlear*, *K. scombri*, *K. sprostonae* (Monogenea), *N. scombri* (Digenea), *C. pelamydis* and *Clavellisa scombri* (Copepoda). *C. pelamydis* revealed the highest prevalence and mean abundance (100% and 17.0, respectively). Comparing the parasite populations from both localities, for their parasitological parameters (occurrence and abundance), significant statistical differences were found for *G. cochlear*, *K. sprostonae* and *C. pelamydis*. Environmental parameters, like water temperature and salinity, maybe related with different parasite distributions recorded.

## Effects of copper exposure on the expression of the metallothionein gene family of *Solanum nigrum* L. plants

**A. Santiago<sup>1,2</sup>, F. Fidalgo<sup>1,2</sup>, J. Teixeira<sup>1,2</sup>**

<sup>1</sup>Center for Biodiversity, Functional & Integrative Genomics (BioFIG)

<sup>2</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

The industrial revolution led to many changes in our world. Unfortunately, one of the consequences of these anthropological activities is the pollution of the environment, in particular the contamination of soils with heavy metals. This is very dangerous for human health and represents a biohazard that can affect water resources and the entire food chain. One of the solutions to this problem is the use of hyperaccumulating plants in a process called phytoremediation [1]. In order to use this process it is firstly necessary to study the mechanisms behind it, which is possible by using both biochemistry and molecular biology tools. These later tools are the best to study metallothioneins (MT), high homologous proteins rich in cysteine, which are suggested to participate in metal homeostasis since they can bind metal ions through their thiol groups, although very little is known regarding this process. There are 4 groups of MTs (MT1, MT2, MT3, MT4) and they are differentially present in the different organs of the plants [2]. To characterize and study the effects of copper (Cu) exposure on MT gene family expression, *Solanum nigrum* L. plants were grown hydroponically over a period of 4 weeks in optimum conditions, during which they were supplemented with 11 ppm Cu (control), 100 µM Cu and 200 µM Cu. Afterwards they were collected, separated into shoots and roots, weighted and finally frozen in liquid N<sub>2</sub> at -80°C for posterior use. The plants were utilized for mRNA extraction using TRIzol (Invitrogen, USA), which was then used to access the effects of Cu exposure in the expression of this plant's MT gene family by a semi-quantitative RT-PCR approach. The MT2 (MT2a+b, MT2c, MT2c+d) and MT3 (MT3, MT3c) subgroups were studied, as those were the ones with defined primers for *S. nigrum*. The results revealed that no MT3 family-related mRNAs were accumulated in all situations analyzed. Within the MT2 family, their related mRNAs were present in all situations studied. For both MT2a+b and MT2c types there was an increased mRNA accumulation in the 100 µM Cu situation relatively to control, and a noticeable decrease in the 200 µM Cu relatively to the 100 µM treatment. Therefore, it is possible to conclude that the MT3 family is not expressed in *S. nigrum* plants and it is not Cu-responsive; that MT2 types are constitutive in shoots and roots, as they are expressed in control plants, MT2a and MT2c responding to a moderately high concentrations of Cu, but as in the 200 µM situation there was a decrease in their accumulation it may suggest that perhaps the MT2d type appears to compensate the decrease in MT2c in this situation by responding to higher concentrations of Cu.

### References:

- [1] Lasat M.M. (2000), *Phytoextraction of Metals from Contaminated Soil: A Review of Plant/Soil/Metal Interaction and Assessment of Pertinent agronomic issues*, Journal of Hazardous Substance Research, Volume Two, no. 5, pp. 1-25.
- [2] Hassinen V.H., Tervahauta A.I., Schat H., Kärenlampi S.O. (2011), *Plant Metallothioneins – Metal Chelators with ROS Scavenging Activity?*, Plant Biology, no. 13, pp. 225-232.

## Effects of proton pump inhibitors on co-cultures of human osteoclasts and breast cancer cells

**S. Reis<sup>1,2</sup>, M.H. Fernandes<sup>1</sup>, J. Costa-Rodrigues<sup>1</sup>**

<sup>1</sup> Laboratório de Farmacologia e Biocompatibilidade Celular, Faculdade de Medicina Dentária, Universidade do Porto, Portugal.

<sup>2</sup> Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal.

Proton pump inhibitors (PPIs) are widely used in many pathological conditions, like, for example, gastroesophageal reflux, dyspepsia and peptic ulcers. The mechanism of action relies in the inhibition of  $H^+/K^+$  ATPases, responsible for gastric acid secretion. Osteoclasts are specialized cells that solubilize extracellular bone matrix through the production of acid, by proton pumps that belong to the same family as the gastric ones. Thus, PPIs appear as good candidates to modulate osteoclast resorption activity, particularly in conditions that are associated with a hyperactivation of osteoclasts, like it happens, for example, in bone osteolytic metastasis. Breast cancer is one of the most frequent tumours that originate bone metastasis, usually osteolytic metastasis. In this context, this work intended to characterize the effects of three PPIs on human osteoclastogenesis in co-cultures of human osteoclasts and breast cancer cells.

Osteoclastic precursors were isolated from human peripheral blood and were co-cultured with two different breast cancer cell lines (T47D and SK-BR-3). Cell cultures were treated with different concentrations ( $10^{-7}$ - $10^{-3}$  M) of omeprazole, esomeprazole and lansoprazole. Cell cultures were characterized throughout a 21 day period for total protein content, tartarate-resistant acid phosphatase (TRAP) activity, TRAP+ multinucleated cells and the presence of cells with actin rings and expressing vitronectin and calcitonin receptors.

The presence of breast cancer cells, particularly T47D cells, greatly induced osteoclastogenesis. The tested PPIs induced a dose-dependent inhibition of osteoclast development. The osteoclastogenic inhibition was verified at levels higher than  $10^{-6}$  M for the three PPIs. Although the highest concentrations seemed to be toxic for osteoclastic cells, the inhibition observed at lower concentrations appeared to be due to specific effects on the osteoclastogenic process, rather than to a significant decrease on the cellular viability.

Taken together, PPIs have the ability to decrease human osteoclastogenesis, when osteoclastic precursors were co-cultured with breast cancer cells. Understanding the subjacent mechanisms can open new perspectives in the utilization of such compounds in pathological conditions characterized by a hyperactivation of osteoclastic cells.

# Energy Content of Two flatfish from the Dutch Wadden Sea: the flounder *Platichthys flesus* and the plaice *Pleuronectes platessa*

**A.F. Nogueira<sup>1</sup>, V. Freitas<sup>3</sup>, H. van der Veer<sup>3</sup> and J. Campos<sup>2</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> CIMAR/ CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Portugal.

<sup>3</sup> Royal Netherlands Institute for Sea Research, PO Box 59, 1790 AB Den Burg Texel, The Netherlands.

The European flounder (*Platichthys flesus*) and the European plaice (*Pleuronectes platessa*) are two common flatfish in the Dutch Wadden Sea with commercial interest, especially for plaice. Like many other marine species, usually they store body reserves during spring and summer, when food is more abundant, which are depleted during autumn and winter when food is limited. It is then expected that during warmer periods their energy or caloric content will be higher than during the colder seasons.

The present work resulted from a previous IJUP11 project (102) on seasonal variations on the nutritional status of the flounder population from Minho estuary, Portugal, and followed the same methodology. The two flatfish species were collected monthly at the Dutch Wadden Sea to evaluate their energy content during autumn/winter. All individuals were measured for total and standard length, wet weighed, inspected for sex determination and dissected and eviscerated; eviscerated weight was also registered. Stomachs were preserved frozen for later analyses of stomach content. The otoliths were removed for posterior analyses about age and to link age with energy content. Each individual was dried for 72h at 60°C to determine the dry weight. The samples must be well dried to obtain good results with the calorimeter. Afterwards, the dried material was homogenized using a blender. The powdered samples were put into tightly closed bottles. About 0.5g of dried sample was used for the calorimetric analyses. A bomb-calorimeter which measures the heat liberated when a sample is combusted was used to determine the energy content of the samples because it is quicker, less expensive and more appropriate for large sample sizes. Results were used to compare between species, sexes, ages and sampling dates. Preliminary results suggest that the flounder population is older and presents more energy content, almost the double, than plaice.

# Energy reserves of the brown shrimp *Crangon crangon* (Linnaeus, 1758)

**C. Moreira<sup>1,2</sup>, M. J. Almeida<sup>1</sup> and J. Campos<sup>1</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental – University of Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal

The brown shrimp *Crangon crangon* (Linnaeus, 1758) is a highly abundant epibenthic crustacean along European shallow waters from Norway to Morocco and throughout the Mediterranean and Black Seas [1]. With this large range of distribution and being a species that spends most of the life cycle in estuarine environments *C. crangon* is exposed to a great range of abiotic parameters and food availability. Environmental changes in temperature and food conditions will affect the energy available for the different physiological processes and determine rates of growth and reproduction.

When lacking food animals have to rely on their reserves. These reserves can be mobilized by several paths, stored in several organs and in different compounds. Besides the class of reserves mobilized, the sequence of substrates used varies considerably [2]. Cuzon and Ceccaldi [3] suggested that *C. crangon* uses glycogen as a first resource and protein as a last but other authors have suggested that proteins are the main reserve of this crustacean [4].

In order to estimate the maintenance costs of brown shrimp from Minho estuary and determine how their reserves are allocated, and in this way contributing to establish the parameters for the Dynamic Energy Budget (DEB) theory, 180 shrimps were measured, weighed, placed in individual cages inside three experiment aquaria and kept without food. Animals were kept in starvation until the last animal died or was sacrificed. Six individuals from each aquarium were sacrificed, measured and weighed every week; at day zero also 6 animals from the 3 aquaria were sacrificed to be used as initial estimates. About 3 sacrificed shrimps were used for calorimetric determination and 3 for total proteins using isotope-ratio mass spectrometry (IRMS) and total lipids applying a commercial kit (Spinreact). At the end 67 individuals were analyzed for calorimetry and 72 for proteins and lipids.

## References:

- [1] Campos, J. and van der Veer, H. (2008), *Autoecology of Crangon crangon (L.) with an emphasis on latitudinal trends*, Oceanography and Marine Biology: An Annual Review 46, 65-104
- [2] Sánchez-Paz, A., García- Carreño, F., Hernández-López, J., Muhlia-Almazán, A. and Yepiz-Plascencia, G. (2007), *Effect of short-term starvation on hepatopancreas and plasma energy reserves of the Pacific white shrimp (Litopenaeus vannamei)*, Journal of Experimental Marine Biology and Ecology 340, 184–193
- [3] Cuzon, G. and Ceccaldi, H. J. (1973), *Influence de la stabulation à jeun sur le metabolisme de la Crevette Crangon crangon (L.)*, Biologie Animale
- [4] Comoglio, L., Smolko, L., Amin, O. (2005) *Effects of starvation on oxygen consumption, ammonia excretion and biochemical composition of the hepatopancreas on adult males of the False Southern King crab Paralomis granulosa (Crustacea, Decapoda)*, Comparative Biochemistry and Physiology, Part B 140, 411–416

## Evaluation of partition coefficients and protein binding affinity of nimesulide incorporated in ionic liquid systems

**Ana M. Azevedo, Diogo M. Ribeiro, Paula C.A.G. Pinto, José L.F.C. Lima, Marlene Lúcio, Salette Reis, M. Lúcia M.F.S. Saraiva**

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Ionic liquids (ILs) are salts with reduced volatility and inflammability and tunable properties that are being widely used in several fields in replacement of conventional organic solvents. Recently, several authors started debating the utilization of these compounds in the pharmaceutical field [1]. One of the most interesting possibilities is the incorporation of poorly soluble drugs in ILs based systems. As a result, ILs from several classes have been proposed as possible drug solvents and eventually as drug carriers of both hydrophilic and hydrophobic drugs [2]. The possibility of engineering the properties of ILs by manipulating the anion-cation combination, in association with their solvent properties and in some cases water-miscibility are considered promising characteristics regarding the applicability of ILs as solvents or carriers of pharmaceutical drugs. The research in this field has been mainly focused on the release profile of drugs from systems including ILs or mixture of ILs [3]. It is then urgent to perform a deeper evaluation on the pharmacokinetics and pharmacodynamics of drugs incorporated in formulations including ILs in order to predict their *in vivo* efficiency and safety.

In this work we focused on the well known anti-inflammatory drug nimesulide and studied its behavior in terms of protein binding affinity and partition coefficient in ILs systems. The protein binding affinity studies were based on the quenching of human serum albumin fluorescence in association with models of drug-protein binding in the equilibrium. The interaction of the drug-IL system with a biomimetic phospholipid bilayer composed of hexadecylphosphocholine enabled the calculation of partition coefficients ( $K_p$ ) by derivative spectroscopy. With these studies we intended to predict the bioavailability of nimesulide in these novel systems and further increase the possibilities of application of ILs as drug solvents/carriers in the pharmaceutical industry.

### References:

- [1] Moniruzzaman, M., Goto, M. (2011) *Ionic liquids: future solvents and reagents for pharmaceuticals*, J. Chem. Eng. Jpn., 44, 370 – 381.
- [2] Mizuuchi H., Jaitely V., Murdan S., Florence A.T. (2008) *Room temperature ionic liquids and their mixtures: potential pharmaceutical solvents*, Eur. J. Pharm. Sci., 33, 326 – 331.
- [3] Jaitely V., Mizuuchi H., Florence A.T (2010) *Current-stimulated release of solutes solubilized in water-immiscible room temperature ionic liquids (RTILs)*, J. Drug Targ., 18, 787–793.

## Heavy metal toxicity in *Planctomycetes*

**C. Flores<sup>1</sup>, R. Carneiro<sup>1</sup>, P. Ferreira<sup>1</sup>, M. I. Amorim<sup>1,2</sup> and O. M. Lage<sup>1,3</sup>**

<sup>1</sup>Department of Biology, Faculty of Sciences, University of Porto

<sup>2</sup>CGUP, University of Porto

<sup>3</sup>CIIMAR, University of Porto

The phylum *Planctomycetes* is a particularly interesting phylum whose members are ubiquitous bacteria that revealed several striking features that distinguish them from other bacteria. They have unique properties such as a peptidoglycan-less proteinaceous cell wall, intracellular compartmentalization and a mode of reproduction via budding. This work focused on understanding the effect of the toxicity of heavy metals in cultures of *Rhodopirellula* strain LF2. The metals studied were cadmium, chromium, copper and nickel. The difference in the cellular viability after 30 min exposure to a series of metal concentrations (5.9 – 586.6  $\mu\text{M}$ ) was analyzed using the drop plate method. In water as base medium, adverse signs of toxicity were observed for 29.3  $\mu\text{M}$  chromium, copper and nickel, and 293.3  $\mu\text{M}$  cadmium. Complete loss of viability occurred at 293.3  $\mu\text{M}$  for chromium and copper while in nickel it just occurs to 586.6  $\mu\text{M}$ . Furthermore, the kinetics of the toxic effects of the metals was assessed up to 60 minutes in water and in medium M13. Effects of toxicity in water were observed after exposure for 5 min to 58.7  $\mu\text{M}$  chromium (and growth inhibition after 10 min), for 5 min to 58.7  $\mu\text{M}$  copper (and growth inhibition after 30 min), for 60 min to 58.7  $\mu\text{M}$  cadmium (and no growth inhibition obtained) and no growth inhibition for 44  $\mu\text{M}$  nickel. No heavy metals harmful effects were observed when the kinetic assay was done in M13 medium highlighting the importance of metal lability in these studies. *Rhodopirellula* strain LF2 seems to be more sensitive to chromium, followed by copper and then nickel. Cadmium was the less toxic of the four metals. Differences in metal susceptibility could be due to specific interference in fundamental metabolic pathways or the metal scavenging capacity of this planctomycete. Further studies at genomic, proteomic and metabolomic level are needed to clarify heavy metals toxicity in *Planctomycetes*.

## Human osteoclastogenesis over microstructured and nanostructured hydroxyapatite surfaces

**S. Carmo<sup>1,2</sup>, J. Costa-Rodrigues<sup>1</sup>, F.J. Monteiro<sup>2</sup>, M.H. Fernandes<sup>1</sup>**

<sup>1</sup> Laboratório de Farmacologia e Biocompatibilidade Celular, Faculdade de Medicina Dentária, Universidade do Porto, Portugal.

<sup>2</sup> Faculdade de Engenharia, Universidade do Porto, Portugal.

The bone tissue has an extracellular mineralized matrix, composed essentially by hydroxyapatite (HA). Due to this, synthetic hydroxyapatite appears as a potentially good biomaterial for bone regeneration applications because it displays good bioactive and osteoconductive properties. Nevertheless, it presents a slow resorption rate and its mechanical characteristics are not always suitable for the proposed applications. In order to improve their biological properties and with the advent of micro- and nanoscale technology, nano-structured HA (nanoHA) is being increasingly studied and applied, revealing a high potential in many bone regeneration applications. Nanoscaled materials display improved performances due to their large surface to volume ratio and especially to their surface reactivity (unusual chemical/electronic synergistic effects). In particular, the properties of nanoHA as compared to microphased HA (microHA), such as surface grain size, pore size and wettability may control protein interactions (like adsorption, conformation and bioactivity) and thus interfere with cellular responses. This potential to modulate the cellular behavior has generated a landslide of research with nanoscaled biomaterials in the orthopedic field. In this work, the behavior of osteoclastic cells cultured over nanoHA and microHA disks was evaluated and compared.

NanoHA and microHA disks were characterized by scanning electron microscopy and atomic force microscopy. Osteoclast precursors (PBMC) were isolated from human peripheral blood and seeded over the biomaterials. When indicated, cultures were treated with inhibitors of MEK, NFkB, PKC, p38 and JNK signaling pathways. Cell cultures were assessed at days 14 and 21 for tartarate-resistant acid phosphatase activity, presence of cells with actin rings and expressing vitronectin and calcitonin receptors, and ability to resorb HA.

It was observed that nanoHA disks displayed surface characteristics distinct of microHA, with an average grain size of 70 nm (average grain size of microHA was 330 nm). Moreover, the osteoclastogenic behavior of cell cultures was modulated by the biomaterial properties, and significant differences were found among the intracellular signaling pathways involved in the observed cellular response.

In conclusion, osteoclastogenesis is influenced by the surface properties of HA, in particular by its grain size. An understanding of how this modulation occurs opens the possibility to the design new biomaterials to be used in bone tissue regeneration strategies.

# Impact of dietary protein sources on lipid content and fatty acid profile of Senegalese sole juveniles (*Solea senegalensis* Kaup, 1858)

T.J.R. Fernandes<sup>a</sup>, R.C. Alves<sup>a,b</sup>, T. Souza<sup>a</sup>, A. Costa<sup>a</sup>, L.M.P. Valente<sup>c</sup> and M.B.P.P. Oliveira<sup>a</sup>

<sup>a</sup> REQUIMTE/Department of Chemical Sciences, Faculty of Pharmacy, University of Porto

<sup>b</sup> REQUIMTE/ ISEP, School of Engineering, Polytechnic of Porto

<sup>c</sup> CIMAR/CIIMAR, Interdisciplinary Centre of Marine and Environmental Research and ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto

Although aquaculture industry has expanded in Europe over the last decades, few marine species have contributed to this growth and a matching increase of fish feed production is required to sustain high rates of production. The use of vegetable protein sources (instead of animal ones) in diets for aquaculture fish has large sustainability and safety advantages. The quantity and quality of body fat stores vary widely among fish species and depend mainly on dietary factors [1].

In this study, we aimed to assess the impact of different vegetable diets on fat content of muscle, liver, fin, skin and head of juvenile Senegalese sole (*Solea senegalensis* Kaup, 1858) and also the fatty acid profile of muscle and liver of this fish species. The animals considered in this study (6 groups) were fed for 92 days under controlled conditions, using diets with different levels of vegetable protein (increasing amounts up to 75% of total protein using different mixtures of soy, wheat, corn, peas and potatoes). The fish were caught after a 24 hours fast. Samples were collected and immediately frozen at -80°C. Lipid content was determined by the Folch method and fatty acid profile was analyzed by GC-FID after methylation.

The liver was the preferential local for fat deposition (5.5 to 10.8% of fat) followed by the fins (3.4 to 6.7% fat). Increasing levels of plant protein in the diets induced increased levels of total lipids in the liver. The sole muscle is lean (2.4 to 4.0% fat) and total lipids were similar among treatments. The fatty acid profile of liver varied significantly among treatments. Protein plant diets induced higher levels of C16:1 and C18:2 n-6 and a reduction of ARA and EPA. The fatty acid profile of the muscle also evidenced higher levels of C18:2 n-6 but ARA and DHA remained similar among treatments. The substitution of fishmeal by plant protein is hence possible without major differences on the nutritional value of the main edible portion of the fish – the muscle.

References:

[1] Borges P., Oliveira, B., Casal, S., Dias, J., Conceição, L. and Valente, L.M.P. (2009), *Dietary lipid level affects growth performance and nutrient utilisation of Senegalese sole (Solea senegalensis) juveniles*, British Journal of Nutrition, 102, 1007-1014.

## Acknowledgements

This work was carried out by Project PROAMBIENTE for enhancing SMEs organizational and management structure to enter foreign markets. PROAMBIENTE has the financial support of QREN and Programa Operacional Regional do Norte – ON2, supported by the European fund for regional development FEDER. T. Fernandes thanks the research grant from this project. R. Alves is thankful to FCT for a post-doctoral grant (SFRH/BPD/68883/2010) financed by POPH - QREN - Tipologia 4.1 - Formação Avançada, subsidized by FSE and MCTES. This work has been supported by FCT through grant no. PEst-C/EQB/LA0006/2011.

# Influence of calcium in staurosporine-induced cell death in *Neurospora crassa*

**R. Vieira<sup>1,2,3</sup>, A. P. Gonçalves<sup>1,2</sup>, A. Videira<sup>1,2</sup>**

<sup>1</sup> Institute for Molecular and Cell Biology (IBMC), University of Porto, Porto, Portugal.

<sup>2</sup> Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto, Porto, Portugal.

<sup>3</sup> Faculty of Sciences of the University of Porto (FCUP), Porto, Portugal.

Mitochondria is a complex organelle whose interconnected functions include control of programmed cell death (PCD) and calcium storage [1]. Dysregulation of mitochondrial  $\text{Ca}^{2+}$  handling triggers the cascade of events that lead to cell death [2].

Staurosporine induces PCD and various mutant strains of the filamentous fungus *Neurospora crassa* are highly sensitive to this substance, in particular, the knockout mutants for the mitochondrial  $\text{Ca}^{2+}$ -dependent NADPH dehydrogenase *nde1* and for the mitochondrial complex I subunit *nuo51* [3], pointing to their involvement in PCD.

The measurement of intracellular  $\text{Ca}^{2+}$  is possible through heterologous expression of aequorin, a  $\text{Ca}^{2+}$ -sensitive photoprotein.

We are particularly interested in the relationship between mitochondria,  $\text{Ca}^{2+}$  and PCD, therefore, this work initially consists in the construction of a vector containing the aequorin gene fused to a mitochondrial signal sequence and its transformation in *Neurospora* strains in order to express the protein in mitochondria and enable  $\text{Ca}^{2+}$  measurement in this organelle. Then, mitochondrial and cytoplasmic  $\text{Ca}^{2+}$  signals will be determined after the induction of PCD with staurosporine. These determinations will be made using luminometry in different culture conditions of the fungus and in presence or absence of various inhibitors of  $\text{Ca}^{2+}$ -modulating molecules.

This work is ongoing. So far, the mitochondrial signal sequence of *su-9* (ATP synthase subunit 9) was amplified by PCR and cloned into an intermediate plasmid vector.

We also tested the sensitivity to staurosporine of different knockout mutants for proteins involved in  $\text{Ca}^{2+}$  signaling and identified some resistant or sensitive mutant strains. This further indicates a clear influence of  $\text{Ca}^{2+}$  signaling status in staurosporine-induced PCD.

Our project focuses on seeking PCD mediators in *Neurospora*. It consists in unraveling the relationship between cell death, mitochondria and  $\text{Ca}^{2+}$  homeostasis. It presents a broad interest since the results are expected to contribute not only to the understanding of fungal mechanisms but also to human biology and medicine.

## References:

[1] Contreras, L., Drago, I., Zampese, E., *et al.* (2010), *Mitochondria: The calcium connection*, *Biochim Biophys Acta*, 1797 (6-7), 607-618.

[2] Drago, I., Pizzo, P. and Pozzan, T. (2011), *After half a century mitochondrial calcium in- and efflux machineries reveal themselves*, *EMBO J.*, 1-7.

[3] Videira, A., Duarte, M. (2002), *From NADH to ubiquinone in Neurospora mitochondria*, *Biochim Biophys Acta*, 1555 (1-3), 187-191.

# Influence of porins OmpF and OmpC in the influx of sparfloxacin in *Escherichia coli*

**V. Claro<sup>1</sup>, R. G. Saraiva<sup>1</sup>, P. Gameiro<sup>1</sup> and M. J. Feio<sup>1</sup>**

<sup>1</sup>REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Portugal.

The use of antibiotics to treat bacterial infections is nowadays fairly common, but the increasing misuse of these drugs leads to the development of resistance by bacteria and renders treatments as ineffective [1]. So, it is essential to understand how antibiotics act within microorganisms and how they can be more efficient and less prone to mechanisms of resistance. The present study focus on porins, as these are channel proteins of the outer membrane of gram-negative bacteria that facilitate the passage of antibiotics and other solutes into the microorganism and are known to play a role in antibiotic resistance. Two porins are analysed, OmpF and OmpC, the most abundant porins of *E. coli*.

In this study, minimal inhibitory concentrations (MIC) were determined [2, 3] for different strains of *E. coli*: JF568 (OmpC<sup>+</sup> OmpF<sup>+</sup>), JF701 (OmpC<sup>-</sup> OmpF<sup>+</sup>) and JF703 (OmpC<sup>+</sup> OmpF<sup>-</sup>). The antibiotic used was sparfloxacin (spar), a third generation fluoroquinolone, as well as copper-complexed variants binary copper(II):sparfloxacin<sub>2</sub> and ternary copper(II):sparfloxacin:phenantroline.

**Table 1** – Values of MICs (µM) with sparfloxacin and its copper complexes.

<i>E. coli</i>	<i>JF568</i>	<i>JF701</i>	<i>JF703</i>
	OmpC <sup>+</sup> OmpF <sup>+</sup>	OmpC <sup>-</sup> OmpF <sup>+</sup>	OmpC <sup>+</sup> OmpF <sup>-</sup>
Sparfloxacin	0.062	0.062	0.117
Cu(II):spar (1:2)	0.016	0.008	0.124
Cu(II):spar:phen (1:1:1)	0.062	0.062	0.124

The presented results, on Table 1, demonstrate that OmpF has a great contribution to the influx of sparfloxacin and its complexes into the cell – the absence of this porin in *E. coli* JF703 leads to a significant increase in the MIC; the same does not happen, however, in the absence of OmpC in the JF701 strain. Also, looking at the results for the binary complex, we can see that the values of MIC are generally lower than those of free sparfloxacin; in fact, it is important to realize that at pH=7.4 and at these concentrations almost all the complex is dissociated and the effect we perceive is that of twice the free antibiotic. Therefore, comparing the two porins, it is shown that OmpF seems to have a higher impact on the susceptibility of *E. coli* to sparfloxacin.

## References:

- [1] Sosa, Anibal (2006). The threat of antibiotic-resistant bacteria and the development of new antibiotics in *Antimicrobial Resistance in Bacteria*, ed. Carlos F. Amabile-Cuevas, Horizon Bioscience, U.K., pp. 7-9.
- [2] Saraiva, R. G., Lopes, S., Ferreira, M., Novais, F., Pereira, E., Feio, M. J. and Gameiro, P. (2010). *Solution and biological behavior of enrofloxacin metalloantibiotics: A route to counteract bacterial resistance?* Journal of Inorganic Biochemistry 104, pp. 843-850.
- [3] Clinical and Laboratory Standards Institute (2009). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard*.

# Involvement of *Planctomycetes* in the nitrogen cycle

**T. Torres<sup>1</sup>, J. Bondoso<sup>1,2</sup> and O.M. Lage<sup>1,2</sup>**

<sup>1</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto

<sup>2</sup>CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental – Universidade do Porto

Preservation of the natural environment depends heavily on the roles played by microorganisms in the biogeochemical cycles and in maintaining the chemical balance of the available nutrients and the waste products of metabolism. *Planctomycetes*, a phylum of Bacteria that possess a combination of morphological, biochemical and genetic characteristics that truly distinct them from other bacteria and are unforeseen in prokaryotic organisms, play an important role in the ecosystems due to their physiological diversity and ubiquity in many habitats [1]. Despite the reported widespread distribution of *Planctomycetes*, precise knowledge of their ecological role in the environment is still very much unknown, mostly because of the relatively few species present in pure culture. Here we aim to extend our knowledge on the role of *Planctomycetes*, on the nitrogen cycle.

Several strains of *Planctomycetes*, belonging to different species and genera, isolated from the north coast of Portugal [2] were analysed in order to study their ability of reduce nitrate to nitrites by conventional tests and assess denitrification by gas chromatography measuring the gases nitrous oxide (N<sub>2</sub>O) and dinitrogen (N<sub>2</sub>). Results showed that the production of nitrite from nitrate is not a widespread feature in this phylum even in strains belonging to the same species. Of all 10 strains tested, only one strain, *Rhodopirellula* spp. strain UF6 was clearly positive for the production of N<sub>2</sub>O, but not N<sub>2</sub>.

The sequenced genomes of *Planctomycetes* available in the databases were screened for the presence of the genes involved in the nitrogen cycle. Genes related to the dissimilatory nitrate reduction to ammonium (DNRA) were found in the genomes of marine *Planctomycetes*. Freshwater and terrestrial *Planctomycetes* seem to lack these genes.

Amplification of genes *nirK*, *nirS* and *nosZ* in strains from the OJF collection with non-sequenced genomes were attempted. The *nirK* gene was successfully amplified in strains UF6, FC18, FF15 and Pd1 and *nosZ* was only amplified in strain, FC18.

The results obtained in this study proved that heterotrophic *Planctomycetes* are involved in the nitrogen cycle in two of the pathways: dissimilatory nitrate reduction to ammonium and denitrification.

[1] Ward, N., Staley, J. T., Fuerst, J. A., Giovannoni, S., Schlesner, H. & Stackebrandt, E. (2006). The order Planctomycetales, including the genera Planctomyces, Pirellula, Gemmata and Isosphaera and the Candidatus genera Brocadia, Kuenenia and Scalindua. . In *The Prokaryotes: a Handbook on the Biology of Bacteria*, vol. 7, pp. 757-793. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer

[2] Lage, O.M. & Bondoso J. (2011). *Planctomycetes* diversity associated with macroalgae. *FEMS Microbial Ecology*. 78: 366–375.

# Localization of inhibitory A<sub>1</sub> and facilitatory A<sub>2A</sub> adenosine receptors on motor nerve terminals of rats with Experimental Autoimmune Myasthenia Gravis (EAMG) by confocal microscopy

C. Costa, M.F. Ferreira, D. Almeida, P. Correia-de-Sá & L. Oliveira

Laboratório de Farmacologia e Neurobiologia, UMIB / Instituto de Ciências Biomédicas de Abel Salazar (ICBAS) – Universidade do Porto, Portugal.

At the rat neuromuscular junction, adenosine acts predominantly as an inhibitory signal (via A<sub>1</sub> receptors) under resting conditions, whereas during high frequency neuronal firing synaptic accumulation of the nucleoside facilitates neuromuscular transmission through the activation of excitatory A<sub>2A</sub> receptors on motor nerve terminals [1]. Previous findings from our group showed that tonic activation of A<sub>2A</sub> receptors on motor nerve terminals may overcome neuromuscular tetanic fade [2]. This outcome suggests that activation of adenosine A<sub>2A</sub> receptors might be of clinical interest to preserve neuromuscular transmission in pathological motor endplates, like in *Myasthenia gravis*.

Experimental autoimmune myasthenia gravis (EAMG) has been routinely used as a model of human *Myasthenia gravis*. Among the various techniques designed at generating EAMG animal models, we focused our attention on Wistar rats that have been immunized with 50 µg of R97-116 in CFA, a synthetic peptide corresponding to a specific region of the  $\alpha$  subunit of the rat nicotinic AChR (day 0) [3]. Thirty-days later the animals were boosted with the same peptide in IFA. Control animals received CFA emulsion without the peptide; animals of the naive group were not submitted to any kind of treatment. Control and EAMG animals were evaluated for signs commonly associated with *Myasthenia gravis*, such as (1) tetanic failure (fatigue) of diaphragm muscle contractions induced by indirect nerve stimulation (intermittent 50 Hz-bursts) and (2) increases in the activity of type I adenosine deaminase (ADA) in the serum. Neuromuscular morphology and receptors expression were assessed by immunofluorescence confocal microscopy (Olympus FV 1000, Japan).

Fatigue of nerve-evoked diaphragm contractions was more intense ( $P<0.05$ ) in EAMG rats than in control and naive animals. As expected, EAMG animals exhibited higher activity ( $72\pm4$  U/mL,  $n=3$ ) of type I ADA in the serum as compared with control ( $24\pm2$  U/mL,  $n=3$ ) and naive ( $16\pm4$  U/mL,  $n=3$ ) littermates. Fluorescence labeling of perisynaptic Schwann cells with S100, motor nerve terminals with synaptophysin and VACHT (vesicular acetylcholine transporter), and nicotinic acetylcholine receptor clusters with  $\alpha$ -bungarotoxin conjugated with tetramethylrhodamine (Rhod- $\alpha$ -BTX) showed abnormal swelling of axons and myelin, abnormal tortuosity and branching of nerves, abnormal area of motor endplates (both type I and type II), and widening of synaptic clefts in EAMG animals, as compared with both control and naive groups (see *e.g.* MacDermot, 1960, *Brain*, **83**, 24-36). Careful examination of the fluorescent labeling with Rhod- $\alpha$ -BTX and immunoreactivity against adenosine A<sub>1</sub> and A<sub>2A</sub> receptors in all animal groups suggests that adenosine receptors are localized predominantly in the presynaptic component, *i.e.* in regions appearing to “crosslink” synaptic buttons. We found no evidence to suggest localization of adenosine receptors on skeletal muscle fibers.

Data indicate that immunization with a single peptide fragment which sequence is homologous to a region of  $\alpha$  subunit of the rat nicotinic AChR is instrumental to reproduce the pathophysiological features of human *Myasthenia Gravis* in Wistar rats. Evidence for the expression of presynaptic inhibitory A<sub>1</sub> and facilitatory A<sub>2A</sub> receptors at the neuromuscular junction of EAMG rats opens new perspectives for pharmacological intervention and, hence, minimization of the neuromuscular transmission deficits in myasthenics.

[1] Correia-de-Sá *et al.*, 1996, *J. Neurophysiol.*, **79**, 3910-19 [2] Oliveira *et al.*, 2004, *J. Physiol. (Lond.)*, **560**,157-158 [3] Baggi *et al.*, 2004, *J. Immunol.*, **172**,2697-2703.

Work supported by FCT (FEDER funding, PTDC/SAU-FCF/108462/2008 and UMIB-215/94) and by Univ. Porto / Santander Totta.

## New polymorphisms within the non-coding region of the *OTC* gene

A. Silva<sup>1,2</sup>, L. Azevedo<sup>1,2</sup>, A. Amorim<sup>1,2</sup>

<sup>1</sup> IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal.

<sup>2</sup> Faculty of Sciences of the University of Porto, Portugal

The ornithine transcarbamylase gene is located in the short arm of the X-chromosome, specifically in the Xp21.1 [1]. The *OTC* gene contains 10 exons and 9 introns [2] and encodes the second enzyme of the urea cycle [3]. Discovering polymorphisms within the intronic region of *OTC* will allow the design of a complementary molecular diagnostics technique for the OTC deficiency, which is especially significant considering that routine PCR-based tests are only effective in approximately 80% of the cases [4].

In this work, several polymorphic markers within the non-coding region of the gene were selected using the Ensembl database. Primers were designed and evaluated according to the reference sequence (ENSG00000036473) using the Primer 3, Oligocalc and BLAT utilities. The fragments were amplified by PCR and validated by automatic sequencing. The results were analyzed using the programs Sequencing Analysis and Geneious.

Preliminary data revealed two new indels and two microsatellites with high heterozygosity. These new polymorphisms in combination with others previously documented are distributed along the entire genomic region and can thus be useful as markers to define haplotypes that can be associated with the OTC deficiency. Moreover, because large genomic rearrangements are common among OTC patients, this set of markers will be used as a complementary diagnostic strategy to detect such rearrangements.

### References:

- [1] Lindgren, V., Martinville, B., Horwich, A. L., Rosenberg, L. E. and Francke, U. (1984) *Ornithine Transcarbamylase locus mapped to band Xp21.1 near to Duchenne Muscular Dystrophy Locus*. Science, 226, 698–700.
- [2] Hata, A., Tsuzuki, T., Shimada, K., Takiguchi, M., Mori M., Matsuda, I. (1988) *Structure of the human ornithine transcarbamylase gene*. Journal of Biochemistry, 103, 302-308.
- [3] Tuchman, M., McCullough, B., Yudkoff, M. (2000) *The molecular basis of ornithine transcarbamylase deficiency*. European Journal of Pediatrics, 159, 196-198.
- [4] Tuchman, M., Morizono, H., Rajagopal, B.S., Plante, R.J., Alewell, N.M. (1997) *Identification of 'private' mutations in patients with ornithine transcarbamylase deficiency*. Journal of Inherited Metabolic Diseases, 20, 525–527.



Posters

# Posters from Thursday, 23<sup>rd</sup>



## Acute toxicity of the antibiotic cephalixin to the freshwater cladoceran *Daphnia magna*

**Joana Vieira<sup>1,2</sup>, Fernanda Marques<sup>1,2</sup>, Lúcia Guilhermino<sup>1,2</sup>**

<sup>1</sup> ICBAS - Institute of Biomedical Sciences of Abel Salazar, University of Porto, Department of Populations Studies, Laboratory of Ecotoxicology, Lg. Prof. Abel Salazar, 2, 4099-003, Porto, Portugal.

<sup>2</sup> CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Laboratory of Ecotoxicology and Ecology, University of Porto, Rua dos Bragas, 289, 4050 Porto, Portugal.

In recent years special attention has been given to pharmaceuticals as relevant environmental contaminants of aquatic ecosystems [1]. Their excessive use to prevent and treat human and veterinary diseases, results in the contamination of aquatic ecosystems, raising concern on potential adverse effects on wild organisms. In fact, in addition to biological activity of pharmaceuticals and of some of their metabolites and environmental degradation products, some of these substances may have a considerable persistence in the aquatic environment. Antibiotics are of special concern because they are able to induce genetic resistance in both target and non-target species [2]. The ecotoxicological effects of a large number of antibiotics are not known, as well as their acute toxicity to organisms with important ecological functions. Thus, the objective of the present study, was to investigate the acute toxic effects of cephalixin, an antibiotic widely used, to the cladoceran *Daphnia magna*, a relevant primary consumer of freshwater ecosystems [2].

To assess the effects of cephalixin to *D. magna*, acute bioassays were carried out following the OCDE guidelines [3] with some modifications. Briefly, tests were conducted in temperature (20±1°C) and photoperiod (16h light: 8 h dark) controlled rooms up to 96h. Different concentrations of cephalixin and appropriate controls were tested. Twenty organisms were used per treatment, being exposed in groups of 5 per 200ml glass beaker filled with 50 ml of ASTM hard water [4]. Mortality was recorded at each 24h and parameters for test validity purposes were also monitored. Tests were carried out with and without medium renewal.

The results indicate that acute toxicity of cephalixin to *D. magna* is in the high ppm range and thus ecotoxicological concerns are mainly on the potential chronic toxicity of the substance. Therefore, chronic bioassays to assess the effects of the antibiotic on growth, reproduction and population growth rate should be carried out.

### References:

- [1] Daughton, C.G. and Ternes, T.M. (1999), *Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?* Environmental Health Perspectives, 107 (6).
- [2] Wollenberger, L., Halling-Sorensen, B., Kusk, K. O. (2000), *Acute and chronic toxicity of veterinary antibiotics to Daphnia magna* Chemosphere 40 (7) 723-730
- [3] OECD (2004), *Test No. 202: Daphnia sp. Acute Immobilisation Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing.
- [4] American Society for Testing and Materials (ASTM) (1980). *Standard Practice for conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians*, Report E-790-80. ASTM, Philadelphia.

# An electrochemical approach to the study of the phase solubility of MCPA - Methyl- $\beta$ -cyclodextrin inclusion complex

**M. Martins**<sup>1,2</sup>, **J. Garrido**<sup>1,2</sup>, **F. Borges**<sup>1</sup> and **E.M Garrido**<sup>1,2</sup>

<sup>1</sup> CIQ/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

<sup>2</sup> Department of Chemical Engineering, School of Engineering, ISEP, Polytechnic Institute of Porto, 4200-072 Porto, Portugal

Pesticides stand out as one of the major developments of the twentieth century. During the past decades, however, concern has arisen as to the extent their presence in the environment poses a threat to wildlife and mankind. A severe problem often encountered is leaching of the pesticide molecules to deep soil layers and thus reduction of the herbicidal efficacy and migration to nontarget areas. One approach to solve this problem is to design controlled-release formulations, based on cyclodextrin complexes, which could decrease the dose and rate of release of the active ingredient.

Cyclodextrins are cyclic organic molecules formed by enzymatic modification of starch. The most important structural feature of these compounds is a hydrophobic cavity which is able to accommodate host inclusion complexes with different guest molecules. These complexes offer a variety of physicochemical advantages including the possibility to increased water solubility, chemical solution stability and bioavailability.

The phase-solubility diagrams obtained according to the Higuchi and Connors method [1], based on UV-Vis spectrophotometry, allow estimating the molar ratio and the apparent stability constants of the inclusion complexes formed. Nevertheless, if the compound under study is electroactive then it is possible to design a voltammetric procedure in order to obtain the formation constants and the host-to-guest ratio of the inclusion complexes.

The aim of this study was to investigate the formation of inclusion complexes of MCPA with methyl- $\beta$ -cyclodextrin (Fig. 1) and the determination of association constants of the complex formed by using an electrochemical method. The results obtained will be presented and discussed.

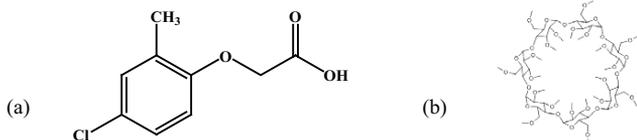


Figure 1 – (a) MCPA and (b) Me- $\beta$ -cyclodextrin.

## References:

[1] Higuchi, T.; Connors, K.A. (1965), *Phase-solubility techniques*. Adv. Anal. Chem. Instrum., 4, 117–212.

## Acknowledgements:

Financial support from U. Porto/Santander Totta and from Fundação para a Ciência e Tecnologia FCT/MCTES project PTDC/AGR-AAM/105044/2008, National Funds also co-financed by the European Community Fund FEDER through COMPETE–Programa Operacional Factores de Competitividade (POFC), is gratefully acknowledged.

# Antioxidant enzymes in response to metalaxyl stress in *Solanum nigrum* L. cell suspension cultures

A. de Sousa, J. Teixeira and F. Fidalgo

Center for Biodiversity, Functional & Integrative Genomics (BioFIG)  
Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

Phytoremediation is a bioremediation process that uses plants and their associated microbes to clean up toxic elements such heavy metals and persistent organic compounds from contaminated areas (soils, groundwater and sediments) [1]. Metalaxyl is a systemic fungicide with a protective and curative action used to control diseases caused by fungi of the order Peronosporales and therefore widely used in agriculture. This fungicide is stable to a wide range of pH, light and temperature conditions and consequently it has a tendency to accumulate in soil and groundwater representing a serial concern not only to the environment but also to public health [2]. Plant tissue cultures of *Solanum nigrum* L. have been used as a model system applied in phytoremediation research [3,4]. Following previous results [5,6], the aim of the present study was to evaluate the antioxidant enzymes response of *S. nigrum* cell suspensions cultures against the stress induced by metalaxyl. Green and friable calli were used to establish cell suspensions in Murashige & Skoog medium (1962) supplemented with 2 mg/L 2,4-D and 0.5 mg/L BA, at pH 5.7. Cells were propagated on a rotary shaker (120 rpm), in the dark, at 25°C. To evaluate the effect of metalaxyl on the antioxidant enzymes, cells were transferred to identical MS medium supplemented with 0 µM; 71.60 µM and 143.20 µM of metalaxyl and cell samples were collected at 312 h (13 days). Superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11) activities were increased, particularly at the higher concentration of metalaxyl tested (143.20 mM), however, non-denaturing PAGE showed that metalaxyl did not change any enzyme isoform patterns in which seven SOD isoenzymes, two CAT isoenzymes and nine APX isoenzymes. Globally, the data showed that the presence of the fungicide in the culture medium affected all the antioxidant enzymes evaluated, on a concentration-dependent manner. In conclusion, the enhanced activity of these enzymes suggested that the enzymatic antioxidant system is involved in the defense response that enables *S. nigrum* cells to cope with metalaxyl-induced stress. In addition, the results provided useful information for the understanding of the specific physiological role of each antioxidant enzyme in cell suspension cultures of *S. nigrum* in response to metalaxyl exposure.

## References:

- [1] Pilon-Smits E. (2005). *Annu. Rev. Plant Biol.* 56: 15-39.
- [2] Sukul, P., Spiteller, M. (2000). *Rev. Environ. Contam. Toxicol.* 164: 1-26.
- [3] Rezek, J. *et al.* (2007). *Chemosphere.* 88: 1221-1227.
- [4] Kucerová, *et al.* (2000). *Plant and Soil.* 225: 109-116.
- [5] Sousa A *et al.* (2011a). *IJUP' 11 - 4<sup>th</sup> Meeting of Young Researchers of U.Porto.* Porto. Portugal.
- [6] Sousa A *et al.* (2011b). *IJUP' 11 - 4<sup>th</sup> Meeting of Young Researchers of U.Porto.* Porto. Portugal.

# Bioassays to study the ecotoxicity of soils contaminated by pharmaceuticals

**D. Rede**<sup>1</sup>, **S. Sousa**<sup>1</sup>, **C. Antão**<sup>2</sup>, **F. Oliva-Teles**<sup>3,4</sup> and **C. Delerue-Matos**<sup>1</sup>

<sup>1</sup>REQUIMTE, Instituto Superior de Engenharia do Porto, Politécnico do Porto, Portugal.

<sup>2</sup> Departamento de Microbiologia/Ecotoxicologia, Equilibrium, Laboratório de Controlo de Qualidade e de Processos, Portugal.

<sup>3</sup> Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal.

<sup>4</sup> CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Portugal.

Pharmaceuticals started to be seen as micro pollutants in the early 90s [1]. When improperly discharged in the environment and after being used and excreted (unaltered or metabolized) by humans and animals this kind of pollutants can contaminate waters and soils [2]. Environmental toxicity can be assessed by physical-chemical tests or by bioassays [3]. The latter uses living organisms (biomarkers) to evaluate the overall toxicity of contaminated soils, waters or sediments. Ibuprofen (IB) is an anti-inflammatory, non-steroid that also can be administered as a painkiller or antipyretic. It is one of the most sold pharmaceutical products in the whole world, what justifies its great presence in the environment being selected for this study.

The present work evaluates the impact caused in the soil by IB and sewage waters from a hospital. This evaluation was made through bioassays using lettuce seeds var. Buttercrunch (*Lactuca sativa*) [4]. For that it was used sandy soil in a Petri dish adding the seeds and hydrating the soil with contaminating solution. All conditions were replicated 3 times. The Petri dishes were placed in a germination chamber at  $24 \pm 2$  °C with a photoperiod of 48 h dark followed by 16 h light and 8 h of dark until the end of the test. Finally the number of emergence of seedling in each Petri dish was counted; shoot, root and leaf length was measured; and the number of surviving plants showing an atypical appearance was counted.

Contaminated soils by IB showed a reduction in the number of germinated seeds and also in the medium length of the plant. The obtained results for hospital sewage waters indicate a toxic effect by the decrease of germination and the root length.

## Acknowledgments:

This work has been supported by Fundação para a Ciência e Tecnologia through grant no. PEst-C/EQB/LA0006/2011 and Project PTDC/ECM/103141/2008.

## References:

- [1] Meisel, M., Costa, M. and Pena, A. (2009) *Regulatory approach on environmental risk assessment. Risk management recommendations, reasonable and prudent alternatives*, Ecotoxicology, 18 (8) 1176 - 81.
- [2] Gielen G.L.H.P (2007), *The fate and effects of sewage-derived pharmaceuticals in soil*, PhD thesis, University of Canterbury.
- [3] Hughes, W.W. (1996), *Essentials of Environmental Toxicology: The Effects of Environmentally Hazardous Substances on Humana Health*, Washington DC: Taylor & Francis.
- [4] US EPA (1996), *Protocols for short term toxicity screening of hazardous wastesites*, EPA 600/3-88/029.

## Bioremediation: possible application for the elimination of oil buried in beaches affected by oil spills

**J. Pontes<sup>1</sup>, A. P. Mucha<sup>2</sup>, I. Reis<sup>2</sup>, M. Clara P. Basto<sup>1</sup>, C. Marisa R. Almeida<sup>2</sup>**

<sup>1</sup> CIMAR/CIIMAR, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> CIMAR/CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal

Large quantities of petroleum enter the environment every year by leaking from storage tanks and pipelines, or released in accidental spills. An example is the *Prestige* tanker, which in November 2002, spilled about 70000 tons of oil, affecting a large area, with economics and ecological impacts. Recent research, on sandy beaches affected by this oil spill, showed the persistence of buried oil in the sand at depths up to 4 m [1].

Bioremediation is an effective approach to clean spilled oil in all type of coastal environments, such as rocky, coarse and sandy beaches. Moreover, this is a technique simple to maintain, cost-effective and applicable into large area but, to our knowledge, it has still not been evaluated for buried oil.

In this study we assess, in controlled laboratory experiments, bioremediation efficiency for buried oil. For that, three treatments were tested: (A) natural attenuation (inherent capacity of oil degradation in sediments) (B) biostimulation (addition of oleophilic fertilizer S200) and (C) bioaugmentation (inoculation of an autochthonous enriched-specific consortium of oil-degraders microorganisms plus S200). The experiments were carried out in a microcosm system composed of 9 microcosms (each one with 60 cm height and 20 cm of diameter), three per treatment, containing beach sand (collected in a beach in the NW of Portugal), in which a layer of sand was artificially contaminated with crude oil and buried at 30 cm depth. This system mimics tidal cycles (each one with 6 h) with natural seawater. Sediment from the contaminated layer was sampled at 0, 1, 2, 4, 8, 16 and 24 weeks. Total petroleum hydrocarbons (TPHs) were analyzed in all samples by FTIR spectrophotometry and gas chromatography (GC/FID).

Analyses are still in course but preliminary results showed that microbial degradation of TPHs was occurring in all treatments. In fact, after 1 week TPHs presented already a 50% reduction. Differences among treatments were only observed after 4 weeks, with bioaugmentation presenting a higher TPHs decline. The marked effect of bioaugmentation occurs mainly in the 8th week of the assay, when degradation of TPHs reached 80%. Results already obtained indicate that bioremediation can also be a suitable treatment for the remediation of buried oil.

Acknowledgments:

This work was partially funded by Fundação para a Ciência e Tecnologia (FCT), Portugal, through the project ERA-AMPERA/0003/2007

References:

[1] Bernabeu, A.M., Rey, D., Rubio, B., Vilas, F., Dominguez, C., Bayona, J.M. and Albaigés, J., (2009), *Assessment of Cleanup Needs of Oiled Sandy Beaches: Lessons from the Prestige Oil Spill*, Environmental Science Technology, 43(7): 2470-2475

# Characterization of the cholinesterases present in specific tissues of *Corbicula fluminea* (Mollusca: Bivalvia)

**J. Rocha**<sup>1,2</sup> and **L. Guilhermino**<sup>1,2</sup>

<sup>1</sup> ICBAS – Institute of Biomedical Sciences of Abel Salazar, University of Porto, Department of Populations Studies, Laboratory of Ecotoxicology, Lg. Prof. Abel Salazar, 2, 4099-003 Porto, Portugal

<sup>2</sup> CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Laboratory of Ecotoxicology and Ecology, University of Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal

The activity of cholinesterases enzymes (ChE) have been widely used as environmental biomarker. For field biomonitoring studies and ecotoxicological bioassays, it is very important to characterize the forms present in the organism to be used as sentinel species in the field or as test organism in bioassays, as well as in the specific tissue to be used to make the determinations of cholinesteratic activity [1]. Therefore, with the view of using *Corbicula fluminea* as sentinel species and as test organism in ecotoxicity bioassays, the objective of this study was to characterize the ChE present in specific tissues of this species. The characterization of ChE in this species was done previously in the whole body but it was not completely conclusive [2].

A general biochemically-based framework was used [1]. Briefly, in *in vitro* assays, the effects of the selective inhibitors serine sulfate, a strong inhibitor of all ChE enzymes, iso-OMPA, a selective inhibitor of vertebrate pseudocholinesterases, and BW284C51, a selective inhibitor of invertebrate on the ChE activity of each of the tissues was investigated. Additionally, the enzymatic activity towards the substrates acetylthiocholine, butyrylthiocholine and propionylthiocholine was investigated in a typical range of concentrations. Overall, the results indicate differences among tissues regarding the type of ChE present and levels of activity. These results are discussed in relation to the use of *C. fluminea* as test organism and sentinel species.

Aknowledgements: This study was carried out in the scope of the project “*NISTRACKS - Processes influencing the invasive behaviour of the non indigenous invasive species Corbicula fluminea (Mollusca: Bivalvia) in estuaries – identification of key genetic and environmental factors*” (FCT: PTDC/AAC-AMB/102121/2008). Joana Rocha has a BIC grant in the scope of the project.

## References:

- [1] Garcia, L.M., Castro, B., Ribeiro, R., Guilhermino, L., (2000), *Characterization of cholinesterase from guppy (Poecilia reticulata) muscle and its in vitro inhibition by environmental contaminants*, Biomarkers 5(4), 274-284.
- [2] Mora, P., Fournier, D., Narbonne, J.F., (1999), *Cholinesterases from the marine mussels Mytilus galloprovincialis Lmk. and M. edulis L. and from the freshwater bivalve Corbicula fluminea Muller*, Comparative Biochemistry and Physiology Part C 122, 353–361.

# Chemical oxidation of amoxicillin in contaminated water using potassium permanganate

**S. Machado, S.L. Pinto, H.P.A. Nouws, J.P. Grosso, J.T. Albergaria, C. M. Delerue-Matos**

REQUIMTE, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200 - 072, Porto, Portugal

Amoxicillin is one of the most widely consumed antibiotics and is used to treat and prevent infections in humans and animals [1]. This consumption, combined with inadequate disposal habits, has led to contamination of distinct environmental matrices, such as effluents (from hospitals or wastewater treatment plants), surface and groundwater, soils, and sediments [2]. Therefore remediation actions are required. Chemical oxidation processes are commonly used to degrade a wide variety of contaminants in aqueous systems, namely through the use of potassium permanganate, hydrogen peroxide, or Fenton's reagent [3].

This work describes the use of potassium permanganate as oxidant to degrade ibuprofen in water. The main objectives of this study were: i) to evaluate the influence of pH on the degradation reaction; and ii) to establish the optimum ibuprofen:potassium permanganate ratio that guarantees an efficient and fast remediation.

The obtained results led to the conclusion that: i) acidic conditions (pH=3) presented faster and more efficient treatments; and ii) the best ibuprofen:potassium permanganate ratio was 1:6 for experiments at pH 3 and 1:9 for experiments at pH 7 and 10, presenting, after a 30-min reaction, degradation efficiencies higher than 94% in all cases.

## Acknowledgements

This work has been supported by Fundação para a Ciência e a Tecnologia through grant no. PEst-C/EQB/LA0006/2011 and Project PTDC/ECM/103141/2008. J.G. Pacheco thanks the Fundação para a Ciência e a Tecnologia for financial support through his pos-doc grant (SFRH/BPD/73943/2010).

## References:

- [1] Bailón-Pérez, M.I., García-Campana, A.M., Cruces-Blanco, C., Iruela, M.O. (2008), *Trace determination of b-lactam antibiotics in environmental aqueous samples using off-line and on-line preconcentration in capillary electrophoresis*, Journal of Chromatography A, 1185, 273-280.
- [2] Watkinson, A.J., Murby, E.J., Kolpin, D.W., Costanzo, S.D. (2009), *The occurrence of antibiotics in an urban watershed: From wastewater to drinking water*, Science of Total Environment, 407, 2711-2723.
- [3] Tekin, H., Bilkay, O., Ataberk, S.S., Balta, T.H., Ceribasi, I.H., Sanin, F.D., Dilek, F.B., Yetis, U. (2006), *Use of Fenton oxidation to improve biodegradability of a pharmaceutical wastewater*, Journal of Hazardous Materials B, 136, 258-265.

## Construction of a family of criteria to evaluate the sites invaded by the species *Corbicula fluminea* – preliminary results

Marcela Oliveira<sup>1,2</sup>, José Rui Figueira<sup>2,3</sup> and Lúcia Guilhermino<sup>1,2</sup>

<sup>1</sup>ICBAS – Institute of Biomedical Sciences of Abel Salazar, University of Porto, Department of Population Studies, Laboratory of Ecotoxicology, Lg. Prof. Abel Salazar, 2, 4099-003 Porto, Portugal

<sup>2</sup>CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Laboratory of Ecotoxicology and Ecology, University of Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal

<sup>3</sup>CEG-IST – Technical Superior Institute, Av. Rovisco Pais, 1049-001 Lisboa, (Associate member at LORIA Laboratory, Nancy, France).

Due to their invasive and dispersal potential, some species of *Corbicula* can cause important ecological and economical impacts, such as changes in food webs, bioaccumulation of environmental contaminations, competition with native bivalves and serious biofouling problems (e.g. in dams)[1]. The species *Corbicula fluminea* has been present in the freshwater tidal ecosystem of the Minho River estuary (NW Portugal) at least since 1989. More recently, the species was also found in the corresponding area of the Lima River estuary [2], where the invasive behavior of the population has been considerably different from the invasive behavior of the Minho estuary populations [3]. Thus, the comparison of these two populations may provide most important insides on the factors influencing the invasive behavior of the species to be used to control its invasions and mitigate negative impacts. Therefore, the main objective of this work was to develop a multiple criteria methodology to assess and evaluate the risk of invasions by *Corbicula fluminea* in freshwater tidal areas of estuaries in temperate regions.

A methodology was developed in order to take into account four main steps: the description of the context, the formulation of the problem, the construction of a formal model for aggregating the criteria and adding the different zones for risk categories, and finally, built results to produce recommendations [4]. The major task consists of gathering all the aspects, characteristics and parameters related to the environmental point of view that can lead to classify a zone into a risk class [4]. Finally, a model of the criteria risk classification should be presented. In the present communication, the general framework and preliminary steps of the methodology will be presented and discussed.

Acknowledgments:

This study was carried out in the scope of the project “NISTRACKS – Processes influencing the invasive behavior of the non indigenous invasive species *Corbicula fluminea* (Mollusca: Bivalvia) in estuaries – identification of the key genetic and environmental factors (FCT: PTDC/AAC-AMB/102121/2008).

References:

- [1] Sousa, R., Antunes, C., Guilhermino, L., *Annales de Limnologie - International Journal of Limnology*, 44: 85–94.
- [2] Souda, R., Antunes, C., Guilhermino, L. 2006. *Annales de Limnologie – International Journal of Limnology*, 42: 165–171.
- [3] Sousa, R., Dias, S., Antunes, C. 2007. *Annales de Limnologie – International Journal of Limnology*, 44: 303–313.
- [4] Almeida-Dias, J., Figueira, J.R., Roy, B.2010. *European Journal of Operational Research*, 204: 565 – 580.

# Contribution of the plant/rhizosediment system to the phytoremediation of metals

**Marta Nunes<sup>1</sup>, Ana P. Mucha<sup>2</sup>, Carlos R. Gomes<sup>1</sup>, C. Marisa R. Almeida<sup>2</sup>**

<sup>1</sup> CIMAR/CIIMAR, Faculdade de Ciências, Universidade do Porto, Porto, Portugal.

<sup>2</sup> CIMAR/CIIMAR, – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal

Salt marshes have an important ecological role. However, they receive important anthropogenic inputs of contaminants and their great sensitivity makes them very difficult to clean. It is already known that phytoremediation is a low damaging and high cost-effective method for the recovery of contaminated areas. Some salt marsh plants have already shown to have potential for metal phytoremediation in estuarine areas (e.g [1]), being important to study strategies to enhance that potential, an aim embraced in the PHYTOBIO project. In the frame of that project, the aim of this work was to evaluate how the plants *Juncus maritimus* and *Phragmites australis* responded in a short time to a Cd contamination and how metal was distributed between plant and sediment after the contamination.

For that, plants of both species from Rio Lima estuary were sampled together with the sediment involving their roots, placed in vessels and maintained in greenhouses, exposed to natural environmental and light conditions. Similar vessels were prepared with uncolonized sediments. A nutritive saline solution was added to all vessels through an automated irrigation system (2 daily cycles each one with two 6 h periods: one of flood and another of draught) to mimic the tides and maintain plants at optimum nutritional conditions. After 2 weeks of acclimation, all vessels were spiked with a saline cadmium solution (20 mg L<sup>-1</sup> of Cd, as CdCl<sub>2</sub>). Solution was in contact with the system sediment/root plant for about 6h, being the vessels afterwards disassembled. For that, plants' aboveground tissues were separated from belowground structures, which in turn were carefully separated from the sediment. Samples were put to dry until constant weight, after which cadmium content was determined. Cadmium determinations were carried out, according to Almeida *et al.* [1], in sediments and in plants' roots, rhizomes, stems and leaves.

Results showed that both plants were able to uptake, in a short period of time, a considerable amount of cadmium, indicating that *J. maritimus* and *P. australis* have potential for cadmium phytoremediation in salt marsh areas. Most cadmium was accumulated in plants belowground tissues. For *J. maritimus* no metal translocation to the aboveground structures was observed, whereas for *P. australis* an eight fold increase on aboveground cadmium levels was observed, indicating different plants response to cadmium contamination.

In the future the long time response of plants to cadmium contamination will be evaluated.

## ***Acknowledgments***

This work was partially funded by Fundação para a Ciência e Tecnologia (FCT), Portugal, through the project PTDC/MAR/099140/2008.

## **References:**

[1] Almeida C. M. R., Mucha A. P., Vasconcelos M. T. S. D. (2004). Influence of the sea rush *Juncus maritimus* on metal concentration and speciation in estuarine sediment colonized by the plant. *Environmental Science and Technology* 38: 3112-3118.

# Cultivation and reproduction of the marine sponge *Hymeniacion perleve*: Evidences of horizontal transmission of cyanobacteria

**S. Pereira<sup>1,2,3</sup>, A. Regueiras<sup>2,3</sup> and V. Vasconcelos<sup>2,3</sup>**

<sup>1</sup> Abel Salazar Institute of Biomedical Sciences, University of Porto, Portugal.

<sup>2</sup> Laboratory of Ecotoxicology, Genomics and Evolution, Interdisciplinary Centre of Marine and Environmental Research, CIIMAR/CIMAR, University of Porto.

<sup>3</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal

Sponges are sessile animals, mainly marines, with relevant ecological functions in their environment. These invertebrates are known as a source of new bioactive compounds with pharmaceutical and toxicological relevance. It is considered that some of these compounds may result from associations between sponges and other organisms, being cyanobacteria one of the most relevant symbionts. In spite of the recognition of these symbiotic associations, the scientific data about how the symbiosis occur is still scarce. The purpose of this research is to optimize techniques of culturing sponges in laboratory in order to study how the symbiosis appear and their ecological importance.

*Hymeniacion perleve* was selected because it is one of the most common intertidal sponge species of Northern Portuguese coast. Optimization of sponge cultures will be performed in what concerns with light, temperature and food. Once the maintenance of sponges in laboratory is achieved, cyanobacterial strains from cultures of symbiotic and free living forms will be introduced in the aquariums to test the incorporation of these as symbionts. The quantification of chlorophyll a [1] and ficobiliproteins [2] complemented with molecular methods as DGGE [3] will enable to verify the ratio between the gain/loss of cyanobacterial symbionts and their effects on the survival rate of sponges. Different luminosity intensities studies will also be conducted to determine their importance in these associations. The methodologies used in the present work will permit to understand the relevance of cyanobacterial symbionts in intertidal sponges of the northern portuguese coast. Simultaneously, the reproduction of sponges in captivity will be tested, for the acquisition of larvae. The larvae will also be submitted to cyanobacterial infection, to verify the possibility of infection in an early stage.

In the terminus of this study, is expected to produce scientific data capable of demonstrate, for the first time, the infection of sponges by marine cyanobacteria from the temperate waters of the North Atlantic.

## References:

[1] Erwin, P.M. and Thacker, W. (2007), *Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages*, Journal of the Marine Biological Association of the United Kingdom, 87, 1683-1692.

[2] Suda, S.; Liu, Y.; He, J.; Hu, Z.; Hiroki, M. and Watanabe, M.M. (1998), *Morphological, biochemical and physiological characteristics of Lyngbya hieronymusii var. hieronymusii (Oscillatoriales, Cyanobacteria)*, Phycological Research, 46, 51-55.

[3] Usher, K.M.; Fromont, J.; Sutton, D.C.; and Toze, S. (2004), *The Biogeography and Phylogeny of Unicellular Cyanobacterial Symbionts in Sponges from Australia and the Mediterranean*, Microbial Ecology, 48, 167-177.

## Development of a sequential injection analysis system for the evaluation of aquatic pollutant's toxicity

**Susana P.F. Costa, Paula C.A.G. Pinto, José L.F.C. Lima, M. Lúcia M.F.S. Saraiva**

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

In the last decades there was a growing public and scientific awareness about the impact of chemicals on the environment and human life. This general concern led the scientific community to invest on the development of novel strategies not only for the identification of environmental pollutants, mainly aquatic ones, but also for the evaluation of their toxicity [1]. In this field, the analysis of natural waters and effluents by means of bioassays can provide directly information about their toxicity [2]. Most of these methods employed to evaluate the environmental risk of a substance in an aqueous media are based on inhibition assay. Different microorganism, enzymes and algae have been used in these inhibition measurements. Among these, the *Vibrio fischeri* assay and associated analytical techniques have already proved to be potentially suitable for the monitoring of water and wastewater toxicity. This is a cost-effective and well-established assay based on the use of the marine photobacterium *Vibrio fischeri*, a self maintained bioluminescence unit [2].

In this work, we developed an automated *Vibrio fischeri* assay based on sequential injection analysis aiming the screening of aquatic pollutant's toxicity. The assay was based on the reduction of bacterial bioluminescence in the presence of test compounds. Cu(II) was used as a positive control during the optimization and analysis steps. In each cycle, 50  $\mu\text{L}$  of *Vibrio fischeri* suspension were aspirated between two aliquots (25  $\mu\text{L}$  each) of test compound. After flow reversal the aspirated zones were kept in contact in a reaction coil for 3 minutes. Then, the reaction zone was sent to the detector and a signal proportional to the inhibition extent was obtained. The automated assay assured the precise control of the contact time between *Vibrio fischeri* and test compound by means of a simple protocol. Furthermore, a significant reduction of the assay costs was achieved through automation mainly by a drastic reduction of the volume of bacterial suspension and test compound. By comparison with a microplate assay the developed methodology proved to be a good option for the evaluation of (eco)toxicity with reduction of time and increase of robustness and repeatability ( $n=10$ ;  $\text{rsd}<1.1\%$ ).

References:

[1] Walker, C.H. (2006) *Ecotoxicity testing of chemicals with particular reference to pesticides*, Pest Manag Sci., 62, 571–583.

[2] Parvez, S., Venkataraman, C., Mukherji, S. (2008) *Toxicity assessment of organic pollutants: Reliability of bioluminescence inhibition assay and univariate QSAR models using freshly prepared Vibrio fischeri*, Toxicology in Vitro, 22, 1806–1813.

This work has been supported by Fundação para a Ciência e a Tecnologia through grant no. PEst-C/EQB/LA0006/2011 and Reitoria da Universidade do Porto.

# Electrochemical nanosensor for simultaneous determination of MCPA and its metabolite 4-chloro-2-methylphenol

**V. Rahemi<sup>1,2</sup>, M. Martins<sup>1,2</sup>, J. Garrido<sup>1,2</sup>, F. Borges<sup>1</sup>, C.M.A. Brett<sup>3</sup>, E. Garrido<sup>1,2</sup>**

<sup>1</sup> CIQ / Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

<sup>2</sup> Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto, 4200-072 Porto, Portugal.

<sup>3</sup> Department of Chemistry, Faculty of Sciences and Technology, University of Coimbra, 3004-535 Coimbra, Portugal.

Pesticides are used worldwide for weed control in the conventional production of agricultural crops as well as in non-agricultural areas. Pesticide residues are frequently detected in surface and groundwater near or below areas where there is intensive use, with possible negative consequences for ecological and public health [1]. MCPA (4-chloro-2-methylphenoxyacetic acid) has been one of the most often-used phenoxy acid herbicides against dicotyledonous plants over the last 50 years. It is easily degraded by soil microorganisms (particularly in warm and moist conditions) via 4-chloro-2-methylphenol to ultimately harmless forms. This intermediate phenol compound is more hazardous to human health than the parent phenoxyacetic acid herbicide [2]. Hence, the determination of both compounds in different matrices is a key component of any strategy of environmental management and control.

In recent years carbon nanotubes have been widely used in the field of electrochemistry for their excellent performance and because of their mechanical and unique electronic properties that lead to a broad range of applications including chemical sensors and biosensors [3].

In the present work, a multi-walled carbon nanotubes (MWCNTs) composite film has been prepared by electrochemical polymerization of aniline containing  $\beta$ -cyclodextrin on the surface of a glassy carbon electrode. The modified electrode was applied to the electrochemical determination of MCPA and its metabolite 4-chloro-2-methyl phenol. The results obtained will be presented and discussed.

## References:

- [1] Sørensen, S. R., Schultz, A., Jacobsen, O. S., Aamand, J. (2006), *Sorption, desorption and mineralisation of the herbicides glyphosate and MCPA in samples from two Danish soil and subsurface profiles*, Environmental Pollution, 141(1), 184–194.
- [2] Crespin, M.A., Gallego, M., Valcarcel, M., Gonzalez, J. L. (2001), *Study of the Degradation of the Herbicides 2,4-D and MCPA at Different Depths in Contaminated Agricultural Soil*, Environ. Sci. Technol, 35(21), 4265-4270.
- [3] Iijima S., Ichihashi T. (1993), *Single-shell carbon nanotubes of 1-nm diameter*, Nature, 363 (6430), 603–605.

## Acknowledgements

Financial support from U. Porto/Santander Totta and from Fundação para a Ciência e Tecnologia FCT/MCTES project PTDC/AGR-AAM/105044/2008, National Funds also co-financed by the European Community Fund FEDER through COMPETE–Programa Operacional Factores de Competitividade (POFC), is gratefully acknowledged.

# Green synthesis of zero-valent iron nanoparticles using vine leaves

**S.L. Pinto, S. Machado, M.C. Ribeiro, Y. Pereira, J.P. Grosso, J.T. Albergaria, C. M. Delerue-Matos**

REQUIMTE, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200 - 072, Porto, Portugal

The utilization of zero-valent iron is increasing substantially, mostly because of the efficiency of this material. Most recently, it is being used on a nano-sized scale. These nanoparticles can be synthesized using sodium borohydride, mechanical grinding, or green synthesis [1].

The green synthesis is based on the reduction of iron(III) by a polyphenol-containing extract obtained from different plant materials or plant processing waste streams. It is known that many natural products such as fruits, vegetables, and herbs contain significant concentrations of polyphenols [2].

This work studied the synthesis of zero-valent iron nanoparticles through the utilization of vine leave extracts. The main objectives of this study were: i) to optimize the extraction process, regarding temperature and contact time; measuring the “antioxidant power” by the Ferric Reducing Ability Plasma (FRAP) method; ii) to optimize the leaves-weight:solvent-volume ratio; and iii) to optimize the iron(III):extract volume ratio, considering the amount of produced nanoparticles, measuring the turbidance of the solution containing the nanoparticles.

The obtained results led to the conclusion that: i) the extract with the highest antioxidant concentration was obtained after a 20-min extraction at 90°C; ii) the best extraction efficiency was obtained using 3.7g of leaves per 100 mL of water; and iii) the solution with the highest concentration of nanoparticles was obtained by mixing 300  $\mu\text{L}$  of 0.100 mol  $\text{L}^{-1}$  iron (III) solution per mL of extract.

## Acknowledgements

This work has been supported by Fundação para a Ciência e a Tecnologia through grant no. PEst-C/EQB/LA0006/2011 and Project PTDC/ECM/103141/2008. J.G. Pacheco thanks the Fundação para a Ciência e a Tecnologia for financial support through his pos-doc grant (SFRH/BPD/73943/2010).

## References:

- [1] Hoag, E.H., Collins, J.B., Holcomb, J.L., Hoag, J.R., Nadagouda, M.N., Varma, R.S. (2009), *Degradation of bromothymol blue by 'greener' nano-scale zero-valent iron synthesized using tea polyphenols*, Journal of Materials Chemicals, 19, 8671-8677.
- [2] Fu, L., Xu, B.T., Xu, X.R., Gan, R.Y., Zhang, Y., Xia, E.Q., Li, H.B. (2011), *Antioxidant capacities and total phenolic contents of 62 fruits*, Food Chemistry, 129, 345-350.

## Magnetic susceptibility of soils close to a highway

A.Vidal<sup>1</sup>, H. Sant'Ovaia<sup>2</sup> and J. Marques<sup>3</sup>

<sup>1,2,3</sup> Department of Geosciences, Environment and Spatial Planning, Faculty of Sciences, Porto University, Portugal.

This study was conducted in the North of Portugal, in an area adjacent to the highway A-28, which connects Porto and Caminha, more precisely in the area of Mindelo.

The main purpose of this study is to analyse the spatial variation of the magnetic susceptibility in soil samples collected at different distances from the highway in order to assess the influence of the road traffic in the magnetic characteristics of the soils.

Two types of samples were collected at each point: a surface sample, representative of the O horizon (organic), and another representative of the A horizon (mineral). Samples from horizon A were divided in two size fractions (< 2 mm and < 0,063 mm).

The measurement of the mass magnetic susceptibility values ( $\chi$ ) was performed using the AGICO Kappabridge KLY-4S device with the SUMEAN software.

The results show that, in the O horizon, there is a steady decrease in the values of the mass magnetic susceptibility with the increasing distance from the highway which reflects the influence of the road traffic as source of soil magnetic particles.

On the A horizon, the mass magnetic susceptibility variation also reflects the soil features, however the existence of different values of magnetic susceptibility may be related to the different origins of the soils.

## Marine cyanobacteria toxicology: toxic effects on marine invertebrates, marine microalgae, bacteria and cytotoxic effects on human cells

Sofia Costa<sup>1,2</sup>, Margarida Costa<sup>1</sup>, Pedro Leão<sup>1</sup>, João Costa-Rodrigues<sup>3</sup>, Helena Fernandes<sup>3</sup>, Vitor Vasconcelos<sup>1,2</sup>, Rosário Martins<sup>1,4</sup>

- 1 CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal
- 2 Faculdade de Ciências, Universidade do Porto, Departamento de Biologia, Rua do Campo Alegre, Edifício FC4, 4169-007 Porto, Portugal.
- 3 Faculdade de Medicina Dentária, Universidade do Porto, Rua Dr. Manuel Pereira da Silva, 4200-393 Porto, Portugal.
- 4 Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, Rua Valente Perfeito 322, 4400-330 Vila Nova de Gaia, Portugal.

Cyanobacteria are well known for their ubiquity, morphological and physiological properties and for the production of toxic compounds. Recently, the production of bioactive compounds with pharmacological applications has led to an increasing interest in the study of these organisms [1]. Until about a decade the study on cyanobacteria was focused on its occurrence and toxicity in freshwater ecosystems. In recent years the interest in the study of these organisms in the marine environment increased, partially due to its potential in biotechnology interest.

This study had as main objectives the study of the ecotoxicology and the pharmacological potential of cyanobacteria from the genera *Cyanobium*, *Leptolyngbya* and *Synechococcus* isolated from the Portuguese coast. From freeze dried biomass, a crude extract was obtained with a methanol and dichloromethane solution, and after three more fractions with hexane, ethyl acetate and methanol. In order to evaluate the potential toxic effects of cyanobacteria on other marine organisms, bioassays were performed on the crustacean *Artemia salina* and fertilized eggs of the sea urchin *Paracentrotus lividus* and on the marine microalgae *Nanochloropsis* sp.. In order to evaluate the potential production of interesting pharmacological compounds, bioassay were performed with the bacteria *Pseudomonas* sp. and cytotoxicity assays were performed using human lung, breast and colon carcinoma cell lines and normal fibroblasts. In terms of ecotoxicology, results with *Artemia salina*, *Paracentrotus lividus* and *Nanochloropsis* sp revealed low or no toxicity of the cyanobacteria strains. No evident toxic effects were also registered on the assay with *Pseudomonas* sp.. With human carcinoma cell lines, a decrease in cells viability with some extracts of some of the cyanobacteria strains was observed. The cytotoxic effect was less evident in fibroblast, which accentuates the importance of cyanobacteria as producers of compounds with anticancer applicability. From the crude extract and fractions prepared, the fraction obtained using ethyl acetate revealed the highest percentage of inhibition of tumor cell growth, and is therefore promising in terms of isolation of bioactive compounds.

### References:

- [1] Tan, L. T., 2007. *Bioactive natural products from marine cyanobacteria for drug discovery*. *Phytochemistry* 68(7), 954-979.

# Physiological and biochemical response of *Oriza sativa* L to toxic *Microcystis aeruginosa* extracts

C. Azevedo<sup>1</sup>, V. Vasconcelos<sup>1,2</sup> and A. Campos<sup>1,2</sup>

<sup>1</sup> Centro Interdisciplinar de Investigação Marinha e Ambiental, CIIMAR/CIMAR, Rua dos Bragas 289, 4050-123, Porto, Portugal

<sup>2</sup> Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, Rua do Campo Alegre, 4069-007 Porto, Portugal

Toxic cyanobacteria proliferation is regarded as a serious source of pollution and contamination of fresh water habitats due to the fact that, in particular conditions, cyanobacteria produce and release biologically active secondary metabolites, at levels considered toxic for most organisms [1, 2]. The process has, therefore, significant impact on the use of water for recreational purposes, fishing, drinking, plant irrigation and agriculture [1, 2, 3-5]. The aim of this project is to assess negative effects (physiological stress, growth impairment, lowered photosynthetic efficiency, bioaccumulation) on rice plants caused by exposure to toxic cyanobacteria extracts.

In order to understand if the toxin microcystin (MC) induces physiological stress, 20-day-old plants were exposed to an extract of *Microcystis aeruginosa* during 48 hours or 7 days, with MC at 1, 50 and 300 µg/L. Photosynthetic efficiency, plant biomass and GPx and GST activities were determined in the control and treatments. An assay on 8-day-old plants is also in progress, and a proteomics analysis of the exposure assays done so far is to be performed. To determine whether the toxin is bioaccumulated, HPLC or LC-MS analysis of the tissues will be used.

The results obtained so far, from enzymatic analysis of GPx and GST, indicate no significant differences between control groups and the exposed ones, as seen in the example assay in figure 1. The same can be said for the observations on biomass and photosynthetic efficiency.

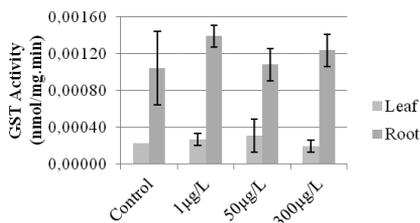


Fig. 1 - GST Activity in 20-day-old rice plants (021111) - 48hour exposure to microcystin.

From the results obtained until now, we may conclude that no negative effects arise from exposure of rice plants to the tested concentrations of MC in *Microcystis aeruginosa* extract. Future results from proteomics analysis will provide further insight on physiological stress, and results for HPLC/LC-MS will provide information on bioaccumulation.

## References:

- [1] Saqrane, S., Ghazali, I.E., Oudra, B., Bouarab, L., Vasconcelos, V. (2008) *Effects of cyanobacteria producing microcystins on seed germination and seedling growth of several agricultural plants*, Journal of Environmental Science and Health Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 43, 443-451.
- [2] Tyler A N, Hunter P D, Carvalho L, Codd G A, Elliott J A, Ferguson C A, Hanley N D, Hopkins D W, Maberly S C, Mearns K J, Scott E M. (2009) *Strategies for monitoring and managing mass populations of toxic cyanobacteria in recreational waters: a multi- interdisciplinary approach*, Environmental Health, 8 (Suppl 1), S11.
- [3] Zhang H-j, Zhang J-y, Hong Y, Chen Y-x. (2007) *Evaluation of organ distribution of microcystins in the freshwater phytoplanktivorous fish Hypophthalmichthys molitrix*, Journal of Zhejiang University SCIENCE B, 8 (2), 116-120.
- [4] Ibelings B W, Chorus I. (2007) *Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: A review*, Environmental Pollution, 150, 177-192.
- [5] Mohamed, Z., Al Shehri, A. M. (2009) *Microcystins in groundwater wells and their accumulation in vegetable plants irrigated with contaminated waters in Saudi Arabia*, Journal of Hazardous Materials, 172, 310-315.

# Soil microorganism selection for amoxicillin biodegradation

I. Bragança, S. Sousa, S. Martins, V. F. Domingues and C. Delerue-Matos

<sup>1</sup>REQUIMTE, Instituto Superior de Engenharia do Porto, Politécnico do Porto,  
Rua Dr. António Bernardino de Almeida, 431, 4200 – 072 Porto

The identification and remediation of contaminated soils is nowadays beginning to be done all worldwide, due to the aware by the scientific community of environmental implication. The contaminants that can be found at each location are closely related with human activities that operate at the site. A high proportion of hazardous waste sites are co-contaminated with organic pollutants. The antibiotics are extensive applied for prevention and treatment of microbial infections in human and veterinary medicine, where  $\beta$ -lactam antibiotics such as amoxicillin are widely used [1, 2]. An important aspect related to the presence of antibiotics in the environment is the possibility of inducing resistance in bacterial strains [3].

Bioremediation is the elimination or immobilization or inactivation of contaminants from a polluted environment operated by biological action. In the light of this definition, it is clear that, for bioremediation to proceed successfully is fundamental organisms that will be able to withstand the existing levels of pollution and transform it into non-toxic compound [4].

The aim of the present work is to study the biodegradation of amoxicillin in a model soil. Different soil samples were collected and it was made a microorganism extraction and selection by adding amoxicillin as only carbon source. The selected microorganisms that are resistant to amoxicillin were placed in contact with an amoxicillin solution and biodegradation was investigated with respect to amoxicillin degradation by determination of the Total Organic Carbon and by HPLC.

Some tests achieved a concentration of amoxicillin much lower than the control test with some soil bacteria have. The results reveal that the microorganisms in this study may have significant potential for aerobic biodegradation of amoxicillin.

## References

- [1]. Bergheim, M., Helland, T., Kallenborn, R., and Kummerer, K. (2010) *Benzyl-penicillin (Penicillin G) transformation in aqueous solution at low temperature under controlled laboratory conditions*, Chemosphere 81, 1477-1485.
- [2]. Kotzerke, A., Fulle, M., Sharma, S., Kleineidam, K., Welzl, G., Lamshoft, M., Schloter, M., and Wilke, B. M. (2011) *Alterations in total microbial activity and nitrification rates in soil due to amoxicillin-spiked pig manure*, J. Plant Nutr. Soil Sci. 174, 56-64.
- [3]. Andreozzi, R., Caprio, V., Ciniglia, C., De Champdore, M., Lo Giudice, R., Marotta, R., and Zuccato, E. (2004) *Antibiotics in the environment: Occurrence in Italian STPs, fate, and preliminary assessment on algal toxicity of amoxicillin*, Environmental Science & Technology 38, 6832-6838.
- [4]. Soares, A. A., Albergaria, J. T., Domingues, V. F., De Marco, P., and Delerue-Matos, C.: *Sequential Soil Vapor Extraction and Bioremediation processes applied to BTEX contaminated soils*. In: *Fungi as Bioremediators*. vol. New York: Springer: Verlag Berlin-Heidelberg.

## Acknowledgments

The authors are grateful to Fundação para a Ciência e Tecnologia (Project PTDC/ECM/103141/2008 and through grant no. PEst-C/EQB/LA0006/2011) for the material support for this work.

# **Studies on *Arundo donax* development for utilization in constructed wetlands for saline aquaculture effluent treatment**

**A.-T. Gonçalves<sup>1</sup>, J. Jesus<sup>2</sup>, I. Mina<sup>3</sup>, M.-T. Borges<sup>1,2</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> CIIMAR, University of Porto, Portugal

<sup>3</sup> Department of Biology, School of Sciences, University of Minho, Portugal

Saline aquaculture effluents are characterized by high salinity combined with considerable amounts of inorganic nutrients [1]. The discharge of untreated aquaculture effluents causes severe environmental damages but conventional treatment methods are inefficient for this type of wastewaters. Thus, it is essential to find alternative treatment processes to apply in these cases. Constructed wetlands (CWs) are artificial systems designed to simulate the natural processes of water treatment [2]. Plants growing in CWs are fundamental in the treatment process. *Arundo donax* is a halo-tolerant, perennial plant [3] that has been used for CWs treating domestic wastewater. Plantation protocols employing stems are needed, namely for saline situations. The aim of this study is to assess the development of *A. donax* stems for later successful transplantation and use in CWs. The response of newly rooted stems to different conditions was also preliminarily tested.

Forty-two *A. donax* stems (with or without roots) were collected in the wild and put in fresh water to adapt to indoors conditions. Development was assessed weekly using six growth categories. After a 40 days growth period, stems with new roots sufficiently developed were put individually in triplicate containers under different conditions. The variables considered were: salinity (0, 15 and 25 ppt), substrate (presence/absence) and nutrient solution (presence/absence). The percentage of chlorotic/necrotic leaves and the number of new shoots were registered every 5 days.

For the first test, survival rate was 32.2% for stems collected without roots (group A) and 100% for stems collected with some roots (group B). Of the surviving stems, 22.2% of group A and 71.4% of group B stems reached the highest root development. Thus, stems initially with some roots are more likely to reach the adequate root network volume for transplantation. Newly rooted plants seemed to be affected by salinity, showing an increase in chlorotic/necrotic leaves from 50.3% at 0 ppt, to 100% at the highest salinity tested (which reached 35 ppt after 30 days, possibly reflecting evaporation effects). In freshwater, plants developed 80% of the total number of new shoots registered in the test. The presence of substrate or nutrient solution did not seem to have any influence on plant growth. The time of the year and the hydraulic retention time chosen probably influenced the results obtained, suggesting the need of further studies.

## References:

- [1] Lin, Y., Jing, S., Lee, D. and Wang, T. (2002), *Nutrient removal from aquaculture wastewater using a constructed wetland system*, *Aquaculture* **209**:169-184
- [2] Vymazal, J., Greenway, M., Tonderski, K., Brix, H. and Mander, U. (2006) *Constructed wetlands for wastewater treatment*, *Ecological Studies*, vol.190:69-96
- [3] Ceotto, E., Di Candilo, M. (2010) *Shoot cuttings propagation of giant reed (*Arundo donax* L.) in water and moist soil: The path forward?* *Biomass and Bioenergy* **34**:1614-1623



# The influence of benthic macroinvertebrates on the degradation of organic matter in river systems

A. Ribeiro<sup>1,2</sup> and N. Vieira<sup>1,2</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> CIMAR/CIIMAR – Centre of Marine and Environmental Research, University of Porto, Portugal.

Leaf litter input from riparian landscapes has been identified as both a major energy flow to stream ecosystems and as a food source for stream macroinvertebrates. In this study the benthic macroinvertebrate community was used to evaluate the decomposition of organic matter in river systems.

The aim of this work was to study the decomposition of organic matter using the benthic macroinvertebrate colonization. The research was developed over five months (January 2011 to May 2011) in four different sites, distributed along the River Ferreira terminal area, northern Portugal. A litter bag experiment was used to examine the role of macroinvertebrate communities in the processing of organic material on the river. Litter bags were placed in the water and collected every 7, 15, 30, 60, 90 and 120 days.

In litter bags was observed a high abundance of Oligochaeta and Chironomidae, which are characterized by being detritivorous-herbivores and filtering collectors, respectively, indicating the benthic macroinvertebrate more involved on the decomposition of organic matter.

These results contributed to increase current knowledge about benthic macroinvertebrate communities and may serve as incentive for future research works.

# Toxicity of the pesticide mancozed to the Asian Clam (*Corbicula fluminea*).

**O. Araújo<sup>1,2</sup>, P. Vilares<sup>2</sup>, and L. Guilhermino<sup>1,2</sup>**

<sup>1</sup> ICBAS – Institute of Biomedical Sciences of Abel Salazar, University of Porto, Department of Population Studies, Laboratory of Ecotoxicology Lg. Prof. Abel Salazar 2, 4099-003 Porto, Portugal

<sup>2</sup> CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Laboratory of Ecotoxicology and Ecology, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

The Asian clam *Corbicula fluminea* (Müller, 1774) is an invasive species that has been colonizing several ecosystems around the world, causing important ecological and economic damages, particularly in the Europe and US. This clam, was first reported in the freshwater tidal area of the Minho River estuary in 1989<sup>[1]</sup>. At the present, it is a major component of the benthic fauna in terms of density and biomass<sup>[2]</sup> and it has been identified as one of the major causes contributing for the decline of several native molluscan species, some of which are now facing the risk of local extirpation<sup>[2]</sup>. Regularly, in the summer, the population of Minho estuary has rapid die-offs that make environmental conditions considerably challenging particularly for more sensitive species, and accelerates the loss of biodiversity of the benthic community. Apparently, these massive mortality events are related with the increase of temperature and reduction of the river flow in the summer, and the consequent alterations of some crucial abiotic factors (such as dissolved oxygen and redox potential) and potentially also with the increase of the concentration of pollutants<sup>[3]</sup>. Since *C. fluminea* populations has the capability of recovering rapidly from the die-offs while native species are not able to, it is important to know the factors that may contribute to these episodic events to support management actions. Therefore, the main goal of the present study was to investigate the toxic effects of the pesticide mancozeb on *C. fluminea*, to assess the sensitivity of this species to a model compound widely used in agriculture, including in crops potentially surrounding freshwater ecosystems colonized by the species. The hypothesis behind (to be tested during the MSc Thesis) is that chemical pollution may contribute to the summer mortality events that have been observed in this species. Bioassays were conducted in laboratory conditions in temperature (20 + - 1°C) (16h light: 8h and photoperiod controlled rooms dark). Animals collected in the in the Minho estuary were acclimatized to laboratorial conditions for at least 2 weeks. Bioassays were conducted for 2 weeks in laboratory conditions in temperature (20 + - 1°C) (16h light: 8h and photoperiod controlled rooms dark), with adequate food being provided. Semi-static conditions were used. Groups of animals were exposed to different concentrations of the pesticide and adequate controls were included in the experimental design. At the end of the bioassays, several biomarkers involved in neurotransmission, anti-oxidant defenses, energy production, among others were used. Significant effects were found in several enzymes involved in crucial functions. The results are discussed in relation to the economic and ecological potential impacts.

**Acknowledgements:** This study was carried out in the scope of the project “NISTRACKS - Processes influencing the invasive behavior of the non indigenous invasive species *Corbicula fluminea* (Mollusca: Bivalvia) in estuaries – identification of key genetic and environmental factors” (FCT: PTDC/AAC-AMB/102121/2008).

[1] Araújo, R., Moreno, D., Ramos, M.A. (1993), *The Asiatic clam Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae) in Europe. American Malacological Bulletin, 10, 39-49; [2] Sousa, R., Rufino, M., Gaspar, M. Antunes, C., Guilhermino, L. (2008), *Abiotic impacts on spatial and temporal distribution of Corbicula fluminea* (Müller, 1774) in the River Minho estuary, Portugal. Aquatic Conservation, 18, 98-110; [3] Ilarri, M., Antunes, C., Guilhermino, L., Sousa, R. (2011), *Massive mortality of the Asian clam Corbicula fluminea in a highly invaded area*. Biological Invasions, 13, 277-280. DOI: 10.1007/s10530-010-9833-5; [4] Gagné, F., Blaise, C., André, C., Pellerin, J. (2007), *Implication of site quality on mitochondrial electron transport activity and its interaction with temperature in feral Mya arenaria clams from the Saguenay Fjord*. Environmental Research, 103, 238-246.

# Zircons from metagreywacke of the “Complexo Xisto-Grauváquico”: constraints from petrographic and lithogeochemical data

**D. Teixeira<sup>1</sup>, M. A. Ribeiro<sup>1,2</sup> and H. C. B. Martins<sup>1,2</sup>**

<sup>1</sup> DGAOT – FCUP, Rua do Campo Alegre, 4169-007 Porto, Portugal, daniel\_rft@hotmail.com

<sup>2</sup> Centro Geologia, DGAOT – FCUP, R. Campo Alegre, 4169-007 Porto, Portugal, maribeir@fc.up.pt, hbrites@fc.up.pt

This study aims to understand the petrographic and lithogeochemical features of the metagreywacke samples belonging to the “Complexo Xisto-Grauváquico” (“CXG”), as support of subsequent mineralogical study of zircons. The “CXG” is geographically distributed over the north and center of Portugal and also extends to Spain, outcropping in a great area in the Central Iberian Zone (CIZ) the axial geotectonic zone of the Iberian Massif [1-2]. We present a detailed petrographic and lithogeochemical study of four samples of the “Grupo das Beiras” [3] (samples Alv 34, Alv51, Alv55 and TR6) and of one sample of “Grupo do Douro” [1,4] (sample T1). The petrographic results suggest that the samples are at different metamorphic conditions: “Grupo das Beiras” in chlorite zone and “Grupo do Douro” in higher metamorphic condition, specifically in biotite zone. The samples of “Grupo das Beiras” have a clastic texture, with a recrystallized clay quartz matrix. The matrix foliation is parallel to the stratification and is marked by the concentration of organic matter, with an anastomosed character, controlled by the size of quartz porphyroclasts. Some plagioclase, muscovite and biotite porphyroclasts also occur. The sample of “Grupo do Douro” is a micaschist, with biotite porphyroblast and a quartz feldspar glauconitic matrix [5]. Three of the samples of “Grupo das Beiras” (Alv 34, Alv 51 and TR6) present very similar lithogeochemical composition, in both major and trace elements. The light rare earth elements (LREE) content is very homogeneous, unlike the heavy rare earth elements (HREE) that are more variable and dependent on the zircon content [6]. One sample, with higher Zr content (Alv51), was selected for zircons concentration [7]. BSE imaging of the zircons, carried out at CEMUP, have shown euhedral zircons, with oscillatory zoning, that may be an indicator of igneous rocks provenance.

## References:

- [1] Sousa, M. B. (1983). *Considerações paleogeográficas e ensaio de correlação do Grupo do Douro com as formações da ZCI*. Memórias e Notícias, 95, p.65-98.
- [2] Solá, A. R., Chichorro, M., Pereira, M. F., Medina, J., Linnemann, U., Hofmann, M., Silva, J. B. (2011). *U-Pb detrital zircon ages from the Beira Group – Implications for the Neoproterozoic evolution of the SW Iberia*. CIG/XVII SG, p119, CD.
- [3] Romão, J.M.C. (2000). *Estudo tectono-estratigráfico do bordo SW da ZCI e as suas relações com a ZOM*. Tese doutoramento, Univ. Lisboa, 231p.
- [4] Ribeiro, M.A., Cuney, M., Noronha, F. (2003). *Geoquímica das unidades litoestratigráficas do CXG – Grupo do Douro*. In: Neiva et al Eds –IV CIG/ XIII SG, DCT, p.158-160.
- [5] Teixeira, D. (2011). *Estudo petrográfico e mineralógico de metagruvaques do “Complexo Xisto-Grauváquico*. Estágio de 1º Ciclo/Licª em Geologia, GDAOT-FCUP, 53p.
- [6] McLennan, S.M. (1989). *Rare earth elements in sedimentary rocks: influence of provenance and sedimentary process*. Rev. Minera., 21, p. 169-200.
- [7] Abreu, J., Martins, H.C.B., 2010. *Separação de concentrados de zircão para estudos geocronológicos*. In: Flores, D & Marques, M. (eds). Memórias nº 14, CD, p. 981-989.

# **Denoising of phonocardiograms: A study of current algorithms and approaches**

**Nuno Silva and Miguel Coimbra**

Department of Computer Science, Faculty of Sciences, University of Porto, Portugal.

One of the main obstacles that stalls the widespread use of phonocardiograms (PCGs) in modern day medicine are the various noise components they invariably contain. Although many advances have been made towards automated heart sound segmentation[1] and heart pathology detection and classification, an efficient method for noise reduction would come as a major aid for further development in this field, especially when it comes to working with PCGs collected in realistic environments such as hospitals and clinics.

The use of a digital stethoscope to collect experimental samples of heart sounds in realistic conditions results in highly noise contaminated PCGs. Besides the fact that these environments are often crowded, the very method of data gathering (the use of a transducer for heart sound recording) contributes to the addition of background noise. Time-variant conditions such as the patient's build and posture, position and pressure of the stethoscope's head over the patient's body, possible presence of clothes have also to be taken in consideration. Thus, only an adaptive algorithm that takes all these aspects into account will successfully increase signal-to-noise ratio (SNR). After extensive testing of commonly used PCG noise reduction algorithms, such as traditional band-pass filtering, least-mean squares[2], discrete wavelet transform[3], and their variants, we propose to further develop/combine those who exhibit better results.

Current experiments exhibit an initial comparison of algorithmic efficiency in different environments and have served to better identify certain aspects of the problem, such as the frequency bands that heart sounds occupy, frequent and problematic noise types and deficiencies that arise in the process of sample gathering.

Using state of the art denoising techniques, this work aims at developing a PCG noise reduction algorithm that can be incorporated in the preprocessing stage of PCG gathering by qualified professionals in hospitals and clinics using a digital stethoscope. We believe this to be an important step towards reaching the ambitious goal of creating an automated heart sound segmentation and heart pathology detection system.

## References:

- [1] Liang, H.; Lukkarinen, S.; Hartimo, I.; "*Heart sound segmentation algorithm based on heart sound envelogram*", Computers in Cardiology 1997, vol., no., pp.105-108, September 1997.
- [2] Samir B. Patel, Thomas F. Callahan, Matthew G. Callahan, James T. Jones, George P. Graber, Kirk S. Foster, Kenneth Glifort, and George R. Wodicka, "*An adaptive noise reduction stethoscope for auscultation in high noise environments*", J. Acoustic Soc. Am. 103, 2483 (1998).
- [3] Sheila R. Messer, John Agzarian, Derek Abbott, "*Optimal wavelet denoising for phonocardiograms*", Microelectronics Journal, Volume 32, Issue 12, December 2001, Pages 931-941, ISSN 0026-2692.

## From Thermoelectric Materials to Thermoelectric Devices

M.Rosmaninho<sup>1</sup>, O. Moutinho<sup>1</sup>, A.M.Pereira<sup>1</sup>, J. Ventura<sup>1</sup> and J.P. Araujo<sup>1</sup>

<sup>1</sup> IFIMUP-IN and Physics Department of FCUP, University of Porto, Portugal.

In the last years, large efforts have been made to discover alternatives to traditional energy production processes, particularly on refrigeration and the power generation.

One of the most promising alternatives is the use of thermoelectric devices, since it is based on recovering thermal energy. Several sources can be found in our surrounding World such as several machines, transportation facilities and even human body.

Usually, the TE device is constituted by semiconductor systems that can directly convert electricity in thermal energy (for refrigeration) or recover wasted heat and convert it into electrical power, thus providing a clean energy form in a non-mechanical process.

By far the most widely used TE materials are  $\text{Bi}_2\text{Te}_3$  and  $\text{Sb}_2\text{Te}_3$  alloys for near room-temperature (RT) applications. Nevertheless, new materials are emerging as alternative and M-Si(Ge) based materials gained renewed interest due to its large figure of merit discovered recently. Furthermore, since it is a magnetic material it enables the production of a multifunctional device.

In this work, we synthesize several samples based on M-Si(Ge) system, namely FeSi, FeGe, CoGe, CoSi, MnSi e MnGe by using the traditional arc-melting method. The samples were further magnetically and structurally characterized by SQUID and XRD respectively. Later, thin film materials with FeGe composition with different thickness were produced. Studies of the structural characterization and morphology were performed using the methods of XRD and SEM/EDS.

In parallel to materials production, microdevice design studies are being conducted namely by using AutoCAD software to produce lithography masks to further develop the device.

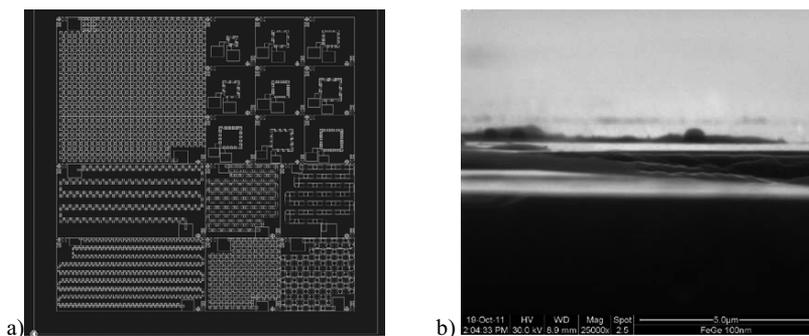


Fig. 1- a) Schematic representation of the mask created for the production of micro devices and b) Image of a 100nm FeGe thin film.

## Iron meteorites and the formation of the early Solar System

**B. Alves<sup>1,2</sup>, M. Côto<sup>1,2</sup> and T. Seixas<sup>1,2</sup>**

<sup>1</sup> Department of Physics and Astronomy, Faculty of Science, University of Porto, Portugal.

<sup>2</sup> Centre for Geophysics of the University of Coimbra, University of Coimbra, Portugal.

Iron meteorites are Fe–Ni alloys containing minor amounts of Co, P, S, and C. The 10 largest irons, which each weigh more than 10 tons (e.g., [1]), are meter sized and most were large single crystals of taenite (fcc Fe–Ni) after solidification and at high temperatures in their parent bodies. Most iron meteorites exhibit a Widmanstätten pattern which can be used to determine their cooling rate. The Widmanstätten pattern develops as a two-phase intergrowth of kamacite (□ bcc, ferrite) and taenite (⊠fcc, austenite), and forms by nucleation and growth of kamacite from taenite during slow cooling of the parent body [2].

Many studies suggest that iron meteorites may have been derived originally from bodies as large as 1000 km or more in size that accreted and melted early, even before the parent bodies of chondrites. As a consequence, it was claimed by [3] that these bodies accreted not in the asteroid belt but closer to the Sun at 1–2AU where planetesimals are thought to have accreted faster. Thus, the parent bodies of the iron meteorites could have been much more diverse than those of the chondrites and the irons may tell us more about the bodies that accreted to form the terrestrial planets than the chondrites.

In this work, we will undertake a detailed bibliographic review on the state of art of research on iron meteorites and its implications on the formation of the early Solar System.

### References:

- [1] Buchwald, V.F., (1975), *Handbook of Iron Meteorites*, University of California Press, Berkeley, CA.
- [2] Owen, E.A., Burns, B.D., (1939), *X-Ray Study of Some Meteoritic Irons*, *Philos. Mag.* 28, pp. 497–512.
- [3] Bottke, W.F., Nesvorný, D., Grimm, R.E., Morbidelli, A., O'Brien, D.P. (2006), *Iron meteorites as remnants of planetesimals formed in the terrestrial planet region.*, *Nature* 439, pp. 821–824.

# Laboratorial characterization of a Fe-Ni meteorite

**J. Teixeira<sup>1,2</sup>** and **T. Seixas<sup>1,2</sup>**

<sup>1</sup> Department of Physics and Astronomy, Faculty of Science, University of Porto, Portugal.

<sup>2</sup> Centre for Geophysics of the University of Coimbra, University of Coimbra, Portugal.

The past decade has seen a significant expansion both in the interpretative methodologies used to extract mineralogical information from asteroid spectra and other remote-sensing data and in the number of asteroids for which mineralogical characterizations exist [1]. This has allowed significant progress to be made in the identification of meteorite parent bodies. Groupings of meteorites according to petrologic, mineralogical, bulk-chemical, and isotopic properties suggest the existence of 100–150 distinct parent bodies [2].

Most meteorites contain at least some Fe-Ni metal, being these metal-bearing achondrites fertile subjects for metallographic study. Iron meteorites are composed primarily of various alloys of iron and nickel, namely kamacite and taenite [3]. Iron meteorites are derived from the cores of ancient Planets that were destroyed around 4.5 billion years ago by catastrophic impact events during the formation of our Solar System. Most iron meteorites have unique crystalline structures known as Widmanstatten patterns, which are formed by interwoven bands of kamacite and taenite, which can only form over literally millions of years of very slow cooling [4]. It is well known that most iron meteorites can be polished and etched to reveal the Widmanstatten pattern, telling us about the meteorites compositions and how long it took for the planetary core from which the meteorite is derived to cool [4].

Our aim in this work is to study iron meteorite microstructures, namely the Campo del Cielo meteorite, in order to learn about their thermophysical history and their parent asteroids. In order to reveal the complex microstructures of the Campo del Cielo meteorite, metallographic studies will be performed, using optical microscopy. The Campo del Cielo is described as a polycrystalline coarse octahedrite and is classified in Group I. Samples will be etched using Nital, which preferentially reacts with kamacite (low-Ni) relative to taenite (high Ni), allowing the enhancement of kamacite and taenite grain boundaries. The Widmanstatten pattern will be also investigated.

## References:

- [1] Gaffey, M.J., Cloutis, E.A., Kelley, M.S., Reed, K.L. (2002), *Mineralogy of asteroids*. In: Bottke, W.F., Cellino, A., Paolicchi, P., Binzel, R.P. (Eds.), *Asteroids III*. University of Arizona Press, Tucson, pp. 183–204.
- [2] Burbine T. H., McCoy T. J., Meibom A., Gladman B., Keil K. (2002), *Meteoritic parent bodies: Their number and identification*. In: Bottke, W. F. Jr., Cellino, A., Paolicchi, P., Binzel, R. P. (Eds), *Asteroids III*. University of Arizona Press, Tucson, pp. 653–667.
- [3] Rubin, A.E. (1997), *Mineralogy of meteorite groups*. In: Jull, A.J.T, *Meteoritics & Planetary Science* 32, USA, pp. 231-247.
- [4] Yang, J.; Goldstein, J. I. (2004), *Nucleation of the Widmanstatten Pattern in Iron Meteorites*, Lunar and Planetary Science and Exploration, NASA, Goddard Space Flight Center, CASI.

# Magnetic ferrites in Nano-MEMS: from real-time synthesis control to automatic transport devices

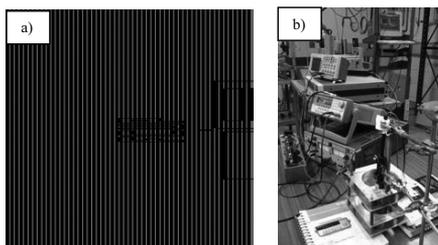
**B. Bordado<sup>1</sup>, C. Fernandes<sup>2</sup>, M. Rocha<sup>2</sup>, M.P. Fernández-García<sup>1</sup>, J.M. Teixeira<sup>1</sup>, C. Pereira<sup>2</sup>, J. Ventura<sup>1</sup>, A.M. Pereira<sup>1</sup>, C. Freire<sup>2</sup>, J.P. Araújo<sup>1</sup>**

<sup>1</sup> IFIMUP and IN – Institute of Nanoscience and Nanotechnology, Department of Physics and Astronomy, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

<sup>2</sup> REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

Magnetic materials at the nanometer scale are very attractive due to their remarkable properties and applications in several fields. Magnetic nanoparticles (MNPs) are particularly applied in the development of devices for micro-automatic energy transport (MTED). The main purpose of these devices is to remove heat from the interior of electric components and/or to create systems for fluid transport that can be subsequently used in microdevices such as lab-on-a-chip. Some prototypes have already been developed but thorough studies on the design of MTED and MNPs with the desired properties deserve attention.

The main purpose of our work is the design of a MTED incorporating magnetic ferrite nanoparticles with tunable and desired physicochemical properties. For that objective, we designed the necessary mask align that is required for the lithographic process (Fig. 1a)). However, one major drawback related with the control of MNPs properties is the reproducibility of the employed synthesis route since it may depend on the type of chemical components, mixing rates, reactants concentrations, sequence of addition, pH, etc. In order to control all the aforementioned crucial variables, real-time control of the synthesis route is of vital importance. To achieve this goal, a novel AC magnetic susceptometer was designed and implemented to monitor in real-time and *in situ*, the chemical synthesis of magnetic ferrite nanoparticles by aqueous co-precipitation method (Fig. 1b). We observed that the magnetic signal increased with time and attained a constant value after 1 h of chemical reaction.



**Fig. 1** – a) Design of mask align b) AC susceptometer implemented to monitor in real-time the chemical synthesis of magnetic nanoferrites by co-precipitation method.

**Acknowledgements:** This work has been supported by Fundação para a Ciência e a Tecnologia (FCT) and FEDER through grant no. PEst-C/EQB/LA0006/2011 and through project ref. PTDC/QUI-QUI/105304/2008 in the framework of Program COMPETE. C.F., M.R., C.P., J.M.T. and A.M.P. thank FCT for their grants. M.P.F.G. thanks the Spanish “Programa Clarín” of “Gobierno del Principado de Asturias” for a post-doctoral grant (POST10-31). This work has also been supported by “Projecto de Investigação Científica na Pré-Graduação 2010, U.P. and Santander Totta”.

# The early stages of the Solar System Evolution: constraints from the small icy worlds

**S. Batista<sup>1,2,3</sup> R. Albuquerque<sup>1,3</sup> and T. Seixas<sup>1,3</sup>**

<sup>1</sup> Department of Physics and Astronomy, Faculty of Sciences, University of Oporto, Portugal

<sup>2</sup> Department of Mathematics, Faculty of Sciences, University of Oporto, Portugal

<sup>3</sup> Centre for Geophysics of the University of Coimbra, University of Coimbra, Portugal

The birth environment of the Sun has been subject of several studies [1]. It is known that stars are born in clusters [2], as it is thought the Sun also did [3]. Nowadays, the architecture of the Solar System is well known: its eight planets and the small icy worlds, which are sculpted by the force of gravity and by the phenomenon of resonances [4]. However, understanding several steps in the early Solar System is a puzzling task. In this scenario, asteroids provide our only *in situ* record of the conditions and processes that the inner portions (~1.8-3.5 AU) of the late solar nebula and the infant solar system have experienced [5]. In addition, comets can also play an important role in this scenario. However, the only way that Humankind can access this material is through meteorites, i.e. extraterrestrial material that comes from the small icy worlds such as asteroids or comets. Trying to link a specific meteorite with a particular asteroid or class of asteroids can help us to understand a bit better the early stages of our Solar System. Actually, as the number of known exoplanets has recently been increasing in the last decade [6], understanding the steps evolved in the formation and evolution of the Solar System can then be extrapolated to understand other planetary systems.

Our aim in this work is to try to establish possible genetic linkages between asteroids and meteorites [7]. We hope to link a specific meteorite to a certain parent or source body. For this purpose, the reflectance spectra of meteorite samples will be compared to spectra of known asteroids available at the Brown University RELAB database.

## References:

- [1] Adams, F. C. (2010), *The Birth Environment of the Solar System*, ARAA, 48, 47-85
- [2] Lada, C. J. and Lada, E. A. (2003), *Embedded Clusters in Molecular Clouds*, ARAA, 41, 57-115
- [3] Bobilev, V., Bajkova, A. T., Myllari, A. and Valtonen, M. (2010), *Searching for possible siblings of the sun from a common cluster based on stellar space velocities*, Astronomy Letters, 37, 8, 550-562
- [4] Jewitt, D. (2009), *Icy bodies in the New Solar System*, Proceedings of the International Astronomical Union, IAU Symposium, 263, 3-16
- [5] Gaffey, M.J., Cloutis, E.A., Kelley, M.S., Reed, K.L. (2002), *Mineralogy of asteroids*. In: Bottke, W.F., Cellino, A., Paolicchi, P., Binzel, R.P. (Eds.), *Asteroids III*. University of Arizona Press, Tucson, pp. 183-204.
- [6] Butler, R. P., Wright, J. T., Marcy, G. W., Fischer, D. A., Vogt, S. S., Tinney, C. G., Jones, H. R. A., Carter, B. D., Johnson, J. A., McCarthy, C., Penny, A. J. (2006), *Catalog of nearby Exoplanets*, ApJ, 646, 1, 505-522
- [7] Burbine T. H., McCoy T. J., Meibom A., Gladman B., Keil K. (2002), *Meteoritic parent bodies: Their number and identification*. In: Bottke, W. F. Jr., Cellino, A., Paolicchi, P., Binzel, R. P. (Eds), *Asteroids III*. University of Arizona Press, Tucson, pp. 653-667.

# The Sandage-Loeb test for dynamical dark energy models

**P.E. Vielzeuf<sup>1,2</sup> and C.J.A.P. Martins<sup>1</sup>**

<sup>1</sup> Centro de Astrofísica, Universidade do Porto, Rua das Estrelas, 4150-762 Porto, Portugal

<sup>2</sup> Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal

The Sandage-Loeb test [1,2] consists of a measurement of the evolution of cosmic redshift obtained by taking quasar spectra at sufficiently separated epochs which provides a direct measurement of the expansion of the universe. It has the unique feature that it does not rely on any assumptions about the geometry or dynamics of the universe, nor on the behavior of gravity. It only assumes that on sufficiently large scales the universe is homogeneous and isotropic.

Although the currently available observing facilities are not yet powerful enough to carry out the test, it is thought that it will become feasible with CODEX, a high-resolution ultra-stable spectrograph that will be installed at the next-generation 39.2 metre European Extremely Large Telescope (E-ELT).

We have studied the performance of this test as a discriminator between different cosmological models, focusing on several classes of dynamical dark energy models that have been proposed as alternatives to Einstein's cosmological constant. We will discuss examples of models to which the test has different sensitivities, and show how those sensitivities can be improved in combination with other cosmological datasets.

## References:

[1] Sandage, A. (1962), *The Change of Redshift and Apparent Luminosity of Galaxies due to the Deceleration of Selected Expanding Universes*, *Astrophys. J.* 136, 319.

[2] Loeb, A. (1998), *Direct Measurement of Cosmological Parameters from the Cosmic Deceleration of Extragalactic Objects*, *Astrophys. J.* 499, L111.

## **Museum of the Resistance**

**Carlos Jorge Silva Mendes**

Faculdade de Letras, Universidade do Porto, Porto, Portugal

This work aims to reflect on the role of the Museum of the Resistance as a *locus* for critical reflection on local and global context. Transnationality of Concentration Camp, historical values and symbolic elements are indicators for the development of a transnational project. I based myself on topics related to the memories issues, since it functions as a fundamental basis and as part of the history of critical support Concentration Campo Tarrafal.

The theoretical approach of this paper is developed from considerations of different types of museums in post-colonial period and the memories issues and reflection of the museums of conscience. Likewise, the theoretical reflections are all associated in the paradigm of museums rebuilders, in the perspective of citizenship education in schools and communities.

Keywords: museum, transnational and citizenship education

## **The Living Book: the myth of the Ideal Perfect Library at *Prosas Portuguezas* of Rafael Bluteau**

**Ana Araújo Rafael**

Faculdade de Letras, Universidade do Porto, Portugal

The present investigation is about the library in Portuguese Literature of the XVII and XVIII centuries. Especially the myth of the ideal perfect library in *Prosas Portuguezas* of Rafael Bluteau.

This study gets a symbolic charge, who is intimately connected with it tripartition.

In a first part we will demonstrate the Man and the human perfection. Rafael Bluteau, as a «living book», through the ideal society (Academy) and the three perfect times (past/present/future).

In a second part we will start with the Library and the divine perfection, to get in to the edge of the mountain (Nature) and the edge of the ziggurat (Art).

In a third part we will expose the Scholar and the knowledge's perfectibility, so we can cross the myth of the ideal perfect library (universal and particular), giving place to a kind of mental Bluteau's Library (the construction of the catalogue, based in a kind of garden's project).

The main purpose of this work is to fuse, as an alchemical step, the Man, the Library and the Scholar. At the amalgam of these three substances, stands the perfection secret, either in the infinite places of the universal library, either in the delimited gardens of the private library.

## Structure and textured panels for modular architectural surfaces

**J. Cordeiro<sup>1</sup>, G. Furtado<sup>2</sup>, M. Lucas<sup>3</sup>, P. Joel<sup>2</sup> and J. Lino<sup>2</sup>**

<sup>1</sup> Faculty of Architecture, University of Porto, Portugal.

<sup>2</sup> Faculty of Engineering, University of Porto, Portugal

In a contemporary culture characterized by new interfaces, it emerges the notion of an interactive environment, which expresses the idea of a multisensory and multidimensional configuration. This project is inspired and reflects the infinite possibilities that interaction stimulates.

The object of this research project is the study of structures and textured panels for modular architectural surfaces, which will adapt to different contexts and spatial configurations. The project is focused on the areas of architecture and design, however the approach requires developing a close relationship with the areas of material sciences and computing.

Broadly speaking, the investigation covers the draft architectural modular interior surfaces including the structure and textured panels. These will be modeled computationally in order to manufacture and subsequently assemble the various components. Therefore, the designed components will respond to functional requirements and resistance, aesthetic form, as well as cost. There is to mention the importance of different authors of which we highlight Agkathidis,<sup>1</sup>. The project, as an holistic system, will pursue the achievement of effective contemporary results, both functionally and aesthetically. There will be a prototype, using various techniques of "manufacturing" (including the aforementioned CAM and rapid prototyping<sup>2</sup>) as well as the integration of interactive dynamics.

Innovative results will be obtained, in terms of design and materiality of the system as well as electronic interfaces, which will be integrated into the surface in order to confer interactivity. The characterization described suggests a modular composition, allowing easy assembly and storage. The surface will be characterized by its innovative design and materiality, as well as playful dynamic performances. Furthermore, an attention to visual and tactile aspects will not be overlooked.

The structure and textured panels of garment will be constructed in several materials, including composites. The "modeling" process includes CAD software and computer systems, related to CAM.

This multidisciplinary research project, involving the design of the system itself including the surface structure and textured panels, aims to achieve a multi-functional configuration that interacts in an appealing, meaningful and dynamic way.

### References:

- [1] agkathidis, asterior, "modular structures in design and architecture", bis, publishers, amsterdam, 2009
- [2] alves, f; jorge lino; braga, F., jorge sousa, simão, manuel; neto, r. jorge de lenor; duarte, t.m. guerra pereira; prototipagem rápida, protoclick, fevereiro 2004, leça do balio.

# Assessing the Efficiency of Portuguese Banks using Data Envelopment Analysis

A. Ribeiro<sup>1</sup>, M. Loureiro<sup>1</sup>, R. Fardilha<sup>1</sup> and A. Brochado<sup>1</sup>

<sup>1</sup> Faculty of Economics, University of Porto, Portugal.

The Portuguese banking sector has been recently subjected to important structural changes associated with the diversification of the supply of financial services and the emergence of new technologies. Furthermore, new challenges have arrived from the actual economic recession scenario.

This study aims at investigating the efficiency performance of the commercial banking sector in Portugal in 2009 and 2010. Moreover, we intend to compare the obtained results with previous studies [1]. A second objective of this paper is to quantify the relative efficiency of domestic banks as compared to that of foreign banks.

Data envelopment analysis is used to identify banks' levels of performance. In order to test for differences of efficiency scores between national and foreign banks hypotheses tests were used. We found an improvement on technical and scale efficiency from 2009 to 2010. Our results also revealed that foreign banks were more efficient both in 2009 and 2010 than national banks. The results should be of interest to management in banking and others studying bank performance trends.

## References:

Figueira, C. , Nellis, J. and Parker, D. (2009). *Banking performance and technological change in non-core EU countries. A study of Spain and Portugal*. Studies in Economics and Finance, Vol. 26 (3), pp. 155-170

## Preliminary forensic science study of dispersion in sawed-off shotgun

**T. Cunha<sup>1</sup>, M. Sousa<sup>1</sup>, A. Lopes<sup>2</sup>, V. Teixeira<sup>3</sup>**

<sup>1</sup>Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

<sup>2</sup>Department of Sociology, Faculty of Arts, University of Porto, Portugal

<sup>3</sup>Department of Shooting and Fire Arms, Judicial Police Directory of North, Porto, Portugal

The evaluation of the dispersion in smooth-bore weapons is fundamental to the forensic research applied to the practice of crimes/accidents. This study focuses on the dispersion in sawed off shotguns.

The main objective is to determine dispersion patterns as a function of the variables studied, framed in a possible scenario of the crime with a gun of this type. Through these patterns we propose to measure the distance of firing of projectiles, as this type of weapon is often used in criminal practices. The cutting of barrels was performed with the objective of substantially reducing the length of the weapon, facilitating their dissimulation.

Were used five key variables in the study of the process of dispersion of multiple projectiles: Length of the barrel; distance to the target; the type of load of ammunition, type of wad of ammunition and the type of lead grains used in each ammunition as multiple projectiles. In order to ensure the accuracy and reliability of the data each shot was repeated three times in order to decrease the effect of sampling error. 3120 shots were made covering the following conditions: barrel size (72.6 , 55.7 , 40.4 and 25 cm); and barrel distance to the target (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 m); Ammunition (Type of load 28, 32, 34 and 36 grams), (Type of wad - plastic cup, plastic cup dispersant, felt and paperboard), (Grains of lead - type 1; 5; 6; 7.5 ; 8 and SG). Paper targets were used to preserve the dispersion pattern traces derivatives of firing. Targets had 1m<sup>2</sup> with 200g of weight in order to ensure the necessary resistance of the role and to prevent degradation.

The measurements led to the following conclusions: after statistical treatment using the techniques of multiple linear regression, consistent and significant results were observed with an high degree of security. A well marked linear dispersion was observed, where the variable "*distance to target*" emerged in relation to the other, as a promoter of the dispersion. On the basis of these results, it was possible to observe the relevance of each variable in the process of dispersion, as well as to establish an intimate relationship between them. In the future, given a pattern from the traces collected, it will be possible to assess how a given shot was carried and to establish the connection between the dispersion and the distance of firing.

## RNA profiling for the determination of *post-mortem* interval

**F. Silva**<sup>1,2</sup>, **R.J. Dinis-Oliveira**<sup>3,4</sup>, **R. Silvestre**<sup>2</sup>

<sup>1</sup> ISCS-N Instituto Superior de Ciências da Saúde, Norte, Gandra, Portugal.

<sup>2</sup> IBMC Institute for Molecular and Cell Biology, Porto, Portugal

<sup>3</sup> Faculty of Medicine, University of Porto, Porto, Portugal

<sup>4</sup> Advanced Institute of Health Sciences – North, CESPU, CRL

The determination of the *post-mortem* interval (PMI) is a major focus of investigation in forensic sciences. Forensic pathologists commonly use a panel of physical markers such as algor, rigor and liver mortis to determine the PMI. Nevertheless, these methods only provide us with a mere approximation, especially during the first hours after death [1]. RNA analysis offer insights into diseases and mechanisms leading to death, and could develop into a valuable tool for diagnosis of the cause of death in forensic pathology. In this context, RNA potential has been studied for several purposes, which include the identification of body fluids, determination of the age of biological stains by means of RNA decay analysis and determination of the PMI [2,3]. Therefore, we hypothesized that a comparative analysis of organ-specific mRNA and miRNA decay might be used as a cooperative indicator of the PMI. The aim of this study was to correlate the degradation of several mRNA transcripts in different organs with the PMI using a murine model. We have performed a twelve hour kinetic analysis of extracted total RNA from the heart, muscle, liver and pancreas with a checkpoint at every single hour during the considered period. The degradation profile of total RNA was evaluated by RNA electrophoresis (Experion™ Bio-Rad) through the RNA integrity number (RIN/RQI) value. The expression levels of several housekeeping genes (Tropomyosin, Albumin, Beta-Actin, GAPDH and 18S rRNA) were analyzed by quantitative real-time PCR and expressed by threshold cycle (Ct) value. Our results clearly demonstrated a time dependent decrease of the RQI values that was nevertheless variable accordingly to the organ. mRNA analysis evidenced an organ specific transcript degradation perfectly corroborated through the respective Ct-PMI and Ct-RQI correlations. A study of such nature might provide a new basis for estimating PMI and eventually become a complementary tool to traditional methods. In the future, we will expand this analysis to miRNA transcripts that has proved its advantages as compared to mRNA profiling [1,4].

[1] Maria de Fátima Terra Pinheiro (org.). (2008), *CSI Criminal*. Edições Universidade Fernando Pessoa. Porto.

[2] C. Courts, M. Madea (2010), *Micro-RNA – A potencial for forensic science?*, Forensic Science International 203, 106-111

[3] M. Bauer, I. Gramlich, S. Polzin, D. Patzelt (2003), *Quantification of mRNA degradation as possible indicator of postmortem interval-a pilot study*, Legal Medicine 5, 220–227

[4] M. Vennemann, A. Koppelkamm (2010), *mRNA profiling in forensic genetics I: Possibilities and limitations*, Forensic Science International 203, 71–75

## A Adegas do Olho é Aqui: A Context of Rural-Urban Symbiosis

F. Dias<sup>1</sup>, G. Marques<sup>1</sup> and S. Sousa<sup>1</sup>

<sup>1</sup> Third year student in the First Cycle of Studies in Sociology, Faculty of Arts, University of Porto, Portugal.

This research aimed to analyse a context of cultural dynamics and social interactions in an urban public space that at the same time is crossed by a strong component of rurality. As A. Giddens [1] refers, the study of the everyday life can be a microscope watch that illuminates the interpretation of systems and broader social institutions.

The methodology of this investigation was based on a qualitative nature that combined, on the one hand, the technique of direct observation, with a set of incursions into the social context for visual observation and written record of the behaviour of the social actors and, on the other hand, the application of *photovoice*, an innovative technique that connects the photographic record and a discussion with the actors of the social context about their own existence.

The selected empirical context was *A Adegas do Olho é Aqui*, located at Flores street, near the São Bento train station. It's a century year old restaurant that has strong traits of a tavern, but that at the same time manages to have a family atmosphere, a traditional and rustic ambience and with unique sounds and smells

Regarding the customers profile, the observations pointed to a place mostly attended by male individuals, aged 18-60 years, from various social backgrounds and with diverse symbolic universes [2].

In regards to the main conclusions, it was notorious that the main sociability held within the establishment was dominated mostly by rural aspects, but that still managed to intertwine with the urban context where it is located, and so being a good example of integration on the rural-urban axis. It also demonstrated the empirical applicability of the theatrical metaphor of E. Goffman [3], one of the main theoretical foundations of this work. It was possible to identify the concepts of actor, audience, stage, backstage and front space in which the employees worked, structured routines and interacted with the customers.

References:

- [1] Giddens, Anthony (2009), *Sociologia*. 7<sup>th</sup> ed, Fundação Calouste Gulbenkian, Lisbon.
- [2] Berger, Peter L; Luckman, Thomas (1999), *A construção social da realidade*, Dinalivro, Lisbon.
- [3] Goffman, Erving (1993), *A apresentação do eu na vida de todos os dias*, Relógio d'Água, Lisbon.

## **Development a GIS-Based online Database for Municipality Emergency Planning Service**

**L. Martins<sup>1,2</sup>, M. Santos<sup>1,2</sup>, C. Bateira<sup>1,2</sup>, F. Matos<sup>1</sup>, M. Teixeira<sup>1</sup> S. Moreira<sup>1</sup>, A. Costa<sup>1</sup>**

<sup>1</sup> Department of Geography, Faculty of Arts, University of Porto, Portugal.

<sup>2</sup> DyNat/CEGOT - Natural Processes Assessment, Modelling and Mapping/Centro de Estudos em Geografia e Ordenamento do Território

The study of Natural and technological Hazards in the context of Emergency Planning and Civil Protection, is one of the biggest concerns of contemporary societies.

Although the natural dynamic has become highly destructive, Mankind has promoted the acceleration and development of this type of processes.

The aim of Emergency Plans is to reduce the vulnerability of potential disasters. Therefore, the constitution of an integrated database (i.e Agents, support resources, occurrences records) becomes crucial in risk mitigation process.

The Database was structured through Microsoft Access®, and GIS platform. The basic information was gathered from the Operational Programs of Emergency Plans, institutional information available on internet and field work using the differential GPS.

The data base is composed by Civil Protection Local Agents , entity wich relates with the Vehicles (Forest Fire Fighting, Urban Fire Fighting, water support equipment, Medical Emergencies, etc.), Assistance Spaces for Emergency Support, Emergency Support Buildings and Other Equipment. The database preparation intended to register as much information as possible, obtained by all the means of support, mainly the Civil Protection Local Agents.

Subsequently, with recourse to the XML/HTLM language, this database was transposed for the opensource web platform server Google Maps®. Each element was georeferenced in WGS84 coordinates system and geometrically represented by point connected with the information contained in the database (address, contacts, type of equipment, characteristics, capacity, etc.).

It was also linked through the online database, information about road accidents, associating to each black spots information about location, number of accidents, number of seriously injured and slightly injured number of dead's, photos and road characteristics. The main advantage of this database is to gather and make available all the information concerning means of support and the Civil Protection Local Agents.

It can be easily accessed through the form option, where one can perform searches, queries and obtain the reports. On the other hand, there may be a constant updating by each entity with responsibilities in Municipal Emergency Planning, in order to easily contribute to modify and maintain whenever possible updated information of this service, through an common internet account under the coordination of a Municipal Service of Civil Protection technician that previously has validated all the information and then publish the final reporting. POSTER

## J. J. Forrester, knowledge and contribution in the nineteenth century cartography of the Douro region

M. Ribeiro<sup>1</sup>, M. Carvalho<sup>2</sup>, and P. Henriques<sup>3</sup>

<sup>1, 2 and 3</sup> Department of Geography, Faculty of Letters University of Porto, Portugal.

Joseph James Forrester lived in Portugal in a less favourable time, nevertheless he became interested in the Douro region in a very particular way. This young Scotsman, saw, recorded, made known, analyzed, and looked to the future of the Douro region: the extension of the wine region to the Upper Douro, a more diverse and better wine production and also the normalization of flow of the Douro river.

It was this wine merchant who, besides having the Douro demarcated, investigated the problem of vineyards and disapproved the producers who adulterated the wine quality in times of economic crisis, saying the Douro internationally during the thirty years that remained here.



J.J. Forrester (1809-1861), came to live in Porto in 1831 (at 22 years old) in the house of his uncle James Forrester at the Largo da Ramada Alta (housing that has been demolished). His uncle worked at the firm *Offley, Webber, Forrester & C.*<sup>a</sup> since 1803, however the company existed since 1737. In 1853 J.J. Forrester moved to V.N. de Gaia. Toponyms in Porto (20 April 1940), V. N. de Gaia and Régua have been assigned to this personality.

The interest and passion for the Douro region is demonstrated by the numerous expeditions along the river. For that he ordered the construction of a large and luxurious “Rabelo” boat, where he spent hours drawing and redrawing the banks of the Douro.

In addition of being dealer and winemaker he was also cartographer, watercolorist, photographer, painter. The liberal and painter João Batista Ribeiro inspired him.

In 1855 he received the title of Baron of Forrester in recognition of “*transcendent value to this country*” shaped in his mapping work. His work is not extensive but it is of enormous cartographic value: Geological Map of the Douro river banks, Topographical charter of the Douro river course and Alto Douro wine region map.

So, after taking knowledge of the J.J. Forrester cartographic work it can be shared the view of the deputy Joaquim Vieira de Magalhães, in 1843 when he classified as “*an excellent and perfectly finished work, rich by the abundance of original information, (and) by its accuracy and beauty of execution*”. It can be considered that his cartographic contribution as well as pioneering was important to showcase the region, including the navigability along the Douro river.

### References:

Forrester e a Cartografia Vinhateira do Douro”, com Didiana Branco, in Barão de Forrester. Razão e Sentimento. Uma História do Douro, 1831-1861, coordenação de Isabel Cluny, Peso da Régua, Museu do Douro, 2008, p. 68-85.

LIMA, José Joaquim da Costa, (1940) *O Cachão da Valeira e o Regime Económico-Demográfico da Região do Douro*, Porto.

# Progressive floods along the valley of the Arda river: implications on the land planning in the Arouca municipality (Portugal)

**I. Marafuz**<sup>1</sup>

<sup>1</sup> Department of Geography, Faculty of Letras, University of Porto, Portugal.

Floods are a global phenomenon with significant impact in terms of human and economic losses (Jonkman & Vrijling, 2008). In order to mitigate these events, some methodologies have been developed to delimit flooded areas, according different return periods.

The most commonly used methods employ reasoning and mathematical formulas that allow the pre-determination of peak flood flows. Thus, initially in our case study, we used the kinematics formula of Giandotti, however, the calculated values were too much for the size watershed of the Arda river basin (168 km<sup>2</sup>).

Therefore, we used a statistical method widely used in Portugal – the Loureiro method – that takes into account the area of the basin and two regional parameters with values set by the author, according the area of the country and the return period considered (Lencastre & Franco, 1984). Although, the method does not consider some important features such as the watershed time of concentration or duration of precipitation, the results are close to reality.

The following procedure involved the topographic survey of the cross sections of the river and of the flood plain, using a total station and a high precision GPS in areas of lower visibility where the presence of vegetation was an important obstacle. In order to refine the data obtained by these two devices, it was adapted a reference system relating the local topographic surveying with three geodesic vertices placed very near of the study area.

The river channel and floodplain characteristics are extremely important as they constitute the basis for the calculation of hydraulic parameters used in determining the free surface flow (Manning-Strickler formula).

Finally, we used a range of optimization available in Excel spreadsheet (Solver), in order to obtain the heights of the water flow corresponding to the previously calculated for each section.

The result, gives us a map with the perimeters of flooding on the alluvial plain of the Arda river, for the return periods of 10, 50 and 100 years. The flood perimeters delimited reveal the presence of homes in flooded areas, a fact confirmed by photographs obtained in local newspapers regarding the 2001 centennial flood. The methodological approach and the mapped results turn out to be an important contribution to the planning and evaluation of surface water resources.

## References:

- [1] Jonkman, S.N. and Vrijling, J.K. (2008), *Loss of life due to floods*. Journal of Flood Risk Management. Vol. 1. pp. 43-56.
- [2] Lencastre, A. and Franco, F. M. (1984), *Lições de Hidrologia*. Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa.

## Road networks – contributes for a multi-risk analysis

**A. Costa<sup>1</sup>, C. Bateira<sup>1,2</sup>, E. Pacheco<sup>1</sup>, F. Matos<sup>1</sup>, L. Martins<sup>1,2</sup>, M. Teixeira<sup>1</sup>, M. Santos<sup>1,2</sup> and S. Moreira<sup>1</sup>**

<sup>1</sup> Department of Geography, Faculty of Arts, University of Porto, Portugal.

<sup>2</sup> DyNat/CEGOT - Natural Processes Assessment, Modelling and Mapping/Centro de Estudos em Geografia e Ordenamento do Território

The territory is constantly changing, so rapidly that the different agents that are responsible for its planning aren't able to keep up with the changes. In this context, the knowledge about the road network becomes important for territory planning by the local authorities.

Using the road network of Trofa municipality as a starting point (in Oporto district, in the north of Portugal), mapped using 2008 fieldwork, some initial goals were set, namely the update of the road network, the analysis of drivers' bad behavior and the conservation condition of the networks.

Employing Geographic Information Systems (GIS) we chose to first perform the road network update, using aerial photos orthorectified shot in later dates to the road network production. In the next step we used GPS in the field work to get the road track and directions [1]. This initial work allowed several vectorization errors to be corrected in the original cartography and as a result we got a road network with an updated morphology.

With the traffic data provided by 'Estradas de Portugal', traffic accidents data given by GNR and the drivers' behavior observation we were able to analyze the accident number at each node, the total traffic belonging to the two main roads of the municipality (EN 14 and EN 104) and improper behavior from the road users.

This analysis allowed us to draw some conclusions, namely, that EN 14, one of the most important roads of the municipality, has a monthly traffic superior to 400 000 vehicles, from which about 20% are driven above the speed limit, which helps to justify the fact that 90% of the registered accidents are collisions, since the increase of the velocity, reduces substantially the vision angle and the reaction time from the drivers [2].

On the other hand, the joining of two important roads, like EN 14 and EN 104, in the urban center of the analyzed municipality, causes frequently traffic congestions and accidents. Applying a small spatial modeling exercise, where we used the kernel interpolator [3], it was possible to check that a great part of the road accidents occur next to the urban space of Trofa, precisely around the crossroad of the two roads mentioned above.

References:

[1] A. Fathi, J. Krumm, Detecting Road Intersections from GPS Traces, in: S.I. Fabrikant, T. Reichenbacher, M. VanKreveld, C. Schlieder (Eds.) *Geographic Information Science*, Springer-Verlag Berlin, Berlin, 2010, pp. 56-69.

[2] A. Costa, J. Macedo, *ENGENHARIA DE TRÁFEGO: CONCEITOS BÁSICOS*, CCDRN, Porto, 2008.

[3] A. Okabe, T. Satoh, K. Sugihara, *Int. J. Geogr. Inf. Sci.*, 23 (2009) 7-32.

# **Tectonic Geomorphology in the Fornelo Valley (Amarante) and some implications for planning**

**H. Teixeira**<sup>1</sup>

<sup>1</sup> Department of Geography, Faculty of Arts University of Porto, Portugal.

This paper presents the results of a tectonic geomorphologic study, focused on the valley of the Fornelo river, a tributary of the Tâmega river. The area under analysis is between the city of Lixa (Felgueiras municipality) and the interfluvium to the northwest where the Teixeira village is located (Baião municipality), separating this sector from the Douro valley.

Based upon a general analysis of the geomorphologic context related to this old shear zone, an interpretation is proposed for the present-day morphology. Therefore, an evaluation of the importance that structural conditioning implies on present day topography is performed.

In order to achieve this, some field work was carried out supported by intensive deskwork treatment of data, both oriented towards the interpretation of the terrain morphology in its relation with the structural characteristics of the area.

The methodologies applied enable the production of several maps exploring the potential of GIS technologies, notably in the definition of structural lineaments. Still in this context, a new geomorphologic map and an inventory of springs represent important results, allowing the definition of the main tectonic compartments. Through the integration of all collected data, a main deformation zone was also identified, called the PDZ (principal displacement zone) of the Fornelo fault.

The main conclusions show that the relief of this area is controlled by an inherited tectonic structure, namely, by three fundamental faults that determine the main topographic structures and the configuration of the main river network, according to the directions NW-SE, NE-SW, and NNE-SSW. The valleys of the Fornelo river as well as some of its tributaries are mostly structural fracture valleys, presenting themselves as some depressions of sigmoid pattern which add up along the shear zone.

# Transit-Oriented Development – a methodological test for the city of Porto

**R. Fernandes**<sup>1</sup>

<sup>1</sup> Department of Geography, Faculty of Arts, University of Porto, Portugal, argf84@gmail.com

Defined as a mixed centre of high densities and different uses, namely residential, of commerce and services, and in which core there's a transportation hub that can be easily reached from the nearest residential areas [1], the Transit-Oriented Development (TOD) can be defined as a principle concerned with the development of the cities and the planning that should lead to a more efficient land use and the use of the transportation patterns. This principle is based on three key elements: land uses, high densities, and transportation networks.

Reanalysing the TOD concept within the consolidated urban areas, having as main goal the contribution to a reflection regarding urban mobility, we have selected the main indicators for the construction of TOD localization scenarios, and we tested a methodology in order to identify these scenarios in the city of Porto. There were made various simulations, where GIS technologies were used, in two different levels of analysis, in a set of combinations of variables. Regarding the Greater Porto, the test integrated two variables, which were the population density and the areas that are served by the existing transportation structures, taking into consideration some particular aspects such as time and distance. Regarding the city of Porto, the simulations that were made came with the addition of one more variable of great complexity – the demand generators density.

The scenarios that were obtained reveal that, within the Greater Porto's area, the municipality of Porto is the one that gathers the best conditions for identifying TOD areas, followed by the municipalities of Matosinhos, Maia, and Vila Nova de Gaia. However, the most important piece of information to retain from these scenarios is the identification of Porto's city centre as the area which has the highest TOD potential, i.e., it's the area where there are, simultaneously, the majority of the elements that were taken into account within the variables that were used.

In fact, the variables of population density and transportation structures worked as the most decisive elements in the identification of the TOD areas, while the role of the demand generators density was to refine the larger scale simulations.

This methodological test allows us to delineate ideas and recommendations concerning the spatial planning in urban areas with a strong population density and a strong historical background, namely concerning the importance of reinforcing the residential spaces in areas that are potentially ideal for the localization of the TOD's, and/or concerning the importance of conducting detailed studies that allow to build a TOD network which is capable of integrate the means and modes of transportation in a perspective of mobility promotion.

References:

[1] Littman, T. (2011), *Evaluating Criticism of Smart Growth*, Victoria Transport Policy Institute, Victoria.

## Functional and pharmacological studies of p53 family proteins: screening for selective small-molecule modulators

M. Leão<sup>1</sup>, D. Costa<sup>1</sup>, J. Soares<sup>1</sup>, C. Bessa<sup>1</sup>, C. Pereira<sup>1</sup> and L. Saraiva<sup>1</sup>

<sup>1</sup>REQUIMTE, Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Portugal

Several functions initially attributed to p53 only are now shared by other members of the p53 family. The p53 family consists of three members, p53, p63 and p73, which give rise to multiple protein products from both alternative promoter and mRNA splicing. Transcription of p63 and p73 can occur from two distinct promoters resulting in two types of proteins with opposing functions: p53-like proteins with a N-terminal transactivation (TA) domain (TAp63 and TAp73) and proteins lacking the N-terminal TA domain ( $\Delta$ Np63 and  $\Delta$ Np73). Additional complexity is caused by the alternative splicing of p63/p73 transcripts, generating a variety of TA and  $\Delta$ N isoforms (being  $\alpha$ ,  $\beta$  and  $\gamma$  the most relevant variants) [1]. This high complexity of the p53 family and pathway, particularly the coexistence of several isoforms in a same mammalian cell, has hampered the knowledge of the biology and pharmacology of the p53 family members.

To circumvent this limitation, in the present project, the yeast *Saccharomyces cerevisiae* individually expressing human p53, TAp63 $\alpha$ , TAp63 $\beta$ , TAp73 $\alpha$ , Tap73 $\beta$  or  $\Delta$ Np63 $\alpha$  was used. Our previous work showed that human p53 caused a marked yeast growth inhibition associated with S-phase cell cycle arrest [3]. Also, as reported in mammalian cells [2], we showed that human MDM2/MDM4 (major endogenous negative regulators of p53 activity) reduced the p53 effects on yeast growth/cell cycle. In this work, the expression of TAp63 $\alpha$ , TAp63 $\beta$ , TAp73 $\alpha$ , Tap73 $\beta$  or  $\Delta$ Np63 $\alpha$  in yeast was confirmed by Western blot analysis. Moreover, the effects of these proteins on yeast cell growth and death have been analysed and compared with those obtained with p53. Finally, the effects of MDM2/MDM4 on the activities of these p53 family members are under study.

In conclusion, with an independent analysis of major p53 family members we expect to provide new insights about their functional and molecular profiles, as well as to accelerate the discovery of small-molecule modulators of each p53 family member.

### References

- [1] Vilgelm A., El-Rifai W., Zaika A. (2008), *Therapeutic prospects for p73 and p63: rising from the shadow of p53*, Drug Resistance Updates, 11 (4), 152-163.
- [2] Toledo F and Wahl G. (2007), *MDM2 and MDM4: p53 regulators as targets in anticancer therapy*, The International Journal of Biochemistry and Cell Biology, 39 (7-8), 1476-1482.
- [3] Coutinho I., Pereira G., Leão M., Gonçalves J., Côrte-Real M. and Saraiva L. (2009), *Differential regulation of p53 function by protein kinase C isoforms revealed by a yeast cell system*, FEBS Letters, 583 (22), 3582-3588.

This work was supported by REQUIMTE (grant no. PEst-C/EQB/LA0006/2011), FCT and FEDER funds through the COMPETE program under the project FCOMP-01-0124-FEDER-015752 (ref FCT PTDC/SAU-FAR/110848/2009), and by U.Porto/Santander Totta. M. Leão (SFRH/BD/64184/2009) and J. Soares (SFRH/BD/78971/2011) are recipients of FCT fellowships.

# IN THE RAT BRAIN CORTEX NMDA RECEPTOR-EVOKED NORADRENALINE RELEASE IS INHIBITED BY ADENOSINE A<sub>2A</sub> RECEPTORS: MECHANISMS INVOLVED

<sup>1</sup>Diva Silva, <sup>1</sup>Diana Pinho, <sup>1</sup>Filipa Sardo, <sup>1</sup>Clara Quintas, <sup>1</sup>Glória Queiroz

<sup>1</sup>Laboratory of Pharmacology, Department of Drug Sciences, REQUIMTE, Faculty of Pharmacy, University of Porto, PORTO

In the rat brain cortex, noradrenaline (NE) release can be stimulated by activation of local NMDA receptors [1]. In some striatal neurons, NMDA receptors located in cell bodies are modulated by activation of adenosine A<sub>2A</sub> subtype which inhibited the opening of these channels [2]. The aim of this study was to investigate, in the rat brain cortex, whether NE release evoked by NMDA receptor-activation is also modulated by adenosine receptors, namely the A<sub>2A</sub> receptor subtype and the intercellular signalling pathway activated.

Cortical occipital-parietal slices from male Wistar rats were incubated with 0.1 μM of [<sup>3</sup>H]-NE, superfused with a physiological solution and stimulated by two bolus (S<sub>1</sub> and S<sub>2</sub>) of 30 μM NMDA applied by 2 min. In all experiments Mg<sup>2+</sup> was omitted from the superfusion medium being replaced by equimolar concentration of NaCl. The [<sup>3</sup>H]-overflow was used as an indicator of NE released and the effects of drugs were calculated as S<sub>2</sub>/S<sub>1</sub> ratios and expressed as % change (increase or decrease) from respective controls as mean ± S.E.M from *n* slices.

NMDA-evoked NE release was abolished by omitting Ca<sup>2+</sup> from the medium and by blockade of voltage-sensitive Na<sup>+</sup> channels with 0.3 μM tetrodotoxin, indicating that is dependent on vesicle exocytosis. Adding 1.2 Mg<sup>2+</sup> or blocking NMDA receptors with the non-selective ionotropic glutamate receptor antagonist kynureate (0.5 mM) or the selective antagonist MK 801 (50 μM) also abolished NMDA-evoked NE release.

Under these conditions, the selective adenosine A<sub>1</sub> agonist CPA (1 μM) and the selective adenosine A<sub>2A</sub> agonist CGS 21680 (0.1 μM) inhibited NE release by 38±5 % (n=6; *P*<0.05) and 31±5 % (n=6; *P*<0.05), respectively. The effect of CPA was abolished by the selective A<sub>1</sub> receptor antagonist DPCPX (30 nM) whereas the effect of CGS 21680 was abolished by the selective A<sub>2A</sub> receptor antagonist SCH 58261 (30 nM). Neither DPCPX (30 nM) nor SCH 58261 (30 nM) changed NMDA-evoked NE release. The inhibition of NMDA-evoked NE release caused by CGS 21680 (0.1 μM) was not changed in the presence of the protein kinase A (PKA) inhibitor H-89 (1 μM; 28±5 %, n=6, *P*<0.05). However it was abolished by the phospholipase C (PLC) inhibitor U-73122 (1 μM) to 3±5 % (n=6, *P*<0.05) but not by its inactive analogue U-73343 (1 mM; 30±5 %, n=6) and it was also abolished by the protein kinase C (PKC) inhibitor RO 32,0432 (1 mM; 2±4 %, n=6, *P*<0.05) and by the calmodulin kinase II inhibitor (CAM kinase II), KN-93 (3 μM) to 7±2 % (n=6, *P*<0.05).

The results show that adenosine A<sub>1</sub> and A<sub>2A</sub> receptors inhibit NMDA-evoked NE release in cortical brain slices. It is also suggested that the A<sub>2A</sub> receptors are coupled to an intracellular signalling mechanism that involves the PLC-PKC pathway and activation of CAM kinase II, as observed in the striatal neurons [2].

[1] Kittner H et al. *Prog. Brain Res.*, 1999, 120:223-235.

[2] Wirkner K et al., *Br. J. Pharmacol.*, 2000, 130:259-269.

*This work was supported by Fundação para a Ciência e a Tecnologia through grant N°. PEst-C/EQB/LA0006/2011 e Projetos pluridisciplinares para iniciação à investigação científica da UP.*

## ***In vitro* radical scavenging potential of *Colocasia esculenta* (L.) Shott.**

**A. M. Silva<sup>1</sup>, R. F. Gonçalves<sup>1</sup>, P. Valentão<sup>1</sup>, D. Santos<sup>2</sup>, J. Baptista<sup>3</sup> and P. B. Andrade<sup>1</sup>**

<sup>1</sup> REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal.

<sup>3</sup> Madeira Rochas, Madeira, Portugal.

Natural products discovery and medicine are closely linked by the use of medicinal species for thousands of years. Studies primarily using herbal extracts were the basis of the first drugs and, nowadays, it is still one of the strategies followed by researchers to discover new bioactive compounds from plants already used in traditional medicine. *Colocasia esculenta* (L.) Shott. is an annual herbaceous plant with a long use in traditional medicine in several countries across the world, especially in the tropical and subtropical regions. In Azores islands it is commonly called 'Inhame dos Açores'. It has been used in the treatment of various ailments, such as asthma, arthritis, diarrhea, internal hemorrhage, neurological and skin disorders [1]. The generation of reactive oxygen species (ROS) accompanies these disorders, triggering or being produced by them and leading to oxidative stress, which can be harmful for the human cells. Antioxidant compounds can scavenge these reactive species, providing protection against their deleterious effects.

The antioxidant potential of the aqueous extracts of two varieties of *C. esculenta* (giant white and red) was checked. A concentration-dependent effect was noticed against DPPH (IC<sub>50</sub> = 197 and 533 µg/mL for giant white and red, respectively), nitric oxide (IC<sub>50</sub> = 2311 and 935 µg/mL for giant white and red, respectively) and superoxide (IC<sub>50</sub> = 118 and 136 µg/mL for giant white and red, respectively) radicals (Fig. 1).

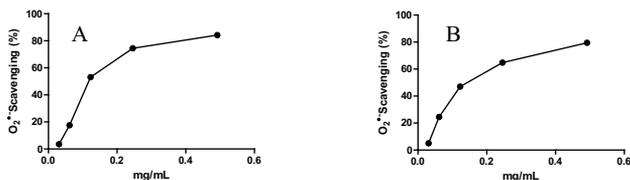


Fig. 1. Scavenging activity of *C. esculenta* aqueous extracts against superoxide radical: (A) giant white variety; (B) red variety. Results correspond to mean  $\pm$  standard error of three assays, performed in triplicate.

The results evidence differences in the scavenging capacity against the different reactive species, though both varieties exhibited best activity against superoxide radical. Further studies are being developed to characterize the metabolic composition of the extracts.

### References:

[1] Prajapati, R., Kalariya, M., Umbarkar, R., Parmar, S. and Sheth N. (2011) *Colocasia esculenta*: A potent indigenous plant. *International Journal of Nutrition, Pharmacology, Neurological Disorders*, 1, 90-6.

# **Influence of lidocaine hydrochloride and bovine serum albumin on physical characteristics and stability of thermo-responsive Pluronic® F127 hydrogels**

**S. Silva, M. H. Amaral, P. A. L. Lobão**

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Thermo-responsive polymers are macromolecules that undergo sol-gel transition in response to temperature changes<sup>[1]</sup>. Pluronics® or Poloxamers are thermo-responsive triblock copolymers with a central hydrophobic chain of polyoxypropylene (PPO) and two identical lateral hydrophilic chains of polyoxyethylene (PEO). Pluronic® F127 has been the most widely used thermo-responsive polymer in drug delivery studies.<sup>[2]</sup> Aqueous Pluronic® F127 solutions display low viscosity at ambient temperature but exhibit a sharp increase in viscosity following a small temperature rise, forming a semi-solid gel at body temperature<sup>[3]</sup>. The aim of this study was to evaluate the influence of two hydrophilic compounds, lidocaine hydrochloride and bovine serum albumin (BSA) on the physical characteristics and stability of Pluronic® F127 hydrogels. A comparative study with a control hydrogel (without active substance) was also carried out.

Hydrogels were prepared by dispersing the thermo-responsive polymer (20%, w/w) in cold water or in 1% (w/w) aqueous solutions of lidocaine hydrochloride or BSA, using a stirrer *Heidoph RZR 2041* (Germany). The dispersions were placed at 5-8°C during 48 hours and then were stored in an oven at 37°C in order to acquire semi-solid consistency. The physical characterisation and stability over time of hydrogels was evaluated after 8 and 30 days of storage by analysis of texture (firmness and adhesiveness) using a texture analyser *Stable Micro Systems, TA-Xt2i* (UK) and rheological behaviour using a viscosimeter *Thermo Haake VT-550* (Germany).

The results of rheological studies showed that all the formulations presented a reofluidificant or shear thinning behaviour (decrease of viscosity with the increase of shear rate). After 30 days of storage at 37°C the gel containing BSA showed a significant decrease of viscosity comparing to the hydrogel with lidocaine hydrochloride and the control. These results were in agreement with those obtained for texture analysis because the hydrogel containing BSA also showed a greater decrease of firmness and adhesiveness after 30 days of storage.

In conclusion, BSA seems to produce a greater change in physical characteristics and stability of Pluronic® F127 hydrogel than lidocaine hydrochloride.

## References:

- [1]Bawa,P.;PillayV.;Choonara,Y.;Toit,L.(2009) *Stimuli-responsive polymers and their applications in drug deliver*, Biomedical Materials 4 1-15.
- [2]Ricci,E.J.; Bentley,M.V.L.B.; Farah,M.;Bretas ,R.E.S.; Marchetti,J.M. (2002) *Rheological characterization of Poloxamer 407 lidocaine hydrochloride gels*, European Journal of Pharmaceutical Sciences 17 161–167.
- [3]Al-Tahami, K., Singh, J. (2009) *Smart Polymer based delivery systems for peptides and proteins*, Recent Patents on Drug Delivery & Formulation 1 65-71.

# Is the presence of BRAF mutation a marker of aggressive Papillary Thyroid Carcinoma?

A. Gaspar da Rocha<sup>1</sup>, Yuliana Eremina<sup>1</sup>, Ricardo Celestino<sup>1</sup>, P. Soares<sup>1,2</sup>

<sup>1</sup> Cancer Biology Group, Institute of Molecular Pathology and Immunology of the University of Porto,

<sup>2</sup> Department of Pathology, Medical Faculty, University of Porto

The activating mutation in the B-type Raf kinase (BRAF) encoded gene is the most common genetic alteration in thyroid cancer [1,2]. BRAF is a potent activator of the mitogen-activated protein kinase (MAPK) pathway and BRAF V600E mutation has been observed in 18 to 87% of thyroid cancers, mostly in Papillary Thyroid Carcinoma (PTC) [3,4].

Some studies suggest an association between the presence of BRAF V600E mutation and poor prognostic factors, such as older age, male gender, and lymph node metastases. However, several investigators have not found association between the presence of BRAF mutation and aggressive thyroid cancer phenotype [1,5]. This is relevant since if this association exists it can change the therapeutic management of the patients with thyroid cancer.

In this study, we intended to evaluate the prevalence of genetic mutations in BRAF and NRAS in a series of PTC and their nodal metastases, verify the association between the presence of mutations in these genes in primary tumor and corresponding metastases, and ascertained whether the mutation in the BRAF gene is related to more aggressive clinicopathological features of the tumors.

The study was made by analyzing 113 cases of thyroid carcinoma and their lymph node metastases comprising a total of 214 samples. DNA was extracted from microdissected paraffin-embedded samples. Genomic regions of interest for NRAS and BRAF were separately amplified by PCR and then subject to automated sequencing. The clinicopathological features included in the study were patient age, histotype, tumor size, vascular invasion, extrathyroidal extension, growth pattern, presence/absence of capsule, thyroiditis, multicentricity and inflammatory infiltrate. Statistical analysis was performed using the SPSS version 20.

The observed frequency of mutations in the BRAF gene in PTC was 47%, which is consistent with the frequencies described in the literature. A slightly lower prevalence of BRAF mutation was observed in the lymph node metastases: 34.5%. It was observed that 28% of patients showed discordance between the presence/absence of BRAF mutations in the tumor and the corresponding metastases ( $p=0.000$ ). The frequency of mutations observed in NRAS was 1.8% both in primitive tumors and metastases.

It was observed that the presence of mutations in the BRAF gene is not related to the dimensions of the primary tumor ( $p=0.990$ ) nor the existence of extrathyroidal extension ( $p=0.753$ ). However, it was found a statistically significant relationship (both in primary tumor and metastases) between the presence of BRAF mutation and vascular invasion ( $p=0.014$  and  $p=0.004$ , respectively) and histologic pattern ( $p=0.000$  and  $p=0.003$ , respectively).

Further studies, including follow-up data, are necessary in order to clarify if BRAF mutated PTC are more aggressive tumors.

[1] Chakraborty A, Narkar A, Mukhopadhyaya R, Kane S, D'Cruz A, Rajan MG. BRAF V600E Mutation in Papillary Thyroid Carcinoma: Significant Association with Node Metastases and Extra Thyroidal Invasion. Endocr Pathol. 2011 Nov 22.

[2] Xing M. BRAF Mutation in Papillary Thyroid Cancer: Pathogenic Role, Molecular Bases, and Clinical Implications. Endocr Rev. 2007 Dec;28(7):742-62.

[3] Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-54.

[4] Trovisco V, Couto JP, Cameselle-Teijeiro J, et al. Acquisition of BRAF gene mutations is not a requirement for nodal metastasis of papillary thyroid carcinoma. Clin Endocrinol (Oxf) 2008;69:683-5.

[5] Lee JH, Lee ES, Kim YS. Clinicopathologic Significance of BRAF V600E Mutation in Papillary Carcinomas of the Thyroid a Meta-analysis. Cancer. 2007 Jul 1;110(1):38-46.

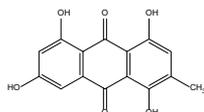
# Marine sponge-associated fungus *Eurotium cristatum*: a great source of anthraquinone derivatives

E.C.,Cardoso<sup>1,2</sup>, B. Macedo<sup>1,2</sup>, A.P. Almeida<sup>1,3</sup>, S. T. Dethoup<sup>4</sup>, N.G.M, Gomes<sup>5,6</sup>, M. Pinto<sup>1,2</sup>, A. Kijjoa<sup>5,6</sup>

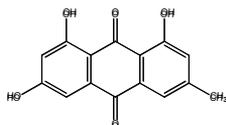
<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP);

<sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal; <sup>3</sup>LAEQUIFAR, Universidade Severino Sombra, RJ, Brasil; <sup>4</sup>Department of Plant Pathology Faculty of Agriculture, Kasetsart University, Bangkok, Thailand; <sup>5</sup>Instituto de Ciências Biomédicas de Abel Salazar; <sup>6</sup>CIIMAR, Universidade do Porto, Portugal.

Since always Nature was the main source of pharmacologically active compounds. The marine environment is recognized as an immeasurable source of natural products with a great potential for human therapies [1]. Our main goal is to study the chemistry and the antitumor potential of new secondary metabolites obtained from marine sponge-associated fungi collected from the Thailand sea, one of the richest in biodiversity in the world. In a previous study of our group with the extract ethyl acetate extract (ECE) of the marine-derived fungus *Eurotium cristatum*, four substances (compounds 1-4) were isolated. Two of them, physcion (compound 2) and erythroglaucon (compound 4), are anthraquinones [2-3]. Curiously, the HPLC study showed that physcion was found in high amount in ECE [3]. Here, we describe the isolation of two other substances, from the same extract. Successive purifications led to the isolation of compounds 5 and 6 (figure 1). Preliminary data indicates that the structure of compounds 5 and 6 are the anthraquinones, catenarin and emodin, respectively. Catenarin (5) was previously isolated from *Eurotium amstelodami* and *E. cristatum* from terrestrial source [4-5]. Emodin (6) was isolated from the mycelium of *Eurotium rubrum* [4]. To the best of our knowledge we are the first group to isolate these anthraquinones from the marine sponge-associated fungus *E. cristatum*.



Catenarin



Emodin

**Figure 1:** catenarin and emodin

[1] Blunt, J. W. *et al.* (2011), *Natural Product Report*, 28: 196-268.

[2] Almeida, A.P. *et al.* (2010), *Journal of Natural Pharmaceuticals*, 1: 25-29.

[3] Macedo, B. *Eurotium cristatum* isolated from the marine sponge *Mycale* sp.: Chemical and Biological Studies. Oporto: Faculty of Pharmacy from Oporto University; 2011. 121 p.

[4] Anke, H. *et al.*, (1980), *Archives of Microbiology*, 126: 223-230.

[5] van Eijk, G.W. (1972), *Cellular and Molecular Life Sciences*, 29 (5): 522-523.

Acknowledgments: CEQUIMED-UP (PEst-OE/SAU/UI4040/2011), FERDER, POCL, U.Porto/Santander Totta, for financial support.

## Mitochondrial metabolism and dynamics following exposure to deacetylase inhibitors in neurons

P. Guedes-Dias, R. Maia-Amaral and J. M. A. Oliveira

*REQUIMTE*, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Epigenetic modulatory drugs, namely, lysine deacetylase (KDAC) inhibitors, are putative neuroprotective compounds [1]. Their efficacy and neuroprotective mechanisms, however, remain controversial. Common mechanistic hypothesis include short-term effects (tubulin acetylation) and gene expression changes (inc. metabolic regulation). Here we further explore the pharmacological properties of KDAC inhibitors in cortical (*Co*) and striatal (*St*) neurons, testing their effects upon excitotoxic injury and modulation of mitochondrial metabolism and dynamics.

Primary cortical and striatal neurons were derived from E17-19 Wistar rat embryos and used at 10-14DIV, following short (2hr) or prolonged (3DIV) exposure to selective KDAC inhibitors (10 $\mu$ M AGK2, 10 $\mu$ M EX527 and 1 $\mu$ M MS275). Mitochondrial metabolism was inferred from resazurin metabolism in 96well plates, also used for estimating excitotoxic injury (10 $\mu$ M NMDA, 4hr, + 5 $\mu$ M MK801, 1hr). Mitochondrial dynamics was assessed by fluorescence videomicroscopy in neurons labelled with Fura-2 and Mitotracker. Data are mean $\pm$ SEM, *n* independent experiments.  $P < 0.05$ , ANOVA.

2hr exposure to AGK2, EX527 and MS275 induced a >40% decrease in resazurin metabolism in cortical neurons, whereas only AGK2 evoked a similar effect in striatal neurons. This decrease was not observed following 3DIV exposure, suggesting a transient effect. NMDA exposure decreased resazurin metabolism by ~50% both in control conditions and in neurons treated with KDAC inhibitors for 2hr or 3DIV, suggesting no protection from excitotoxic injury. Ratios of motile/total mitochondria were higher in control cortical vs. striatal neurites (*Co*: 0.32 $\pm$ 0.01; *St*: 0.25 $\pm$ 0.02, *n*=5;  $P < 0.05$ ). Total number of individual mitochondria/mm, however, was higher in control striatal vs. cortical neurites (*Co*: 250 $\pm$ 22; *St*: 288 $\pm$ 22, *n*=5;  $P < 0.05$ ), suggesting increased fission. The reduction of resazurin metabolism (taken as primarily mitochondrial in neurons) evoked by 2hr AGK2 exposure was not associated with differences in motile/total mitochondria in cortical or striatal neurites (*Co*: 0.29 $\pm$ 0.01; *St*: 0.22 $\pm$ 0.004, *n*=3-5;  $P > 0.05$  vs. control) and evoked a trend towards increase in mitochondria/mm in both neuron types (*Co*: 280 $\pm$ 31, *St*: 345 $\pm$ 31, *n*=3-5,  $P > 0.05$  vs. control), which fully reversed following 3DIV exposure (*Co*: 187 $\pm$ 26, *St*: 284 $\pm$ 6, *n*=2-5;  $P > 0.05$  vs. control).

Reversible effects of KDAC inhibitors on resazurin metabolism suggest short-term effects on neuronal mitochondrial metabolism, associated with mitochondrial fission. A plausible explanation for AGK2 findings is the transient inhibition of mitochondrial dehydrogenases via competition at substrate (e.g. NADH) binding sites.

Acknowledgements: Fundação para a Ciência e a Tecnologia (SFRH/BD/72071/2010, Guedes-Dias P); Universidade do Porto and Santander Totta, IJUP2010 #195.

References:

[1] Kazantsev, A.G. and Thompson, L.M. (2008) *Therapeutic Application of Histone Deacetylase Inhibitors for Central Nervous System Disorders*, Nature Reviews Drug Discovery, 7 (10), 854-68.

# Necrosis induced by carbon tetrachloride in the liver of a model organism: the zebrafish (*Danio rerio*)

**J. Coelho**<sup>1,2</sup>, **E. Rocha**<sup>2,3</sup>, **C. Cruzeiro**<sup>2</sup>, **A. Silva**<sup>2</sup>, **P. Silva**<sup>2,3</sup>

<sup>1</sup>Faculty of Sciences, University of Porto (U.Porto), Portugal.

<sup>2</sup>Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), CIMAR Associated Laboratory, University of Porto (U.Porto), Portugal

<sup>3</sup>Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto (U.Porto), Portugal

Most acute and chronic liver diseases are characterized by an excessive rate of death of both hepatocytes and other types of liver cells. During the last decades substantial progress has been made in the understanding of the necrotic signaling mechanisms in hepatocytes. Most of the experimental findings were obtained in rats and mice, using carbon tetrachloride (CCl<sub>4</sub>) as liver injury inductor. Fish models such as zebrafish (*Danio rerio*) or medaka (*Oryzias latipes*) were more recently used to study liver pathology. The generation of a fish model with a consistent pattern of hepatocellular necrosis is still lacking. The creation of such a model could contribute with new insights into the toxicological research. The aim of this study was, therefore, to analyze the liver histologic changes in response to a high dose of CCl<sub>4</sub>. For this purpose, adult zebrafish of both sexes were exposed to CCl<sub>4</sub> during 96 hours. A stock solution of 50 ml/L CCl<sub>4</sub> was prepared in ethanol at the beginning of the exposure. During the experiment, the stock solution was daily diluted in water, and used for the total renewal of the test solution in the aquaria. Final concentration of ethanol in the aquaria, in both treatment and control groups, was 0.001%. A control solvent-free group was added. A qualitative analysis was made in the liver, at light microscopy. Data point that the acute exposition to CCl<sub>4</sub> induced changes in hepatocytes of both zebrafish males and females. Those morphologic changes do not appear to be specifically located within the liver and included: cellular and nuclear pleomorphism, hydropic vacuolation and ballooning degeneration. Those changes assumed higher expression in the female liver, where perfectly defined circular foci of basophilic hepatocytes were exclusively found and in some cases associated to a necrosis area. In conclusion, these preliminary data showed that CCl<sub>4</sub> induced necrosis in the zebrafish liver in a random way. We now need to confirm the accuracy and reproducibility of this result, using both qualitative and quantitative approaches. We also intend to develop a liver dose-response regarding different CCl<sub>4</sub> concentrations. Herein, liver impacts of this toxicant varied with sex, therefore highlighting the importance of establishing different histopathologic endpoints for males and females in further toxicological studies. This is a very interesting result because of its biomedical and aquatic environmental implications, and consequently further investigation is warrant to understand the functional mechanisms behind the sex-related toxicity differences.

Acknowledgments: This work was financially supported by FEDER funds through the Competitiveness and Trade Expansion Program (COMPETE) and by National Funds via Fundação para a Ciência e a Tecnologia (FCT) – project ref. PTDC/MAR/70436/ 2006.

KEYWORDS: Liver; *Danio rerio*; Histology; Necrosis; Fish.

## ***Neosartorya spinosa*, a marine fungi isolated from the sponge *Rhizodermia* sp.**

**A. R. Faria<sup>1,2</sup>, F. Durães<sup>1,2</sup>, A. P. Almeida<sup>1,3</sup>, T. Dethoup<sup>4</sup>, M. H. Vasconcelos<sup>5,6</sup>, M. Pinto<sup>1-2</sup>, A. Kijjoa<sup>7</sup>**

<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP);

<sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal; <sup>3</sup>LAEQUIFAR, Universidade Severino Sombra, RJ, Brasil; <sup>4</sup>Department of Plant Pathology Faculty of Agriculture, Kasetsart University, Bangkok, Thailand; <sup>5</sup>Departamento de Ciências Biológicas, Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Portugal; <sup>6</sup>Cancer Drug Resistance Group, IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal; <sup>7</sup>Instituto de Ciências Biomédicas de Abel Salazar and CIIMAR, Universidade do Porto, Portugal.

The marine environment is one of the richest sources of new compounds and has gained considerable interest in the last years, considering the big number of isolated bioactive compounds. In particular, marine fungi are marine organisms with apparently great potential since they produce a vast and unique number of secondary metabolites [1]. Our main goal is to study the chemistry and the antitumor potential of new secondary metabolites obtained from fungi from the Thailand sea [2-3], one of the richest in biodiversity in the world. We present some preliminary results from the chemical and biological studies of the crude extract of *Neosartorya spinosa*, a marine fungus isolated from the sponge *Rhizodermia* sp. collected from Mu Ko Similan, Pang-Nga Province, in the Andaman Sea, Thailand. The crude extract was evaluated by the SRB assay in order to study its growth inhibitory activity on three human tumor cell lines: breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and melanoma (A375-C5). Preliminary results showed that the crude extract of *N. spinosa* was active in all three cell lines, with the values of GI<sub>50</sub> = 55.7 µg/mL, 60.3 µg/mL and 89.0 µg/mL for MCF-7, NCI-H460 and A375-C5 cells, respectively. The ethyl acetate extract of *N. spinosa* was extracted using water (EAE-NS, 24.5 g). Two fractions were obtained, the soluble (EAE-NS-1) and the insoluble one (EAE-NS-2). The fraction EAE-NS-2 was purified by chromatography, using a silica gel column (25 x 4 cm; 350 g of silica). Four hundred fractions (200 mL) were collected and joined in 25 groups, according to the chromatographic behavior. The purification of groups 2 and 7, using chromatography and crystallization, allowed the isolation of compounds **1** and **2**, respectively. Structural elucidation of these compounds is in progress. To the best of our knowledge we are the first team to study the chemical and antitumor potential of this marine fungus.

### References:

- [1] Blunt, J.W. *et al.* (2011), Natural Product Report, 28: 196-268.
- [2] Almeida, A.P. *et al.* (2010), Journal of Natural Pharmaceuticals, 1: 25-29.
- [3] Pinheiro *et al.* (2011), Phytochemistry Letters, *in press*.

Acknowledgments: CEQUIMED-UP (PEst-OE/SAU/UI4040/2011), FERDER, POCI, U.Porto/Santander Totta, for financial support.

## Neuregulin attenuates pulmonary endothelial dysfunction in an experimental model of pulmonary hypertension

Adão R<sup>1</sup>, Maia-Rocha C<sup>1</sup>, Mendes-Ferreira P<sup>1</sup>, Mendes MJ<sup>1</sup>, Cerqueira RJ<sup>1</sup>, Castro-Chaves P<sup>1</sup>, De Keulenaer GW<sup>2</sup>, Leite-Moreira AF<sup>1</sup>, and Brás-Silva C<sup>1,3</sup>

<sup>1</sup> Department of Physiology, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> Laboratory of Physiology, University of Antwerp, Belgium.

<sup>3</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

Neuregulin-1 (NRG-1) is implicated in the maintenance and structural integrity of the cardiovascular system [1]. No studies have determined the effects of NRG-1 in pulmonary vasculature, in health or disease. Pulmonary arterial hypertension (PAH) is characterized by a complex proliferation and dysfunction of the endothelium and pulmonary vascular remodeling [2]. Therefore, the role of this work was to evaluate the effects of a NRG-1 chronic treatment on pulmonary endothelial dysfunction in an animal model of pulmonary arterial hypertension (PAH).

Male Wistar rats (180-200g) randomly received monocrotaline (MCT, 60mg/Kg, sc) or vehicle. After 14 days, animals from these groups were randomly assigned to receive treatment with either NRG-1 (4µg/Kg/day, ip) or vehicle. The study resulted in 3 groups: control (CTRL, n=8); MCT (n=8); MCT+NRG (n=5). 21 to 24 days after MCT administration, animals were anesthetized, heart and lungs were excised *en bloc* and pulmonary arterial rings were isolated and mounted in a myograph. Endothelial function was determined by a dose-response curve to acetylcholine in phenylephrine pre-contracted rings. After the experimental protocol arterial rings were stored in formalin (10%) for histological analysis. Only significant results are presented (mean±SEM, p<0.05).

MCT animals presented PAH associated with endothelial dysfunction, has shown by a decreased relaxation, mediated by acetylcholine in phenylephrine pre-contracted rings, when compared with the CTRL group (MCT vs CTRL: 35.41±4.02% vs 86.27±1.85%). Treated animals (MCT+NRG) presented a significant improvement in endothelial function (48.31±5.69%). Histological analysis revealed vascular remodeling in arterial rings of MCT animals when compared with the CTRL group, as shown by an increase in tunica media thickness (MCT vs CTRL: 53.24±1.84mm vs 31.33±0.83mm), tunica media area (MCT vs CTRL: 104.50±7.48mm<sup>2</sup> vs 67.85±3.93mm<sup>2</sup>) and the tunica media area/lumen area ratio (MCT vs CTRL: 41.23±1.48% vs 31.97±2.99%). Treated animals presented a significant decrease in vascular remodeling as shown by improvements in all parameters studied (34.26±0.91mm, 75.64±5.10mm<sup>2</sup> and 29.56±2.46% respectively).

NRG-1 chronic treatment significantly reduced the severity of PAH associated physiopathological processes, namely endothelial dysfunction and vascular remodeling. These results suggest that the NRG-1 system has a crucial role in vascular function, specifically in PAH, proving to be a potential therapeutical target.

[1] De Keulenaer, G.W., Doggen, K. and Lemmens, K. (2010). *The vulnerability of the heart as a pluricellular paracrine organ: lessons from unexpected triggers of heart failure in targeted ErbB2 anticancer therapy*. Circ Res, 106, 35-46.

[2] Rhodes, C.J., Davidson, A. and Gibbs, J.S. (2009) *Therapeutic targets in pulmonary arterial hypertension*. Pharmacol Ther, 121, 69-88.

# **Nutritional Risk and Survival in Cancer Patients Undergoing Percutaneous Endoscopic Gastrostomy in the Instituto Português de Oncologia do Porto Francisco Gentil, Entidade Pública Empresarial**

**R. Barbosa<sup>1</sup>, TF. Amaral<sup>2</sup> and P. Alves<sup>3</sup>**

<sup>1</sup> Student at Faculty of Ciências da Nutrição e Alimentação, University of Porto, Portugal.

<sup>2</sup> Associate Professor at Faculty of Ciências da Nutrição e Alimentação, University of Porto, Portugal.

<sup>3</sup> Director of Serviço de Alimentação e Nutrição in IPOFG Porto, Portugal

**Introduction:** Undernutrition in cancer patients is recognized as an obstacle to treatment and rehabilitation of these patients and often goes beyond the solutions offered to minimize their effects. To determine whether the nutritional status of patients undergoing percutaneous endoscopic gastrostomy (PEG) influence their survival, the occurrence of complications of PEG, as well as the weight evolution and length of stay with .

**Methods:** A retrospective analysis of clinical charts from 173 patients who underwent to PEG procedure, in 2010 at IPOFG, EPE was conducted. It was possible to evaluate the nutritional risk by MUST in 152 patients.

**Results:** At PEG placement, 65.8% of patients were classified as being at High Nutritional Risk (HNR), but there was no difference in survival between them and those who had Low/Medium Nutritional Risk (LMNR). Although no statistically significant differences, patients with high nutritional risk had a lower survival. The frequency of major and minor complications was 6.4% and 27.2%, respectively. When compared in two groups to weight loss that elapsed between the placement of the PEG and the last weight recorded, it appears that patients BMRN lose more weight than those of HNR (5.6 kg vs. 1.1 kg) (< p 0.05).

**Conclusion:** Given the high frequency of HNR during the PEG placement and unfavorable outcomes observed, the minimization of the undernutrition effects in cancer patients with PEG may undergo by an early nutritional intervention, considering more frequently the prophylactic PEG placement.

## **Optimization of an experimental protocol for chorionic villi culture in prenatal diagnosis**

**R. Ribeiro<sup>1,2</sup>, V. Lima<sup>1</sup> and S. Dória<sup>1</sup>**

<sup>1</sup> Department of Genetics, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> Faculty of Sciences, University of Porto, Portugal.

Human Cytogenetic is a relatively recent science that has practical application in various fields namely in the Prenatal Diagnosis. There are different methods of Prenatal Diagnosis, among which stands out the Chorionic Villous Biopsy, because it allows to culture cells at 10-12 weeks of gestation. However, it is still necessary to culture cells for at least 7 to 10 days before harvesting. In the conventional protocol, tissue from chorionic villous are only subjected to a digestion with a Collagenase enzyme before culture. In this work, we describe an experimental protocol that allows to reduce the cells culture time, using a combination of two enzymes (Trypsin and Collagenase) and a Desoxyribonuclease.

Further optimization is still needed in order to improve the quality of the metaphases obtained, however the experimental protocol described in this work has proved to be of great application for routine cytogenetic analysis.

## P2Y<sub>6</sub> RECEPTORS EXPRESSED BY ACTIVATED MICROGLIA INHIBIT ASTROGLIAL PROLIFERATION BY INDUCING NITRIC OXIDE MEDIATED APOPTOSIS

<sup>1</sup>Diana Pinho, <sup>1</sup>Clara Quintas, <sup>1</sup>João Magalhães, <sup>1</sup>Jorge Gonçalves, <sup>1</sup>Glória Queiroz

<sup>1</sup>Laboratory of Pharmacology, Department of Drug Sciences, REQUIMTE, Faculty of Pharmacy, University of Porto, PORTO

Microglia P2Y pyrimidine receptors trigger phagocytosis, the release of cytokines and of other messengers that modulate astrogliosis [1,2]. Using cultures of astrocytes with activated microglia, we investigated the influence of P2Y pyrimidine on the astroglial proliferation and the mechanisms involved. In co-cultures of astrocytes and LPS (0.1 µg/ml) activated microglia were investigated the effects of several pyrimidine nucleotides in cell proliferation by measuring methyl-[<sup>3</sup>H]-thymidine incorporation. P2Y<sub>6</sub> receptor expression was evaluated by western blot analysis and its cellular localization by double-immunofluorescence; microglia were labelled with anti-OX42 antibody followed by incubation with Alexa Fluor, P2Y<sub>6</sub> receptors were labelled with a rabbit anti-P2Y<sub>6</sub> antibody and revealed by TRITC. The release of nitrates plus nitrites was measured and used as an index of NO release. Cell death was evaluated by LDH release and Tunnel assays.

UDP-glucose caused no effect, but PSB 0474 (0.01-10 µM), UTP and UDP (0.01-1 mM) inhibited astroglial proliferation up to 43 ± 2 % (n=10, P<0.05). The inhibitory effects of UTP, UDP and PSB 0474 were prevented by the selective P2Y<sub>6</sub> antagonist MRS 2578 (1 µM). Western blot revealed that P2Y<sub>6</sub> receptors were expressed in these cultures and the experiments of double-immunofluorescence revealed that they were mainly co-localized with microglia. The inhibition caused by UDP (1 mM; 44 ± 2 %, n=25, P<0.05) was mimicked by the NO donor, SNP (0.1 mM; 37 ± 7 %, n=6, P<0.05), not changed by pertussis toxin (PTX 0,1 µg/ml; 41 ± 2 %, n=6) but it was abolished by the phospholipase C (PLC) inhibitor U 73122 (1 µM; 2 ± 4 %, n=6, P<0.05), but not by its inactive analogue U 73343 (1 µM; 40 ± 4 %, n=6) and by the protein kinase C inhibitor (PKC) RO 32,0432 (1 µM; 3 ± 5 %, n=6, P<0.05). In addition, the effect of UDP (1 mM) was abolished by the nitric oxide synthase inhibitor (NOS) L-NAME (0.1 mM; 7 ± 3 %, n=6, P<0.05), an effect that was partially reversed by L-arginine (3 mM; 28 ± 6 %, n=6, P<0.05). The guanilcyclase inhibitor ODQ (10 µM; 6 ± 4 %, n=6, P<0.05) and the protein kinase G (PKG) inhibitor KT 5823 (1 µM; 6 ± 3 %, n=6, P<0.05) also abolished the effect of UDP. After 48 h incubation, the selective P2Y<sub>6</sub> agonists, PSB 0474 (10 µM) and UDP (1 mM), increased NO release by 190 ± 20 % (n=8, P<0.05) and 177 ± 14 % (n=8, P<0.05), respectively. The effect of both agonists on NO release was abolished by MRS 2578 (1 µM), U 73122 (1 µM) and by RO 32,0432 (1 µM).

All pyrimidine agonists that inhibited astroglial proliferation induced cell death by apoptosis.

In conclusion, P2Y<sub>6</sub> receptors are highly expressed in activated microglia and activate the PLC-PKC pathway that lead to an increase in NO production through NOS activation. The increase in NO production observed under these conditions may be responsible for the increase in cell death by apoptosis induced by the pyrimidine nucleotides causing inhibition of astroglial proliferation.

[1] Koizumi *et al.* (2007) UDP acting at P2Y<sub>6</sub> receptors is a mediator of microglial phagocytosis. *Nature* 446:1091-1095. [2] Di Virgilio *et al.* (2009) Purinergic signalling in inflammation of the central nervous system. *Trends Neurosci.* 32:79-87.

*This work was supported by Fundação para a Ciência e a Tecnologia through grant N.º. PEST C/EQB/LA0006/2011, the project PTDC/SAU-TOX/115397/2009 and by Projetos pluridisciplinares para iniciação à investigação científica da UP, financed in 2010-2011.*

## *Padina pavonica* (L.) Thivy: the secret story

**M. Taveira, A. Magalhães, C. Barbosa, C. Elipe García, C. Soares, D. Barrigas, I. Jesus, L. Teixeira and T. Saraiva**

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Marine organisms offer chemical structures, some of them unique and rather unusual, which could be the basis for semi-synthesis or serve as molecular models for pharmaceutical industry. Besides their fundamental role in aquatic ecosystems, the potential of algae as good sources of bioactive compounds is well known.

*Padina pavonica* (L.) Thivy (Phaeophyta) is a brown algae with a world-wide distribution, being found in the shores of tropical or moderate temperature areas. This species is easily recognized in its natural habitat by its deposits of calcium carbonate in concentric layers. For this study an ethanol extract was prepared and evaluated regarding chemical composition and biological activity.

HPLC/DAD analysis revealed the presence of 3 carotenoids. By GC/MS 9 compounds from several classes were detected: 1 amino acid, 6 fatty acids, 1 sterol and 1 phenol.

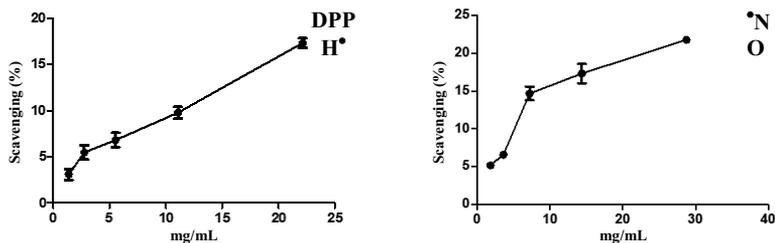


Fig. 1. Antioxidant activity of *P. pavonica* ethanol extract against DPPH• and nitric oxide (•NO). Data are presented as mean  $\pm$  standard error of three assays performed in triplicate.

Antioxidant capacity was assessed against DPPH and nitric oxide radicals, and a concentration dependent effect was observed (Fig. 1). However, no antimicrobial activity was noticed against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and *Bacillus cereus*.

Thus, this matrix revealed to be a source of compounds with biological activity, deserving to be further explored as an alternative by both pharmaceutical and food industries.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade and Patrícia Valentão.

# Penetration of different components of dental amalgam and composite resin in the interface between restoration and dentin

**S. Lopes<sup>1</sup>, A. Portela<sup>1</sup>, A. Moreira<sup>1</sup>, M. Vasconcelos<sup>1</sup>**

<sup>1</sup> Biomaterials Department, Faculty of Dental Medicine, Porto University, Portugal.

**Introduction and Objectives:** The dental amalgam and composite resin are both restorative materials regularly used in dentistry. It is known that mercury (one of dental amalgam's elements), is usually found diluted in saliva after restoration [1, 2]. Since mercury's vapor is released into the oral cavity, it is important to know whether there will be the passage of this or other constituents to the dental tissue. According to Wei [3], pigmentation frequently occurs in the enamel and dentin which is in contact with dental amalgam, as a result of a penetration of some of its constituents to the dental tissue. Regarding to composite resin, according to Anusavice [4], although they are rare, allergic reactions might occur in dental tissue due to poor polymerization of this restorative material, and related with close localization of the pulp [5-7]. This research aims to evaluate the penetration of different constituents of dental amalgam and composite resin, in the interface area with the dentin.

**Materials and methods:** 10 posterior teeth (upper and lower 1st, 2nd and 3rd molars), previously extracted due to complications not associated with dental caries, which structure was found intact, were restored with dental amalgam (n=5) and composite resin (n=5). To make a proper evaluation of the variants under study, the scanning electron microscopy (SEM) associated with X-ray microanalysis (EDS) was performed in the restored dental surface.

**Results and conclusion:** In dental amalgam it was detected the passage of tin and mercury from the restoration to the interface area, and these values differed depending on the samples with or without restorative material. The method used to assess the penetration of composite resin, was not the most appropriate. However, it was considered that if there is penetration, this would not be significant.

## References:

- [1] Haikel Y, Gasser P, Salek P, Voegel JC. Exposure to mercury vapor during setting, removing, and polishing amalgam restorations. *J Biomed Mater Res.* 1990 Nov;24(11):1551-8.
- [2] Berglund A. An in vitro and in vivo study of the release of mercury vapor from different types of amalgam alloys. *J Dent Res.* 1993 May;72(5):939-46.
- [3] Wei SH, Ingram MJ. Analyses of the amalgam-tooth interface using the electron microprobe. *J Dent Res.* 1969 Mar-Apr;48(2):317-20.
- [4] Anusavice KJ, Phillips RWSodm. *Phillips' science of dental materials.* 11th ed. ed. St. Louis, Mo. ; [Great Britain]: Saunders; 2003.
- [5] Mohsen NM, Craig RG, Hanks CT. Cytotoxicity of urethane dimethacrylate composites before and after aging and leaching. *J Biomed Mater Res.* 1998 Feb;39(2):252-60.
- [6] Yoshii E. Cytotoxic effects of acrylates and methacrylates: relationships of monomer structures and cytotoxicity. *J Biomed Mater Res.* 1997 Dec 15;37(4):517-24.
- [7] Geurtsen W, Lehmann F, Spahl W, Leyhausen G. Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. *J Biomed Mater Res.* 1998 Sep 5;41(3):474-80.

# Prenylated Xanthonic Derivatives: Optimization of synthetic methodologies and evaluation of physicochemical and biological properties

J. Almeida<sup>1</sup>, R. Castanheiro<sup>1,2</sup>, M. Lúcio<sup>3</sup>, S. Reis<sup>3</sup>, M. Pinto<sup>1,2</sup>

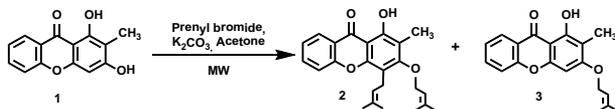
<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Portugal.

<sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal.

<sup>3</sup>REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal.

Xanthone derivatives, namely prenylated xanthenes (PXs) have been found to exhibit interesting activities such as anti-inflammatory, antibacterial, antioxidant and antitumor [1,2]. The presence of prenyl groups becomes an important structural factor taking into account the influence on the physicochemical properties, including lipophilicity, and the overall stereochemistry of the molecule, which may create additional interactions with biological targets [1,2]. Therefore, PXs can represent excellent models for the development of new and more effective drugs, being the introduction of prenyl groups in scaffold of "hit" compounds one of the strategies used in our research group (CEQUIMED-UP) [3].

One of the major aims of this project is to synthesize a small library of PXs derivatives through the application of classical and "non-classical" synthetic methodologies, namely microwave-assisted organic synthesis and heterogeneous catalysis. Until now, we have obtained the PXs **2** and **3**, by the reaction of 1,3-dihydroxy-2-methylxanthone (**1**) with prenyl bromide under microwave irradiation (**Fig.1**).



**Fig.1.** General Procedure for the synthesis of PXs by MW irradiation.

Subsequently the synthesized compounds will be evaluated for their physicochemical parameters. These features will allow to predict the pharmacokinetic behavior of PXs as well as a better understanding of their interaction with biological targets [4].

PXs will be also evaluated for their antioxidant activity and for the inhibition of membrane located enzymes that might be involved in inflammation, namely phospholipase A2 and cyclooxygenases. From the results of the biological evaluation, we expect to obtain new compounds with potential antioxidant and anti-inflammatory activities, as well as the possibility of study the structure-activity relationship (SAR).

Acknowledgments: CEQUIMED-UP (PEst-OE/SAU/UI4040/2011), FERDER, POCI, for financial support.

References:

- [1] Pinto, M., *et al.*, (2005), *Curr. Med. Chem.*, 12, 2517-2538.
- [2] Pinto, M. and Castanheiro, R., (2009) "Natural Prenylated Xanthenes: Chemistry and Biological Activities" in *Natural Products: Chemistry, Biochemistry and Pharmacology*, Ed. Brahmachari, G., Narosa Publishing House PVT. LTD., Nova Deli, India, Ch. 17, pp.520-676.
- [3] Pinto, M. and Castanheiro, R. (2009), *Curr. Org. Chem.*, 13(12), 1215-1240.
- [4] Ferreira, H., *et al.* (2003), *Anal Bioanal Chem.*, 377, 293-298.

# Privileged xanthonic structures as inspiration for synthesis of new inhibitors of growth of human tumor cell lines

**R. Marques<sup>1</sup>, D. Torres<sup>1</sup>, R. Castanheiro<sup>1,2</sup>, M. H. Vasconcelos<sup>1,3,4</sup>, M. Pinto<sup>1,2</sup>**

<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Portugal.

<sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal.

<sup>3</sup>IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal.

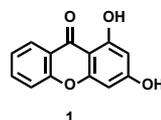
<sup>4</sup>Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal.

Xanthenes show interesting biological activities associated with their tricyclic scaffold depending on the nature and/or position of the different substituents [1]. A relationship between activity and the existence of prenyl groups in key positions on the xanthone scaffold was associated with some biological activities particularly antitumor [2].

Therefore, prenylated xanthenes could represent an excellent model for the development of new and more effective drugs, particularly in the improvement of antitumor activity, and for this reason the introduction of prenyl groups in "hit" compounds, has been one of the strategies used in CEQUIMED-UP [3].

The central aim of this project is to synthesize some analogues of xanthenes that have demonstrated promising antitumor activity, namely prenylated derivatives.

The synthetic approach to obtain these compounds is through the application of classical and "non-classical" methodologies, namely microwave-assisted organic synthesis and heterogeneous catalysis. In the present work, we have synthesized 1,3-dihydroxyxanthone (**1**), through the Grover, Shah and Shah reaction using microwave energy as heating source and also applying Eaton's reagent (P<sub>2</sub>O<sub>5</sub>/CH<sub>3</sub>SO<sub>3</sub>H) as the condensation agent [4]. The next step will be the synthesis of a small library of prenylated xanthone derivatives, using **1** as starting material to carry out some molecular modifications, by the introduction of prenyl, dimethylpyran and/or furan groups.



Subsequently the synthesized compounds will be tested for their effect on the *in vitro* growth of some human tumor cell lines. From the results of the biological evaluation, we expect to obtain new compounds with improved antitumor activity, as well as the possibility of study the structure-activity relationship (SAR).

Acknowledgments: CEQUIMED-UP (PEst-OE/SAU/UI4040/2011), FERDER, POCI, for financial support.

References:

- [1] Pinto, M., *et al.*, (2005), *Curr. Med. Chem.*, 12, 2517-2538.
- [2] Pinto, M. and Castanheiro, R., (2009) "Natural Prenylated Xanthenes: Chemistry and Biological Activities" in *Natural Products: Chemistry, Biochemistry and Pharmacology*, Ed. Brahmachari, G., Narosa Publishing House PVT. LTD., Nova Deli, India, Ch. 17, pp.520-676.
- [3] Pinto, M. and Castanheiro, R. (2009), *Curr. Org. Chem.*, 13(12), 1215-1240.
- [4] Pillai, *et al.* (1986), *J. Org. Chem.*, 51(5), 717-723.

## Production and purification of a nucleocapsid protein from a novel canine norovirus in *Saccharomyces cerevisiae*

C. Pereira<sup>1,3</sup>, E. Lopes<sup>3</sup>, J. R. Mesquita<sup>2,3</sup>, J. Soares<sup>3</sup>, M. S. J. Nascimento<sup>2,3</sup>, L. Saraiva<sup>1,3</sup>

<sup>1</sup>REQUIMTE, <sup>2</sup>CEQUIMED-UP, <sup>3</sup>Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Portugal.

Human noroviruses (NoVs) are recognized as the most frequent cause of foodborne outbreaks of acute gastroenteritis and of sporadic enteric illness [1]. Though the most important mode of transmission is person-to-person contact, zoonotic transmission has been recently suggested [2]. In 2007, studies focusing in the search of NoVs in dogs led to the discovery of a novel canine NoV in the North of Portugal [3]. This constituted a relevant achievement in the emerging field of canine NoVs, compelling to explore the ecology and biology of this new virus, particularly a possible zoonotic transmission. Since this is a new virus, with no commercial Virus-Like Particles (VLP)-based Enzyme Immunoassay (EIA) available, the development of an in-house EIA will be crucial. To establish this assay, a substantial amount of VLPs to be used as an antigen is required. The yeast *Saccharomyces cerevisiae* has been extensively used for the expression of viral proteins, including viral core and nucleocapsid proteins [2]. Hence, the aim of this work was to use *S. cerevisiae*, as a cost-effective and high-level expression system, to produce sufficient amounts of the novel NoV capsid protein, VP1 (which self-assembles into VLPs), to be used as antigen in the in-house EIA assay. With this goal, the VP1 gene was cloned into a multicopy yeast expression vector (pRS) and the protein was fused, at the C' terminus, to a 6His tag. 6His tag allows not only the detection and quantification of recombinant proteins in Western blot (using a commercial anti-His) but also, and most importantly, to perform a one-step purification of VP1 by immobilized metal affinity chromatography (IMAC). The production of VP1 in yeast was optimized for the strain and growth conditions used and the purification performed by IMAC was analyzed by Coomassie blue staining of SDS-PAGE and Western-blot. The obtained product corresponded to an expected molecular weight of 63 kDa and the yield obtained was 1.38 mg/g wet weight of yeast. The immunological properties of the recombinant VP1-6His protein were evaluated by Western blot and by in-house EIA and its efficiency proved by confirming the serology of several canine samples. Together, in this work a fast and efficient system of production and purification of VP1 is presented.

### References

- [1] Patel, M.M., Hall, A.J., Vinje, J. and Parashar, U.D. (2009), *Noroviruses: a comprehensive review*, Journal of Clinical Virology, (44), 1-8.
- [2] Widdowson M.A., Rockx, B., Schepp, R., Poel, W.H., Vinje, J., van Duynhoven, Y.T. and Koopmans, M.P. (2005), *Detection of serum antibodies to bovine norovirus in veterinarians and the general population in the Netherlands*. Journal of Medical Virology, (76), 119-28.
- [3] Mesquita, J.R., Barclay, L., Nascimento, M.S.J. and Vinje, J. (2010), *Novel norovirus in dogs with diarrhea*, Emerging infectious diseases, (16), 980-982.

We thank REQUIMTE (grant no.PEst-C/EQB/LA0006/2011), FCT (PTDC/CVT/113218/2009), POCI, FEDER and U.Porto/Santander Totta for financial support. J.R. Mesquita (SFRH/BD/45407/2008) is recipient of a FCT fellowship.

## Revealing sea secrets: *Holothuria forskali* Chiaje

**R. F. Gonçalves, A. R. Lucas, C. Castro, C. Fernandes, I. Manzanares, J. A. C. Barbosa, J. T. Costa, M. Sousa and L. R. Silva**

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Interest on marine organisms as sources of bioactive compounds has been rising. They developed defense mechanisms that allow them to survive in hostile conditions, like extreme temperatures, pressure, light and salinity variations. Sea cucumbers (Echinodermata) are important components of marine ecosystems, being found all over the oceans. Some of them have medicinal and nutritional interest. In the present study the knowledge on *Holothuria forskali* Chiaje was expanded concerning its metabolic profile and biological potential.

GC/MS analysis of an ethanol extract allowed the determination of 23 compounds from distinct chemical classes: 7 amino acids, 14 fatty acids and 2 sterols (Fig. 1). This extract was tested for anti-radical activity against DPPH and nitric oxide, but no effect was noticed. The same happened when antimicrobial activity was assessed against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and *Escherichia coli*.

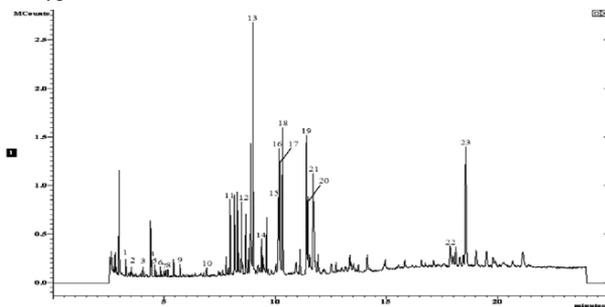


Fig. 1. GC/MS chromatogram of the ethanol extract of *H. forskali*. (1) alanine; (2) glycine; (3) valine; (4) leucine; (5) proline; (6) serine; (7) nonanoic acid; (8) threonine; (9) decanoic acid; (10) dodecanoic acid; (11) tetradecanoic acid; (12) pentadecanoic acid; (13) hexadecanoic acid; (14) heptadecanoic acid; (15) linolenic acid; (16) linoleic acid; (17) oleic acid; (18) stearic acid; (19) arachidonic acid; (20) eicosapentaenoic acid; (21) eicosaenoic acid; (22) 5- $\alpha$ -colestan-3- $\beta$ -ol; (23) 5- $\alpha$ -colestan-3- $\beta$ -ol similar.

A chloroform:methanol extract was also prepared. GC/MS analysis showed a composition similar to that of the ethanol one. However, this extract displayed some antibacterial activity against *B. cereus* (MIC > 62.5 mg/ml).

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the 5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy, University of Porto, under the responsibility of Paula Andrade and Patrícia Valentão.

# Ribavirin is a poor inhibitor of norovirus replication in the murine model

J. Rocha-Pereira<sup>1,2</sup>, A.R. Tiago<sup>1</sup>, M.S.J. Nascimento<sup>1,2\*</sup>

<sup>1</sup>Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal \*Corresponding author: saojose@ff.up.pt

<sup>2</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal

Human noroviruses are the number one agent of foodborne gastroenteritis outbreaks worldwide but are also estimated to be responsible for 200 000 deaths each year due to severe childhood diarrhea in developing countries [1]. However, no antiviral drug is available for treatment or prevention of norovirus illness. Considering the tremendous burden of this disease in public health, the discovery of anti-norovirus drugs is in high demand.

To date, few compounds with anti-norovirus activity were reported [2,3]. Among these, ribavirin was the only available antiviral drug with *in vitro* activity against norovirus as shown in studies with the Norwalk replicon model [2]. Ribavirin is a 30-year-old antiviral which is currently approved for treatment of chronic infections with hepatitis C virus, for respiratory syncytial virus and Lassa fever virus.

The aim of the present work was to evaluate the anti-norovirus activity of ribavirin through another *in vitro* model, using the cultivable murine norovirus (MNV) as a surrogate for human norovirus. The antiviral effect of ribavirin was measured by: (i) infectivity by classic plaque reduction assay, (ii) virus-induced cytopathic effect (CPE) through a colorimetric assay (MTS/PMS) and (iii) viral RNA synthesis by qRT-PCR.

Results showed that ribavirin presented a modest anti-norovirus activity, reducing 50% of viral infectivity (150  $\mu$ M) and viral RNA copies by 2 log<sub>10</sub> (200  $\mu$ M). However, no reduction of virus-induced CPE was observed when cells were treated and MNV-infected at the same time. Only when cells were treated prior to MNV-infection, an inhibition (30%) of virus-induced CPE was achieved (100  $\mu$ M). Overall, the modest *in vitro* antiviral activity against norovirus associated with the significant cytotoxicity presented by this drug at concentrations  $\geq$ 200  $\mu$ M limits the use of ribavirin as an antiviral against norovirus. The study of structurally related compounds could however have a better outcome and help the discovery of novel antivirals against norovirus.

## References:

- [1] Patel M.M. et al. (2008) *Emerg Infect Dis* Vol.No. 8 pp. (1224-1231).
- [2] Chang K.O. and George D.W. (2007) *J Virol* 81(22): 12111-8
- [3] Rocha-Pereira J. et al. (2010) *Bioorg Med Chem* 18(12): 4195-201.

## Acknowledgments.

FCT (SFRH/BD/48156/2008, PhD grant for J. Rocha-Pereira). Herbert W. Virgin for the generous provision of the MNV

## ***Satureja parvifolia*: bioactive compounds and antioxidant activity**

**R. Cabana<sup>1</sup>, P. B. Andrade<sup>2</sup>, P. Valentão<sup>2</sup>, C. I. Viturro<sup>1</sup> and L. R. Silva<sup>2</sup>**

<sup>1</sup> PRONOA/UNJu, Faculty of Engineering, National University of Jujuy, Argentina.

<sup>2</sup> REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

*Satureja parvifolia* (Phil.) (Lamiaceae) is an abundant shrub in the Andean region of South America. This species is used in food as aromatic plant and in traditional medicine against gastric troubles, stomach aches, haemorrhages, stimulant, colds and female sterility [1]. It is also known as an aphrodisiac, emmenagogue and used against altitude sickness [2]. However, very little is known about their phytochemical composition.

The aim of this work was to evaluate the biological activities (antioxidant and acetylcholinesterase inhibition) and to establish the metabolic profile of three different extracts (hydrolate, infusion and ethanol extract) of plants collected at three distinct places (samples HN 10 05, HN 11 06 and HN 11 01) in Jujuy Province (Argentina). Antioxidant activity was assessed by DPPH, nitric oxide and superoxide radicals scavenging microassays. Phenolic compounds were analyzed by HPLC-DAD.

The screening by the DPPH<sup>•</sup> scavenging assay revealed HN 11 01 as the most active sample, being this material selected for further analysis.

The antioxidant capacity of three analysed extracts was distinct, although all of them displayed good potential: the infusion proved to be the most effective against DPPH and nitric oxide, while the hydrolate was the most interesting against superoxide radical. In addition, ethanol extract showed the strongest inhibitory effect on acetylcholinesterase.

Regarding phenolic composition, some qualitative and quantitative differences were noticed among the three analyzed extracts, infusion being the richest one. All extracts presented hydroxycinnamic acids and flavonoids, with the latter being predominant. Rosmarinic acid was the main compound in both infusion and hydrolate, while in ethanol extract luteolin-4'-*O*-glucoside was the most abundant.

### References:

- [1] Viturro, C.I., Molina, A.C., Guy, I., Charles, B., Guinaudeau, H., Fournet, A. (2000), *Essential oils of Satureja boliviana and S. Parvifolia growing in the region of Jujuy, Argentina*, Flavour and Fragrance Journal, 15(6), 377-382.
- [2] Bandoni, A., Mendiondo, M., Rondina, R. and Coussio, J. (1972), *Survey of argentine medicinal plants I. Folklore and phytochemical screening*, Lloydia, 3, 69-78.

# Specific IgE and IgG to recombinant *Aspergillus fumigatus* allergens in patients with allergic bronchopulmonary mycosis, asthma and cystic fibrosis. A pilot study.

**AS. Moreira<sup>1</sup>, O. Sokhatska<sup>1</sup>, AM. Pereira<sup>2</sup>, A. Ferreira<sup>3</sup>, M. Beltrão<sup>1</sup>, A. Amorim<sup>4</sup>, MC. Palmares<sup>1</sup>, R. Araujo<sup>5</sup>, L. Delgado<sup>1,2,6</sup>.**

<sup>1</sup> Department of Immunology, Faculty of Medicine, University of Porto, Portugal. <sup>2</sup> Immunology Lab., Department of Clinical Pathology, Centro Hospitalar São João, Porto, Portugal. <sup>3</sup> Department of Immuno-Allergology, Centro Hospitalar São João, Porto, Portugal. <sup>4</sup> Department of Pneumology, Centro Hospitalar São João, Porto, Portugal. <sup>5</sup> Institute of Molecular Pathology and Immunology of the University of Porto, Portugal. <sup>6</sup> Centre for Research in Health Technologies and Information Systems of the University of Porto, Portugal. *UP Projectos Pluridisciplinares 2010, n.130*

Fungal spores are detected in indoor and outdoor environments and are now generally recognized as important causes of respiratory allergies [1]. A seasonal pattern for asthma deaths was suggested in young adults with asthma, some studies describing asthma death peaks when mold spore rates were highest [2]. Fungal allergic reactions affect the lower respiratory tract more frequently than do pollen allergies, but do not show a clear seasonal pattern as other allergies [3]. A significant portion of the atopic population does have underlying sensitivities to fungal spore allergens, although subjects sensitized to a single fungal species are quite rare and the management of fungal allergy is challenging. Allergic bronchopulmonary mycosis (ABPM) is an allergic pulmonary disorder caused by hypersensitivity to *Aspergillus fumigatus* (Asp f), clinically manifesting as chronic asthma, recurrent pulmonary infiltrates, and bronchiectasis [4]. An elevated level of Asp f-specific antibodies (sIgE and sIgG) is considered a good diagnostic marker of ABPM [5-6], a condition also seen in cystic fibrosis patients.

**Materials and Methods:** We selected five representative cases of fungal allergy, with ABPM, bronchiectasis, asthma or lung aspergiloma. To uncover a possible local IgE response to Asp f allergens, we also measured sIgE in the induced sputum [7] of four patients with cystic fibrosis, all with negative serum sIgE. Total and sIgE and sIgG to Asp f recombinant allergens (Asp f1 - mitogillin family of cytotoxins, Asp f2 - fibrinogen binding protein, Asp f3 - peroxisomal-like protein, Asp f4 - intracellular protein and Asp f6 - superoxide dismutase) were determined by a fluoro-enzyme-immunoassay (CAP-FEIA, Phadia®, Sweden). To evaluate test results, the responses of patient samples are directly compared to the responses of total IgE and IgG calibrators.

**Results and Discussion:** sIgE and sIgG to an all-extract of Asp f (m3) and its major antigens (Asp f) of the selected cases are presented on the Table. The dominant specificities are highlighted (bold). For sIgE Asp f4 reactivity is seen in most cases and in ABPM Asp f3 seems the dominant response (although not exclusive). sIgE is absent to any allergen in the Aspergiloma sample, in spite of a vigorous sIgG response.

sIgG to Asp f1 seems dominant, although the IgG response is more polyclonal, with positivity detected to all antigens. In spite of measurable IgE and eosinophil cationic protein (ECP) in the induced sputum of the four cystic fibrosis cases, no local sIgE to *Aspergillus fumigatus* was detected.

In

CASES	<i>Specific IgE kU/L</i>						<i>Specific Ig G mg/L</i>					
	m3	Aspf1	Aspf2	Aspf3	Aspf4	Aspf6	m3	Aspf1	Aspf2	Aspf3	Aspf4	Aspf6
ABPM	71.00	7.94	13.60	<b>22.00</b>	16.80	<0.10	36.00	<b>14.00</b>	3.85	2.12	3.57	4.13
ABPM	83.50	23.50	3.59	<b>39.30</b>	24.90	0.41	13.90	<b>5.99</b>	4.70	2.29	3.32	4.64
Bronchiectasis	5.57	<0.10	<0.10	<0.10	0.12	<0.10	60.80	ND	ND	ND	ND	ND
Asthma	2.03	0.97	0.18	<0.10	1.61	<0.10	49.70	<b>12.30</b>	6.69	2.97	5.35	7.37
Aspergiloma	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	195.00	<b>54.90</b>	49.00	11.00	5.89	21.80

ND – Not done (insufficient sample)

conclusion, the pattern of sIgE and sIgG to recombinant allergens of Asp f is different in several lung disorders associated with mold sensitization. It seems promising for the diagnostic work-up of these patients.

**References:** [1] Oliveira M, Delgado L, et al. (2010) Fungal spores from Pleosporales in the atmosphere of urban and rural locations in Portugal. *J Environ Monit*, pp.1187-1194. [2] Campbell MJ, Holgate ST, Johnston SL. (1997) Randomised controlled trial of a general practice programme of home based exercise to prevent falls in elderly women. *BMJ*. 315, p. 1012. [3] Horner WE, Helbling A, et al. (1995) Fungal allergens. *Clin Microbiol Rev*. 8(2), pp. 161-79. [4] Lazarus AA, Thilagar B, McKay SA. (2008) Allergic bronchopulmonary aspergillosis. *Dis Mon*. 54(8), pp.547-64. [5] Agarwal R. (2009) Allergic bronchopulmonary aspergillosis. *Chest*. 135(3), pp. 805-26. [6] de Oliveira E, Giavina-Bianchi P, et al. (2007) Allergic bronchopulmonary aspergillosis: diagnosis remains a challenge. *Respir Med*. 101(11), pp. 2352-7. [7] Araujo LM, Palmares C, Beltrão M et al. Validation of total and specific IgE measurements in induced sputum. *J Investig Allergol Clin Immunol* (submitted).

# Shift of the adenosine $A_1/A_{2A}$ receptor balance regulating $Ca^{2+}$ influx in the hippocampus and neocortex of patients with mesial temporal lobe epilepsy (MTLE) towards the activation of excitatory $A_{2A}$ receptors

C. Teixeira, S. Guerra-Gomes, M. Mendes, F. Ferreirinha, M.G.B. Lobo, J.M. Cordeiro & P. Correia-de-Sá

Laboratório de Farmacologia e Neurobiologia, UMIB, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Portugal.

Excitatory glutamatergic synapses are modulated by adenosine (ADO) through its inhibitory action on voltage operated calcium channels (VOCC) via  $A_1$  receptors. The widespread distribution and high levels of expression of  $A_1$  receptors in the CNS render ADO an effective “endogenous anticonvulsant” action controlling brain excitation. Conversely, ADO may facilitate  $Ca^{2+}$  influx through VOCC by activating less abundant  $A_{2A}$  receptors, which are believed to promote excitability in confined synapses. Hence, in the normal brain ADO exerts an ambivalent action both as a widespread “fail-safe” mechanism and a localized “amplifier” at expense of a single molecule. Therefore, unbalanced ADO signals may have clinical implications in the pathophysiology of epilepsy. Controversy exists, however, on the role of ADO on epileptic brain tissue perhaps because ADO neuromodulation resultant from the balance between inhibitory  $A_1$  and excitatory  $A_{2A}$  receptors might be compromised.

In this study, we investigated the ADO modulatory balance between inhibitory  $A_1$  and excitatory  $A_{2A}$  responses in hippocampus and adjacent neocortex synaptosomes of drug-resistant MTLE patients submitted to surgery as compared to a control group (non-MTLE patients and cadaveric individuals undergoing forensics evaluation). Procedures were all approved by the Ethics Committees of CHP-HGSA, IML-DN and ICBAS. Synaptosomes were incubated with the calcium-sensitive (Fluo4-NW) dye and placed in a microplate reader to measure fluorescence, while  $A_1$  and  $A_{2A}$  protein expression was evaluated by Western Blot analysis.

Inactivation of endogenous ADO with adenosine deaminase (ADA, 0.5 U/ml) increased by  $28\pm 4\%$ , ( $n=2$ ) veratridine (VT,  $10\ \mu\text{M}$ )-induced calcium influx ( $[Ca^{2+}]_i$ ) into synaptosomes of cadaveric hippocampus, but it reduced  $[Ca^{2+}]_i$  accumulation in the neocortex and hippocampus of MTLE patients by  $13\pm 8\%$  ( $n=6$ ) and  $11\pm 5\%$  ( $n=5$ ), respectively. The effect of ADA (0.5 U/ml) was mimicked by the selective  $A_{2A}$  receptor antagonist, ZM 241385 (50 nM). Furthermore, the  $A_1$  receptor agonist, R-PIA (100 nM), decreased ( $\sim 7\text{-}30\%$ )  $Ca^{2+}$  uptake by synaptosomes of cadaveric and non-MTLE hippocampus and neocortex depolarized with VT ( $10\ \mu\text{M}$ ), but this compound enhanced  $[Ca^{2+}]_i$  accumulation in the hippocampus of MTLE patients. Blockade of  $A_1$  receptors with DPCPX (10 nM) prevented the inhibitory effect of R-PIA (100 nM), but kept unaltered R-PIA-induced  $Ca^{2+}$  uptake in MTLE hippocampus. The  $A_{2A}$  receptor agonist, CGS21680C (3-10 nM), decreased (by 2 to 22%) the uptake of  $Ca^{2+}$  into brain tissues from cadaveric and non-MTLE patients. CGS21680C (3-10 nM), consistently facilitated  $[Ca^{2+}]_i$  accumulation (by 3.5 to 14.4%) in the hippocampus and neocortex from MTLE patients. Western blot analysis showed that the expression of the inhibitory  $A_1$  receptor was significantly reduced both in the hippocampus and neocortex of MTLE patients as compared to cadaveric controls. Contrariwise, the  $A_{2A}$  protein expression in the hippocampus and neocortex of MTLE patients increased (by 2-12 fold) as compared to cadaveric controls. These changes were more obvious in synaptosomal fractions (4-fold enriched in synaptophysin terminal marker), but they were also detected in non-neuronal cells (total lysates, 8-fold enriched in GFAP marker).

Data suggest that the adenosine  $A_1/A_{2A}$  receptor activation balance regulating  $Ca^{2+}$  influx in the hippocampus and neocortex of MTLE patients is shifted towards a predominant activation of excitatory  $A_{2A}$  receptors. Accordingly, a significant decrease in the expression of inhibitory  $A_1$  receptors with a concomitant upregulation of excitatory  $A_{2A}$  receptors was observed in the hippocampus and neocortex of MTLE patients as compared to cadaveric controls.

*Work supported by FCT (FEDER funding, PIC/IC/83297/2007; UMIB-215/94 and FSE-POPH-QREN) and by Univ. Porto / Santander Totta.*

# Solid lipid nanoparticles (SLN) for Miconazole encapsulation: preparation, *in vitro* release and long-term stability studies

Sá, A.<sup>1</sup>, Ferreira, D.C.<sup>1</sup>, Sousa Lobo, J.M. <sup>1</sup>, Silva, A.C.<sup>1,2</sup>

<sup>1</sup> Laboratory of Pharmaceutical Technology/Centre of Research in Pharmaceutical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

<sup>2</sup> Faculty of Health Sciences, Fernando Pessoa University, Porto, Portugal

**Introduction:** Several limitations could be responsible for the therapeutic *in vivo* failure of drugs [1]. Regarding oral delivery, some molecules are not suitable for administration by means of conventional formulations, which could be responsible by poor patient's acceptance and, sometimes, discontinuation of the treatments [2]. Nonetheless, concerning their advantages, the lipid-based drug delivery-systems, the so-called solid lipid nanoparticles (SLN), gained attention during the last 20 years [3]. The aim of this work was to develop a new SLN formulation for incorporation of Miconazole (MIC), an antifungal drug often used for the treatment of oral candidiasis. Lipid screening tests, particle sizes analysis, encapsulation parameters, *in vitro* drug release and long-term storage stability studies were performed.

**Methods:** Placebo and MIC-loaded SLN formulations were prepared by ultrasound technique. The mean particle size and polydispersity index (PI) were assessed by photon correlation spectroscopy. The encapsulation parameters were obtained indirectly, separating the non-encapsulated MIC by filtration/centrifugation followed by UV/VIS quantification. The *in vitro* MIC release profile from SLN was compared with a commercial oral formulation, applying the dialysis bag diffusion technique, during 12h.

**Results:** All formulations showed particles with sizes in the nanometer range, with PI values lower than 0.4, which is reasonable for oral delivery [1]. Moreover, they showed stable colloidal sizes during storage at different temperatures (4, 25 and 40°C). Very satisfactory EE (98%) and LC (20% results were obtained, which means that the prepared SLN systems are effective for MIC encapsulation). In contrast to the commercial formulation, a controlled release profile was observed for MIC-loaded SLN during 12h, which supports the idea that the drug is solubilised in the lipid matrix of SLN.

**Conclusions:** Suitable placebo and MIC-loaded SLN formulations were developed. The systems showed stable colloidal sizes during storage, high encapsulation parameters and an *in vitro* controlled drug release. The release studies should be performed for longer periods, in order to assess the time need for the total MIC release from SLN. Therefore, we conclude that the developed SLN formulations are promising carriers for oral delivery of MIC. Further studies should be done in order to assess long-term stability for longer periods and correlations between *in vitro/in vivo* performances.

## References:

- [1] W. Mehnert, K. Mader (2001), *Solid lipid nanoparticles: production, characterization and applications*, Adv Drug Deliv Rev (47), pp. 165-196.
- [2] Z. Cai, Y. Wang, L.J. Zhu, Z.Q. Liu (2010), *Nanocarriers: a general strategy for enhancement of oral bioavailability of poorly absorbed or pre-systemically metabolized drugs*, Curr Drug Metab (11), pp. 197-207.
- [3] R.H. Muller, R. Shegokar, C.M. Keck (2011), *20 Years of Lipid Nanoparticles (SLN and NLC): Present State of Development and Industrial Applications*, Curr Drug Discov Technol (8), pp. 207-227.

## Spiny sea-star (*Marthasterias glacialis* Linnaeus): beyond what we see

A. P. Oliveira, F. M. Martins, H. Tavares, J. Cêrca, M. V. Osório, R. J. Pereira, R. Pereira, S. Morgado and V. Mota

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Last decades have seen an increase of research in marine organisms as sources of molecules with pharmacological properties. More than 14000 compounds deriving from marine organism were described and about 15 natural products of marine origin are under clinical trials, mainly for cancer, pain and inflammatory diseases. *Marthasterias glacialis* Linnaeus (Phylum Echinodermata, Class Asteroidea) is a sea-star sensitive to environmental conditions, being found in waters of higher salinity and pH, namely in the Atlantic ocean, Mediterranean sea, English channel and North sea. In this work its metabolite composition and bioactivities were explored.

HPLC/DAD analysis of an ethanol extract allowed the determination of three carotenoids (Fig. 1). In addition, 25 compounds, comprising amino acids, fatty acids and sterols, were identified by GC/MS.

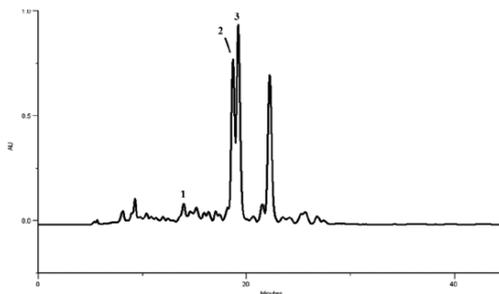


Fig. 1. HPLC/DAD chromatographic profile of *M. glacialis* ethanol extract. Detection at 450 nm. (1) astaxanthin; (2) lutein; (3) zeaxanthin.

This extract was tested for antioxidant capacity against DPPH and nitric oxide, but a reduced protective effect was observed. On the other hand, it displayed antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and *Bacillus cereus*, being the latter the most susceptible species.

These results suggest that this macro invertebrate may have interest for pharmaceutical industry as source of bioactive compounds.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the 5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy, University of Porto, under the responsibility of Paula Andrade and Patrícia Valentão.

## Trace elements in human brain: age-related changes and anatomic region specific differences

**P. Ramos<sup>1</sup>, N. Pinto<sup>2</sup>, A. Santos<sup>2</sup> and A. Almeida<sup>1</sup>**

<sup>1</sup> REQUIMTE, Department of Chemical Sciences, Laboratory of Analytical Chemistry and Physics-Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> Service of Legal Medicine, Faculty of Medicine, University of Porto, Portugal. Forensic Sciences Centre, National Institute of Legal Medicine, Portugal.

As a result of the increased average life expectancy, and, hence, the increasing percentage of elderly people, it has also significantly increased the prevalence of the most feared disorders of this age group population: the neurodegenerative diseases [1].

Although its causes are not yet well defined, several neurodegenerative diseases (ND) have been subject of intensive research in last decades in an attempt to understand the mechanisms underlying the processes of neurodegeneration.

Despite all the questions related to this emerging public health problem that remain with no answer, the progress has been evident. One of the factors identified as responsible for the development of ND is the alteration of homeostasis of some trace elements (TE) in certain areas of the brain [2, 3].

Based on this background, we are developing a project aiming to understand the role of TE in brain functions, particularly their implication in neurodegeneration.

Since ND are strongly age-related and there is a specific region of the Central Nervous System that seems to be particularly affected in each disease, the main objectives are to study: 1) the changes on TE levels in human brain in relation to age; 2) the regional anatomic differences of TE levels within the brain; 3) the differences between individuals with and without evidence of ND.

In this postmortem study, human brain from healthy individuals and individuals with documented neurodegeneration (e.g., Alzheimer, Parkinson, Amyotrophic Lateral Sclerosis, Huntington, Acquired Hepatocerebral Degeneration, and Wilson's disease patients) are being collected. Trace elements will be determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-MS) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS) after samples microwave-assisted acid digestion.

### References:

[1] World Population Ageing 2009; available from: <http://www.un.org/esa/population/publications/WPA2009/WPA2009-report.pdf> (accessed on January 2012).

[2] Hozumi I, Hasegawa T, Honda A, Ozawa K, Hayashi Y, Hashimoto K et al., *Patterns of levels of biological metals in CSF differ among neurodegenerative diseases*, Journal of the Neurological Sciences, 2011; 303: 95-99.

[3] Paine S, Lowe J, *Approach to the post-mortem investigation of neurodegenerative diseases from diagnosis to research*, Diagnostic Histopathologic, 2011; 17:5: 211-216.

## Tracing the emerging pathogen *Escherichia albertii*

C. Sousa<sup>1</sup>, J. Pires<sup>1</sup>, Â. Novais<sup>1</sup> and L. Peixe<sup>1</sup>

<sup>1</sup> REQUIMTE-Department of Microbiology, Faculty of Pharmacy, University of Porto, Portugal

*Escherichia albertii* was firstly recognized as a new species belonging to the genus *Escherichia* in 2003, frequently being misidentified as *Hafnia alvei*, *Salmonella enterica*, *Escherichia coli*, *Shigella boydii* or *Yersinia ruckeri*. It appears to be a foodborne pathogen found either in animals and humans.

In this work we assess the presence of *E. albertii* in a collection of Portuguese isolates from different sources. A large collection of isolates ( $n= 350$ ), presumptively identified as *H. alvei*, *S. enterica* or *E. coli*, obtained from different sources (human clinical isolates, piggeries, aquacultures and food), was assayed.

Phenotypic characterization included lactose fermentation, motility, indole production, and the Voges-Proskauer test. Genotypic tests included the identification of specific polymorphisms in *lysP* and *mdh* genes by a diagnostic PCR multiplex, and the identification of the *eae* gene or the *malB* operon, specific for *E. coli* species, by simplex PCR. *E. albertii* (CCUG46494T) and *E. coli* (ATCC25299) were used as controls.

Twenty-two nonmotile and non-lactose fermenters isolates, mostly from aquacultures and piggeries, were considered presumptive *E. albertii* isolates. Nevertheless, PCR reactions don't confirm this identification, stressing the relevance of genotypic characterization. Further studies based on a genotypic approach and using different animal species should be conducted in order to estimate the presence of *E. albertii* in natural reservoirs, in Portugal.

## Ubiquinone analogues and histone deacetylase inhibitors in an induced zebrafish model of mitochondrial dysfunction

B. R. Pinho<sup>1,2</sup>, A. Fonseca-Silva<sup>1</sup>, M. M. Santos<sup>3</sup>, P. Valentão<sup>2</sup>, P. B. Andrade<sup>2</sup> and J. M. A. Oliveira<sup>1</sup>

<sup>1</sup> REQUIMTE, Dep. Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

<sup>3</sup> CIIMAR, Environmental Toxicology Lab, University of Porto, Portugal

Friedreich's ataxia (FRDA) is caused by frataxin deficiency and associated with neurological and cardiac manifestations. Mitochondrial frataxin is required for normal iron-sulfur cluster assembly. Its deficiency causes mitochondrial dysfunction and oxidative damage, leading to impaired energy production. Ubiquinone (UQ) analogues (e.g. idebenone-IDB and decylubiquinone-DCB) and histone deacetylase inhibitors (HDACi) are putative therapeutics in FRDA. UQ analogues may act as antioxidants and improve electron flow through the mitochondrial respiratory chain. HDACi may facilitate transcription of frataxin or other transcripts associated with mitochondrial biogenesis and energy production [1]. Experimental support for this therapeutic potential, however, stems primarily from *in vitro* studies in cellular or subcellular systems. In the present study we use an *in vivo* mitochondrial dysfunction paradigm causing cardiac abnormalities in zebrafish, and test the efficacy of UQ analogues and HDACi in rescuing these phenotypes. Mitochondrial dysfunction was induced by acute treatment of zebrafish (56 hours post fertilization; hpf) with mitochondrial inhibitors: rotenone (RTN), myxothiazol (MYX), antimycin (ANT), or oligomycin (OLG); the resulting effects on heartbeat and circulation were recorded at 10 min intervals. IDB and DCB were co-exposed with mitochondrial inhibitors at 56 hpf. HDACi (trichostatin-TSA, MS275, valproate-VPA, EX527, and AGK2) were pre-incubated from 4-56 hpf to allow for gene expression changes prior to the assay with mitochondrial inhibitors. RTN decreased the heart rate after 30 min of exposure, arresting circulation and heartbeat in 50% of the embryos ( $n=60$ ) after 98 min and 144 min exposure, respectively. TSA, MS275, AGK2 and EX527 did not attenuate the toxicity induced by RTN, whereas VPA and OLG delayed the asystole induced by RTN in 27% and 18%,  $n=40$ ,  $P<0.05$ , respectively. IDB and DCB delayed the arrest in circulation (61% and 79%, respectively) and heartbeat (52% and 104%, respectively),  $n=40$ ,  $P<0.05$ . IDB and DCB did not protect the zebrafish embryos from toxicity induced by MYX or ANT. The protection by IDB and DCB against RTN-induced mitochondrial dysfunction is probably due to increased ATP production. Indeed, OLG delayed the RTN-induced heartbeat arrest, suggesting that ATP synthase reversal limits heart energy availability. Also, no protection was verified against ANT, suggesting that putative IDB's or DCB's antioxidant activity against ANT- (or RTN-) induced superoxide formation plays no role in the protection. Among HDACi, only VPA attenuated RTN's acute cardiovascular toxicity. Currently, we are testing protection against chronic toxicity.

Acknowledgments: Fundação para a Ciência e a Tecnologia (SFRH/BD/63852/2009, Pinho BR); Universidade do Porto & Santander Totta, IJUP2010 #195.

References: [1] Mancuso, M., Orsucci, D., Choub, A. and Siciliano, G. (2010), *Current and emerging treatment options in the management of Friedreich ataxia*, *Neuropsychiatric disease and treatment*, 6, 491-499.

## Using yeast to study the role of ceramide pathway in the regulation of mammalian protein kinase C isoforms

**C. Bessa<sup>1a,2</sup>, M. Leão<sup>1a</sup>, J. Gonçalves<sup>1b</sup>, M. Corte-Real<sup>4</sup>, V. Costa<sup>2,3</sup> and L. Saraiva<sup>1a</sup>**

<sup>1</sup>REQUIMTE, <sup>a</sup>Department of Biological Sciences, Laboratory of Microbiology,

<sup>b</sup>Department of Drug Sciences, Laboratory of Pharmacology, Faculty of Pharmacy,  
University of Porto, Portugal

<sup>2</sup>IBMC, Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal.

<sup>3</sup>ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Department of Molecular Biology,  
University of Porto, Portugal

<sup>4</sup>Molecular and Environmental Biology Centre, University of Minho, Campus de Gualtar,  
Braga, Portugal

Ceramide is one of the most extensively studied bioactive sphingolipid involved in many cellular responses, including cell proliferation and death [1]. The connection between the ceramide pathway and protein kinase C (PKC), a family of serine/threonine kinases, has already been established by several works [2]. However, the role of ceramide in the regulation of individual PKC isoforms is still not well understood. Hence, with this work we intend to study the effect of the ceramide pathway on the activity of distinct PKC isoforms considered major players in carcinogenesis. Our previous works showed that the yeast *Saccharomyces cerevisiae* is an efficient cell system for functional and molecular studies of individual mammalian PKC isoforms [3]. Based on this, in the present study, we used yeast cells deficient in *Isc1*, a neutral sphingomyelinase that enables the cell to produce ceramide by degradation of complex sphingolipids, individually expressing the mammalian classical PKC $\alpha$ , novel PKC $\delta$  or atypical PKC $\zeta$ . As observed with wild-type (wt) strain, PKC $\alpha$  and  $\zeta$  did not interfere with the growth of *isc1 $\Delta$*  cells. However, contrarily to that observed with wt strain, PKC $\delta$  inhibited the growth of *isc1 $\Delta$*  cells, an effect associated with a G2/M cell cycle arrest.

In conclusion, the results obtained show that the ceramide pathway distinctly interferes with the activity of PKC isoforms in yeast. Moreover, since PKC $\delta$ -induced growth inhibition was not observed in the wt yeast strain, this suggests that the reduction of intracellular levels of ceramide led to the activation of PKC $\delta$ . This may therefore indicate that ceramide is an inhibitor of PKC $\delta$ .

### References

[1] Hannun, Y.A., and Obeid, L.M. (2008). Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol* 9, 139-150.

[2] Kajimoto, T., Shirai, Y., Sakai, N., Yamamoto, T., Matsuzaki, H., Kikkawa, U., and Saito, N. (2004). Ceramide-induced apoptosis by translocation, phosphorylation, and activation of protein kinase Cdelta in the Golgi complex. *J Biol Chem* 279, 12668-12676.

[3] Pereira, C. *et al.*, (2012). New insights into cancer- and neurodegeneration-related proteins provided by yeast models. *FEBS J (in print)*.

We thank REQUIMTE (grant no. PEst-C/EQB/LA0006/2011), FCT funded project (PTDC/BIA-BCM/69448/2006), and U.Porto/Santander Totta. M. Leão (SFRH/BD/64184/2009) is recipient of a FCT fellowship.

# UV Filter Encapsulation Using Aero-Dynamically Assisted Jet Extrusion

**M. Costa, A. Brito, P.C. Costa, P. Sousa e Silva, M.H. Amaral, P.A. Lobão, I.F. Almeida, J.M. Sousa Lobo**

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal.

2-Ethylhexyl *p*-methoxycinnamate (EHMC) is the most widely used UV filter in sunscreens. EHMC is an organic filter which protects skin against UV-induced sunburn, photoaging, immunosuppression and skin cancer [1]. However, this filter has demonstrated to undergo photodegradation, which reduces its photoprotection and may even act as photo oxidant when in contact with skin [2]. Therefore, efforts have been made to develop techniques for protecting UV filters.

In this work, alginate microparticles (MP) incorporating EHMC were prepared using the extrusion process with aerodynamically assisted jetting (AAJ) methodology. AAJ is a phenomenon exploring a pressure differential for forming a liquid jet, which subsequently generates a myriad of droplets [3] that jellifies in contact with a CaCl<sub>2</sub> solution. This way microparticles obtained are below the average range of few hundred microns obtained by simple extrusion process [4]. In order to increase the EHMC incorporation into the alginate microparticles the surfactant polysorbate 80 (P80) was used at different amounts. The assay of EHMC was made with a HPLC method.

The average diameter for the produced alginate MP alone was around 20(±6) μm, 39(±13) μm for the alginate MP incorporating EHMC and 27 μm for the three batches of alginate MP incorporating EHMC using increasing percentages of P80. P80 demonstrated to decrease the average size of the particles, independently of the percentage used. However, when increasing the percentage of P80 the size of EHMC droplets inside each MP decreased as droplets were about 13(±6) μm without the surfactant, 7(±3) μm when using 2% P80, 4(±0.3) μm when using 4% P80 and 3(±0.5) μm when using 6% P80. The assay gave on average of 16% of encapsulated UV filter in hydrated MP, not demonstrating significant difference between the use of EHMC alone or with P80. Each MP has an average 58% of water.

The AAJ method allowed the preparation of small size microparticles. Further studies are required to confirm the effect of encapsulation on the EHMC photostability.

References:

- [1] Wyde, M., *2-Ethylhexyl p-methoxycinnamate*, National Toxicology Program. <http://ntp.niehs.nih.gov/files/5WydeBSCReview-EHMC.pdf> (accessed: 12-01-2012).
- [2] Hojerová, J., Medovčíková, A. and Mikula, M. (2006), *Photoprotective efficacy and photostability of fifteen sunscreen products having the same label SPF subjected to natural sunlight*, International Journal of Pharmaceutics, 307 (2), 123-8.
- [3] Gañan-Calvo, M. and Barrero, A. (1999), *A Novel Pneumatic Technique to Generate Steady Capillary Microjets*, Journal of Aerosol Science, 30, 117-125.
- [4] Arumuganathar, S., Suter, N., Walzel, P. and Jayasinghe, S.N. (2009), *Aerodynamically assisted jetting and threading for processing concentrated suspensions containing advanced structural, functional and biological materials*, Journal of Biotechnology, 4 (1), 64-72.

# Waist-to-Height Ratio as a possible Health Index for Swedish Children

**Patricia Rowcliffe, Sara Rodrigues, Eric Poortvliet, Bruno Oliveira, Agneta Yngve**

Faculdade de Ciências da Nutrição e da Alimentação, Universidade do Porto, Portugal

**Background:** Central body fat has been reported to better predict risk for cardiometabolic diseases than total body fat. Recently, Waist-to-Height Ratio (WHtR) has been proposed to be a conveniently age-independent, simple and rapid screening tool for assessing this kind of risk in different populations.

**Aims:** To investigate whether WHtR is age or gender dependent; to describe the prevalence of Swedish children at risk using 0.5 as a cutoff point; to examine the relationship between this index and Body Mass Index (BMI).

**Methods:** The study was a cross-sectional design and included a random sample of 1804, 7-9 year old Swedish children (53% boys and 47% girls) from 45 primary schools. WHtR was calculated by waist circumference divided by height on the bases of standard anthropometric measurements. *Independent Sample t test* were performed to determine whether WHtR was gender dependent. *Pearson's correlation* between WHtR and age was assessed. The number of Swedish children at risk according to this method (using 0.5 as a cutoff point) was described and these results were then compared with the number of children considered at risk by using BMI as a risk assessment tool. The level of agreement between the two indexes was obtained by *Cohen's k*.

**Results:** No significant differences for WHtR between genders were found. There was a very weak correlation between WHtR and age ( $r = -0.061$ ,  $p=0.01$ ). Much fewer children were considered at risk according to WHtR (6.8%) compared to BMI (22.1%). The level of agreement between these two indexes was low ( $k=0.382$ ,  $p<0.001$ ). Nevertheless, after merging the overweight children together with the ones classified by BMI as having a normal weight, there was a much higher agreement between this index and WHtR ( $k=0.693$ ,  $p<0.001$ ).

**Conclusions:** WHtR was found to be age and gender independent in the evaluated Swedish population and therefore may be a simple health index for children. However, WHtR and BMI may produce differing predictions for disease risks. Hence, longitudinal data are needed to examine WHtR's relation to disease.

**Keywords:** Waist-to-Height ratio, Body Mass Index, Children, Abdominal obesity, Risk

---

## Whole blood manganese: Reference range and Mn levels in several diseases states

**R. Ferreira<sup>1</sup>, C. Soares<sup>1</sup>, P. Ramos<sup>1</sup>, L. Araújo<sup>2</sup>, M. Duro<sup>3</sup> and A. Almeida<sup>1</sup>**

<sup>1</sup> *REQUIMTE*, Department of Chemical Sciences, Laboratory of Analytical Chemistry and Physics-Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> Clinical Laboratory A.V. Lda., Arcos de Valdevez, Portugal.

<sup>3</sup> Department of Biological Sciences, Laboratory of Biochemistry, Faculty of Pharmacy, University of Porto, Portugal.

Manganese is a well-known essential trace element for humans. It is necessary for a variety of metabolic functions and, particularly, it is a cofactor for the mitochondrial antioxidative enzyme superoxide dismutase (Mn-SOD). On the other hand, it has long been known that Mn is a neurotoxic substance (the brain is particularly susceptible to Mn excess) [1].

Our group has been studying the levels of Mn in several disease states, specifically: 1) HIV-infected/AIDS patients; 2) patients with chronic renal failure undergoing hemodialysis therapy; and 3) patients suspected of having acquired hepatocerebral degeneration (to aid in the diagnosis, in this case).

Clinical laboratory data are typically interpreted against the so-called reference (or “normal”) range (interval), usually defined as the range of values into which 95% of non-diseased (“normal”) individuals will fall [1]. Therefore, the accurate definition of the reference intervals is of crucial importance. In this context, and because a variety of factors can influence reference intervals (from samples collection procedures, storage conditions and laboratory methods used to sex, age, genetic background or exposure to environmental factors) [1], different populations may need different reference intervals and clinical laboratories are strongly encouraged to establish their own, in order to reflect the specificities of the population to which they will be applied.

Our recent work has been addressed to this topic too. Briefly, it allowed us: 1) to define our “normal” range for Mn (calculated as: 4.0-15.1 µg/L), 2) to obtain evidence of decreased Mn levels in HIV-infected/AIDS patients [3] and 3) to obtain evidence of increased Mn levels in haemodialysis patients [4].

References:

[1] Santamaria, A.B. (2008), *Manganese exposure, essentiality & toxicity*, Indian Journal of

[2] Jhang, J.S., Sireci, A.N. and Kratz, A. (2012), *Postanalysis: medical decision making*, in McPherson, R.A. and Pincus, M.R., “Henry’s Clinical Diagnosis and Management by Laboratory Methods, 22<sup>nd</sup> edition, Saunders Elsevier, Philadelphia, p. 82.

[3] M. Duro, A. Almeida, C. Soares, R. Medeiros and I. Rebelo (2011), *Trace element status in a Portuguese population of HIV/AIDS patients – Evidence for a decreased blood manganese concentration*, Proc. of the 7<sup>th</sup> International Conference on Instrumental Methods of Analysis - Modern Trends and Applications (IMA2011), Chania, Crete, Greece, September 18-22, 2011, p. 63.

[4] A. Almeida, L. Araújo, C. Soares and A. Santos (2011), *Trace element status in a Portuguese population of haemodialysis patients – Evidence for increased whole blood manganese and decreased serum zinc concentrations*, idem, p. 134.

# Parasitology of the Brown Shrimp *Crangon crangon* Linnaeus, 1758

C. Dias<sup>1</sup>, J. Campos<sup>2</sup> and P. Luttkhuizen<sup>3</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> CIMAR/ CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Portugal.

<sup>3</sup> Royal Netherlands Institute for Sea Research, PO Box 59, 1790 AB Den Burg Texel, The Netherlands.

Phylogeographic studies are important to reveal the historical processes that may be responsible for the geographic distribution of a species. The past geological history of a species' present geographic range includes several events which can have caused changes in its distribution. The Late Quaternary, in the last 130,000 years, which was a very important period of geological and geomorphological changes that occurred due to global and regional changes in climate, was dominated by Ice Ages. The most recent glaciation event, the Last Glacial Maximum (LGM), dates back to around 20,000 years. During the LGM, the species were pushed as further south due to sea ice conditions. When ice melted, the species re-colonized northern areas. In the present European coast this resulted in two separated basins: the Mediterranean and the Atlantic.

Phylogeographic groups may be genetically recognizable. In the case of the brown shrimp *Crangon crangon* which is an abundant epibenthic species in European shallow systems, populations are very well structured along the geographic range with four groups: northeastern Atlantic, western Mediterranean, Adriatic Sea and Black Sea. Besides direct phylogeographic studies, i.e., studying the target-species' phylogeography, also the investigations focusing on the species' parasites can provide an insight on the events responsible for the present geographic distribution. Almost no information exists on *C. crangon*'s parasites. Therefore, as a first step to contribute to this issue, an attempt was made to investigate the occurrence of parasites in brown shrimp populations and to identify the species.

Brown shrimp samples were collected from several locations spanning the species' distribution. Muscle tissue was inspected for the presence of parasites. These were preserved in alcohol 96% and later DNA extraction was performed, followed by PCR and DNA sequencing. In this work it was found for the first time one trematode species that parasites the brown shrimp muscle tissue in populations from the Dutch Wadden Sea.

# Pattern discovery in Gene expression

A.F.Goncalves<sup>1</sup>, V.S.Costa<sup>1</sup>, I.M.Ong<sup>2</sup>

<sup>1</sup> Department of Computer Science, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Great Lakes Bioenergy Research Center, University of Wisconsin, Madison.

Nowadays, more than ever, bioinformatics has an important role on biology research. In this article we present a new approach to discover connectivity patterns between genes and how they relate to each other (activation/repression).

We use LeProblog[1] for this task, as implemented on Yap-Prolog. Our dataset includes the results of 5 experiments on yeast (in a stress state and non-stress state). We choose this dataset due to the fact that the transcriptome and proteome change dynamically as cells respond to environmental stress [2]. We defined a Prolog model that describes the genes in a pathway, the way genes can connect to each other, and a couple of rules and knowledge that we think that is necessary to build a correct network of genes.

The results that we have obtained so far seem quite and promising. We were able to generate a correct graph of the Hog1 MAPK transcriptional network [3], and also number of relations between pairs of genes that exist and documented on public databases. At this moment we are

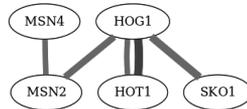


Fig.1

realistic  
(Fig.1)  
a good  
are  
using

correlations between the genes with temporal data, but an implementation with temporal data is already being developed and tested. We believe that through using temporal data and also through the inclusion of non-coding RNA in our model, we will obtain even better results and maybe find some connections and patterns related to the non-coding RNA. These non-coding RNAs appear to comprise a hidden layer of internal signals that control various levels of gene expression in physiology and development, including chromatin architecture/epigenetic memory, transcription, RNA splicing, editing, translation and turnover. RNA regulatory networks may determine most of our complex characteristics play a significant role in disease and constitute an unexplored world of genetic variation [4].

The presenting author would like to acknowledge his grant related to the Horus Project financed by FCT, and also all the support and help given by the second and third authors.

## References:

- [1] L. De Raedt, A. Kimmig, and H. Toivonen, ProbLog: A probabilistic Prolog and its application in link discovery, IJCAI 2007, Proceedings of the 20th International Joint Conference on Artificial Intelligence, Hyderabad, India, pages 2462-2467.
- [2] M Violet Lee, Scott E Topper, Shane L Hubler, James Hose, Craig D Wenger, Joshua J Coon, Audrey P Gasch (2011), A dynamic model of proteome changes reveals new roles for transcript alteration in yeast, Molecular Systems Biology
- [3] Andrew P Canaldi, Tommy Kaplan, Ying Liu, Naomi Habib, Aviv Regev, Nir Friedman, Erin K O'Shea (2008), Structure and function of a transcriptional network activated by the MAPK Hog1, Nature Genetics
- [4] John S. Mattick, Igor V. Makunin (2006), Non-coding RNA, Human Molecular Genetics, Vol.15

## Phylogeny of the Genus *Crangon* Fabricius, 1798

**L. Ferreira<sup>1</sup>, J. Campos<sup>2</sup> and P. Luttkhuizen<sup>3</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> CIMAR/ CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Portugal.

<sup>3</sup> Royal Netherlands Institute for Sea Research, PO Box 59, 1790 AB Den Burg Texel, The Netherlands.

The shrimps of the genus *Crangon* Fabricius, 1798 only occur in the Northern Hemisphere both in the northwestern and northeastern coasts of the Pacific and Atlantic Oceans, in the littoral and sublittoral. The higher number of *Crangon* species in the Pacific suggests that they originated from this area.

The *Crangon* species are characterized by a relatively short rostrum, one or two median spines in the carapace, which can be absent, one ventral spine on the merus of the first pereopod, and an arthrobranchia on the third maxilliped. Besides these recognized morphological similarities between the 20 members of this genus, a few studies have identified some common features between members of the genus, but the species have largely been ignored in taxonomic and phylogeographic studies. Therefore, in this work we attempted to contribute to clarify the phylogeny of the genus *Crangon*.

A total of 45 shrimps of 12 species of the genus *Crangon* and 16 shrimps of 8 genus were obtained from the Naturalis collection, from collections of other researchers and from sampling. All shrimps were individually stored in 96% ethanol. DNA was extracted from a tissue sample taken from under the third pleonite using the GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma®). The DNA was quantified by spectrophotometry. The amplification was done with 2 primers: the primers for the Second Internal Transcribed Spacer (ITS-2) which were designed based on sequences of four different shrimp species and used to amplify the ITS-2 gene; and the universal 16S Primers 16Sar and 16Sbr which were used to amplify a 500-600 basepairs fragment from 16S rRNA. After sequencing, the sequences forward and reverse were edited manually with the software Sequencing Analysis (Applied Biosystems) and assembled using the program AutoAssembler (Applied Biosystems) into consensus sequences.

# Roof nesting gulls (*Larus michaellis*) of downtown Porto: An eco-ethological approach

A. Cajão<sup>1</sup>, J. Neto<sup>1</sup> and P. Fontoura<sup>1,2</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Eco-Ethology Research Unit (FCT 331/94)

The Yellow-legged gull, *Larus michaellis*, is a Mediterranean gull that, during the last decades, has been colonizing urban areas, including the city of Porto, nesting in rooftops. In these areas, the opportunistic and omnivorous diet, the limitless nesting habitat and the lack of natural predators, seem to be factors responsible for a high reproductive success and consequently for an exponential growth of gull colonies that are now considered as a pest. Damage on buildings and cars; aggression to humans; possible transmission of pathogens, are examples of some problems that can be attributed to this urban gulls.

Unfortunately the knowledge about the particularities of the biology of the roof nesting Yellow-legged gull colonies in the urban area of Porto, an indispensable tool for the implementation of management and deterring strategies, is very scarce. So, included in a multidisciplinary program under the coordination of the Parque Biológico de Gaia, we intend to examine the breeding biology of the urban birds. Using eco-ethological methods, such as point census and systematic observations of behavioral categories, the importance in numbers of the breeding population; their structure and movements, and also the breeding behavior and reproduction success, will be estimated.

In this study, some preliminary results regarding the structure and size of the Yellow-legged gull population in the city of Porto are presented. Point counts (during 5') were carried out downtown, in two central areas (Cordoaria and S. Bento) each one with 4 sub-sampling stations. Five census campaigns were performed during autumn 2011 (October – December). Additionally, the behavior of the gulls was registered.

The results showed that the temporal evolution of the total number of individuals was very stable despite small fluctuations caused by weather conditions, period of the day, etc. Actually, a mean number of individuals/20' observation of 77.4 ( $\pm 23.0$ ; min. 45; max. 105) in the area of Cordoaria, and 79.2 ( $\pm 12.7$ ; min. 61; max. 96) in Batalha were estimated. Structurally, the population is strongly dominated by adults (more than 90%) in both areas. It is interesting to note that a very young juvenile (a fledgling about 60 days old) was observed in November 4 at S. Bento, showing that, in Porto, the reproduction period may extend until the end of August. During the studied period the majority of the individuals were observed alone, in some cases displaying agonistic behaviors, such as the *jabbing*, against other conspecifics. However, the presence of couples, with male and female resting very close each other, was common.

The amount of data obtained in the aim of this study is still very scarce, avoiding any kind of conclusion. However, the preliminary results obtained (stable numbers of individuals, low proportion of juveniles and the presence of couples during autumn) suggest that the Yellow-legged gull population of Porto (downtown) is mainly resident, requiring specific management strategies.

# Spatial Ecology and Behaviour of Free-roaming Cats (*Felis catus*) in Urban Environment (Porto, Portugal)

A. Gomes<sup>1</sup>, A. Valente<sup>1,2</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal

<sup>2</sup> Eco-Ethology Research Unity, I. & D. - FCT - N° 331/94

Rua do Campo Alegre, Edifício FC4, 4169-007 PORTO, PORTUGAL

Free-roaming cats include the domestic that are not confined to a yard or house [1] and those who have no owners and who are accidentally or deliberately provided with food, and are commonly known as feral cats; these latter can be further distinguished as semiferal or strays, if socialized toward humans [2]. Published data about feral cat is available in some countries, but in Portugal little is known about the ecology of urban cat populations; the nocturnal behaviour of these mammals, along with their fugitive reaction, makes data collection problematical and is probably one of the reasons for the lack of studies. Such knowledge is however fundamental in order to control feral cat colonies and to avoid the public health problems. One of the reasons to start this study, as far as we know the first in Portugal, is (1) understanding free-roaming cats (*Felis catus*) spatial distribution and what are the determining factors and (2) estimate the size of cat populations and of the number and constitution of cat colonies. We also intend to determine the home-range, using a telemetry approach, of both feral and pet cats.

Monthly observations (both during the day and the night) in a small study area (ca 1.76 km<sup>2</sup>), in the city of Porto, began in November of 2011. Each cat/colony observed is photographed and some information is registered (time, location, behaviours).

Only a preliminary characterization (distribution, abundance and population structure) is possible for now. The analysis of cat spatial distribution revealed that they have a wide distribution in the study area but it seems obvious the relationship that exists between human neighbourhood feeding spots and cat colony location; so cat presence tends to be near major house areas and not in nearby scrublands. Our data is still insufficient but it seems that cat movements (in and out of the study area) might be limited by urban artificial barriers, namely major traffic roads and train/metro lines. So far 110 different free-roaming cats and 12 different colonies were registered in our database; free-roaming cat density might be larger than expected.

The free-roaming cats show a considerable density and wide distribution in the study area. Our estimate must however be considered with caution as they are based on a small number of observations. Additional research is needed to better understand cat spatial ecology. Such information is fundamental when analyzing the risk of disease spreading, both human and animal, and in the research of sterilization programs.

## References:

[1] Slater, M.R. (2001), *The role of veterinary epidemiology in the study of free-roaming dogs and cats*, Preventive Veterinary Medicine, 48 (4), 273-286.

[2] Jones, A.L. and C.T. Downs (2011), *Managing feral cats on a university's campuses: how many are there and is sterilization having an effect?*, Journal of applied animal welfare science, 14 (4), 304-20.

# Species-specific PCR detection and real-time PCR quantification for the authentication of cooked hams

Graciete da S. Santos, M. Beatriz P.P. Oliveira, Isabel Mafra

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal. (E-mail: isabel.mafra@ff.up.pt).

Lately, the concern with consumer's health due the existence of potentially allergenic foods has been growing. Soybean is reported to be the vegetable protein most widely used in the meat industry, including the production of cooked ham, because of the high prices of animal proteins. This ingredient is also included in the list of allergenic foods that are required to be declared according the Directive 2007/68/EC. In fact, it has been described that amounts of soy below 0.1% and 1% (w/w) can lead to allergic reactions in sensitive consumers [1].

Various methods have been applied for identification of species in foods, mainly techniques based on the analysis of proteins, electrophoretic or chromatographic methods. More recently, DNA molecules, due to its higher stability when compared to proteins and its ubiquity in every type of cell, have been the target for species identification. The polymerase chain reaction (PCR) presents a fast, sensitive and specific method.

In this work, we ascertained the authenticity of twenty-one commercial samples of cooked hams and bologna, in compliance with the labelling statements, using species-specific PCR assays. For that purpose, specific primers for soybean detection targeting the lectin gene were used and binary reference mixtures with known amounts of texturized hydrated soybean protein in pork's meat (0.1% to 50%) were prepared. To identify animal species often found in this type food, namely pork (*Sus scrofa*), turkey (*Meleagris gallopavo*) and chicken (*Gallus gallus*) species-specific PCR primers targeting *cytb* gene were used.

The results for soybean detection showed that from the 21 samples tested, three were not according the labelling since undeclared soybean was identified with strong PCR bands. The identification of meat species showed that for pork all the samples were in accordance with labelling. Regarding poultry species, some samples showed the presence of undeclared chicken meat. To estimate the amount of soybean, quantitative real-time PCR assays based on the measurement of fluorescence increments using EvaGreen dye were also performed. A calibration standard curve was obtained using the  $\Delta\Delta C_t$  method, allowing the detection and quantification of soybean. The estimated values showed that the majority of meat samples in which soybean was detected, contained more than 5% of added soybean protein.

## References:

[1] Koppelman, S.J., Lakemond, C.M.M., Vlooswijk, R. and Hefle, S.L. (2004), *Detection of soy proteins in processed foods: literature overview and new experimental work*, J. AOAC Int. 87, 1398.

## Studies on DNA, fertility, proteins and allergenicity of *Platanus* spp. pollen following *in vitro* exposure to Ozone

**R. Neves<sup>1</sup>, R. Sousa<sup>2</sup>, A. Pessoa<sup>3</sup>, L. Duque<sup>2</sup>, H. Ribeiro<sup>2</sup>, M. Pereira<sup>1,3</sup>, I. Abreu<sup>1,2</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Grupo Ambiente do Centro de Geologia, University of Porto, Portugal.

<sup>3</sup> BioFig – Center for Biodiversity, Functional and Integrative Genomics, Portugal.

The increase of allergic respiratory symptoms has been related to the interaction between air pollutants and pollen allergens. Among the atmospheric pollutants, Ozone is one of the most relevant air pollutants. Therefore, the objective of this work is to evaluate the effects of this gas on *Platanus* spp. pollen, focusing on changes in DNA, fertility, proteins and, particularly, its allergenicity.

*Platanus* spp. pollen collected directly from the anther was exposed to concentrations of O<sub>3</sub> below, equal and above the standard values for the protection of human health. Fertility was evaluated through viability tests using Trypan Blue Dye and through *in vitro* germination in an optimized liquid medium, at 25°C, in the dark. Biochemical and immunochemical methods (SDS-PAGE, Western Blotting) were performed in order to analyze differences in the protein profile and in the pollen allergenicity using sera from polysensitized patients to *Platanus* spp.. Genomic DNA was also extracted in order to verify any electrophoretic profile variations. The results were compared with the ones obtained using non-exposed pollen.

A decrease in the viability and germination rates was gradually verified from the non-exposed pollen to the pollen with highest concentration of O<sub>3</sub> and protein content also slightly decreased with the increase of O<sub>3</sub>. Polypeptide profiles from SDS-PAGE didn't reveal any significant differences, however, immunochemical assays indicated higher IgE recognition in patient sera to *Platanus* spp. pollen in all exposed samples in comparison to non-exposed pollen. No variation in the genomic DNA electrophoretic profiles was verified.

Further molecular biology approaches, such as procedures based on mass spectrometry and RT-PCR, will be performed with the aim of identifying potential allergenic pollen proteins and obtaining recombinant proteins for future allergenic tests.

# Study of genetic polymorphisms implicated in sensitivity to taste in African populations

**M. Oliveira<sup>1,2</sup>, C. Valente<sup>1,2</sup>, L. Alvarez<sup>2</sup>, A. Amorim<sup>1,2</sup>, MJ Prata<sup>1,2</sup> and S. Quental<sup>2</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Portugal.

From the Neolithic, most human societies abandoned the hunting and gathering practices, replacing them for agriculture and pastoralism. This change was accompanied by the decrease of diet diversity and the consequent health problems associated to food deficit and new habits [1]. The introduction of new food and behaviors may have led to changes in biological processes, acting like a selective pressure leading to genetic adaptations related to food metabolism and even taste sensitivity [2], the latter explained by the role of genetics in certain food preferences. Nowadays, there are five known taste qualities: salty, sour, sweet, bitter and umami [3], despite the underlying genetic mechanisms of each of them being at different stages of knowledge.

The aim of this study is the genetic characterization of African agriculturalists and pastoralists' populations, through the analysis of single nucleotide polymorphisms (SNPs) in genes known to be implicated in phenotypes related to taste. According to that, SNPs were selected in four genes: TAS1R1 and TAS1R3, belonging to the gene family TAS1R, responsible for umami and sweet tastes; and TAS2R16 and TAS2R38, associated to bitter perception and included in the gene family TAS2R. The interest in African populations relies in several reasons, among which is their life style diversity, since hunter-gatherers, agriculturalists and pastoralists are still present in Africa. Furthermore, African populations remain very scarcely characterized from the genetic point of view.

This study focused two agrarian samples from Angola and Mozambique that included individuals who are Bantu speakers; and a sample representative of a pastoralist life style, composed of Ugandan individuals who speak an Eastern Nilotic language.

In order to detect the polymorphisms, traditional PCR techniques were used to amplify fragments that contain the chosen SNPs. The genotyping of SNPs was done using a SNaPshot reaction kit (Applied Biosystems) and a specific probe to each SNP.

The results obtained will be used to assess patterns of diversity within each population and between them, as well as to evaluate whether those patterns might be related to differences in life style between the population samples analyzed.

## References:

- [1] Jobling, MA *et al.* (2004) Agricultural expansions. *Human Evolutionary Genetics* (Jobling, MA, Hurles, ME and Tyler-Smith, C, eds), pp. 299-306, Garland Publishing
- [2] Luca, F *et al.* (2010) Evolutionary Adaptations to Dietary Changes. *Annu Rev Nutr* 30: 291-314
- [3] Bachmanov, AA and Beauchamp, GK (2007) Taste receptor genes. *Annu Rev Nutr* 27: 389-414





Posters

# Posters from Friday, 24<sup>th</sup>



## **“B” TEAM: a stage on development process of young soccer players**

**P. Mané**<sup>1</sup>

<sup>1</sup> Faculty of Sport, University of Porto, Portugal.

In a country seen as a real Europe's entrance platform for players from Africa and South America, it's worrying that Portuguese professional leagues (1st and 2nd League) are mainly composed by foreign athletes (53.23%) [1], with fewer players coming out from youth teams for their first squads.

Talent identification and development is a complex but vital process both for financial and sportive clubs survival. However, it only gets an effective meaning when it's made a real wager in those young talents, giving them the opportunity to maximize their skills in the first squads. The transition between youth and senior soccer (first squads) it's a very important stage to youth players, being, for many, an extremely hard barrier to cross. The competitive gap between youth and first squads is seen as the main reason for the almost nil bet in youth talents, existing a special need to create competitive opportunities for kids.

This document presents a challenging project framed in the perspective of progressive integration of young soccer players in first squad of an 3rd National Division team, with an appeal to the constitution of one more formative stage, assuming this clearly as a transition phase: the “B” team.

Recognizing the existing limitations, as well the inability to compete with powerful clubs, this document also defines concrete measures for talent identification, based on cooperation with the local community, particularly with educational establishments.

This project is seen as a guideline for the growth and sustained development of the club, in a clear attempt to transform it in a footballers formative center at the geographic area where is inserted, looking for a sportive and financially profit of the existing human resources. Given the relevance of the project to the club development, the main lines base on the construction of own club identity/philosophy, using the synergy between the coach staff in the pursuit of a common gold: contribute to the youth's soccer development, in order to supply the first squad.

### References:

[1] LPFP. *Estatísticas da Liga*. Accessed in: 26/10/2011, in: [http://www.lfpf.pt/futebol/pages/estatisticas.aspx?stat=Player\\_Nationality&epoca=20102011](http://www.lfpf.pt/futebol/pages/estatisticas.aspx?stat=Player_Nationality&epoca=20102011).

## A reflection of the history of sports adapted

**A. Pinho<sup>1</sup>, A. Reyes<sup>1</sup>, R. Farias<sup>1</sup>**

<sup>1</sup> Department of Adapted Physical Activity, Faculty of Sport, University of Porto, Portugal.

According to Rechineli et al. (2008) “ since the beginning of the human’s history, the types of behavior towards people with disabilities were of disposal, destruction and contempt, characterized by the body and its brands.”

The methodology was a literature review, built on a scientific articles removed from online databases.

For Melo (2009), in the Middle ages with disabilities were seen as being possessed by demons. These were in particular places, such as orphanages, asylums, prisons, among others. In the Renaissance, there arise indications of research on the issue of disability. And for Carmo (1991, p.25) meant a landmark in the field of rights and duties of the disabled.

According to Richineli (2008), it was after the World War II that positive attitudes have happened to these people. Started in rehabilitation’s hospital programs for injured wars as a way to reintegrate them into society.

The neurologist Ludwing Guttman, in England, created the National Center for Spinal Cord Injuries at the Stoke Mandeville’s Hospital, which treat the wounded in World treatment of these individuals were basketball, archery, darts and biliards. (Cashman & Darcy, 2008).

For Costa (2004), two trends emerge in this period, with a medical focus, by Guttman, using the sport as rehabilitation; and other sports with a focus that came from U.S. as a means of social integration. Throughout history these two currents intersect forming common goals.

Scientific studies have proliferated in the eighteenth and nineteenth centuries, reaching its peak in the twentieth century, a period marked by social reforms and wars, the early government interests in issues relating to disabled people, especially in education, psychology and medicine. (Rechineli, 2008)

### References:

- [1] Rechineli, A., Porto, E., & Moreira, W. (2008), *Corpos deficientes, eficientes e diferentes: uma visão a partir da educação física. Revista Brasileira Educação Especial*, 14 (2), 293-310.
- [2] Melo, T., & Freitas, A. (2009), *Educação Física Adaptada, uma prática de possibilidades no contexto escolar*. Revista Digital, Buenos Aires, ano 14, n. 136, sep. 2009. Consult. 07 Dez 2011, disponível em <http://www.efdeportes.com/edf136/educacao-fisica-adaptada-no-contexto-escolar.htm>
- [3] Carmo, A. (1991), *Deficiência Física: A sociedade Brasileira Cria, Recupera e Discrimina*. Brasília: Secretaria dos Desportos/PR, pp. 230.
- [4] Cashman, R., & Darcy, S. (2008), *Os Jogos Paraolimpicos de Sydney 2000*. Austrália: Walla Walla Press.
- [5] Costa, A., & Sousa, S. (2004), *Educação Física e Esporte Adaptado: história, avanços e retrocessos em relação aos princípios da integração/inclusão e perspectivas para o século XXI. Revista Brasileira de Ciências do Esporte*, Campinas, 25 (3), 27-42.

# **Abdominal Circumference: Rate of risk factor in sedentary men and women starting with weight training**

**C. Schmidt**<sup>1</sup>

<sup>1</sup> Faculty of Sport, University of Porto, Portugal.

Abdominal obesity is the increase of the fat tissue in the abdominal region which is considered a risk of morbidity, representing great danger when compared to other forms of body fat distribution. By measuring the abdominal circumference, it is possible to obtain a correlation between the abdominal fat and health risks.

This study was made on a group of 104 individuals, 58 sedentary men and 56 sedentary women, over 40 years-old, starting a workout program at a local gym between January 1<sup>st</sup> and December 31<sup>st</sup> 2010. The measurement chosen for this study was the abdominal circumference (CA), which was included in the anthropometric measurements made in physical evaluation process. The goal of this process was to verify in incidence of risk factors associated with abdominal circumference in sedentary men and women.

The average of men's abdominal circumference was 99.6 cm with standard deviation of 10.7 cm; whereas, in women it was 90.6 cm with standard deviation of 10 cm. In the present study, the frequency of the related risk to health and the abdominal circumference was 91.1% of women and 72.5% of men. Among these, 53.6% of women and 51.7% of men were considered as a great risk to health. Because this incidence was very high, it is essential for the person to have a physical evaluation to identify or simply warn them the high risk.

# **An Intergenerational School-based Program of Physical Activity: An Exploratory Research Project**

**S. Carrapatoso<sup>1</sup>, J. Carvalho<sup>1</sup> and A. L. Pereira<sup>2</sup>**

<sup>1</sup> Research Center in Physical Activity, Health and Leisure (CIAFEL)

<sup>2</sup> Faculty of Sports, University of Porto

The main objective of the Intergenerational Programs is to encourage meaningful contact among generations that result in significant benefits for both young and old subjects [1]. Among the intergenerational programs, those that involve Physical Activity context seem to be of special importance since they can offer unique and multidimensional benefits related with health and fitness [2]. However, besides the potential benefits, the area of Intergenerational Physical Activity (IPA) is new and unexplored. In fact, to the best of our knowledge, few studies have assessed the relative effectiveness of IPA programmes with regard of their impact on the promotion of physical activity levels and in the development of positive attitudes between generations. . Furthermore, in some of those scarce studies strong conclusions cannot be made due few quantity and low quality of published empirical studies. To address this gap, we developed a research project expecting to bridge some methodological weaknesses of the intergenerational physical activity studies. We will develop a school-based intergenerational physical activity program consisting in two/time week of physical activity sessions with students and grandparents of pre-primary school. During this program, we will apply a qualitative approach to address social representations toward aging and older adults combined with objective quantitative measurements of physical activity levels and physical fitness of the older participants in the program. A participant and a direct observation will be carried out through 10 sessions which will be complemented by semi-structured interviews and focus group, conducted with the participants in the beginning and in the end of the program. In order to inspect the qualitative results, content analysis technique and interpretation will be applied. With this research project we pretend to provide valuable information for school-based intergenerational programs based on Physical Activity and the related (re) constructions of social representations of aging and older adults.

1] Chodzko-Zajko, W. J. (1999), *Improving quality of life in old age: the role of regular physical activity*, The Aging Male, 2 (4), 218 - 222

[2] Flora, P. K. and Faulkner, G. E. J. (2007), *Physical Activity*, Journal of Intergenerational Relationships, 4 (4), 63 - 74.

## Backstroke technical characterization of 11-13 years old swimmers

A. Silva<sup>1</sup>, P. Figueiredo<sup>1</sup>, S. Soares<sup>1</sup>, J. P. Vilas-Boas<sup>1</sup>, R. J. Fernandes<sup>1</sup>

<sup>1</sup> University of Porto, Faculty of Sport, CIFI2D, Portugal

The technical ability in stroke organization has traditionally been evaluated through changes in velocity, Stroke Rate (SR), Stroke Length (SL) and Stroke Index (SI) [3]. Last decade special attention has been given to the modifications in the temporal organization of arm stroke phases. First for front crawl and after for backstroke, it was proposed the Index of Coordination (IdC) [1]. IdC quantifies the lag time between the propulsive phases of each arm, expressing three different synchronization modes: (i) opposition (IdC = 0), evidencing continuous motor action through a smooth series of propulsive phases without lag time; (ii) catch-up (IdC < 0), evidencing a lag time between propulsive phases of the two arms; and (iii) superposition (IdC > 0), evidencing an overlap of the propulsive phases. According to our knowledge, no studies were conducted in young swimmers. So, the aim of the study was to characterize the backstroke technique of young swimmers performing at very high intensity.

One hundred and fourteen swimmers, 60 pubertal (24 boys and 36 girls) and 54 post-pubertal (34 boys and 20 girls) performed 25-m front crawl at 50-m pace. Two underwater cameras were used to assess general biomechanical parameters (velocity, SR, SL and SI) and IdC).

Table 1. Mean  $\pm$  SD values of velocity, SR, SL, IdC according to genders and maturation.

	Girls (n = 58)		Boys (n = 56)	
	Pubertal (n = 36)	Post-Pubertal (n = 20)	Pubertal (n = 24)	Post-Pubertal (n = 34)
Velocity (m.s <sup>-1</sup> ) <sup>a, b</sup>	1.06 $\pm$ 0.14	1.13 $\pm$ 0.14	1.18 $\pm$ 0.14	1.24 $\pm$ 0.12
Stroke Rate (cycle.min <sup>-1</sup> ) <sup>a</sup>	39.50 $\pm$ 5.88	38.43 $\pm$ 5.92	42.65 $\pm$ 5.46	43.35 $\pm$ 5.80
Stroke Length (m.cycle <sup>-1</sup> ) <sup>b</sup>	1.64 $\pm$ 0.26	1.79 $\pm$ 0.22	1.68 $\pm$ 0.25	1.75 $\pm$ 0.27
Stroke Index (m <sup>2</sup> .s <sup>-1</sup> .cycle <sup>-1</sup> ) <sup>a, b</sup>	1.76 $\pm$ 0.43	2.03 $\pm$ 0.37	2.01 $\pm$ 0.45	2.20 $\pm$ 0.46
Index of Coordination (%)	-9.89 $\pm$ 3.16	-9.77 $\pm$ 2.93	-10.16 $\pm$ 3.60	-10.39 $\pm$ 2.44

Legend: a (boys > girls), b (post-pubertal > pubertal) (P  $\leq$  0.05)

Comparing to a study conducted with adults [2], young swimmers presented lower values in all general biomechanical parameters, having the post-pubertal group closest values of velocity, SL and SI, due to their superior anthropometric and maturational characteristics. Regarding gender, boys showed higher values of velocity, SR and SI than girls. In addition, in accordance to adult studies [2], even at high intensity, age-group swimmers also showed catch-up coordination mode.

### References:

- [1] Chollet D., S. Charlies, and C. Chatard (2000). *A New Index of Coordination for the Crawl Description and Usefulness*. International Journal of Sports Medicine, 21, 54-59.
- [2] Lerda R., and C. Cardelli (2003). *Analysis of Stroke Organization in the Backstroke as a Function of Skill*. Research Quarterly for Exercise and Sport, 74, 215-219.
- [3] Seifert L., M. H. Toussaint, M. Alberty, C. Schnitzler, and D. Chollet, (2010). *Arm coordination, power, and swim efficiency in national and regional front crawl swimmers*. Human Movement Science, 29, 426-439.

## Comparison of manual and pedal time reaction between preferred and non-preferred swimming techniques

C.Cascais<sup>1</sup>, F. Cabral<sup>1</sup>, A. Moraes<sup>1</sup>, R.Fernandes<sup>2</sup>, M. Botelho<sup>3</sup>, O. Vasconcelos<sup>4</sup>

1- Students of Undergraduate Degree of Sciences of Sport, Faculty of Sport, University of Porto, Portugal

2- Department of Swimming, Faculty of Sport, University of Porto, Portugal

3, 4- Department of Learning Motor, Faculty of Sport, University of Porto, Portugal

Time reaction (TR) is the interval between onset of a stimulus and the beginning of the response (Magill, 2007). In swimming, the TR is very important in the start for every events, but more relevant in short races (Maglischo, 1993).

The goal of our study is to analyze the differences in manual (MTR) and pedal speed reaction (PTR), between backstroke swimmers and swimmers of ventral techniques. Our sample was formed by 12 female swimmers of absolute team (juniors and seniors) of Futebol Clube do Porto, six backstroke swimmers and six others techniques swimmers. The mean of the age is  $17,33 \pm 2,06$  years old and the practices years of swimming was  $12,75 \pm 2,77$ . To assess MTR and PTR, we used a TR instrument and collected 32 repetitions for each member. The statistical procedures included the descriptive statistic and the Mann-Whitney test. The significance level was set at  $p \leq 0,05$ .

The main conclusions were: i) there aren't statistic differences in MTR and PTR, for preferred and non-preferred member, between backstroke swimmers and others techniques swimmers; ii) there seems to be a tendency to backstroke swimmers show a greater performance in MTR and PTR, for both members, than the others swimmers; iii) there aren't differences between the two groups in manual and pedal motor asymmetry.

[1] Magill, R.A (2007), *Motor Learning and Control*, McGraw-Hill, New York

[2] Maglischo, E.W. (1993), *Swimming Even Faster* (2<sup>nd</sup> Edition), Mayfield Publishing Company, California

# EFFECT OF THE SPORT IN PEDAL DEXTERITY FOR YOUNG PRACTITIONERS OF BASKETBALL, SWIMMING AND SKATEBOARDING

João Coelho<sup>1</sup>, João Neto<sup>1</sup>, Alexandra Oliveira<sup>1</sup>, Doutor Manuel Botelho<sup>1</sup>, Doutora Olga Vasconcelos<sup>1,2</sup>

<sup>1</sup> Faculdade de Desporto da Universidade do Porto

<sup>2</sup> Responsável pela UC Aprendizagem Motora

Pedal skill is a coordinative and accurate ability which we consider extremely important for this study, especially concerning Basketball, Skateboard and Swimming, sports that often use technical movements with lower limbs.

This hereby research aimed to analyse the effect of each modality practiced in both pedal motor skill and functional motor asymmetry of the practitioners.

Values of pedal skill concerning the preferred foot (PF), the non-preferred foot (NPF) and also the functional motor asymmetry (FMA) within a sample of 15 male individuals, aged 14 years old, with two to three years of practice, were taken in consideration and analysed. For the study and data collection we used the Pedal Tapping Test. By SPSS, version 18.0, the statistical procedures involved descriptive and inferential statistics. The significance level was set at  $p \leq 0.05$ . We may conclude with this research that swimming proves to be the most asymmetric of all, and that skateboard holds the greatest influence, in a short time break (two a three years of practice), in the pedal motor skill of its practitioners both in PF and NPF.

**Key-words:** Pedal Motor Skill, Basketball, Skateboard, Swimming, Functional Motor Asymmetry.

# Improving instruction in a pre-service teacher of Physical Education: presentation of a research project

**E. Vieira<sup>1</sup>, T. Sousa<sup>2</sup> and O. Teixeira<sup>3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal

<sup>2</sup> CIFI<sup>2</sup>D, Faculty of Sport, University of Porto, Portugal

<sup>3</sup> Basic School Júlio Dinis, Portugal

**Introduction:** Instruction plays an important role in the quality of the teaching-learning process. It is vital to focus on key aspects, in order to conduct pupils to the learning of specific and nuclear content. Teachers should face instruction as one of the instruments of pedagogic intervention that they should dominate. The teacher need recognize by analysing deeply the way as this is accomplished with the intention of defining strategies to make possible its evolution. Instruction, namely the *feedback*, is considered as one of the variables that influence the efficiency of the teacher, affecting the teaching and learning process when it isn't properly used. Thus, it is regarded as one basic stage that assures the quality in the transmission of information within instruction [1]. Consequently, it is crucial to analyse if the *feedback* is provided in a specific way, corrective and suitable with the contents of teaching. The focus on the essential is seen as a didactic demand of considerable importance [2]. **Aim:** The main aim of this study is to improve the quality instruction of a pre-service teacher in Physical Education. During this process, for a better understanding of it, the content of instruction will be analysed, not only regarding their purpose and direction, but also concerning their adequacy of critical components of each exercise applied in the class. After explore and understand the information achieved, some strategies to correction and consequent improvement will be outlined in order to improve instruction. In second and third phases, the progress of the study will be repeated in order to new developments. The last one will consist of a final confirmation of the content of instruction. **Methodology:** Action-research will be used as the research methodology. The aim is to change the practice of the participant by trying to improve the intervention of the actors [3]. In this study, the pre-service teacher will be effective of teaching practice at Basic School Júlio Dinis. It is expected that he analyse and transform your instruction in the context, during the academic year 2011-2012. The content of the verbal instruction (speech) of the pre-service teacher will be recorded in audio, using a lapel recorder device. The collected data will be fully transcribed. Two classes will be recorded (one of forty-five minutes and another of ninety-minutes) covering three different sports (gymnastics, football and volleyball). The transcription and analysis will be done immediately after the data collection, so that it can provide valid information for following classes.

## References:

- [1] Rosado, A. & Mesquita, I. (2009). Melhorar a aprendizagem otimizando a instrução, In A. Rosado & I. Mesquita (Eds.), *Pedagogia do Desporto*, Edições FMH, Cruz Quebrada, pp.69-130.
- [2] Bento, J. (2003), *Planeamento e avaliação em Educação Física*, Livros Horizonte, Lisboa.
- [3] Carr, W. & Kemmis, S. (1986), *Becoming critical: Education, knowledge and action research*, Falmer Press, England.

# Laterality and Ballet. The effect of Crossed vs Ipsilateral Hand/Foot Preference on the Static Balance of Classical Dancers

Ana Meneses<sup>1</sup>, Joana Oliveira<sup>1</sup>, Sara Silva<sup>1</sup>,  
Doutor Manuel Botelho<sup>1</sup>, Doutora Olga Vasconcelos<sup>1,2</sup>

<sup>1</sup> Faculty of Sport, University of Porto

<sup>2</sup> Head of Motor Learning course

## Abstract

The present study aims to compare the effect of Crossed vs Ipsilateral Hand/Foot Preference on the Static Balance of Classical Dancers. Our sample comprises 16 female with a chronological age between 14 and 20 years ( $17,00 \pm 1,71$ ), attending to a private ballet school.

Core et al. (1981) [1] and Van Strien (2002) [2] Lateral Preference Questionnaires were applied to identify hand and foot preference. The Flamingo Balance Test (EUROFIT, 1983) [3] was used to evaluate the static balance. Statistical analysis was conducted using PASW Statistics 18.0 and procedures included descriptive statistics followed by the Mann-Whitney U test with a 5% level of significance.

This study allowed us to conclude that: i) ballet dancers showed better performance with their non-preferred foot; ii) ipsilateral subjects presented an average of falls superior to the cross lateral ones; iii) the difference between ipsilateral and cross lateral subjects was not statistically significant with respect to the static balance; iii) however, there was a tendency, like the literature suggests in this domain, for the cross lateral subjects to present a better performance when compared to their ipsilateral counterparts, with regard to the preferred foot. The major conclusion of this study is based on the fact that no significant differences were found between ipsilateral and cross lateral subjects in static balance of classical dancers.

[1] Coren, S. e Porac, C. (1981). *Lateral preferences and human behavior*. New York: Springer-Verlag.

[2] Van Strien, J. W. (2002) – *The Dutch Handedness Questionnaire: FSW*, Department of Psychology, Erasmus University Rotterdam.

[3] EUROFIT (1983) - *Physical Fitness Test Battery* (Council of Europe, 1983). Flamingo Balance Test

## Manual dexterity and functional motor asymmetry in symmetric and asymmetric sports. A study in swimmers and tennis players.

R. Oliveira<sup>1</sup>, T. Ferreira<sup>1</sup>, T. Lourenço<sup>1</sup>, M. Botelho<sup>1,2</sup>, O. Vasconcelos<sup>1,2</sup>

<sup>1</sup>Faculdade de Desporto da Universidade do Porto

<sup>2</sup> Responsável pela UC Aprendizagem Motora

The coordinated movements of the arms play an important role in tennis and swimming. The hand is an essential element for the handling of the racket in tennis and to the performance of the various techniques in swimming. The range of motor experiences in tennis focuses more intensively on the preferred member over the non-preferred one. This no longer applies in swimming, in which the range of experiences is similar for the two members (Maglischo, 2006) [1].

The aim of this study is to investigate the influence of practicing symmetrical or asymmetrical sports on manual dexterity and function motor asymmetry, study carried out in federated tennis players and swimmers. The sample of this investigation comprises 24 high level athletes, 12 tennis players (6 males and 6 females) and 12 swimmers (6 males and 6 females). The participants representing tennis come from different clubs from the north of the country and their ages range between 16 and 22 years old. The swimmers come from a club from the north of the country, too, and their ages range between 15 and 20 years old. To assess manual dexterity we used the *Plate Tapping Test*, Eurofit (1983) [2] and we assessed preferred hand, non-preferred hand and correspondent functional hand asymmetry. The statistical procedures using SPSS version 19 included descriptive statistic (mean and standard deviation) and inferential statistic (Mann Whitney Test). Significance level was set on  $p \leq 0.05$ . The main conclusions were: (i) In the entire sample, an absence of differences according to the groups studied; (ii) In females tennis players, a non-significant tendency to perform better with the non-preferred hand; (iii) Males tennis players were significantly more asymmetric than their counterparts males swimmers.

[1] Maglischo, E. W. (2006). *Swimming Even Faster*. California: Mayfield Publishing Company.

[2] EUROFIT (1983) - *Physical Fitness Test Battery* (Council of Europe, 1983). Plate Tapping.

## **Physical Education as a contribute to the integration of a student with special educational needs**

**M. Sousa<sup>1</sup>; R. Carvalho<sup>2</sup>; P. Batista<sup>3</sup>**

<sup>1</sup>Faculty of Sports of the University of Porto, Portugal.

<sup>2</sup>Paços de Ferreira Secondary School/3, Portugal.

<sup>3</sup>CIFI2D, Faculty of Sports of the University of Porto, Portugal.

This study it's a personal narrative of a Physical Education pre-service teacher in practicum context of master degree in Physical Education teaching, at the academic year 2011/2012. The issue was the process of integration of a student with special educational needs in Physical Education classes. The student in focus attends the 7<sup>th</sup> degree, he has 14 years age and presents in limitations on the cognitive level. Reason why is marked as a student with special educational needs. He is the last of five brothers who have a history of school dropout. In spite belong to the class since last year his integration it still very precarious. It was made an analysis of board diary, developed during the first period of the academic year 2011/2012, relating to strategies and perceptions of the results achieved in the class integration process of the student. The data were submitted to a content analysis taking into account the following defined categories: i) strategies ii) implementation iii) teacher perceptions of the strategies effects. The analysis, including its limitations and strengths, allowed me to select the most appropriate strategies to used; ii) it was visible that the improvement of student's motivational levels, brought improvements in self-esteem, which contributed to a positive social interaction with other students within the class; iii) the understanding of the students needs was the crucial point for that through strategies, in particular the responsibility increased in the classroom, led to positive results. At the end of the study the student showed more motivation for the class activities, and to cooperate with their colleagues purchase their respect. The attendance to lessons has also become a reality. Aspects not seen previously. The lived experience until this moment allowed me to understand that the teacher's role goes far beyond the teaching. Indeed, with regard specifically to the Physical Education teacher, this has the responsibility to contribute to other acquisitions, namely the psychosocial concepts, in which values of sport take presence. This contribution arises as fundamental to students with special educational needs.

Keywords: Integration, Special Educational Needs, Physical Education, Pre-service teacher

## Styles of Supervision in the context of a practicum group: a study with Cooperating Teachers of Physical Education

G. Silveira<sup>1</sup>; P. Gomes<sup>1</sup>; C. Ferreira<sup>1</sup>; A. L. Pereira<sup>1,2</sup>; P. Batista<sup>1,3</sup>

<sup>1</sup>Faculty of Sport, University of Porto, Portugal.

<sup>2</sup>IS-FLUP, Institute of Sociology, Faculty of Arts and Humanities, University of Porto, Portugal.

<sup>3</sup>CIFI<sup>2</sup>D, Centre for Research, Education, Innovation and Intervention in Sport, University of Porto, Portugal.

The issue of pedagogical supervision has been assumed as a research area with increased relevance and is consistently accepted that the qualification process is crucial for the development of teachers in training. If in the past the supervision was seen as a process in which a teacher, more experienced and informed, directed another teacher or a teacher candidate in their professional training [1], nowadays, this process is considered as a formative process that contributes to the development of both: the initial teacher education and the Cooperating Teacher. In this way, the idea of supervision being related to connotations of "leadership", "dirigisme", "levy" and "authority" [2], gradually began to fade, earning more and more a collaborative form of guidance and cooperation. This study aimed to compare the way cooperating teachers perceive their supervision actions in the context of the teaching practice to the ways they conduct them. Two cooperating teachers with great experience in supervision participated in the study and were members of the list of cooperating teachers at the Faculty of Sports at the University of Porto, in the 2010/2011 academic year. The *corpus* of the study included conducting interviews and workshops for pedagogical practice. The semi-structured interview incorporates as main themes the practicum orientation, the reasons for being supervisors and the contributions of the teaching practice. The recordings were made autonomously by cooperating teachers during the workshops on teaching practice. For analyzing data a content analysis approach was used, aided by the use of the QSR NVivo9. The results indicate that during the interviews, the cooperating teachers preferably showed a supportive supervision style, even though one of the participants reveals the existence of a confluence between the interpretive and the supportive styles. During the seminars, it was found that the style of supportive supervision was the only expressed by both.

Acknowledgments: authors acknowledge financial support given by University of Porto and Santander Totta through "Projetos Pluridisciplinares 2010\_código 83"

### References

[1] Alarcão, I. and Tavares, J. (1987), *Supervisão da prática pedagógica: uma perspectiva de desenvolvimento e aprendizagem*, Livraria Almedina, Coimbra.

[2] Vieira, F. (1993), *Supervisão: uma prática reflexiva de formação de professores*, Edições Asa, Rio Tinto.

## Swimming Team Cohesion: differences between gender and competitive levels

C.Cascais<sup>1</sup>, F.Silva<sup>1</sup>, A. Lopes<sup>1</sup> and C.Dias<sup>2</sup>

<sup>1</sup>Students of Undergraduate Degree of Sciences of Sport, Faculty of Sport, University of Porto, Portugal  
<sup>2</sup>Department of Psychology, Faculty of Sport, University of Porto, Portugal

Team cohesion is considered one of the factors that influence the performance [1]. However the strategies to develop it don't care about possible differences between male and female athletes [2]. The goal of our study is to analyze the differences between female and male swimmers and between two competitive levels (juniors and seniors) in the perception of cohesion of one of the best Portuguese swimming teams.

The sample was formed by 37 swimmers, 19 male and 18 female, of absolute category (12 juniors and 20 seniors). The mean of the ages is  $18,22 \pm 3,25$  years old, with  $13,97 \pm 4,04$  years of swimming practice. We used as instrument the Group Environment Questionnaire (Antunes and Cruz, 1995; adapted and translated from Carron and colleagues, 1985) that measures four dimensions: Group Integration-Social (IG-S), Group Integration-Task (IG-T), Individual Attraction to the Group-Social (AI-S) and Individual Attraction to the Group-Task (AI-T). The Individual Attraction represents the attraction that each element feels about the group. The Group Integration expresses the understanding the team as a whole. The statistic procedure included descriptive measures, mean and standard deviation, and the parametric test *t* the student. We used the informatics program SPSS, version 18.0.

The main conclusions were: i) the male swimmers are more cohesive in IG-S and IG-T than female swimmers; ii) there aren't statistic differences in cohesion between juniors and seniors; iii) there seems to be a tendency to seniors swimmers be more cohesive than juniors swimmers.

[1] Carron, A. and Hausenblas, H. A. (1998), *Group Dynamics in Sport*, Morgantown: Fitness Information Technology, USA

[2] Latham, S. A (2007), *Gender differences in collegiate athletes' perceptions of cohesion: a comparison study using mixed methodology*, Capella University, USA

# The contribute of the school resident teachers to the learning process in the practicum context: a self-narrative of a Physical Education pre-service teacher

**P. Moreira<sup>1</sup>, R. Carvalho<sup>2</sup> and P. Batista<sup>3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal.

<sup>2</sup> Secondary School | 3 of Paços de Ferreira, Portugal.

<sup>3</sup> CIF12D, Faculty of Sport, University of Porto, Portugal.

The practicum is usually understood as a fundamental stage on the student's learning process, usually it was the first contact of the future teacher on the school reality. As Lave and Wenger (1991) and Wenger (1998) [1,2] said, professional learning can't be dissociated from the contexts on which the experiences happen and develop, namely the participating on social activities developed by the community on which they are working on. On the teachers learning process the several interactions established between the candidate to teacher and the elements of the school community accept themselves as essentials on their developing process. On the other hand, doing the practicum at a school which was attended by the pre-service teacher as a student, during the junior and secondary school, represent the reunion with teachers who participated on his education. This context determines the type of relationship that the pre-service teacher developed at the practicum context. This study represents the analysis of a personal report by a Physical Education pre-service teacher, from the master degree of Physical Education (PE) on Teaching, at the school year 2011/2012. The focus was the type of interactions established between the pre-service teacher and the PE school's teachers and his contribute to his learning process. The data was the reports written on the board diary developed by the pre-service teacher (the author), during the first semester of the practicum. After locate the relevant information for the study, the corpus of the study was six reports in a total of nine pages which illustrate the interaction established with the PE teachers, namely the ones which occurred on group meetings, informal conversations and sports activities at school. From data the analysis it was clear that: i) PE teachers had a important role in my school integration and in the educational community; ii) the close relation which I established with them was very important to overcome some problems related with the teaching/learning process, namely I share some anguishes and dilemmas. The swap of experiences, resulting from that interaction, allowed me to make more consistent decisions concerning class control strategies, class management and ways to solve some problems; iii) the participation and involvement of the elements of the practicum group on the activities organized by the PE teachers was also influenced by this close relationship with all the teachers and iv) teachers who in the past influenced my learning process kept on giving their contribute, now on a professional level. It is still important to highlight that building the board diary has been a strategy which has enhanced the thinking about developed practices the learning itself, revealing to be an important instrument on the development of my professional skills as a future PE teacher.

## References:

[1] Lave, J., & Wenger, E. (1991). *Situated learning: Legitimate peripheral participation*. Cambridge University Press, New York.

[2] Wenger, E. (1998). *Communities of practice: Learning, meaning, and identity*. Cambridge University Press, New York.

# The effect of practice (futsal and athletics) on pedal reaction time and pedal dexterity in young athletes

**P. Machado<sup>1</sup>, P. Cardoso<sup>1</sup>, P. Brito<sup>1</sup>, M. Botelho<sup>1</sup>, O. Vasconcelos<sup>2</sup>**

<sup>1</sup> Faculdade de Desporto, Universidade do Porto, Portugal.

<sup>2</sup> Responsável pela UC Aprendizagem Motora, Faculdade de Desporto, Universidade do Porto, Portugal

With the rise of national futsal and athletics results, it's ever more important to study young athletes in order to improve at a higher level their performance and making possible to reach the best results. These two sports request important coordinative skills that can be measured using objective tests. Reaction speed [1] and motor-pedal dexterity [2] was previously described and was largely used in this kind of studies. The aim of this study was to compare futsal and athletics young athletes according to their reaction time and motor-pedal dexterity.

We studied 20 male athletes (10 athletics and 10 futsal players) aged between 13 and 15. We assessed their preferred foot reaction speed using the Nelson Foot Reaction Test (NFRT). Dexterity evaluation for the preferred foot (PF), non-preferred foot (NPF) and correspondent functional foot asymmetry (FFA) involved the Tapping Pedal Test (TPT) (number of alternate taps in 10 seconds). As statistical procedures, we used the descriptive statistics, the Mann-Whitney test and the Pearson correlation test. The significance level was set at  $p \leq 0.05$ . Results of NFRT and TPT are presented in table 1.

Table 1: Mean and standard deviation of measured tests (NFRT and TPT) and Mann-Whitney test comparing Athletics and Futsal.

NFRT (ms)		Athletics	Futsal	p
TP (taps in 10s)	PF	40.60±3.57	44.60±6.67	p=0.225
	NPF	37.60±2.50	38.40±6.24	p=0.568
	FFA	3.40±1.71	7.00±2.71	p=.005

We observed statistically significant differences between both groups in the preferred foot reaction time and, according to TP, in the functional foot asymmetry.

Correlation analysis was significant between NFRT and TPT for the total sample ( $r=-0.526$ ,  $p=0.009$ ) and for the athletics ( $r=-0.777$ ,  $p=0.004$ ), when we analysed the groups separately. In this case, negative correlations means that a better performance in foot reaction time is associated with a better performance in pedal dexterity.

With this study we are able to conclude that futsal players presented the fastest foot reaction time but an asymmetric motor function. We suggest a symmetric stimulus of both preferred and non-preferred foot in trainings.

[1] Carvalho, F. (2009). *Avaliação da Destreza e Velocidade de Reacção Manuais em Idosos: estudo comparativo entre idosos praticantes de hidroginástica, idosos não praticantes, e entre os não praticantes, um grupo de idosos exercendo actividades no campo (agricultura) e um outro de sedentários, residentes em Vila Nova de Famalicão*. Monografia. Porto: FADEUP.

[2] Pereira, A. (2010). *Destreza motora e assimetria motora funcional em sujeitos com Síndrome de Down. Efeito do grau de deficiência e do sexo*. Dissertação de Mestrado. Porto: FADEUP.

## **The importance of the group experience for the (re)construction about the pre-service teacher understanding of being a teacher**

**C. Ferreira<sup>1</sup>; P. Mota<sup>2</sup>; P. Batista<sup>3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal.

<sup>2</sup> Almeida Garrett High School, Portugal.

<sup>3</sup> CIFI<sup>2</sup>D, Faculty of Sport, University of Porto, Portugal.

The act of sharing is an essential element to the development of the pre-service teacher in practicum context. So, the student training must be developed within a group that should work as a community of practice, [1], as a learning community. Thus, the aim of this study was to analyze the meanings attributed by the pre-service teacher (the author) to the contribution of the different actors in the process of student training (pre-services teachers, cooperating teacher and supervisor), as well as the other elements of the educational community (colleagues from other professional practicum groups, teachers and students) in the process of professional identity construction. The author made a self-analysis of his/her board diary during the period of student training from 20 September 2011 to 12 January 2012. In the board diary were registered class experiences, practicum group seminars, informal conversations with colleagues from others practicum groups, classes observations and reflections of specific themes.

From the reflection about the written experiences, we can infer that: i) the process of sharing goes far beyond the elements of the practicum group (the community of practice), extending to other elements of the educational community; ii) the cooperating teacher is an essential element in the pre-service teacher learning process, because (a) he/she made a systematic supervising of teaching practice; (b) the knowledge that he/she acquires about the pre-service teacher personal characteristics contributes to give better indications of the development of teaching skills; iii) the others pre-service teachers of the practicum group work as references, because their characteristics and practices are different from ours, and they analyze our performance, particularly through the reflections about the classes observations; iv) informal conversations with students proved to be important elements that led me to question some conceptions and to influence my practice; v) conferences with other practicum groups allow to share knowledge.

From this revisiting of notes about my experiences on professional training was still evident that the board diary is a key element which, by itself, contributes to achieve new and renewed understandings of what is being a teacher and, therefore, for the construction of pre-service teacher professional identity.

### References:

[1] Wenger, E. (1998), *Communities of practice: learning, meaning and identity*. Cambridge: Cambridge University Press.

# The role of anticipatory socialization and practicum experience to the (re)construction of Professional Identity: a study with Pre-service Teachers of Physical Education

P. Gomes<sup>1</sup>; C. Ferreira<sup>1</sup>; P. Batista<sup>1,3</sup>; A. L. Pereira<sup>1,2</sup>

<sup>1</sup> Faculty of Sport, University of Porto, Portugal.

<sup>2</sup> IS-FLUP, Institute of Sociology, Faculty of Arts and Humanities, University of Porto, Portugal.

<sup>3</sup> CIFI<sup>2</sup>D, Faculty of Sport, University of Porto, Portugal.

Among several factors, the process of learning to teach is largely influenced by the characteristics of the pre-service teachers, the quality of learning experiences that are provided for supporting the supervisor, and the school environment [1-3]. The purpose of this study was to describe and to interpret the expectations of pre-service teachers and to understand how they constructed their professional identity as teachers. For data collection semi-structured interviews were conducted with ten pre-service teachers of the Faculty of Sport, University of Porto, in the beginning and in the end of the academic year 2010/2011. The pre-service teachers statements were recorded and transcript. The transcriptions were submitted to thematic analysis, using the Nvivo9. Data showed that: (i) the development of skills and capabilities exceeded their expectations, while the characteristics of the classroom and the school's physical space fell short of expectations; (ii) contrary to initial feeling the pre-service teacher doesn't remember negative moments. The positive moments that they remember were the increasing of motivation and involvement in classes and in extracurricular sports activities from their students; (iii) pre-service teachers initially felt some required tasks as formal requirements, except in the planning process, in particular the M8 of Joan Vickers [4] knowledge structure model, which was considered very useful. They understand its functionality and usefulness; (iv) the investment of their own initiative in class' tasks was a reality. They gained space to be more autonomous and they felt necessity to involve students in extracurricular-activities, because they think that the promotion of culture and sports pleasure in student is a duty; (v) teaching was the task that the pre-service teachers felt more comfortable and the best sensation as teachers was (a) when they observed the students gradual evolution; (b) when students recognized the authority, the leadership and the work of pre-service teacher; (vi) the pre-service teachers value the learners in community practice in school and distinguish the different roles of the faculty supervisor and cooperating teacher. The positive interaction between both was also considered as fundamental.

Acknowledgments: authors acknowledge financial support given by University of Porto and Santander Totta through "Projetos Pluridisciplinares 2010\_código 83"

## References

- [1] Stones, E. (1984), *Supervision in teacher education: A counseling and pedagogical approach*, Methuen, London
- [2] Bullough, R. V.; Jr., Knowles, J. G. and Crow, N. A. (1991), *Emerging as a teacher*, Routledge, London.
- [3] McNally, J., Cope, P., Inglis, B. and Stronach, I. (1994), Current realities in the student teaching experience: A preliminary inquiry, *Teaching and Teacher Education*, 10 (2), 219-231.
- [4] Vickers, J. N. (1992), Gaze control in putting, *Perception*, 21(1), 117-132.

# The Teacher's Active Control as a requirement of a good environment: A self-analysis of a Physical Education pre-service teacher

**V. Ribeiro<sup>1</sup>, R. Carvalho<sup>2</sup> and P. Batista<sup>3</sup>**

<sup>1</sup> Faculty of Sport, University of Porto, Portugal

<sup>2</sup> Secondary School | 3 of Paços de Ferreira, Portugal

<sup>3</sup> CIFI2D, Faculty of Sport, University of Porto, Portugal

This study results from analysis of the personal experiences of a pre-service-teacher, in the student teaching context, in Physical Education master degree, at Faculty of Sport, University of Porto, of the academic year 2011/2012. Taking as reference Rink (1993) [1] statement, which refers that the creation of favorable environmental designs is a valuable instrument for teaching, the purpose of this study was the self-analysis of written personal reflections in a board diary, relating to class teaching, more specifically on teacher active control strategies with the purpose of create a good environment for teaching and, consequently, improve student learning. The corpus of this work was the board diary developed during the first semester of the school year, centered in teaching issues. The content analysis resulting in the following evidences: (i) the active control, contrarily to my initial understanding, beyond the behavior control of the class; (ii) the teacher has to make itself felt in the classroom, communicating with students in a regular and structured way, paying attention to practice all the time, thus demonstrating; (iii) the positive reinforcement became a decisive factor in creating good teaching environment and, consequently, more and better learning. In summary, for me it was evident that is necessary maintain an active practice control in all teaching components, using the vision, the displacements, the physical proximity and constant attention to practice. Moreover, the manner of establishing this control should contribute to the creation of consistent climate and encouragement that improve learning. In this sense, the teacher should stimulate the desired response in students. Finally, I noted that revisiting the board diary made me think about my teaching practice and bring new elements to the improvement of my speech with students, in classroom context. The board diary has proved to be a pedagogical tool extremely useful in the process of my knowledge building as pre-service teacher.

**Key words:** Practicum; Physical Education; Active control; auto- narrative.

References:

[1] Rink, J. (1993), *Teaching Physical Education for Learning* (2<sup>a</sup> Ed.). St. Louis: Times Mosby College Publishing.

## Hand Reaction Speed in Wheelchair Basketball Players

G. Ferreira<sup>1</sup>, J. Silva<sup>1</sup>, J. Vaz<sup>1</sup>, M. Botelho<sup>1</sup>, R. Corredeira<sup>1,2</sup>, O. Vasconcelos<sup>1,3</sup>

<sup>1</sup> Faculdade de Desporto da Universidade do Porto

<sup>2</sup> Responsável do Gabinete de Educação Física Especial

<sup>3</sup> Responsável pela UC Aprendizagem Motora

Sport is seen as an essential tool for integration and social interaction, being possible to say that physical activity may be the way for that disability should be seen as a limitation and not an exclusion factor [1](Guttman, 1976).

With this research we intend to contribute to a better understanding of the reaction speed ability in athletes from Wheelchair Basketball, at the competitive and recreational level, before and after a training session. In this way we hope to understand the differences between these two groups with respect to the ability to react as quickly as possible, in order to develop research programs based on a more scientific source of information and giving the possibility to act and to work with the athletes to reach higher levels of performance.

This study aims to investigate the manual reaction time [2] according to preferred hand (PH), non-preferred hand (NPH) and the correspondent functional hand asymmetry (FHA) [3], in all athletes, comparing two moments (before and after a training session) and two groups (competitive team and recreational team).

The sample comprises 8 males athletes divided in two teams of 4 elements each. The APD-Braga is the competitive group, and the APD – Porto is the recreation group. To assess hand reaction speed we used the Nelson Hand Reaction Test (1965). Statistical procedures include descriptive analysis and the Wilcoxon and Mann-Whitney non parametric tests. Significant level was set at  $p \leq 0,05$ .

The main conclusions show that: (i) According all the sample, there were no differences before and after training. However, the NPH presented a tendency to be better after training; (ii) Analyzing separately the groups, neither recreation team nor competition team showed differences between both moments. Nevertheless in the recreation team, the NPH had a tendency to a better performance after the training session and the FHA decrease in the second moment; (iii) When comparing the groups, we observed, before training, that the competition group presented a significant better performance with respect to the NPH and a tendency to be better with the PH. This group also demonstrates a significant reduced FHA when compared to the recreation group; (iv) After training, there were no significant differences between competition and recreation groups, we only highlight tendency to a better performance in the PH of the competition group.

[1] Guttman, L. (1976). *Sport for the physically handicapped*. Paris: Unesco.

[2] Azevedo, A. S. M. (2008). *Velocidade de reação e destreza manuais em idosos estudo comparativo entre idosos praticantes e não praticantes de atividade física*. Monografia Porto:FADEUP.

[3] Oliveira, P. P. (2010). *Destreza e assimetria motora funcional estudo em idosos de diferentes contextos*. Dissertação de Mestrado Porto: FADEUP.

# Automatic Segmentation of Diabetic Retinopathy Exudates in Retinal Images

**Catarina Carvalho<sup>1,2</sup>, Cátia Silva<sup>1</sup>, Marlene Machado<sup>1,2</sup>, Aurélio Campilho<sup>1,2</sup>**

<sup>1</sup> Departamento de Engenharia Eletrotécnica e de Computadores, Faculdade de Engenharia, Universidade do Porto, Portugal.

<sup>2</sup> INEB – Instituto de Engenharia Biomédica, Divisão de Sinal e Imagem, Campus FEUP, Porto, Portugal.

This study was developed within the Computer Aided Diagnosis course of the Master degree in Biomedical Engineering at FEUP.

The human eye can be affected by several diseases that can lead to blindness or vision defects. One of those diseases is the diabetic retinopathy which causes progressive vision loss ending on blindness. This disease is characterized by the presence of microaneurysms, hemorrhages, exudates, blood vessel dilation and progressive retinal neovascularization [1]. Exudates are yellow deposits of lipid in the retina. Its early detection prevents the patients' blindness. However, manual detection is a slow and monotonous process which leads to a low disease screening. For this reason, the computer science favors the development of new screening methods, improving quality of life.

We propose a novel automatic image processing approach for exudates detection and segmentation based on retinal image analysis. The algorithm for exudates detection is composed of 5 main stages: (1) image preprocessing, for image normalization; (2) processing for removing the vessels and optic disc, (3) candidates detection, to identify the candidates to exudates, (4) candidates classification, using color information, (5) identification of retinopathy risk.

These images were acquired from the database of the Hamilton Eye Institute Macular Edema Dataset (HEI-MED) [2]. To perform the evaluation of our results we compared it to the ones obtained by Giancardo et al. on [3]. The results show that we can improve the performance of the detection exudates phase. Our approach labeled the detected exudates as certainly exudates, high probably exudates and low probability exudates. The strong point of our algorithm is that it is very accurate in the analysis of images without exudates. The classification was effective for the elimination of some false positives.

As conclusion we can say that the proposed approach can be used as a robust screening method. However, it is necessary professional identification of exudates in order to validate the results.

## References:

- [1] Agurto, C.; Murray, V.; Barriga, E.; Murilo, S.; Pattichis, M.; Davis, H.; Russell, S.; Abàmov, M.; Soliz, P. (2010), *Multiscale AM-FM Methods for Diabetic Retinopathy Lesion Detection*, IEEE Trans. Medical Imaging, 29 (2).
- [2] HEI-MED. [Online] <http://vibot.u-bourgogne.fr/luca/heimed.php>.
- [3] Giancardo, L.; Meriaudeau, F.; Karnowski, T. P.; Li, Y.; Tobin Jr., K. W.; Chaum, E. (2011), *Automatic Retina Exudates Segmentation Without a Manually Labelled Training Set*, IEEE, pp. 1396–1400.

# Development of a Cost-Effective Tool for Home-Based Rehabilitation of Stroke Patients

**Carlos Faria<sup>1</sup>, Jorge Siva<sup>1,2</sup>, Aurélio Campilho<sup>1,3</sup>**

<sup>1</sup>Instituto de Engenharia Biomédica, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup>Department of Informatics Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>3</sup>Department of Electrical and Computer Engineering, Faculty of Engineering, University of Porto, Portugal.

Usually, stroke patients take physiotherapy with the help of physiotherapists to evaluate if they are following the rehabilitation protocol properly. However, such people are forced to return to their homes immediately after initial rehabilitation in hospitals without receiving enough treatment [1]. Therefore, more and more interest has arisen towards the development of home-based rehabilitation tools through which patients can keep their rehabilitation exercises and get feedback about their performance.

The main goal of this project is to develop a new tool to help stroke patients performing rehabilitation tasks at home without the presence of a therapist. The project is mainly focused on the Sit-to-Stand (STS) movement and in the rehabilitation of balance. The system is centered around two Nintendo Wii balance boards which have the potential to indicate the patient center of mass (COM) [2]. It also includes a computer equipped with Bluetooth and a webcam that records the movements.

A graphical user interface (GUI) is being built in Matlab for both patient and therapist use. The system comprises three different types of exercises including a free session, a weight-shifting game and a STS movement analysis. One of the balance boards is used to collect data from the upper body COM and the other one collects data from the lower body COM of the patient. In the weight-shifting game, the patients are encouraged to shift their COM for certain points previously established by the therapist in order to recover their balance capabilities. The STS movement analysis is performed when the patient stands up from a chair. Both video and COM data from these exercises are automatically analyzed and a visual feedback about their effectiveness is given to the patients. Finally, all data is saved for future consultation and analysis by the therapist.

Thus, the therapists may obtain a more objective and accurate assessment of the patient's recovery. Furthermore, stroke patients seem to be more excited for these types of rehabilitation exercises [3].

## References:

- [1] Tao, Y. and Hu, H. (2004), *Colour Based Human Motion Tracking for Home-based Rehabilitation*, IEEE Int. Conf. on Systems, Man and Cybernetics, The Hague, 1 (1), 773-778.
- [2] Kennedy, M.W., Schmiedeler, J.P., Striegel, A.D., Crowell, C.R., Villano, M. and Kuitse, J. (2011), *Enhanced Feedback in Balance Rehabilitation using the Nintendo Wii Balance Board*, IEEE 13<sup>th</sup> Int. Conf. on e-Health Networking Applications and Services (Healthcom), Columbia, pp. 162-168.
- [3] Sugarman, H., Weisel-Eichler, A., Burstin, A. and Brown, R. (2009), *Use of the Wii Fit system for the treatment of balance problems in the elderly: A feasibility study*, IEEE Int. Conf. on Virtual Rehabilitation, Haifa, pp. 111-116.

# Development of novel dry multi-pin electrodes for electroencephalogram recording

B. Branco<sup>1</sup>, G. Vieira<sup>1</sup>, P.Chaves<sup>2</sup>, P. Almeida<sup>2</sup>, P. Pedrosa<sup>1,3</sup>, F. Barbosa<sup>2</sup>,  
C. Fonseca<sup>1,3</sup>

<sup>1</sup> Universidade do Porto, Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e de Materiais, Rua Roberto Frias, s/n, 4200-465 Porto, Portugal

<sup>2</sup> Universidade do Porto, Faculdade de Psicologia e Ciências da Educação, Laboratório de Neuropsicofisiologia, Rua Alfredo Allen 4200-135 Porto Portugal

<sup>3</sup> SEG-CEMUC – Department of Mechanical Engineering, University of Coimbra, Rua Luís Reis Santos, 3030-788 Coimbra, Portugal

**Introduction:** Electroencephalography (EEG) is the recording of the brain electrical activity along the scalp [1]. EEG is nowadays the most widely used brain research technique. It provides very good time-resolution of brain activity, and it offers significant advantages over competing state of the art technologies, such as MRI (magnetic resonance imaging) and CT (computerized tomography). However, despite all technological advancements over the last years, EEG signal acquisition still remains a challenge, essentially due to the scalp-electrode signal transfer. The conventional recording set-up generally involves the use of silver/silver chloride electrodes (Ag/AgCl) for signal transduction [1]. These are very reliable but a skin preparation and gel paste application are needed before the exam in order to lower the skin/electrode impedance. This preparation is time consuming, uncomfortable to the patient, it sometimes generated allergic reactions and it requires trained staff.

We propose in this work a novel electrode design with specific shape for hair layer interfusion and reliable skin contact, with a multi-pin shape. The electrode body is made of thermoset polyurethane (PU) onto which a silver layer was deposited. This electrode doesn't require any paste application before the exam, making it appropriate for self-application. In the current work we describe the development of the new electrode and its testing in real EEG tests in human volunteers.

**Experimental:** the electrodes were coated with a silver layer by using an electroless plating method, optimized in this work and inspired in the methodologies described by Takeyasu [2] and Lobodzinski [3]. Briefly, the electrodes substrates are immersed in a 1M solution of AgNO<sub>3</sub> (water/methanol solution (1:1) for 1h and then they are immersed in a NaBH<sub>4</sub> solution (40 mM) for 10 m. Finally, the samples are immersed in a AgNO<sub>3</sub> solution, according with the Tollens procedure for silver plating [2]. At the end the samples are annealed (170°C, 10 m). The Nihon Kohden cap with a home-made accessory are used for the electrode fixation on the scalp. The signal acquisition is performed with an ANT 32 channel bioamplifier.

## **Results and Discussion:**

The developed methodology for the electroless Ag coating of PU takes place in two steps: the deposition of silver seeds on the PU surface and the Ag film formation, by using the Tollens process. The controlled use of methanol as a solvent proved to be essential in order to achieve a partial swelling of the PU structure with the silver solution. Such swelling enabled us to deposit silver not only on the surface but also within a superficial PU layer, what proved to be essential to achieve a good metal-polymer adhesion. The Ag thickness can be controlled from the immersion time and temperature of the Tollens solution. A deposition time of 20h at 40°C allowed to obtain a 20 micron thick silver layer. A thick Ag layer is essential owing to the abrasion process taking place during the exams, which is particularly aggressive due to the small diameter of the pins (about 1 mm). The testing of the electrodes in real EEG signal acquisition is now underway and the first results will be available in the next weeks.

## **Bibliography:**

[1] - M. Teplan, "Fundamentals of EEG measurement", Measurement Science Review 2 (2) (2002).

[2] - N. Takeyasu, T. Tanaka, S. Kawata, "Fabrication of 3D metal/polymer microstructures by site-selective metal coating", Appl. Phys. A (2008) 90, 205–209.

[3] - S. Lobodzinski, M. Laks, "New material for implantable cardiac leads", Journal of Electrocardiology 42 (2009) 566–573.

# Forest fire risk maps in a GIS Open Source environment for Norwest of Portugal

Teodoro, A.C.<sup>1</sup>, Duarte, L.<sup>2</sup>

<sup>1</sup> Geo-Space Sciences Research Centre, Faculty of Sciences, University of Porto, Rua Campo Alegre, 687 4169-007 Porto, Portugal, [amteodor@fc.up.pt](mailto:amteodor@fc.up.pt)

<sup>2</sup> Dep. Geosciences, Environment and Land Planning, Faculty of Sciences, University of Porto, Rua Campo Alegre, 687 4169-007 Porto, Portugal, [up060306005@alunos.fc.up.pt](mailto:up060306005@alunos.fc.up.pt)

**Abstract:** Forest fires threaten natural resources and even human lives in many areas of the world. Portugal suffers from forest fires. The forecast of forest fire risks can be achieved with the use of fire risk zone maps.

**Introduction:** This article presents the results of a research project aimed at producing fire risk maps in a GIS open source environment. The promise of open source is better quality, higher reliability, more flexibility, lower cost, and an end to predatory vendor lock-in.

**Methodology:** This project was developed in QuantumGIS (<http://www.qgis.org/>) platform and the interface is in Python (<http://www.python.org/>). The thematic and individual maps generated are merged into an integrated risk map. The application incorporates seven procedures under a single toolbar. The production of the forest fire risk map comprises several steps and the production of several maps. The probability map incorporates the information of the number of fires that occurs in the last 15 years, for each pixel. After, an annual average is calculated (in %), for each pixel. The susceptibility map contains the slope map and the land cover information (CORINE Land Cover). The mathematical product of these two maps (probability and susceptibility) is the hazard map. The vulnerability map considers the distance from roads and the distance from settlements. The economic value map contains the price (in euros) of the land (for each pixel). This information is given by the local authorities and is public. The mathematical product of the vulnerability map and economic value map gives the potential damage map. Finally, the forest fire risk map is created multiplying the hazard map by the potential damage map. The forest fire risk map comprises six classes: no risk, very low risk, low risk, medium risk, high risk and very high risk.

**Results and conclusion:** This application was tested in three different municipal governments of the Norwest zone of Portugal. The results obtained were similar to the results obtained in a commercial GIS. This application has the advantages of grouping in a unique toolbar all the procedures needed to produce forest fire risk maps and are free for the institution/ user. This work presents several contributions for the area of the GIS open source applications to forest fire management.

## References

- [1] Erten, E., Kurgun, V., and Musaoglu, Ne. (2002), *Forest fire risk zone mapping from satellite imagery and GIS case study*, International Journal of Applied Earth Observation and Geoinformation, 4: 1–10.
- [2] Chuvieco, E., Congalton, R.G., 1989, *Application of remote sensing and geographic information systems to forest fire hazard mapping*, Remote Sensing of the Environment 29, pp.147-159.
- [3] PMDFCI, 2008, *Plano Municipal da Defesa da Floresta Contra Incêndios*, Tecnichal report.

# New methodology to obtain custom prosthesis for rehabilitation of external deformities

**Inês Oliveira<sup>1</sup>, Nuno Leal<sup>2</sup>, António Ferreira<sup>3</sup>, Rui Neto<sup>4</sup>, Ana Reis<sup>5</sup>**

<sup>1</sup> Department of Mechanical Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup> Institute for Mechanical Engineering and Industrial Management (INEGI), Portugal.

<sup>3</sup> Faculty of Medicine, University of Porto, Portugal.

<sup>4</sup> Institute for Mechanical Engineering and Industrial Management (INEGI), Portugal.

<sup>5</sup> Department of Mechanical Engineering, Faculty of Engineering, University of Porto, Portugal.

This work presents a new methodology to obtain customized prosthesis for the rehabilitation of deformities. The goal was to develop a method that could provide faster and less expensive options in the manufacturing of customized prosthesis, and at the same time being able to reproduce the highest degree of details, with the maximum comfort for the patient. It is our goal to surpass the inherent difficulties to both existing methods: “conventional method” [1], which requires a very specialized /skilled prosthetic/anaplastologist, and “digital method”, which relies totally on digital tools [2].

In this paper we describe a case study of a 30 years old woman, that present a resection of “hallux”, the first foot toe. Several solutions were studied together with the medical team. The solution based on implant fixture was abolished since it would imply surgery. A non-invasive solution was selected: silicon prosthesis with retention by “glove”.

The proposed method tries to make use of the advantages of both existing methods, thus presenting a method that we named “combined method”, following these steps:

1. Direct Impression of both feet in Sodium Alginate and laser Surface Scanning of these models;
2. Data processing mirroring of health foot to obtain the amputated toe model using *3-matic* software, followed by sculpture and fitting to the amputated foot with *Blender* software and finally designing inner and outer molds in *3-matic* software;
3. Rapid Prototyping to obtain models for achievement of Silicone Mold and prosthetic Silicone Casting;
4. Prosthesis finishing and fitting.

The proposed “combined method” to obtain customized prosthesis for the rehabilitation showed an encouraging results since it combines the advantages and overcomes the problems that the processes used currently present, particularly regarding the data handling resulting from digital direct scan or files obtain by medical imaging. The direct impression in sodium alginate allow a more accurate impression of the texture of the toe, and the post digital scanning of the produced model (instead of directly from the patient) prevented the problems arising from patient immobilization, overcomes the unfeasibility to scan all the surfaces, and also problems arising from brightness of the skin. The process allows to the attainment of prosthesis with the minimum of contact and discomfort for the patient, disclosing excellent results in terms of aesthetic since prosthesis gotten revealed very similar to the healthful foot of the patient.

References:

[1] Thomas, K. F. “*The Art of Clinical Anaplastology*” (2006).

[2] Jin Sun et al, “*A CAD/CAM system for fabrication of facial prostheses*”, (2010).

# Novel functional nanostructured semiconductors for Dye-sensitized Solar Cells

**A. C. Duarte<sup>1</sup>, A. Varela<sup>2</sup> and H. Aguilar Ribeiro<sup>1,\*</sup>**

<sup>1</sup> Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

<sup>2</sup> Departamento de Ingeniería Química, Instituto Tecnológico de Durango, Mexico

The Dye-sensitized Solar Cell (DSSC) is an unconventional photovoltaic system which provides a technical and economic alternative to traditional solid state photovoltaic devices. Typically, DSSC consist of titanium oxide nanocrystals that are coated with light-absorbing dye molecules and immersed in an electrolyte solution, which is sandwiched between two conductive glass plates or embedded in plastic substrates. Photon-to-current conversion efficiencies of DSSC are strongly dependent on the effective wavelength range for light energy absorption of the photosensitizer (typically in the 380-800 nm range of the solar spectrum), light harvesting within the semiconductor film and electron scavenging from the metal oxide electron acceptor by the electrolyte [1,2]. Here, we present a new semiconductor design, consisting of high-area SiO<sub>2</sub>/TiO<sub>2</sub> nanoparticles sensitized with quantum dots (QD) or ruthenium-based dyes, which were fabricated onto fluorine-doped tin oxide (FTO)-coated glass substrates. The films were characterized by scanning electron microscopy (SEM/EDS), X-ray diffraction, and light and dark electrochemical impedance spectroscopy. Being an insulator, the role of silica nanoparticles is to scatter light, while the mesoporous structure consisting of nanoparticles of TiO<sub>2</sub> offers a high surface area for QDs or dye adsorption [1]. The dual function of light-scattering and enhancement of electron transport properties of the semiconductor is at the basis of a photocurrent density improvement in comparison to similar cells containing TiO<sub>2</sub>-based photoanodes. As a result, enhanced conversion of incident photons into electric current is then foreseen for the “self-light harvesting” dye-sensitized solar cells.

## Acknowledgements

The authors acknowledge the Portuguese Science Foundation (FCT) and the DyeCell project (PTDC/EQU-EQU/101397/2008) for financial support. A. Varela thanks the IAESTE program and LEPAE for the much appreciated facilities and funding. H. Aguilar Ribeiro is grateful to Programa Ciência 2007.

## References:

- [1] Shalom, M., Albero, J., Tachan, Z., Martinez-Ferrero, E., Zaban, A. and Palomares, E. (2010), *Quantum Dot–Dye Bilayer-Sensitized Solar Cells: Breaking the Limits Imposed by the Low Absorbance of Dye Monolayers*, J. Phys. Chem. Lett., 1 (7), 1134–1138.
- [2] Tvrdy, K., Frantsuzov, P. A. and Kamat, P.V. (2010), *Photoinduced electron transfer from semiconductor quantum dots to metal oxide nanoparticles*, PNAS, 108 (1), 29-34.

# Potential use of laccase for the detoxification of phenol pollutant

B. Pinho, C. Costa, A.P.M. Tavares, O. Rodriguez and E.A. Macedo

Laboratory of Separation and Reaction Engineering (LSRE), Department of Chemical Engineering, FEUP, Portugal

Intensive industrial effluents have led to considerable contamination of soil and water with toxic organic pollutants, which are harmful both for the environment and the human health. Although progress has been made over recent years to reduce this problem, numerous contaminated sites still have to be cleaned up. Enzyme-based methods have a minimal impact on ecosystems. They also present some other interesting properties as low energy costs, easy process control and operation over a wide range of pH, temperature and ionic strength. Laccases are oxidative enzymes which have been described to remove hazardous compounds from the environment. They are able to transform a wide range of toxic compounds and are applied as biocatalysts in pulp and paper bleaching, waste-water treatment or soil remediation [1].

Commercial laccase from Novozymes was used as biocatalyst for the degradation of phenol. The degradation conditions by enzymatic catalysis were optimized in aqueous media in a lab-scale stirred batch reactor. The synthetic effluent was prepared with phenol in appropriated buffer solutions, pH varying from 3.0 to 9.0, and different enzyme mediators (ABTS, TEMPO, NHA). All the experiments were carried out at phenol concentration of 50 mg/L. Reaction vials of 5 mL were incubated for 35h at 20°C under stirring. Quantitative analysis of phenol degradation was determined by using HPLC (LaChrom Elite HPLC) with a column RP-18 LichroCART at 25°C.

From the obtained results (Fig. 1), the use of mediators provided only slightly better phenol degradation than laccase alone (degradation > 80% in all cases), thus making the use of mediators unnecessary. The optimum pH value of this oxidative reaction was close to 6.0.

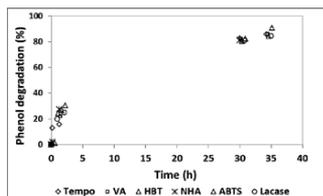


Figure 1 – Degradation of phenol by laccase using different mediators.

## Acknowledgments:

B. Pinho thanks FCT for the Scholarship (BII/LAB/0020/2009).

## Reference:

[1] Husain, M. and Husain, Q. (2008), *Applications of redox mediators in the treatment of organic pollutants by using oxidoreductive enzymes: a review*, Critical Reviews in Environmental Science and Technology, 38, 1-42.

# Technologies for interactive decision support systems for gastroenterology

A. Sousa<sup>1</sup>, C. Gomes<sup>1</sup>, M. Coimbra<sup>1</sup>

<sup>1</sup>Department of Computer Science, Faculty of Science, University of Porto, Portugal

The digestive system is not only essential to our survival but also prone to various diseases such as cancer and thus it's important to minimize the risk of disease. Major diseases in this system are asymptomatic and prolonged, and the word cancer unwanted by anyone. The only way to detect polyps and cancers at an early stage is through routine screening tools using high reliability, in which case these tools are the endoscopies, i.e., in situ visualization of the stomach and intestine.

The rapid and dizzying technological change in recent years allowed digestive endoscopy to extend beyond the mere diagnosis and have a more interventionist role and definitive treatment in clinical situations that are difficult to resolve and often avoiding the use of alternative more penalizing therapies.

The CAGE, funded by FCT, aims to research and develop a Computer Assisted Decision (CAD) system that can be deployed in current gastroenterology rooms, which can support the decision regarding the physician's clinical diagnosis of the patient.

Taking place inside actual video systems implemented inside current gastroenterology rooms, with this project expects explore new ways to assist the physician in assessing the patient's diagnosis.

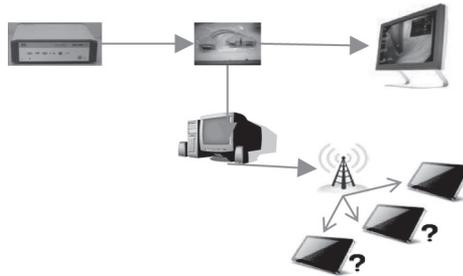


Fig1 CAGE representation

During this MSc thesis research, we will explore technological solutions for providing additional information to a physician during an endoscopy. More specifically, we will investigate how it is possible to capture, store and transmit the endoscopic video wirelessly to a PC, which can then process the image and overlay it with additional information, useful to the physician.

References:

- [1] A. Sousa, M. Dinis-Ribeiro, M. Areia, M. Coimbra, "Identifying cancer regions in vital-stained magnification endoscopy images using adapted color histograms", in Proc. of IEEE ICIP 2009, Cairo, Egypt, Nov 2009.
- [2] Gonzales & Woods, Digital Image Processing, 3rd edition
- [3] Donald Norman, The Design of Everyday Things

## **“Chemistry: A Science Without Borders” – Science Promotion Among Teenagers**

**Daniel Ribeiro, Diana Malheiro, David Silva, Maria D. M. C. Ribeiro da Silva**

Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto  
Rua do Campo Alegre, s/n, P-4169-007 Porto, Portugal.

The relevance of Chemistry in today's Society and its future development is undeniable. Therefore, there is a growing need to invest in the appreciation of scientific education of the general public, with particular focus on young people.

The project “Química: Uma Ciência Sem Fronteiras” (Chemistry: A Science Without Borders), assembled in 2011, was integrated in the Junior University Program – Summer Project. This project intended to contribute to the broad dissemination of the important role of Chemistry, generating the interest of young people and motivating them for its study. This program was held for four weekly cycles, from June to July 2011, and received a total of sixty young 13 to 17 years old students. The students experienced laboratory-practical activities as a way to discover and learn, together with moments of pleasure and even entertainment components.

Considering the STSE (Science, Technology, Society and Environment) perspective, most activities were developed focusing on observation, questioning of reality and the integration of knowledge, within four main themes: "Chemistry and Pollution", "Chemistry and Nutrition", "Chemistry and Magic", and "Chemistry and the Structure of Matter". [1]

The project looked at the importance of implementing rules and safety aspects in the laboratory, [2] in order to provide a responsible atmosphere during the experimental activities, adapted to the scientific grade level of the young students.

It was found a strong commitment and a significant evolution of the majority of the students in terms of autonomy, creativity and responsibility. The students' opinions, expressed in a final survey, reflect the interest of the project for raising awareness on Chemistry topics, emerging also, on the students, a greater incentive to science. It should also be noted that friendly relations were established between students and monitors, inside and outside the laboratory.

### Acknowledgments:

The authors acknowledge A. P. Carvalho and A. V. Reis for all given support. The authors acknowledge Reitoria da Universidade do Porto for supporting the project and for the scholarships awarded to the monitors.

### References:

[1] Ribeiro da Silva, M. D. M. C. (2011), “*A Química: Uma Ciência Sem Fronteiras*”, Verão em Projeto, Universidade Júnior, Faculdade de Ciências da Universidade do Porto.

[2] Hill, P.S. (1995), *Safety Is No Laughing Matter*, Journal of Chemical Education, 72 (12), 1126-1127.

# A structural insight into Tenofovir biological action as a HIV-1 RT inhibitor

**R. M. Ramos<sup>1</sup>, I. S. Moreira<sup>1</sup>, P. A. Fernandes<sup>1</sup> and M. J. Ramos<sup>1</sup>**

<sup>1</sup>REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

HIV-1 reverse transcriptase (HIV-1 RT) is a multifunctional enzyme that is essential for HIV-1 replication as it converts the single-stranded viral RNA into double-stranded linear DNA. Nowadays it is accepted that RT develops two general strategies for resistance to Nucleoside Reverse Transcriptase Inhibitors (NRTIs): (i) discrimination at the time of incorporation and (ii) the excision mechanism, in which RT efficiently incorporates the inhibitor, blocking the DNA synthesis, but it is subsequently removed (excised) unblocking the NRTI-terminated primer [1]. Tenofovir disoproxil fumarate (TDF) is one of the most common used HIV-1 RT inhibitors. In one of the reported crystal structures of TDF in a complex with RT and a DNA template-primer, TDF can adopt two different conformations at the priming site (P-site), in which the adenine base of tenofovir is flipped by 180° from the first conformation [2].

As HIV-1 RT inhibition is such a crucial step in the fight against AIDS it prompted us to investigate the structural and dynamical characteristics behind one of its most common inhibitors: tenofovir. We conducted molecular dynamic (MD) simulations of the complexes of HIV-1 RT with both the natural ligand (deoxynucleoside triphosphate, dNTP) and the different TDF conformers at the P-site in order to understand the factors behind the smaller excision rate of TDF compared to other inhibitors such as AZT. We hypothesize that the flexibility that lead to the different TDF conformations at the P-site prevent the retrotranslocation to the N-site, which is necessary for an ATP-based mechanism of excision, thus reducing the excision rate [3].

## References:

- [1] Sarafianos, S.G., Marchand, B., Das, K., Himmel, D.M., Parniak, M.A., Hughes, S.H., Arnold, E. (2009), *Structure and function of HIV-1 reverse transcriptase: molecular mechanisms of polymerization and inhibition*, Journal of Molecular Biology, 385(3), 693-713.
- [2] Tuske, S., Sarafianos, S.G., Clark, A.D., Ding, J.P., Naeger, L.K., White, K.L., Miller, M.D., Gibbs, C.S., Boyer, P.L., Clark, P., Wang, G., Gaffney, B.L., Jones, R.A., Jerina, D.M., Hughes, S.H., Arnold, E. (2004), *Structures of HIV-1 RT-DNA complexes before and after incorporation of the anti-AIDS drug tenofovir*, Nature Structural & Molecular Biology, 21(23), 6614-6624.
- [3] Ramos, R.M., Moreira, I.S., Fernandes, P.A., Ramos, M.J. - In submission.

## Biometric characterization of squid species available in the Portuguese market

**M. A. Ferreira, M. Oliveira, S. Morais\*, C. Delerue-Matos**

REQUIMTE, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal.\*sbm@isep.ipp.pt

Cephalopods are characterized by a well-developed head with a circumoral arrangement of arms (surrounding the mouth) that bear suckers and/or hooks (except in *Nautilus*). Cephalopods occur almost in all marine habitats of the world. Particularly squids have an elongated body with lateral fins and eight arms with stalked suckers in two or more rows, plus two longer tentacles which have an organized cluster of two to many rows of suckers at the distal end. The flesh of squid is highly appreciated and marketed either fresh, frozen or processed into canned or dried products.

The current study aims to characterize and compare the biometric parameters (length, weight, moisture and total fat content) of the five main squid species available in the Portuguese market, namely *Loligo gahi* and *Loligo reynaudi* from Southwest Atlantic Ocean, *Loligo duvauceli* and *Loligo vulgaris* from Indian Ocean and *Loligo opalescens* from Pacific Ocean.

Fresh squid samples (except *Loligo opalescens* species that was found exclusively frozen) were randomly purchased from different local markets in Oporto metropolitan area during fourteen months. The collection of samples, their processing and biometric characterization were performed in accordance with the EPA Guide No. 823-B-00-07 [1]. Specimens were carefully identified (weight; mantle, tentacle and total length) and manually eviscerated. Moisture was evaluated using 10 g of homogenized sample according to the Portuguese Standard NP 2282-1991 and the Official AOAC method. Moisture content was also evaluated with an infrared balance for comparison and validation purposes of the new methodology. Determination of total fat content was performed accordingly with Ramalhosa et al. [2] using a Microwave Accelerated Reaction System (MARS-X, 1500W, CEM, Mathews, NC, USA; configured with a 14 position carousel and equipped with pressure and temperature sensors).

### Acknowledgements

This work was supported by the Fundação para a Ciência e a Tecnologia through the project PTDC/AGR-AAM/102316/2008 (COMPETE and co-financed by FEDER) and the grant no. PEst-C/EQB/LA0006/2011.

### References:

[1] United States Environmental Protection Agency (2000). *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories*. Vol. 1. Fish Sampling and Analysis. EPA 823-B-00-007, third ed.. Office of Science and Technology, Washington, DC.

[2] Ramalhosa, M. J., Paiga, P., Morais, S., Alves, M. R., Delerue-Matos, C. and Oliveira, M. B. P. (2012). *Lipid content of frozen fish: comparison of different extraction methods and variability during freezing storage*, Food Chemistry, 131, 328–336.

## Characterization of the macromineral content of *Loligo reynaudi*

**E. Silva, M. Oliveira, S. Morais\*, C. Delerue-Matos**

REQUIMTE, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal. \*sbm@isep.ipp.pt

Marine seafood is nutritious and highly essential in a balanced diet, being an important source of proteins and lipids of high biological value, with long chain polyunsaturated fatty acids, and also liposoluble vitamins [1]. Among seafood species, cephalopods represent one of the most important groups captured in Europe presenting a worldwide and Portuguese consumption of 0.6 and 4.1 kg per capita per year in 2007, respectively [2].

The aim of this study was to quantify nutritionally important mineral elements, such as, Ca, Mg, Na and K. The quantification was performed using a high-resolution continuum source flame atomic absorption spectrometer (AnalytikJena 700, Berlin, Germany) equipped with a xenon short-arc lamp operating in a hot-spot mode as a continuum radiation source.

*Loligo reynaudi* fresh samples from southwest Atlantic Ocean were purchased randomly from several markets in O'Porto Metropolitan area (NW Portugal). Sample collection, biometric characterization and preparation were performed in accordance to the EPA Guide No. 823-B-00-07, DL No. 187/2005 and CE Regulation No. 333/2007. Specimens were carefully identified and manually headed and eviscerated. Only the edible tissues (mantle and arms) were preserved. Samples were dried and digested with suprapur nitric acid (65%) in a microwave Mars-X 1500 W (Microwave Accelerated Reaction System for digestion and extraction, CEM Mathews, NC, USA) according to Vieira *et al.* [1].

The calibration curves obtained are linear in the range of 0.20-0.40, 0.05-0.50, 0.25-2.50 and 0.50-1.80 mg/L for Ca, Mg, Na and K, respectively. The week mineral intake of each element achieved by the consumption of this cephalopod species was estimated. This study will be extended to sequential determination of other minor elements in squid samples.

### Acknowledgements

This work was supported by the Fundação para a Ciência e a Tecnologia through the project PTDC/AGR-AAM/102316/2008 (COMPETE and co-financed by FEDER) and the grant no. PEst-C/EQB/LA0006/2011.

### References:

- [1] Vieira, C., Morais, S., Ramos, S., Delerue-Matos, C. and Oliveira, M. B. P. P. (2011), *Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: Intra- and inter-specific variability and human health risks for consumption*, Food and Chemical Toxicology, 49, 923-932.
- [2] Food and Agriculture Organization (2010), *Fish and fishery products: World apparent consumption statistics based on food balance sheets (1961-2007)*, Rome.

## Chemical study from an Azorean macroalgae: *Gelidium microdon*

M. Silva<sup>1-2-5</sup>, A.P. Almeida<sup>1-3</sup>, L.Vieira<sup>4</sup> and A. Kijjoa<sup>4-5</sup>

<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP);

<sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal;

<sup>3</sup>LAEQUIFAR, Universidade Severino Sombra, RJ, Brasil; <sup>4</sup>Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Portugal; <sup>5</sup>CIIMAR, Universidade do Porto, Portugal.

New drugs from natural sources, including macroalgae have been target of pharmaceutical industry interest. Some bioactive compounds as sulfated polysaccharides, phlorotannins and diterpens were isolated from algae [1-3]. This project aims to study the chemical composition and the antitumor potential of marine macroalgae from the Azorean coast, an environmentally healthy habitat, with a high level of biodiversity. We describe the preliminary results obtained from the chemical study of *Gelidium microdon*. The red algae *Gelidium microdon* was collected and identified in April/May of 2010 in the Sea of St Miguel Island – Azores archipelago. The methanolic crude extract of *Gelidium microdon* (151.91 g) was clean up according to the method described by Herz and Hogenaner [4]. The purified extract (4.74 g) was applied on a column chromatography of silica gel 60 (120 g) and eluted with mixtures of petroleum ether, chloroform, acetone and methanol. Three hundred fractions were collected and controlled by analytical thin-layer chromatography with UV detection, with a special emphasis on the compounds with absorption in the  $\lambda = 254$  nm. From the crystallization of group 250-256, using a mixture of petrol/CHCl<sub>3</sub>, one compound was obtained (Compound 1; white crystals; 57.3 mg). Preliminary spectroscopic data (<sup>1</sup>H and <sup>13</sup>C NMR) suggest that compound 1 present an aliphatic chain and a sugar moiety. Some other analysis will be necessary to complete the chemical identification of compound 1.

[1] Smit, A.J. (2004), Journal of Applied Phycology 16: 245-262.

[2] Gupta, S. and Abu-Ghannam N. (2011), Food Science & Technology 22: 315-326.

[3] Pereira, M.G. *et al.* (2005), Carbohydrate Research 340, 2015-2023.

[4] Herz W. and Hogenauer G. (1962), Journal Organic Chemistry 27, 905-910.

Acknowledgments: CEQUIMED-UP (PEst-OE/SAU/UI4040/2011), FERDER, POCl, U.Porto/Santander Totta, PTDC/MAR/100482/2008.

# Co<sub>1-x</sub>Mn<sub>x</sub>Fe<sub>2</sub>O<sub>4</sub> ferrite nanoparticles: preparation and characterization

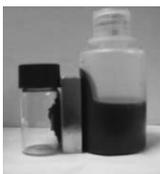
R. Carvalho<sup>1</sup>, T. Ribeiro<sup>1</sup>, D. Silva<sup>1</sup>, C. Fernandes<sup>1</sup>, M. Rocha<sup>1</sup>, C. Pereira<sup>1</sup>, M. P. Fernández-García<sup>2</sup>, A. M. Pereira<sup>2</sup>, J. P. Araújo<sup>2</sup>, C. Freire<sup>1</sup>

<sup>1</sup> REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

<sup>2</sup> IFIMUP and IN – Institute of Nanoscience and Nanotechnology, Department of Physics and Astronomy, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

In the past decades, the synthesis of magnetic nanoparticles (NPs) has been intensively developed not only for its fundamental scientific interest but also for many technological applications such as magnetic data storage, catalysis and medicine [1]. Many biomedical applications such as magnetic hyperthermia and magnetic resonance imaging (MRI) require magnetic NPs with high saturation magnetization values and sizes smaller than 20 nm. In recent studies, mixed ferrites of cobalt(II)-manganese(II) have been reported as a new type of ferrite NPs which may present improved morphological and magnetic properties when compared with the pure ferrites [2].

This work reports the study of the influence of Mn(II) doping in cobalt(II) nanoferrites (Co<sub>1-x</sub>Mn<sub>x</sub>Fe<sub>2</sub>O<sub>4</sub>) on the chemical composition, morphology and magnetic properties of the NPs. Initially, cobalt-manganese nanoferrites with different Co:Mn ratios, Co<sub>1-x</sub>Mn<sub>x</sub>Fe<sub>2</sub>O<sub>4</sub>, where x = 0, 0.1, 0.5 and 1 were prepared by the co-precipitation method using NaOH as precipitating agent [3]. The NPs composition and morphology were characterized by chemical analysis, Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). The FTIR spectra showed the typical bands associated with metal-oxygen vibrations in the range of 600-400 cm<sup>-1</sup>. TEM images showed that pure cobalt ferrite NPs exhibited smaller sizes than the manganese counterpart. All samples presented a magnetic response when an external magnetic field was applied (Fig.1).



**Fig.1 – Magnetic response of nanoferrite to the magnet.**

Acknowledgements: This work has been supported by Fundação para a Ciência e a Tecnologia (FCT) and FEDER through grant no. PEst-C/EQB/LA0006/2011 and through project ref. PTDC/QUI-QUI/105304/2008 in the framework of Program COMPETE. C.F., M.R., C.P. and A.M.P. thank FCT for their grants. M.P.F.G. thanks the Spanish “Programa Clarín” of “Gobierno del Principado de Asturias” for a post-doctoral grant (POST10-31).

## References:

- [1] Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Elst, L. and Muller, R. (2008), *Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications*, Chemical Reviews, 108, 2064-2110.
- [2] Javed, T., Maqsood, A. and Malik, A. (2011), *Structural, electrical and dielectric properties of Co–Mn spinel nanoferrites prepared by co-precipitation technique*, Journal of Superconductivity and Novel Magnetism, 24, 1-8.
- [3] Claesson, E.M. and Philipse, A.P. (2005), *Monodisperse magnetizable composite silica spheres with tunable dipolar interactions*, Langmuir, 21, 9412-9419.

# Computational Study of Electronic and Structural Properties of Molecular Species in Confined Spaces

**J. M. Martins<sup>1</sup> and A. L. Magalhães<sup>1</sup>**

<sup>1</sup> REQUIMTE/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

This work aims to characterize the structural and electronic properties of various molecules when confined in different mediums such as carbon nanostructures and zeolites using a theoretical approach, which can prove to be invaluable in the development of new and cleaner chemical processes with applications in both scientific research, drug development and industrial processes. With the use of a well known, relatively simple,  $S_N2$  Menshutkin reaction we are able to study the effects the confinement medium has over the reaction path and its intermediary states as well as the difference in stability provided by the medium in its charged products.

Building upon work already carried out previously[1-2], our work is based on the employment of high level hybrid ONIOM(DFT/Semi-empirical) theoretical methods for studying reaction mechanisms in confined spaces.

The experimental and theoretical results previously published were reproduced successfully by our methodology. Further calculations such as the Gibbs free energy variation were calculated also for the reaction in gas phase, water and confined space and were found to be within the expected values. The preliminary results obtained by our ONIOM methodology show that it is able to accurately predict the mechanism and energy profile of this type of reactions.

As this is a work in progress, we aim to further evaluate the influence over the reaction of a wide variety of different confinement mediums such as various carbon nanostructures (graphene, stacked graphene, nanotube, multi-walled nanotube, etc) and various zeolites, either naturally occurring ones or man-made.

## References:

[1] Hallsm, M. D. and Schlegel, H. B. (2002), *Chemistry Inside Carbon Nanotubes: the Menshutkin  $S_N2$  Reaction*, Journal of Physical Chemistry B, 106, 1921-1925.

[2] Castejon, H., Wiberg, K. B. (1999), *Solvent Effects on Methyl Transfer Reactions. 1. The Menshutkin Reaction*, Journal American Chemical Society, 121, 2139-2146.

# Contribution for the discovery of a novel class monoamine oxidase B inhibitors based on chromone scaffold as a pharmacological approach to Parkinson's treatment

**D. Martins<sup>1</sup>, J. Reis<sup>1</sup>, A. Fonseca<sup>1</sup>, A. Gaspar<sup>1</sup>, E. Uriarte<sup>2</sup>,  
S. Alcaro<sup>3</sup>, F. Ortuso<sup>3</sup>, F. Borges<sup>1</sup>.**

<sup>1</sup> (1) CIQUP/Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Portugal; (2) Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, Espana, (3) Dipartimento di Scienze Farmacobiologiche, Facoltà di Farmacia, Università “Magna Græcia” di Catanzaro, Campus Universitario “S. Venuta”, Catanzaro, Italy.

Monoamine oxidases (MAOs) are widely distributed enzymes that contain a flavin adenine dinucleotide (FAD) unit covalently bounded to a cysteine residue<sup>1</sup>. Many living organisms possess MAOs and in mammals two isoforms are present, MAO-A and MAO-B, which are located in the outer membrane of the mitochondria. The MAO-B isoform has a crucial role in neurotransmitters metabolism, representing an attractive drug target for neurodegenerative diseases therapy, such as Parkinson's. Parkinson's disease (PD) is a neurodegenerative disorder characterized by a myriad of symptoms that gradually decrease the life quality of the patient. At present, monoamine oxidase inhibitors (MAOI), specifically of MAO-B type, are considered to be beneficial therapeutic drugs. The inadequacy of the current pharmacotherapy and the lack of drugs that can be effective in PD, mainly declined by side-effects, namely the MAO-B inhibitors that are in therapy, are the reasons why the discovery of novel chemical entities (NCE) is still a demand.

Chromones (benzo- $\gamma$ -pyrone) are one of the most abundant groups of naturally occurring heterocyclic compounds. Because of their structural features, and biological properties, they are considered important building blocks in natural product and synthetic organic chemistry areas.

The present project consists on the design and development of a versatile library incorporating a privileged structure based on the benzo- $\gamma$ -pyrone scaffold as a putative shortcut for the early drug-development stage on the discovery of new NCE for the inhibition of MAO-B. Accordingly, a diversity-oriented synthetic methodology was adopted by means of modular syntheses that involve few steps, to obtain structurally varied drug-like compounds. Efforts were done to cover as much chemical space as possible to maximize the likelihood of discovering a novel and patentable lead class of active compounds. The results obtained so far upported by synthetic, biologic and docking studies, pointed out a crucial and undisclosed role of the presence of a carboxamide group in C3 of the pyrone ring that is able to establish hydrogen bond interactions with the active site of the MAO-B enzyme<sup>2</sup>.

## References:

- [1] (a) Reyes-Parada, M., Fierro, A., Iturriaga-Vásquez, A. P., Cassels, B. K. (2005), *Curr., Enzyme Inhib.*, 1, 85; (b) Pacher, P., Kecskeméti, V. (2004), *Curr. Med. Chem.*, 11, 925.  
[2] (a) Gaspar, A., Reis, J., Fonseca, A., Milhazes, N., Viña, D., Uriarte, E., Borges, F. (2011), *Bioorg. Med. Chem. Lett.*, 21, 707; (b) Gaspar, A., Teixeira, F., Uriarte, E., Milhazes, N., Melo, A., Cordeiro, M.N. (2011), Ortuso, F., Alcaro, S., Borges, F., *ChemMedChem.*, 6, 628.

Financial support : PTDC/QUI-QUI/113687/2009 (FCT, FEDER, QREN and COMPETE)



# Desirability-based Multi-criteria Quantitative Structure-Selectivity Relationships of Antimicrobial Peptidomimetics

**Pedro Soares<sup>a</sup>, André Fonseca<sup>a</sup>, Maykel Cruz-Monteagudo<sup>a,b</sup>, Filipe Teixeira<sup>a</sup>, André Melo<sup>a</sup>, Fernanda Borges<sup>a</sup>, Anabela Cordeiro da Silva<sup>c</sup>, M. Natália D. S. Cordeiro<sup>a</sup>**

<sup>a</sup> Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

<sup>b</sup> Applied Chemistry Research Center - Faculty of Chemistry and Pharmacy, & Molecular Simulation and Drug Design Group - Chemical Bioactive Center, Central University of “Las Villas”, Cuba.

<sup>c</sup> Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Microbial drug resistance has achieved a global dimension and an alarming magnitude<sup>1</sup>. Antimicrobial peptides possess unique action mechanisms making peptide antibiotics an attractive therapeutic option against resistant bacteria. However, their high haemolytic activity lack the selectivity required for a human antibiotic<sup>2</sup>. Therefore, additional efforts are needed to develop new antimicrobial peptides that possess greater selectivity for bacterial cells over erythrocytes. We introduce in this work a practical approach to simultaneously deal with these two conflicting properties<sup>3</sup>. The convergence of machine learning techniques<sup>4</sup> and desirability theory<sup>5</sup> allowed us to derive a simple, predictive and interpretable multi-criteria classification rule (m-cCR) for simultaneously handling the antibacterial and haemolytic properties of a set of cyclic  $\beta$ -hairpin cationic peptidomimetics (C $\beta$ -HCPs)<sup>6</sup>. The m-cCR exhibited a prediction accuracy of about 80% on training and external validation sets. Results from a further concordance test showed an excellent 86% of agreement between m-cCR predictions and predictions from independent classifiers for complementary antibacterial and haemolytic activities, respectively; evidencing the reliability of the m-cCR. The m-cCR was also consistent with the general mode of action of cationic peptides that indicates their biological and biophysical relevance. A multi-criteria virtual screening strategy based on the joint use of the m-cCR, desirability, similarity and chemometrics concepts is also proposed. The ability of such virtual screening strategy to prioritize selective (non haemolytic) antibacterial C $\beta$ -HCPs was challenged on training, validation, and overall data. Overall the results suggest that the method is able to rank a selective antibacterial C $\beta$ -HCP earlier than a biologically inactive or non selective antibacterial C $\beta$ -HCP with a probability of *ca.* 0.9. These results allow considering both the m-cCR and the multi-criteria virtual screening strategy as promising chemoinformatics tools suitable for the discovery and development of potent and selective antimicrobial peptides.

## References

- [1] Rossolini, G.M. and Mantengoli, E. (2008), *Antimicrobial Resistance in Europe and Its Potential Impact on Empirical Therapy*, Clin Microbiol Infect, 14 (Suppl. 6), 2-8.
- [2] Kondejewski, L.H., Jelokhani-Niaraki, M., Farmer, S.W., Lix, B., Kay, C.M., Sykes, B.D., Hancock, R.E., and Hodges, R.S. (1999), *Dissociation of Antimicrobial and Hemolytic Activities in Cyclic Peptide Diastereomers by Systematic Alterations in Amphipathicity*, J Biol Chem 274 (19), 13181-13192.
- [3] Cruz-Monteagudo, M., Borges, F., and Cordeiro, M.N.D.S. (2011), *Jointly Handling Potency and Toxicity of Antimicrobial Peptidomimetics by Simple Rules from Desirability Theory and Chemoinformatics*, J. Chem. Inf. Model., 51 (12), 3060-3077.
- [4] Witten, I.H., and Frank, E. (2005), *Data Mining: Practical Machine Learning Tools and Techniques*, 2<sup>nd</sup> Ed., Morgan Kaufmann, San Francisco.
- [5] Derringer, G.; Suich, R. (1980), *Simultaneous Optimization of Several Response Variables*, J. Quality Technol., 12 (4), 214-219.
- [6] Robinson, J.A., Shankaramma, S.C., Jetter, P., Kienzl, U., Schwendener, R.A., Vrijbloed, J.W., and Obrecht, D. (2005), *Properties and Structure-Activity Studies of Cyclic Beta-Hairpin Peptidomimetics Based on the Cationic Antimicrobial Peptide Protegrin I*, Bioorg Med Chem, 13 (6) 2055-2064.

## Development of biosilica nanostructures with immobilized proteases for incorporation in screening methodologies

**A.M.V. Palas, A.D. Costa, M.L.C. Passos, P.C.A.G. Pinto, J.L.F.C. Lima, M.L.M.F.S. Saraiva**

<sup>2</sup> REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Biomimetic mineralization reactions have emerged recently as a versatile tool for generating excellent supports for enzyme stabilization. The formation of the silica-based diatom cell wall has been regarded as one of the most fascinating examples for the controlled production of nanostructured silica in mild conditions [1]. So, while most methods used to synthesize mesoporous, nanostructured and/or hybridized silica materials use harsh conditions [2], when resorting to biosilicification the synthesis occurs in ambient conditions involving only a silica precursor and a biological catalyst, occurring the encapsulation of the enzyme within seconds. The synthesis is economic, rapid and very simple and has been considered a process to mimic, with great potential in the silicon technology. Proteins encapsulated using this approach can be used to catalyze highly specific reactions, to screen for enzyme inhibitors, or to detect the presence of chemical compounds. However, encapsulation efficiency varies with the catalyst-enzyme pair. In fact there is a great versatility of tailoring as by resorting to different catalysts and experimental conditions a variety of sizes and shapes are originated [3]. Thus, optimal encapsulation conditions must be identified for each enzyme before they can be applied in a useful setting.

In this work different biomatrices for the immobilization of two proteases, chymotrypsin and trypsin were tested as well as the stability of the immobilized enzymes. The enzyme leakage, evaluation of the yield of the different immobilization processes and calculation of the loading efficiency were performed. The morphology and surface composition of the solids that were precipitated were studied by SEM-EDS.

For trypsin and chymotrypsin activity determination *N*-benzoyl-DL-arginine-4-nitroanilide and *N*-succinyl-L-phenylalanine-*p*-nitroanilide, respectively, were used. Their hydrolysis form the same chromogenic nitroanilide measured at 405 nm. Inhibition assays for both enzymes were performed with 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride with values reaching 91% for trypsin and 87% for chymotrypsin.

### References:

- [1] Bhatia, R. B., and Brinker, C. J. (2000), *Aqueous sol-gel process for protein encapsulation*, Chem. Mater., 12, 2434-2441.
- [2] Sumper, M., and Brunner, E. (2006), *Learning from diatoms: Nature's tools for the production of nanostructured silica*, Adv. Funct. Mater., 16, 17-26.
- [3] Jin, R.H., Yuan, J.J. (2005), *Synthesis of poly(ethyleneimine)s-silica hybrid particles with complex shapes and hierarchical structures*, Chem. Commun., 1399-1401.

This work has been supported by Fundação para a Ciência e a Tecnologia through grant no. PEst-C/EQB/LA0006/2011 and Reitoria da Universidade do Porto (Concurso 2010).

# Effect of gallic acid and its alkyl esters on the oxidative stability of unsaturated long-chain fatty acids

**N. Preto<sup>1,2</sup>, M. Martins<sup>1,2</sup>, E.M. Garrido<sup>1,2</sup>, F. Borges<sup>1</sup> and J. Garrido<sup>1,2</sup>**

<sup>1</sup> CIQ / Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

<sup>2</sup> Department of Chemical Engineering, School of Engineering, ISEP, Polytechnic Institute of Porto, 4200-072 Porto, Portugal.

Biodiesel is an alternative fuel and extender proposed for applications ranging from on- and off-road compression-ignition (diesel) engine powered vehicles to locomotives, stationary power, heat generation and aviation fuels. Biodiesel is a renewable fuel that is derived from domestic feedstock and described to be environmentally innocuous and safe to handle because it has a relatively high flash point. Biodiesel advantages are also related to the gross heat of combustion, specific gravity and kinematics' viscosity that are comparable to those of corresponding petroleum middle distillate fuels.

Given current estimates for production capacity, recent attention has focused on effects of oxidation caused by contact with ambient air (autoxidation) on biodiesel fuel quality during storage. Maintaining fuel quality of biodiesel and its blends with petroleum middle distillate fuels during long-term storage presents a concern among fuel producers, suppliers and users. The majority of vegetable oil and animal fat feedstocks are triacylglycerols with long-chain (C<sub>16</sub>–C<sub>18</sub>) fatty acid groups attached by ester linkages to a glycerol backbone. To avoid cold weather performance issues, biodiesel derived from such feedstocks must contain a relatively high concentration (80–90 wt.%) of low-melting point mono-alkyl esters; that is, biodiesel must contain 80–90 wt.% unsaturated long-chain fatty acid alkyl esters.

Factors known to affect autoxidation of fatty derivatives include presence (or exclusion) of air, temperature, light, antioxidants, pro-oxidants such as hydroperoxides and metal catalysts. Several approaches for increasing relative resistance to oxidation of fatty derivatives have been shown to be successful for biodiesel. Treatment with oxidation inhibitors is a promising approach because it facilitates the use of existing storage tanks and fuel handling systems without requiring upgrades or redesign.

The aim of this study was to examine the effectiveness of gallic acid and its alkyl esters derivatives (Fig. 1) in increasing the relative resistance to oxidation of biodiesel by inhibiting unsaturated long-chain fatty acid peroxidation.

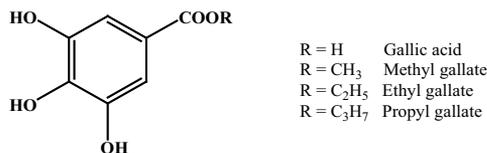


Figure 1 – Structure of gallic acid and its alkyl esters derivatives

The results obtained will be presented and discussed.

## Electrochemical biosensors for carbamate pesticides detection

**F. W. P. Ribeiro<sup>1,2</sup>, S. Morais<sup>1</sup>, M. F. Barroso<sup>1</sup>, P. Lima-Neto<sup>2</sup>, A. N. Correia<sup>2</sup>, M. B. P. P. Oliveira<sup>3</sup>, C. Delerue-Matos<sup>1</sup>**

<sup>1</sup>REQUIMTE, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal

<sup>2</sup>Departamento de Química Analítica e Físico-Química, Centro de Ciências, Universidade Federal do Ceará, Bloco 940 Campus do Pici, 60455-970, Fortaleza - CE - Brazil

<sup>3</sup>REQUIMTE, Dep. Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal

Carbamates constitute a versatile class of compounds used as insecticides, fungicides, nematocides, acaricides, molluscicides, sprout inhibitors or herbicides. Contamination of fruits and vegetables may result from treatment as well as from conditions such as improper use of pesticides, residues from preceding treatments in the soil and cross-contamination. Although carbamates present low bioaccumulation potentials and short-term toxicity (relatively short biological half-lives and are fairly rapidly metabolized and excreted), they are considered hazardous to the environment and human health being included in the priority list released by the United States Environmental Protection Agency.

Different techniques have been employed for carbamates determination. Biosensors are based on the intimate contact between a bio-recognition element that interacts with the analyte of interest and a transducer element that converts the bio-recognition event into a measurable signal. Among the different types of biosensors, the electrochemical sensors are of special interest due to the high sensitivity inherent to the electrochemical detection and the possibility to miniaturize the required instrumentation, thereby making the construction of compact and portable analysis devices possible [1]. Biosensors usually are not able to achieve the sensitivity of chromatographic methods. However, they can serve as a tool for screening of hundreds of samples in a short period of time complementing the existing methods and allowing for a more rapid assessment of problematic environments. Each method has unique advantages which can complement each other [2]. Therefore, this project aimed to develop enzymatic biosensors for carbamates quantification in fruits and vegetables.

### Acknowledgements:

The authors wish to thank the Brazilian and Portuguese research funding institutions CAPES and FCT, respectively, for the financial support through the project “Determinação eletroanalítica de pesticidas em amostras de águas naturais e de alimentos utilizando ultramicroelectrodos/biossensores” (Nº. 313/11). FCT is also acknowledged for the financial support through the project PEst-C/EQB/LA0006/2011.

### References:

- [1] Somerset V, Baker P, and Iwuoha E (2011), *Mercaptobenzothiazole-on-Gold Organic Phase Biosensor Systems: 3. Thick-Film Biosensors for Organophosphate and Carbamate Pesticide Determination*, in Stoytcheva, M. (Ed.) *Pesticides - Strategies for Pesticides Analysis*, InTech, Rijeka, pp 185-204.
- [2] Van Dyk, J. and Pletschke, B. (2011). *Review on the use of enzymes for the detection of organochlorine, organophosphate and carbamate pesticides in the environment*, *Chemosphere*, 82, 291-307.

## Evaluation of anti-Leishmania compound released from polymeric nanoparticles

**M. V. Osório<sup>1</sup>, S. Nogueira<sup>2</sup>, M. A. Segundo<sup>1</sup>, V. L. R. G. Abreu<sup>1</sup>, A. Cordeiro da Silva<sup>2,3</sup>, S. A. C. Lima<sup>2</sup>**

<sup>1</sup> REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal;

<sup>2</sup> IBMC-INEB, Infection and Immunity Unit - Parasite Disease Group, University of Porto, Portugal;

<sup>3</sup> Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

The therapy of infectious diseases caused by protozoan parasites of the trypanosomatid family is a neglected area of research and drug development, including here leishmaniasis, an emerging disease in Portugal. Recently, the anticancer bisnaphthalimidopropyl (BNIP) polyamines compounds have attracted attention due to their potential activity against the parasite *Leishmania infantum* [1]. However these compounds are quite insoluble in aqueous solutions, making their testing and potential development into chemotherapeutics difficult. To overcome this, nanotechnology tools can be applied to provide enhanced formulations, namely nanoparticulate carriers that are able to tailor the spatial and temporal delivery of drugs [2].

In this context, the objective of this work was to develop a fast chromatographic method for determination of bisnaphthalimidopropyl diaaminooctane (BNIPDaoct) and further application in drug release studies. As the target compound exhibits strong fluorescence, fluorimetric detection was implemented in order to reach a low limit of detection (LOD). Furthermore, in order to enhance determination throughput using conventional liquid chromatography equipment, a monolithic column (Chromolith RP-18e, 100 mm × 4.6 mm i.d., Merck) was applied, connected to a Jasco HPLC system. Several parameters were studied, including the flow rate (up to 2 mL min<sup>-1</sup>) and the composition of the mobile phase. The operation conditions chosen comprised a flow rate of 1.5 mL min<sup>-1</sup> (pressure = 2.5 MPa) and a mobile phase containing 40% (v/v) acetonitrile and 60% (v/v) of aqueous buffer (acetic acid/acetate 0.10 mol L<sup>-1</sup>, pH 4.5, 0.010 mol L<sup>-1</sup> octanesulfonic acid). Calibration curves were linear up to 10 μmol L<sup>-1</sup>, with working ranges of 1 to 10 μmol L<sup>-1</sup> (10x gain) and of 2 to 100 nmol L<sup>-1</sup> (1000x gain). The LOD and LOQ were 0.4 and 1.1 nmol L<sup>-1</sup>, corresponding to 7 and 17 fg (20 μL). The developed methodology was successfully applied to monitor the release profile of BNIPDaoct encapsulated in polymeric biodegradable nanoparticles, formulated with poly(D,L-lactide-co-glycolide) by nanoprecipitation, at different pH values.

Acknowledgments: Funding from U.Porto/Santander Totta – project 155/2010 and from FCT through project PEst-C/EQB/LA0006/2011 is acknowledged.

### References:

- [1] Tavares, J., Ouassi, A., Lin, P.K., Loureiro, I., Simarenjeet, K., Nilanjan, R. and Cordeiro-da-Silva, A. (2010), *Bisnaphthalimidopropyl Derivatives as Inhibitors of Leishmania SIR2 Related Protein 1*, *Chem Med Chem*, 5(1),140-147.
- [2] Maurer-Jones, M.A., Bantz, K.C., Love, S.A., Marquis, B.J. and Haynes, C.L. (2009), *Toxicity of Therapeutic Nanoparticles*, *Nanomedicine*, 4(2), 219-241.

## Exploiting bisnaphthalimidopropyl polyamine as an electroactive specie for ISE preparation

**Renato Gil, Célia Amorim, Alberto Araújo, Maria da Conceição Montenegro**

REQUIMTE, Laboratório de Química Analítica e Físico-Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal.

Potentiometry with ion selective electrodes is nowadays a well-established technique with intensive use in many laboratories either carrying out routine analyses or in research. Its attractive features include the simplicity of use, enabling of fast and accurate results, economic in its implementation, non-destructive and environmentally competitive with other traditional methods. The proposal of new electrodes towards different organic and inorganic compounds should fulfill those requirements, and one of the main issues in research is related with the need to provide accurate results, which is dependent on the ISE membrane's ability to discriminate the analyte relative to other ions present in the sample i.e. its selectivity. Useful membranes arise from a careful combination of a hydrophobic charged ion exchanger that pulls same signal ions and not the counter ions through the membrane/sample interface barrier (permselective properties of the membrane) and from a selective hydrophobic complexing agent (called an ionophore) which selectively buffers the activity of target ion in bulk membrane [1]. Different compounds behaving as good ionophores, were proposed mainly for target cationic compounds, once enhanced selectivity of the respective electrodes, relatively to electrodes only based on ion-exchangers embedded in plastic membranes, was shown in literature. On contrary, proposal of ionophore candidates to implement anion-selective electrodes remain relatively limited. In this context, a new (bisnaphthalimidopropyl polyamine) compound has been exploited as ionophore candidate for potentiometric membranes preparation. Several solid contact electrodes, incorporating a plasticized PVC membrane with bisnaphthalimidopropyl polyamine as ionophore in amounts of 1-3% (W/W) relatively to PVC were prepared and evaluated against some common inorganic and organic ions. Preliminary studies suggest that this compound could be used as electroactive specie for potentiometric determination of some anions, namely citrate, periodate, salicylate, thiocyanate, chlorate and perchlorate. The electrodes presented fast response (inferior do 15 s), high potential stability ( $< 2\text{mV/day}$ ) and Nernstian response in the range of  $1.0 \times 10^{-7}$  up to  $1.0 \times 10^{-2} \text{molL}^{-1}$  in aqueous solution.

The work will continue with the optimization of membranes, in order to find the one that provides the most appropriate potentiometric response for given chemical specie.

### References:

1. Amemiya, S., et al., Cationic or anionic sites? Selectivity optimization of ion-selective electrodes based on charged ionophores. *Anal. Chem.*, 2000. 72(7): p. 1618-1631.

### Acknowledgments:

A gratefully acknowledge to Joel Filipe Gomes Oliveira for some laboratory supporting. To Prof. Paul Kong for the supply of the studied compound.

## Extraction and HPLC-DAD determination of chlorogenic acid (CGA) from lamb's lettuce

F. Oliveira<sup>a\*</sup>, Manuela M. Moreira<sup>a</sup>, Aquiles A. Barros<sup>a</sup> and Luís F. Guido<sup>a</sup>

<sup>a</sup> REQUIMTE – Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

\*fabioliveira1990@gmail.com

Lamb's lettuce (*Valerianella locusta*), a member of the family *Valerianaceae* commonly used in salads, has been attaining some relevance as an important source of antioxidant compounds, namely chlorogenic acid (CGA) [1]. Chlorogenic acids are a family of esters formed between certain *trans*-cinnamic acids and (-) quinic acid which have been described as major phenolics in coffee, strawberries, pineapple, apple, sunflower, blueberries. The most common individual chlorogenic acid is 5-O-caffeoylquinic acid (5-CQA) [2]. Reports indicate that CGA compounds have antioxidant properties which are suggested to play an important role in preventing various diseases associated with oxidative stress, such as cancer, cardiovascular, aging and neurodegenerative diseases [3]. The aim of this work was to evaluate the influence of some parameters on the extraction of chlorogenic acid from lamb's lettuce. Thus, an ultrasound extraction of chlorogenic acid was applied to fresh and freeze-dried samples of two different brands (A and B), using two types of solvents (methanol and water). Then, the extracts were analyzed by high-performance liquid chromatography with diode array detection (HPLC-DAD). The results obtained clearly showed that extraction solvent influences the extraction yield. HPLC analysis showed that the methanolic extracts of brand A achieved the highest chlorogenic acid content ( $1434 \pm 111$  mg CGA/100 g DW). The present work also demonstrated that lamb's lettuce brand may significantly impact the CGA content (attained differences of 35%). The results indicate that lamb's lettuce contains higher CGA compared to some medicinal plants, like *Camellia sinensis* as well to a few relevant Brazilian green and roasted coffee cultivars. The results presented in this work prove lamb's lettuce as a very interesting source of polyphenols, and thus a potential health-promoting food.

### Acknowledgements:

Manuela M. Moreira wishes to acknowledge Fundação para a Ciência e a Tecnologia (FCT) for her Ph.D. studentship (SFRH/BD/60577/2009).

### References:

- [1] Grzegorzewski, F.; Rohn, S.; Kroh, L.W.; Geyer, M. and Schluter, O. (2010), *Surface morphology and chemical composition of lamb's lettuce (Valerianella locusta) after exposure to a low-pressure oxygen plasma*. Food Chemistry, 122, 1145-1152.
- [2] Gugliucci, A.; Bastos, D.H.M.; Schulze, J. and Souza, M.F.F. (2009), *Caffeic and chlorogenic acids in Ilex paraguariensis extracts are the main inhibitors of AGE generation by methylglyoxal in model proteins*. Fitioterapia, 80, 339-344.
- [3] Fujioka, K. and Shibamoto, T. (2008), *Chlorogenic acid and caffeine contents in various commercial brewed coffees*. Food Chemistry, 106, 217-221

# Furanic compounds in espresso coffee capsules: validation of a HS-SPME –GC-MS methodology

C. Petisca<sup>1</sup>, Z.E. Martins<sup>1,2</sup>, O. Pinho<sup>1,3</sup> and IMPLVO Ferreira<sup>1</sup>

<sup>1</sup> REQUIMTE – Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> Faculty of Sciences, University of Porto

<sup>3</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

Furan and its derivatives are actually receiving increased attention due to their potential harmful effects on human health. They have been known to occur in thermally processed food and are mainly formed from thermal degradation of carbohydrates, specially reducing sugars. In nature, 2-furfural, furfuryl alcohol and furfuryl ester occurs naturally in many foods and drinks, such as coffee. Nowadays, due to the increasing of the consumption of *espresso* coffee capsules, it became of extremely importance to evaluate the potential harmful compounds present in this type of beverage. In literature there are many articles related to the screening and quantification of furan, but quantification of furanic compounds in *espresso* coffee is still scarce [1-3]

The aim of the present work was to develop and validate a HS-SPME-GC-MS methodology for the simultaneous quantification of the four major furanic compounds in *espresso* coffee capsules, namely, 2-furfural, 2-furfuryl alcohol, 5-methylfurfural and furfuryl acetate. For this purpose, a Headspace – Solid phase microextraction coupled to Gas Chromatography – Mass Spectrometry method was validated. Quality parameters of the method were established, Good linearity with a correlation coefficient ( $r^2$ ) higher than 0.99 was obtained, and low LODs (0.0008 mg L<sup>-1</sup> for 2-furfural, 0.0026 mg L<sup>-1</sup> for furfuryl alcohol, 0.00079 mg L<sup>-1</sup> for 5-methylfurfural and 0.000018 mg L<sup>-1</sup> for furfuryl acetate) and LOQs (0.00232 mg L<sup>-1</sup> for 2-furfural, 0.00767 mg L<sup>-1</sup> for furfuryl alcohol, 0.00238 mg L<sup>-1</sup> for 5-methylfurfural and 0.00015 mg L<sup>-1</sup> for furfuryl acetate) were achieved. The recoveries of furanic compounds were 91.04% for 2-furfural, 97.92% for 2-furfuryl alcohol, 102.13% for 5-methylfurfural and 101.99% for furfuryl acetate. Relative standard deviation (RSD, %) was lower than 5% for intraday assays and lower than 10.6% for interday assays. The results show that a quantitative analysis of the four major furanic compounds in coffee is of extremely importance, due to their possible harmful effects that could be related with dose intake. Considering the high consumption levels of *espresso* coffee by Portuguese population, these compounds should be also monitored and quantified in other processed foodstuffs.

In conclusion, this work allowed for the first time the development and validation of a HS-SPME-GC-MS method for the simultaneous quantification of 2-furfural, furfuryl alcohol, 5-methylfurfural and furfuryl acetate in *espresso* coffee samples.

## References:

- [1] M.S. Altaki, F.J. Santos, M.T. Galceran (2011), *Occurrence of furan in coffee from Spanish market: Contribution of brewing and roasting*, Food Chemistry, 126, 1527–1532.
- [2] L. Giordano, R. Calabrese, E. Davoli, D. Rotilio (2003), *Quantitative analysis of 2-furfural and 5-methylfurfural in different Italian vinegars by headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry using isotope dilution*, Journal of Chromatography A, 1017, 141-149.
- [3] C. Crews, D. Roberts, S. Lauryssen, G. Kramer (2009), *Survey of furan in foods and coffees from five European Union countries*, Food Additives and Contaminants, 2 (2), 95-98.

## Furfural stability in model systems containing ascorbic and citric acids: effect of pH, time and temperature

**A.R. Henriques<sup>1</sup>, C. Petisca<sup>1</sup>, O. Pinho<sup>1,2</sup>, I.M.P.L.V.O. Ferreira<sup>1</sup>**

<sup>1</sup> REQUIMTE – Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

Furanic compounds are highly reactive molecules, giving rise to the formation of reactive oxygen species (ROS); causing ROS-associated damage to proteins, nucleic acids, and cell organelles. In addition, these compounds also contribute to the flavour of foods and beverages. 2-furfural is the most abundant of the simple furanic compounds, due to the conversion of furfuryl esters and furfuryl alcohol. It is also the main degradation product of the hydrolysis of pentoses and is widely accepted as an indicator of flavour changes. Ascorbic acid has been shown as one of the major precursors of furfural formed by thermal decomposition, pressure cooking, roasting or pyrolysis conditions, but studies reporting this furanic compound as a reaction product under roasting conditions are scarce.[1-3]

The aim of the present work was to study the stability of furfural in model systems containing ascorbic acid, citric acid and furfural under different conditions, such as pH(4 and 7), time (0, 30, 120, 180 and 240 min) and temperature (0, 60 and 120°C) of heating. The control was made using only 2-furfural subject to the same conditions as model systems. Reverse phase high performance liquid chromatography (RP-HPLC)/ diode array detection (DAD) with a C18 column at 35 °C was used. The validation of the method was performed with 8 different furfural concentrations, ranging from 0.0313 to 16 mg/L of 2-furfural. Good linearity ( $r^2$  higher than 0.999) was achieved. Limits of detection (LOD) and quantification (LOQ) (based on a signal-to-noise ratio of 3:1 and 10:1) were 0.0054 mg/L and 0.0313 mg/L, respectively. Results showed that for both control and model systems without citric acid and heated at 60 °C, 2-furfural is unstable and it is completely degraded. With addition of ascorbic acid, without citric acid and heated at 120 °C, results show production of 2-furfural in lower amounts, being the higher amount at 240 min of heating. Comparing model systems containing only 2-furfural with citric acid (control) at pH 4 and 7, it is possible to observe a higher production of this furanic compound at pH 4, except for 60 °C 120 min and 120 °C 240 min. Regarding results from models systems with ascorbic acid, citric acid and 2-furfural, it showed that at pH 7 there is a higher production of 2-furfural when comparing to pH 4 and the higher amount achieved is at 120 °C during 240 min. These results show that 2-furfural production is higher in the presence of both citric and ascorbic acid, with the increase of pH, time and temperature of heating. Moreover, these results could be interesting for further research regarding food products that contain both ascorbic and citric acid as food additives.

### References:

- [1] Feron, V.J., Til, H.P., De Vrijer, F., Woutersen, R.A., Cassee, F.R., Van Bladeren, P.J. (1991), *Aldehydes: Occurrence, carcinogenic potential, mechanism of action and risk assessment*, Mutat Research, 259 (3-4), 363-385.
- [2] Almeida, J.R.M., Bertilsson, M., Gorwa-Grauslund, M.F., Gorsich, S., Lidén, G. (2009), *Metabolic effects of furaldehydes and impacts on biotechnological processes*, Applied Microb and Biotechn 82 (4), 625-638.
- [3] Limacher, A., Kerler, J., Conde-Petit, B., Blank, I. (2007), *Formation of furan and methylfuran from ascorbic acid in model systems and food*, Food Add and Contam, 24 (1), 122.

## Gas-diffusion microextraction (GDME): study of influencing parameters on the extraction of aldehydes

**Christiane M. Santos<sup>1</sup>, Inês M. Valente<sup>1</sup>, José A. Rodrigues<sup>1</sup> and Aquiles A. Barros<sup>1</sup>**

<sup>1</sup>Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Gas-diffusion microextraction (GDME) was previously used to analyze aldehydes in beer and wine samples [1, 2]. The process is based on gas-diffusion of semi-volatile and volatile compounds through a hydrophobic membrane to an acceptor solution. This solution is composed by 2,4-dinitrophenylhydrazine (2,4-DNPH) in order to derivatize the compounds [3].

Aldehydes are known to be important organic compounds in environmental atmospheres, alcoholic beverages and industrial materials [4]. These compounds can affect alcoholic beverages' quality as they contribute to overall flavor and are related to aging and color stability.

The aim of this work was to study the effect of some parameters that influences directly the extraction process such as the influence of alcohol content in the extraction of volatile and semi-volatile compounds. Different alcohols were used to see how the increase of the alcohol chain affects the extraction.

The compounds formed by the reaction of aldehydes with the derivitizing agent were analyzed by high-performance liquid chromatography with UV-Vis detection (HPLC-UV).

### Acknowledgements:

This work has been supported by Portuguese Fundação para a Ciência e a Tecnologia (FCT) through grant no. PEst-C/EQB/LA0006/2011. IMV (SFRH/BD/69719/2010) wishes to acknowledge FCT for her PhD grant. The authors also thank Carlos Torres for the skilled manufacturing of the GDME device used in this work.

### References:

- [1] Gonçalves, L.M., Magalhães, P.J., Valente, I.M., Pacheco, J.G, Dostálek, P., Sýkora, D., Rodrigues, J.A., Barros, A.A. (2010), *Analysis of aldehydes in beer by gas-diffusion microextraction: Characterization by high-performance liquid chromatography–diode-array detection–atmospheric pressure chemical ionization–mass spectrometry*, Journal of Chromatography A, 1217, 3717–3722.
- [2] Cruz, M.P., Valente, I.M., Gonçalves, L.M., Rodrigues, J.A., Barros, A.A., *Application of gas-diffusion microextraction to the analysis of free and bound acetaldehyde in wines by HPLC–UV and characterization of the extracted compounds by MS/MS detection*, Analytical and Bioanalytical Chemistry (DOI 10.1007/s00216-011-5664-1).
- [3] Pacheco, J.G, Valente, I.M., Gonçalves, L.M., Rodrigues, J.A., Barros, A.A. (2010), *Gas-diffusion microextraction*, Journal of Separation Science, 33 (20), 3207-3212.
- [4] You, J., Yan, T., Zhao, H., Sun, Z., Xia, L., Suo, Y. and Li, Y. (2009), *A sensitive fluorescence reagent for the determination of aldehydes from alcoholic beverage using high-performance liquid chromatography with fluorescence detection and mass spectrometric identification*, Analytica Chimica Acta, 636 (1), 95-104.

## Interaction of 3,4-dihydroxyxanthone with double stranded DNA

Sara Leirosa<sup>1</sup>, C. Sousa<sup>1,2,3</sup>, M. J. Sottomayor<sup>1</sup>, M. Pinto<sup>2,4</sup>

<sup>1</sup> Centro de Investigação em Química da Universidade do Porto (CIQ-UP), Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal.

<sup>2</sup> Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Porto, Portugal.

<sup>3</sup> REQUIMTE-Departamento de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.

<sup>4</sup> Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.

Xanthenes are polyphenolic compounds which have been reported to have extensive biological and pharmacological activities [1]. The biological activities of these heterocyclic compounds are related with their tricyclic structure and depend on the nature and/or position of the different substituents. In this context, the binding of phenolic compounds to nucleic acids has been recognized as an important mechanism of their actions.

The present work aims to study the interaction of 3,4-dihydroxyxanthone with double stranded DNA, occurring in bulk solution. UV spectroscopy has been used to study the effect of this xanthone on the structure and stability of the DNA molecule, using the characteristics of DNA thermal denaturation as a measure of the effect of the compound on the stability of the double helix.

Absorption spectra, as well as UV melting curves, were recorded for solutions with constant DNA concentration and different concentrations of 3,4-dihydroxyxanthone.

DNA melting experiments were carried out by recording absorbance versus temperature profiles at 260 nm. Absorbance versus temperature curves were obtained both for the DNA solutions with 3,4-dihydroxyxanthone and for the corresponding solutions containing only 3,4-dihydroxyxanthone. DNA denaturation temperature,  $T_m$ , in each solution, was obtained from the curves of fraction of melted base pairs,  $\theta$ , as a function of temperature.

The hyperchromicity of the samples at 260 nm,  $H_{260}$ , was calculated at the denaturation temperature and at a higher temperature, at which it is assumed that the strands of DNA have been totally separated.

UV spectroscopy results evidence a noteworthy interaction of 3,4-dihydroxyxanthone with DNA, changing the stability of the double helix, and suggest the binding of this xanthone to DNA mainly by intercalation. These results are expected to provide a deeper insight into the DNA-binding properties of xanthenes.

### Acknowledgements:

Thanks are due to Fundação para a Ciência e Tecnologia (FCT) for financial support to Centro de Investigação em Química da Universidade do Porto (CIQ-UP) and to CEQUIMED-UP (PEst-OE/SAU/UI4040/2011).

### References:

[1] M.M.M. Pinto *et al*, *Curr. Med. Chem.* (2005), 12(21), 2517-2538.

# Interaction of Chromone with Double Stranded DNA

**Diana Sousa, M. J. Sottomayor**

Centro de Investigação em Química da Universidade do Porto (CIQ-UP), Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal.

Binding of small organic molecules to nucleic acids have attracted considerable interest. Studies of the binding of small molecules to DNA are helpful for better understanding the molecular basis of their bioactivities as well as providing useful guidance for further design of more efficient anticancer drugs. For this reason, characterization of the interaction of small molecular ligands with DNA has been the subject of numerous studies.

Chromones and chromone derivatives are reported to exhibit several biological roles, including antioxidant and free radical scavenging. Compounds that contain the chromone skeleton have been reported to exhibit multiple pharmacological properties, for example antioxidant, antibacterial, antifungal and anticancer.

The aim of the present work is to study the interaction of chromone with double stranded DNA. In order to evaluate the possible effect of chromone on the structure and stability of the DNA molecule, UV spectroscopy has been used to determine the effect of chromone on the process of thermal denaturation of double stranded DNA in aqueous solution.

Absorption spectra, as well as UV melting curves, were recorded for solutions with constant DNA concentration and different concentrations of chromone. DNA melting experiments were carried out by recording the change in absorbance at 260 nm for DNA in the absence and presence of chromone. Absorbance versus temperature curves were obtained both for the DNA solutions with chromone and for the corresponding solutions containing only chromone. The fraction of melted base pairs,  $\theta$ , as a function of temperature was calculated and DNA denaturation temperature,  $T_m$ , in each solution, was obtained from the transition midpoint of the melting curves. The hyperchromicity of the samples at 260 nm,  $H_{260}$ , was calculated at the denaturation temperature and at a higher temperature, at which it is assumed that the strands of DNA have been totally separated.

UV spectroscopy results indicate an interaction of chromone with DNA, changing the stability of the double helix. Taking into account the planar geometry of the chromone molecule, it is likely that this interaction can take place by intercalation into the base pairs of DNA.

## Acknowledgements

Thanks are due to Fundação para a Ciência e Tecnologia (FCT) for financial support to Centro de Investigação em Química da Universidade do Porto (CIQ-UP).

# Iron oxide nanoparticles as heterogeneous catalysts for the oxidation of methylene blue using Fenton reaction

**Mariana Fernandes, Inês Vaz, Mariana Rocha, Carlos Fernandes, Clara Pereira, Susana L.H. Rebelo and Cristina Freire**

REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

Magnetic iron oxide nanoparticles are a class of nanomaterials with tremendous interest in several areas of research, especially in catalysis. [1] In particular, spinel-type ferrite nanoparticles ( $MFe_2O_4$ , where M is a *d*-block transition metal) offer promising perspectives in heterogeneous nanocatalysis due to their high surface area to volume ratio and significant magnetization, which allows their easy separation from the reaction medium by magnetic separation (Fig. 1). [2]

This work reports the synthesis of colloidal magnetite ( $Fe_3O_4$ ) nanoparticles by the co-precipitation method [3] and the application of the nanomaterials as intrinsic heterogeneous nanocatalysts in the degradation of methylene blue pigment through the Fenton reaction. The physicochemical properties of the nanomaterials were characterized by Fourier transform infrared spectroscopy (FTIR) and dynamic light scattering (DLS). The catalytic reaction profile was monitored by UV-Vis spectroscopy. The FTIR spectrum of the sample exhibited typical iron oxide bands and DLS measurements indicated that the hydrodynamic radius was  $\sim 30$  nm. The heterogeneous catalyst showed high catalytic activity in the experimental conditions used in the degradation of methylene blue, since the total degradation of the pigment was observed upon 2 hours of reaction.



Fig. 1 – Magnetic response of  $Fe_3O_4$  colloidal suspension in the presence of a magnet.

**Acknowledgements:** This work was funded by Fundação para a Ciência e a Tecnologia and FEDER through grant no. PEst-C/EQB/LA0006/2011 and through project ref. PTDC/QUI-QUI/105304/2008 in the framework of Program COMPETE. M.R., C.F. and C.P. thank FCT for their grants.

## References:

- [1] Cornell, R. M., (2003), *The Iron Oxides – Structure, Properties, Reactions, Occurrences and Uses*, 1<sup>st</sup> Ed., Wiley-VCH, Weinheim.
- [2] Valdés-Solis, T., Valle-Vigón, P., Alvaréz, S., Marbán, G., Fuertes, A. (2007), *Manganese ferrite nanoparticles synthesized through a nanocasting route as a highly active Fenton catalyst*, Catal. Commun., 8, 2037-2042.
- [3] Massart, R., (1981), *Preparation of Aqueous Magnetic Liquids in Alkaline and Acidic Media*, IEEE Trans. Magn., 17, 1247-1248.

# Jousting Neurodegenerative Diseases Using Novel Lipophilic Antioxidants based on Ferulic and Caffeic Acid

**J. Amorim<sup>1</sup>, N. Leal<sup>1</sup>, I. Encarnação<sup>1</sup>, T. Silva<sup>1</sup>, A. Gaspar<sup>1</sup>  
F. Borges<sup>1</sup>**

<sup>1</sup> CIQUP/ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Neurodegenerative Diseases (ND) are a group of illnesses associated with failure and death of neurons. ND etiology is a complex multifactorial process and a wide range of pathological stimuli have been described to date. Cellular oxidative damage is known to play a vital role on neurodegeneration. Reactive oxygen species (ROS), the cellular effectors of oxidative stress, are extremely deleterious on biological systems when overproduced. Currently, it is believed that targeting the intracellular oxidative damage is a viable therapeutic strategy towards ND like Alzheimer's Disease (AD) and Parkinson's Disease (PD).<sup>[1]</sup>

Phenolic acids, like caffeic and ferulic acid, are a class of natural antioxidants with potent radical-scavenging activity and heavy metal chelating properties, acting as both primary and secondary antioxidants. These compounds can block the formation of ROS and boost the endogenous antioxidant defense mechanisms, reestablishing the oxidative homeostasis. However, these compounds are markedly hydrophilic and are unable to cross membranes and attain their target sites. In fact, their ineffectiveness has been proven in several clinical assays.<sup>[2,3]</sup>

The main goal of this project was the development of new and innovative antioxidants based on caffeic and ferulic acid, adjusting structural and physico-chemical properties that render higher hydrophobicity and a more favorable pharmacokinetic profile, while retaining the original antioxidant activity. The compounds were synthesized in a two-step reaction involving a Pd-catalyzed Suzuki cross-coupling reaction and a Knoevenagel-Doebner condensation and the screening of the antioxidant activity was assessed by DPPH and ABTS radical scavenging assays. The results obtained so far will be presented in this communication.

[1] Roleira, F.M.; Siquet, C., Orrù, E., E. Garrido, M., Garrido, J.; Milhazes, N.; Podda, G.; Martins, F.P., Reis, S.; Carvalho, R.A.; Silva, E.T.; Borges, F. (2010), *Lipophilic Phenolic Antioxidants: Correlation Between Antioxidant Profile, Partition Coefficients and Redox Properties*, *Biorganic & Medicinal Chemistry*, 18, 5816-5825.

[2] Svobodová, A.; Psotová, J. and Walterová, D. (2003), *Natural Phenolics in the Prevention of UV-induced Skin Damage*, *Biomedical Papers*, 147, 137-145.

[3] Gaspar, A.; Garrido, E.M.; Esteves, M.; Quezada, E.; Milhazes, N.; Garrido, J. and Borges, F. (2009), *New Insights into the Antioxidant Activity of Hydroxycinnamic Acids: Synthesis and Physicochemical Characterization of Novel Halogenated Derivatives*, *European Journal of Medicinal Chemistry*, 44, 2092-2099.



# Microwave Organic Reactions Applied to the Synthesis of Novel Monoamino Oxidase Inhibitors

Daniel Chavarria, Fernando Cagide, Alexandra Gaspar, Fernanda Borges

CIQUP/Departamento de Química e Bioquímica, Faculdade de Ciências,  
Universidade do Porto, Portugal.

Microwave chemistry and microwave-assisted organic synthesis remain undeniably effective tools in medicinal chemistry allowing to accelerate drug-discovery, hit-to-lead, and lead optimization programs. The use of microwave-assisted organic synthesis contributes to the development of more economical synthetic routes and to increase the productivity<sup>1</sup>.

In many instances, the use of microwave dielectric heating has been shown to dramatically reduce the processing times, to increase product yields, and to enhance product purity, when compared to conventionally processed experiments. Since several manufacturers of professional-grade equipment have arrived on the scene, and the further development of the technique has proceeded apace (e.g. from multi-mode to single-mode, and above all the use of synthesis robots), one can conclude that the interest on microwave-assisted synthesis continues to grow<sup>1</sup>.

The remarkable biological properties displayed by chromone (4*H*-1-benzopyran-4-one) compounds, either of natural or synthetic origin, have led to a continuous interest on the relevance in drug discovery process of these heterocyclic systems as privileged structures. Actually, our group has been engaged on the synthesis and pharmacologic evaluation of a medium library of chromone carboxamides and has demonstrated that chromone is a valid scaffold for the design of potent, selective, and reversible MAO inhibitors.<sup>2</sup>

In an attempt to improve the synthetic strategies it was decided to study the synthesis chromone carboxamides by using microwave irradiation and a direct amidation process. The developed method was found to be environmentally friendly and allows the formation of the amide bond with a low-price reagent. Furthermore it has been shown to possess several advantages over the conventional including operational simplicity, good performance, and a significant reaction time reduction. Microwave versus conventional synthesis data obtained so far will be presented in this communication.

## References:

[1] Kappe, C.O. and Stadler, A. *Microwaves in Organic and Medicinal Chemistry Wiley- VCH*, Weinheim, 2005.

[2] Gaspar, A., Reis, J., Fonseca, A., Milhazes, N., Viña, D., Uriarte, E., Borges, F., *Bioorg. Med. Chem. Lett.*, 2011, 21, 707; Gaspar A, Silva T, Yáñez M, Vina D, Orallo F, Ortuso F, Uriarte E, Alcaro S, Borges F. *J Med Chem.* 2011,54,5165.

This work was supported by the Foundation for Science and Technology (FCT), Portugal (PTDC/QUI-QUI/113687/2009). A. Gaspar (SFRH/BD/43531/2008) and F. Cagide (SFRH/BPD/74491/2010) thank FCT grants.

# Monovacant polyoxometalates @ MIL-101: synthesis and heterogeneous catalytic studies

André D. S. Barbosa<sup>1</sup>, Filipe A. A. Paz<sup>2</sup>, Baltazar de Castro<sup>1</sup>,  
Salette S. Balula<sup>1</sup> and Luís Cunha-Silva<sup>1</sup>

<sup>1</sup> REQUIMTE & Department of Chemistry and Biochemistry, Faculty of Sciences,  
University of Porto, 4169-007 Porto, Portugal

<sup>2</sup> Department of Chemistry, CICECO, University of Aveiro, 3810-193 Aveiro, Portugal

Metal-organic frameworks (MOFs), also known as coordination polymers, are materials formed by metal centers interconnected by organic molecules (ligands), leading to infinite one-, two- or three dimensional (1D, 2D or 3D respectively) hybrid networks. In addition to the notable structural characteristics, their properties give them high potential for industrial and technological applications, such as heterogeneous catalysis, gas storage and separation, and others.<sup>[1]</sup> In the present work, a porous 3D MOF material, chromium(III) terephthalate, herein named MIL-101, was explored as solid support for the preparation of heterogeneous catalysts.

MIL-101 was prepared by hydrothermal synthesis using terephthalic acid and chromium(III) nitrate nonahydrate, and was characterized.<sup>[2]</sup> Afterwards, two monovacant polyoxometalates  $K_7[PW_{11}O_{39}] \cdot n(H_2O)$  ( $PW_{11}$ ) and  $K_8[SiW_{11}O_{39}] \cdot m(H_2O)$  ( $SiW_{11}$ ) were immobilized in the porous network of MIL-101 leading to the formation of two novel composite materials,  $PW_{11}@MIL-101$  and  $SiW_{11}@MIL-101$ , respectively.<sup>[3]</sup> These materials were characterized by IR spectroscopy, powder XRD and SEM/EDS.

The catalytic performance of the two composite materials was evaluated for the oxidation of geraniol, using hydrogen peroxide as oxidant. Both materials proved to be active heterogeneous catalysts, capable to be reused by several reaction cycles. Higher yield of 2,3-epoxygeraniol was found when  $PW_{11}@MIL-101$  was used, however both catalysts showed 100% of selectivity for this epoxide.

## References:

- [1] S. T. Meek, J. A. Greathouse and M. D. Allendorf, *Metal-Organic Frameworks: A Rapidly Growing Class of Versatile Nanoporous Materials* (2011), *Advanced Materials*, 23, 249-267.
- [2] G. Ferey, C. Mellot-Draznieks, C. Serre, F. Millange, J. Dutour, S. Surble and I. Margiolaki, *A Chromium Terephthalate-Based Solid with Unusually Large Pore Volumes and Surface Area* (2005), *Science*, 309, 2040-2042.
- [3] M. S. S. Balula, I. C. M. S. Santos, M. M. Q. Simões, M. G. P. M. S. Neves, J. A. S. Cavaleiro, A. M. V. Cavaleiro, *A comparative study between Keggin-type phosphotungstates and silicotungstates in the oxidation of cyclooctane with hydrogen peroxide* (2004), *Journal of Molecular Catalysis A: Chemical*, 222, 159-165.

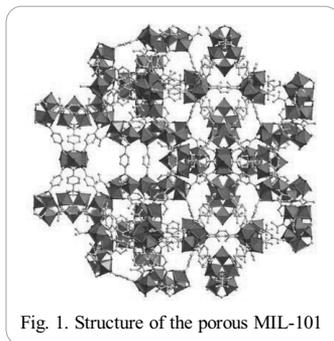


Fig. 1. Structure of the porous MIL-101

# Nickel metallo-organic polymer as a new electrochromic material

**M. Araújo**<sup>1,2</sup>, **D. M. Fernandes**<sup>1</sup>, **J. Fonseca**<sup>2</sup>, **C. Moura**<sup>3</sup> and **C. Freire**<sup>1</sup>

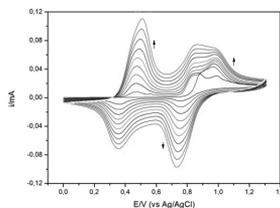
<sup>1</sup> REQUIMTE/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169 – 007 Porto, Portugal

<sup>2</sup> CeNTI – Centre for Nanotechnology and Smart Materials, 4760 – 034 Vila Nova de Famalicão, Portugal

<sup>3</sup> CIQ/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169 – 007 Porto, Portugal

Electrochromism is known as the reversible change of the color resulting from the oxidation or the reduction of a material by electrochemical methods; it results from the generation/disappearance of different visible region electronic bands on switching between redox states[1]. This property has attracted a lot of interest from academia as well as from industry. Nowadays, it is possible to find electrochromic materials in several commercial devices, such as smart windows, anti-glare car rear-view mirrors and controllable light-reflective or light-transmissive display devices for optical information and storage[2]. Electrochromic materials are divided in different classes; the most promising is probably the conducting polymers, which includes the metallo-organic polymers, such as [M(*salen*)]-based polymers (M = transition metal - Cu, Ni, Pd and *salen* = N,N'-bis(salicylidene)-ethylenediamine)[3].

The aim of this work was the preparation of poly[Ni(3-Mesalhd)] by cyclic voltammetry (CV) and the characterization of the electrochromic films. Firstly, the [Ni(3-Mesalhd)] monomer was synthesized and characterized by <sup>1</sup>H NMR and FTIR spectroscopy. Then, its anodic electropolymerization was performed in three different substrates – Pt electrode, transparent glass coated with indium-tin oxide (ITO/glass) – CVs are represented in figure 1 - and transparent flexible polyethylene terephthalate coated with indium-tin oxide (ITO/PET). The electrochemical properties of the resulting polymeric films were studied by CV and the optical properties were characterized by *in situ* UV-visible spectroscopy during the redox switching process.



**Fig. 1.** Cyclic voltammograms of the electropolymerization of [Ni(3-Mesalhd)] in no. ITO/glass, between 0.0 and 1.3 V at 20 mV.s<sup>-1</sup>.

Acknowledgements: This work has been supported by Fundação para a Ciência e a Tecnologia through grant PEst-C/EQB/LA0006/2011. MA and DMF thank ICETA and FCT, respectively, for the grants.

## References:

- [1] Amb, C.M., Dyer, A.L. and Reynolds, J.R. (2011), *Navigating the Color Palette of Solution-Processable Electrochromic Polymers*, Chemistry of Materials, 23, 397-415.
- [2] Mortimer, R.J., Dyer, A.L. and Reynolds, J.R. (2006), *Electrochromic organic and polymeric materials for display applications*, Displays, 27, 2-18.
- [3] Vilas-Boas, M., Freire, C., Castro, B., Christensen, P.A. and Hillman, A.R. (2001), *Spectroelectrochemical Characterisation of poly[Ni(saltMe)]-Modified Electrodes*, Chemistry – A European Journal, 7 (1), 139-150.

# Opening a new avenue in neurodegenerative therapy: development of mitochondria-targeted antioxidants based on benzoic scaffold

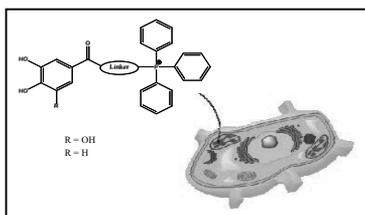
**Catarina Oliveira<sup>1</sup>, Sofia Benfeito<sup>1</sup>, José Teixeira<sup>1</sup>, Pedro Soares<sup>1</sup>, Alexandra Gaspar<sup>1</sup>, Jorge Garrido<sup>1,2</sup>, Fernanda Borges<sup>1</sup>**

(1) CIQ/ Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; (2) Departamento de Engenharia Química, Instituto Superior de Engenharia do Porto, IPP, Porto, Portugal

[catarina.oliveira.fc@gmail.com](mailto:catarina.oliveira.fc@gmail.com); [fborges@fc.up.pt](mailto:fborges@fc.up.pt)

Mitochondria have been implicated in several human diseases, called mitochondrial disorders, such as cardiac dysfunctions, obesity, diabetes, cancer, neurodegenerative diseases, such as Parkinson and Alzheimer, and amyotrophic lateral sclerosis, which affect a significant number of the worldwide population. To understand, neurodegenerative diseases processes are the opposite of cancer: whereas cancer is an uncontrolled proliferation of cells, neurodegeneration is the result of cell death, when necrotic and/or apoptotic processes are intrinsically implicated. Besides, the supplying cellular energy (ATP), mitochondria are involved in a range of other processes, such as signalling, cellular differentiation, cell death as well as the control of the cell cycle, cell growth and reactive oxygen species (ROS) production. In fact, some estimates suggest a daily production of  $10^{11}$  ROS within a typical aerobic cell. For these reasons, it is necessary to find a way to slow the neurodegenerative process progression and, consequently, the ageing processes of the brain.

The aim of our project is the design and synthesis of hydroxybenzoic antioxidant derivatives harbouring positive charges at physiological pH and hence capable of mitochondrial accumulation. In that way they could be applied as potent and selective antioxidant agents throughout specific targeting the mitochondria (Fig. 1).



**Fig. 1** – Lipophilic antioxidant cations aimed to specific targeting of mitochondria.

To achieve the goal the well-known dietary phenolic antioxidants (protocatechuic and gallic acids) were linked to aliphatic lipophilic carbon chains containing at the end the triphenylphosphonium (TPP) cation and, consequently, their antioxidant capacity as well as their lipophilicity of the compounds evaluated. The results obtained so far will be presented in this communication. If the results obtained are favorable, the compounds will be tested in mitochondria and neuronal systems. We hope that in a next future this new therapeutic approach can improve the lifestyle of people suffering with oxidative stress related diseases.

**Financial support:** PTDC/QUI-QUI/113687/2009 (FCT, FEDER, QREN and COMPETE)

## OPENING A NEW AVENUE IN NEURODEGENERATIVE THERAPY: *Lead Optimization of a mitochondria-targeted cinnamic antioxidant*

**S. Benfeito<sup>1</sup>, C. Oliveira<sup>1</sup>, J. Teixeira<sup>1</sup>, P. Soares<sup>1</sup>, A. Gaspar<sup>1</sup>, J. Garrido<sup>1,2</sup>, F. Borges<sup>1</sup>**

(1) CIQ/ Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto;

(2) Departamento de Engenharia Química, Instituto Superior de Engenharia do Porto, IPP, Porto, Portugal

[sofia\\_benfeito@hotmail.com](mailto:sofia_benfeito@hotmail.com); [fborges@fc.up.pt](mailto:fborges@fc.up.pt)

RECENT STUDIES PROVE THAT MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS PLAY A CRUCIAL ROLE IN NEURODEGENERATIVE DISEASES [1].

MITOCHONDRIA ARE A MAJOR SOURCE OF INTRACELLULAR REACTIVE OXYGEN SPECIES (ROS) AND ARE PARTICULARLY VULNERABLE TO OXIDATIVE STRESS. INDEED, OXIDATIVE DAMAGE HAS BEEN SHOWN TO IMPAIR MITOCHONDRIAL FUNCTION AND LEAD TO CELL DEATH, BEING CONNECTED TO MANY DISORDERS SUCH AS ALZHEIMER AND PARKINSON DISEASES [2]. SO, IT IS NOW APPRECIATED THAT BLOCKING MITOCHONDRIAL OXIDATIVE DAMAGE AND ROS PRODUCTION, MAY PREVENT OR SLOW DOWN THE PROGRESSION OF THESE NEURODEGENERATIVE DISORDERS. THEREFORE, THE DEVELOPMENT OF MITOCHONDRIA-TARGETED ANTIOXIDANTS COULD BE AN EFFECTIVE WAY TO BLOCK MITOCHONDRIAL OXIDATIVE DAMAGE. THESE MOLECULES CAN RAPIDLY PERMEATE phospholipid bilayers and accumulate in negatively-charged compartments such as the mitochondrial matrix, driven by the membrane potential [2].

Cinnamic acids (e.g. sinapic, ferulic and caffeic acids) are phenolic compounds present in diet that have been often used as templates for the design and development of new antioxidants. However, the majority of natural antioxidants, studied until the data, had limited success. One of the reasons mentioned for this limitation is related to the fact that the majority of natural antioxidants are distributed throughout the body and only a small fraction penetrates into the target sites, such as mitochondria.

The aim of our project is the design and synthesis of several hydroxycinnamic antioxidant derivatives harboring the positive charge of TPP cations that could be used as potent and selective antioxidant agents throughout the specific targeting of mitochondria.

After the promising results obtained with the lead compound TPP-OH, a caffeic acid derivative linked to a lipophilic TRIPHENYLPHOSPHONIUM CATION (see J. Teixeira et al communication), **IT WAS DECIDED TO DEVELOP A PROJECT RELATED TO ITS OPTIMIZATION IN WHICH CONCERN THE IMPROVEMENT OF THE ANTIOXIDANT EFFICIENCY AND LIPOPHILICITY.** The results obtained so far will be presented in this communication.

Financial support : IPG12 2009/10 and PTDC/QUI-QUI/113687/2009 (FCT, FEDER, QREN and COMPETE)

### References :

[1] Szeto, H.H., (2006), *Mitochondria-Targeted Peptide Antioxidants : Novel Neuroprotective Agents*, The AAPS Journal, 8 (3), 521-531

[2] Murphy, M.P., (2008), *Targeting Lipophilic Cations to Mitochondria*, Biochimica et Biophysica Acta, 1777, 1028-1031

# Optical detection based on the biocatalytic growth of gold nanoparticles

**A.S.P. Brandão, K.L. Marques and J.L.M. Santos**

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

The use of gold nanoparticles (AuNPs) in the development of analytical methodologies is particularly attractive, owing to their unique size-dependent physical and chemical properties [1]. AuNPs exhibit an intrinsic ability to catalyze the reduction of metal ions on the nanoparticles and thereby the enlargement of AuNPs can be employed in different biosensing paths [2,3]. Additionally, AuNPs exhibit an extinction coefficient several orders of magnitude higher than those of traditional organic chromophores allowing the achievement of extremely high sensitivity when using AuNPs-based colorimetric detection.

The main objective of this work is the quantitative optical analysis of biogenic amines, by biocatalytic enlargement of the AuNP seeds following the surface plasmon resonance wave characteristic of the nanoparticles. Biogenic amines enzymatic oxidation generates  $H_2O_2$  that reduces the gold salts. The AuNP seeds act as catalyst for the reduction of  $AuCl_4^-$  by  $H_2O_2$  resulting in the enlargement of the particles and enhanced absorbance features.

The experimental procedure for the catalytic growth of AuNP seeds consists in the addition of  $H_2O_2$  to a phosphate buffer solution that includes  $HAuCl_4$ , AuNP seeds of approximately 4 nm, stabilized by citrate, and cetyltrimethylammonium chloride as surfactant. As the concentration of  $H_2O_2$  increases the absorbance is intensified. The effect of several physico-chemical parameters, such as reagents concentrations, pH and time for reaction development, in the catalytic growth of AuNPs was evaluated.

The developed procedure will be applied to the determination of biogenic amines in food products.

Acknowledgements: Financial support from Fundação para a Ciência e Tecnologia (FCT) through grant n°. PEst-C/EQB/LA0006/2011, as well as from Reitoria da Universidade do Porto was greatly appreciated.

## References:

- [1] Daniel, M.C. and Astruc, D. (2004), *Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-size-related Properties, and Applications toward Biology, Catalysis, and Nanotechnology*, Chemical Reviews, 104 (1) 293-346.
- [2] Shlyahovsky, B., Katz, E., Xiao, Y., Pavlov, V. and Willner, I. (2005), *Optical and Electrochemical Detection of NADH and of NAD<sup>+</sup>-Dependent Biocatalyzed Processes by the Catalytic Deposition of Copper on Gold Nanoparticles*, Small, 1 (2) 213-216.
- [3] Zayats, M., Baron, R., Popov, I. and Willner, I. (2005), *Biocatalytic Growth of Au Nanoparticles: From Mechanistic Aspects to Biosensors Design*, Nano Letters 5 (1) 21-25.

## **Optimization of an analytical method for PAHs determination in estuarine sediments**

**B. Pires<sup>1</sup>, P. Carvalho<sup>1</sup>, C. Marisa R. Almeida<sup>2</sup>, M. Clara P. Basto<sup>1</sup>,**

<sup>1</sup> CIMAR/CIIMAR, Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Portugal

<sup>2</sup> CIMAR/CIIMAR, - Interdisciplinary Centre of Marine and Environmental Research Associated Laboratory, University of Porto

Polycyclic aromatic hydrocarbons (PAHs) are a group of highly lipophilic chemicals that are present ubiquitously in the environment as pollutants. Estuarine areas are exposed to a wide range of pollutants, particularly petroleum hydrocarbons, such as PAHs, pollutants that may adversely affect these habitats, including marshes characteristic of these areas. Sixteen PAHs have high level of toxicity to the organisms and are classified as priority pollutants (US EPA priority PAHs). Due to the hydrophobicity of these PAHs, they rapidly become associated with inorganic and organic suspended particles and subsequently are deposited/ adsorbed on sediments.

The main aim of the research work in progress is the study of the potential effect of the rhizosphere of salt marsh plants in the hydrocarbons distribution and remediation in estuarine environments. In order to achieve this goal, the optimization, in this laboratory, of methods for analysis of PAHs in sediments was necessary.

In a preliminary stage, a chromatographic method was adapted from the literature. After that, the focus was the optimization of extraction parameters to allow the chromatographic quantification of PAHs from the complex matrix of sediments. To achieve this, real sediment samples and reference material were extracted with several solvents and the efficiency of ultrasonic bath extraction and microwave extraction were compared. Detection and quantification of PAHs was performed using headspace solid phase microextraction coupled to gas chromatography with mass spectrometry detection, after hydrocarbons extraction from the sediment. After validation, the methodology will be applied to estuarine sediment samples for PAHs contamination determination and to study the influence of salt marsh plants on the distribution and remediation of PAHs.

# Optimization of the covalent linkage between cyclodextrins and textile substrates by experimental design techniques

R.Neto<sup>1,2</sup>, Cardoso, AP<sup>2</sup>, C.Silva<sup>2</sup>, C.Gomes<sup>2</sup>

<sup>1</sup> Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Centre for Nanotechnology and smart materials

Cyclodextrins are cyclic oligosaccharides consisting of ( $\alpha$ -1,4)-linked  $\alpha$ -D-glucopyranose units and contain a somewhat lipophilic central cavity and a hydrophilic outer surface. The natural  $\alpha$ -,  $\beta$ - and  $\gamma$ -cyclodextrin ( $\alpha$ CD,  $\beta$ CD and  $\gamma$ CD) consist of six, seven, and eight glucopyranose units, respectively. Due to the chair conformation of the glucopyranose units, the cyclodextrins are shaped like a truncated cone rather than perfect cylinders. Because of this arrangement, the interior of the cone is not hydrophobic, but considerably less hydrophilic than the aqueous environment and thus able to host other hydrophobic molecules. In contrast, the exterior is sufficiently hydrophilic to impart cyclodextrins (or their complexes) water solubility.

Cyclodextrins are able to form host-guest complexes with hydrophobic molecules given the unique nature imparted by their structure. As a result, these molecules have found a number of applications in a wide range of fields such as pharmaceutical applications for drug release, food industry for odor retention and more recently in textile industry for odor control [2].

In this work an optimization of the covalent linkage between  $\beta$ -cyclodextrins and a textile substrate was performed, with the objective of optimizing costs in the immobilization process. The optimization was performed by using design of experiments techniques (DOE), which is a tool that can be used for optimization of the experimental parameters [3].

In this work a full factorial design was used to study the influence of four different variables, namely time of reaction, concentration of sodium hydroxide, concentration of cross-linking agent and concentration of  $\beta$ -cyclodextrin.

The immobilized samples were characterized to quantify the total amount of  $\beta$ -cyclodextrins coupled to the substrate surface [4]. Others techniques were used to study the changes in the surface morphology, like ATR-FTIR (Attenuated total reflectance-Fourier transform infrared spectroscopy), AFM (atomic force microscopy) and SEM (Scanning Electron Microscopy).

With this technique, a model was obtained to quantify the amount of  $\beta$ -CD linked to the substrate, and the influence of the variation of the four studied parameters in the process was quantified. An optimum point was attained where an amount of  $\beta$ -CD/g of substrate was available at the surface. At this point, the surface morphology was characterized, indicating a clear coupling between  $\beta$ -CD and the textile substrate.

## References:

- [1] F.V.Manakker, T.Vermoden, C.F. Van Nostrum and W.E Hennink, "Cyclodextrin-Based Polymeric Material: Synthesis, Properties, and Pharmaceutical/Biomedical Applications", Bio Macromolecules 2009, Vol 10, number 12.
- [2] J.Szejtli, "Introduction and general overview of cyclodextrin chemistry", Chem.Rev. 1998, 98,1743-1753
- [3] C.J.S.M.Silva, I.C.Roberto, "Optimization of xylitol production by *Candida guilliermondii* FTI 20037 using response surface methodology", Process Biochemistry 36 (2001) 1119-1124
- [4] M.S.S. Cunha-Filho, L.C.L Sá-Barreto, "Utilização de ciclodextrinas na formação de complexos de inclusão de interesse farmacêutico" Ver. Cienc Farm. Basica Apl., V.28, n.1, p:1-9, 2007

# Phosphomolybdates immobilized onto functionalized silica nanoparticles as new photochromic materials

**T. Pinto<sup>1</sup>, A. Monteiro<sup>1</sup>, L. S. Ribeiro<sup>2</sup>, D. M. Fernandes<sup>1</sup>, C. Pereira<sup>1</sup>, O. S. G. P. Soares<sup>2</sup>, S. S. Balula<sup>1</sup>, M. F. R. Pereira<sup>2</sup>, C. Freire<sup>1</sup>**

<sup>1</sup> REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> Laboratory of Catalysis and Materials (LCM), Associated Laboratory LSRE/LCM, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

Photochromic materials have been the focus of intensive investigations for several decades due to their potential applications in optical switches, optical memories and chemical sensors. Several available photochromic compounds are based on organic molecules, polymers and inorganic oxides such as the early transition-metal polyoxoanions, generally termed polyoxometalates (POMs) [1]. Among POM properties, the ability to reversibly accept and release a large number of electrons with marginal structural rearrangement producing the mixed-valence colored species makes them suitable for the development of photochromic materials [2,3].

In this work, photochromic phosphomolybdate materials based on functionalized silica nanoparticles and the parent Keggin anion  $[\text{PMo}_{12}\text{O}_{40}]^{3-}$  ( $\text{PMo}_{12}$ ) or the lacunary anion  $[\text{PMo}_{11}\text{O}_{39}]^{7-}$  ( $\text{PMo}_{11}$ ) were prepared and characterized. These hybrid materials, denoted as f-SiO<sub>2</sub>-PMo<sub>12</sub> and f-SiO<sub>2</sub>-PMo<sub>11</sub>, were fabricated by a two-step procedure: (i) synthesis of the functionalized nanosilica by co-condensation between tetraethoxysilane (TEOS) and dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride (C18NTMS), following a methodology adapted from literature [4], and (ii) immobilization of the POMs onto the functionalized nanosilica by reflux in acetonitrile. All the materials were characterized by FTIR and UV-Vis spectroscopies, which confirmed the successful immobilization of the phosphomolybdate species onto the functionalized silica nanoparticles. Furthermore, the hybrid nanomaterials exhibited a color change from green to blue upon UV irradiation ( $\lambda=254$  nm).

**Acknowledgements:** This work has been supported by Projecto de Investigação Científica na Pré-Graduação 2010, U.P. and Santander Totta and by Fundação para a Ciência e a Tecnologia (FCT) and FEDER through grant no. PEst-C/EQB/LA0006/2011 and through project ref. PTDC/CTM/108820/2008 in the framework of Program COMPETE. TP, DF and CP thank FCT for their grants.

## References:

- [1] Pardo, R., Zayat, M. and Levy, D. (2011), *Photochromic organic-inorganic hybrid materials*, Chem. Soc. Rev., 40, 672–687.
- [2] Luo, X. and Yang, C. (2011), *Photochromic ordered mesoporous hybrid materials based on covalently grafted polyoxometalates*, Phys. Chem. Chem. Phys., 13, 7892–7902.
- [3] Yamase, T. (2008), *Photo- and electrochromism of polyoxometalates and related materials*, Chem. Rev., 98, 307–325.
- [4] Kobler, J., Möller, K., Bein, T. (2008), *Colloidal Suspensions of Functionalized Mesoporous Silica Nanoparticles*, ACS Nano, 2, 791–799.

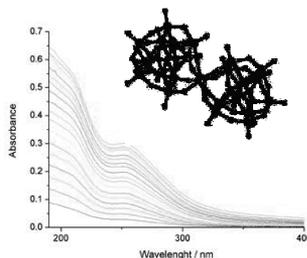
# Preparation and characterization of Europium-sandwich polyoxometalate. Incorporation into Multilayer Films

**D. Julião, D. M. Fernandes, S. S. Balula and C. Freire**

REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

Polyoxometalates (POMs) represent a well-known class of metal-oxygen clusters with a vast variation in structure, size, composition and properties. The synthesis of lanthanide-containing POMs has been subject of increasing interest due to attractive properties of photoluminescence and magnetism: [1] POMs containing  $\text{Eu}^{3+}$  are of special interest as they can produce strong fluorescence. Multifunctional thin multilayer films incorporating lanthanide-POMs have been investigated due to their potential applications in microelectronics optics, sensors and display technologies. [2] Electrostatic layer-by-layer (LbL) self-assembly is one of the methods used to fabricate thin films with thickness control at the nanometer scale.

In the present work, the synthesis and characterization of europium-sandwich POM will be present. The potassium salt of europium-sandwich POM  $\text{K}_{13}[\text{Eu}(\text{SiW}_{11}\text{O}_{39})_2] \cdot x\text{H}_2\text{O}$  ( $\text{Eu}(\text{SiW}_{11})_2$ ) was prepared by the reaction of the monovacant  $[\text{SiW}_{11}\text{O}_{39}]^{8-}$  and  $\text{Eu}^{3+}$  in 2:1 proportion. This compound was then incorporate into a thin film by layer-by-layer self-assembled method, using poly(ethylenimine) (PEI) as polycation. The growth process of the self-assembled films was monitored by UV-vis spectroscopy, Figure 1. The characteristic absorption bands observed in the UV-vis spectra confirm the incorporation of  $\text{Eu}(\text{SiW}_{11})_2$  into the LbL film; furthermore the linear increase of *Abs* vs. number of bilayers suggested a regular growth process for the LbL film.



**Figure 1:** UV-vis absorption spectra of  $(\text{PEI}/\text{Eu}(\text{SiW}_{11})_2)_n$  multilayers for  $n = 1-17$  absorbed on a quartz slide.

## Acknowledgments:

This work has been supported by Fundação para a Ciência e a Tecnologia through grant no. PEst-C/EQB/LA0006/2011.

## References:

- [1] Bassil, B.S., Kortz, U. (2010), *Recent Advances in Lanthanide-Containing Polyoxotungstates*, *Z. Anorg. Allg. Chem.*, 636, 2222.
- [2] Liu, S., Tang, Z. (2010), *Polyoxometalate-based Functional Nanostructured Films: Current Progress and Future Prospects*, *Nano Today*, 5, 267.

# Preparation of metallic nanoparticles functionalized with peptides for applications in biosensors

C. Loureiro<sup>1</sup>, R. Franco<sup>2</sup>, P. Eaton<sup>1</sup>, E. Pereira<sup>1</sup>

<sup>1</sup> REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto

<sup>2</sup> REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa

Bionanotechnology arises as a new branch of nanotechnology and with it comes a large number of applications originated by the huge interest in nanomaterials, particularly in nanoparticles due to their characteristics and the fact that biological molecules like proteins bind easily to the surface.

In the present work bionanoconjugates of gold nanoparticles and laccase were prepared. Laccase from *Rhus vernicifera* is an oxidoreductase that belongs to the multinuclear copper-containing oxidases. It catalyses the monoelectronic oxidation of a variety of substrates in the presence of molecular oxygen, and contains a single set of copper ions of three distinct types [1]. Laccases find several applications in industry, including detoxification of industrial effluents from paper, textile and petrochemical industries, bioremediation agent to clean up herbicides and pesticides, etc. The nanoparticles were synthesized by Turkevich method [2] and functionalized with a pentapeptide of sequence CALNN that binds to the nanoparticle surface by chemisorption of the cysteine thiol group, providing a negative charge to the functionalized nanoparticles through the carboxylate groups of the free peptide. These functionalized nanoparticles allow a better adsorption of the enzyme as well as a better colloidal stability of the bionanoconjugates. The bionanoconjugates of nanoparticles with laccase were prepared by two types of binding strategies: electrostatic interaction (direct adsorption to the surface of functionalized nanoparticles) and covalent coupling, in order to assess which binding strategies is more appropriate to maintain the enzymatic activity in the bionanoconjugates.

Determination of the enzymatic activity of laccase was performed by ultraviolet-visible spectrophotometry, assuming a Michaelis-Menten type enzymatic kinetics. These assays were conducted in the pH range 6-8 and were compared with the results obtained for the free enzyme under the same experimental conditions. It was found that the catalytic efficiency of laccase (immobilized by both binding strategies) in bionanoconjugates doesn't vary significantly with pH, in contrast with the behavior of the free enzyme. However, it was found that laccase, when immobilized by electrostatic interaction, has a higher catalytic efficiency compared to the free enzyme (except for pH 7.5) as well as for the laccase immobilized, in bionanoconjugates, by covalent coupling. Thus, it was found that the electrostatic interaction is a better binding strategy, in this case, since the enzyme exhibited a better enzymatic activity. The results obtained indicate that bionanoconjugates could advantageously replace laccase in industrial processes where optimal pH conditions for the free enzyme (pH = 7.5) are inconvenient or impossible to use.

## References:

[1] Agostinelli, E., Cervoni, L., Giartosio, A., Morpurgo, L., The Journal of Biochemistry 1995, 306, 697-702.

[2] Kimling, J. *et al.*, The Journal of Physical Chemistry B 2006, 110, 15700-15707.

# Rapid simultaneous potentiometric determination of sodium and chloride in bread

**N. Lopes<sup>1</sup>, O. Vaz<sup>1</sup>, A. Plácido<sup>1</sup>, J. Magalhães<sup>2</sup>, H. Nouws, T. Teles<sup>1</sup> and C. Delerue-Matos**

<sup>1</sup> REQUIMTE, Instituto Superior de Engenharia do Porto, Portugal

<sup>2</sup> REQUIMTE, Department of Chemical Engineering, University of Porto, Portugal

Current high salt (sodium chloride) intake of populations worldwide has many negative consequences for human health. Because bread is an important part of the daily diet of most people, a reduction in salt content of bread is a good way to lower the intake. Therefore, the Portuguese government has set new legislation [1] concerning the salt level in bread. The legislation now limits the amount of salt in bread to 1.4 g/100g. There are several methodologies to determine the amount of sodium chloride in foods and are either based on the determination of sodium or on the determination of chloride. In the analysis of sodium the most widely used methods are atomic absorption spectrometry [2] and atomic emission spectrometry [3]. For the analysis of chloride mainly classical argentometric titrations with visual or potentiometric indication are applied. The majority of the available reference procedures [4] are based on one of the previously mentioned methods.

In this work, a method for the simultaneous determination of sodium and chloride based on direct potentiometry was developed to replace the time-consuming reference methods. For this purpose, all-solid-state chloride and sodium ion selective electrodes (ISE's) based respectively on tetraphenylporphyrin manganese (III) chloride complex and 4-*tert*-butylcalix[4]arene-tetraacetic acid tetraethyl ester were constructed and evaluated. The ISE's displayed Nernstian behaviour, with average slopes of -59 and 58 mV decade<sup>-1</sup>, in the concentration range from 10<sup>-4</sup> to 10<sup>-2</sup> M. To evaluate the performance of the ISE's for salt determination, bread samples from several cities in the North of Portugal were analysed by direct potentiometry with the ISE's and by the reference methods [4].

The results obtained suggest that the methods based on direct potentiometry are an adequate alternative to the reference methods for the determination of salt in bread.

## Acknowledgments:

This work has been supported by Fundação para a Ciência e Tecnologia through grant no. PEst-C/EQB/LA0006/2011.

## References:

- [1] Ministério da Agricultura, do Desenvolvimento Rural e das Pescas. Lei n.º 75/2009 de 12 de Agosto de 2009. Diário da República. 1.ª Série, n.º 155, p: 5225-5226.
- [2] Julshamm, K., Lea, P., and Norli, H.S. (2005), *Determination of Sodium in Foods by Flame Atomic Absorption Spectrometry after Microwave Digestion: NMKL Interlaboratory Study*, Journal of AOAC International, 88 (4), 1212-1216.
- [3] Khouzam, R.B., Lobinski, R. and Pohl, P. (2011), *Multi-element analysis of bread, cheese, fruit and vegetables by double-focusing sector-field inductively coupled plasma mass spectrometry*, Analytical Methods, 3 (9), 2115-2120.
- [4] *Approved Methods of The AACC*, (9<sup>th</sup> ed.), American Association of Cereal chemists, St. Paul, MN.

## Simultaneous quantification of cholesterol, vitamin E, total and fatty acids profile in *Octopus*: method validation

**A. Costa<sup>1</sup>, E. Mendes<sup>1</sup>, R. Cruz<sup>1</sup>, S. Morais<sup>2</sup>, and S. Casal<sup>1</sup>**

<sup>1</sup>REQUIMTE – Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

<sup>2</sup>REQUIMTE – Department of Chemical Engineering, Superior Institute of Engineering, Porto Polytechnic Institute, Portugal

Sea food is recognized as an important source of nutrients of high biological value. Among them, lipids encompass important compounds, increasingly recognized as crucial for an adequate health status, and include the fatty acid profile, sterols, and vitamins. Their amounts, however, are highly variable, depending on specie, gender, age, season, geographical region, among others, making their accurate nutritional characterization a huge task. The main objective of this work was to develop a sample preparation procedure that could be used simultaneously for the determination of all these parameters, therefore reducing time, costs and environmental impact. Octopus was chosen due to its high consumption patterns in our country and insufficient data on its nutritional characterization.

Different extractive conditions were tested, including sample state (fresh, lyophilized) and amounts, extractive solvents, derivatization conditions, and adequate amounts of antioxidants and internal standards. The final methodology uses around 200 mg of lyophilized sample and requires solvent extraction with dichloromethane/methanol, based on the classical Folch method. Vitamin E is evaluated directly in the lipid extract by NP-HPLC-FLD. After alkaline hydrolysis and methylation of the fatty acids, the extract is used for the evaluation of total cholesterol by HPLC-UV, under the same chromatographic conditions used for vitamin E, and for the fatty acid profile by GC-FID.

The method presents a large linear working range, and the results obtained for the analyzed samples are similar to those reported in literature. The method is highly reproducible, with precisions below 5% (RSD) for  $\alpha$ -tocopherol and cholesterol, and below 6% for fatty acids and total fat. Accuracy was tested by recovery tests, being higher than 95% for vitamin E and cholesterol.

The method developed is effective for routine analysis, with a substantial reduction in sample preparation time and organic solvent consumption, with a direct impact on economic and environmental costs. Although being tested only for Octopus samples, this method reveals a great potential for application in diverse food samples.

**Acknowledgments:** This work was developed with funding from the Project FCT PTDC/AGR-AAM/102316/2008, co-financed by COMPETE/FEDER/OE.

## **Spectrophotometric determination of nilutamide by multipumping**

**Mélodie M. Carvalho, David S. M. Ribeiro, João L. M. Santos and João A. V. Prior**

Requimte, Department of Chemistry Sciences, Faculty of Pharmacy, University of Porto, Portugal.

The automatic systems of flow analysis have innumerable applications in diverse social and economic fields, namely in the quality control of pharmaceutical products. One of the quality control tests of pharmaceutical formulations is the quantification of the active substance. The execution of this analytical parameter is of most importance due to possible chemical modifications of the drug throughout its storage or derived from production errors during manufacturing, like for example, in the incorporation of substances in a different ratio different from the one defined for the final product.

The antiandrogen nilutamide is used to prevent tumor development observed in the first phase of the administration of analogues of gonadotropin-releasing hormone in patients with prostate cancer. The administration is orally. Adverse effects include diarrhea, nausea and vomiting and reversible changes in renal function as well as decreased libido and gynaecomastia. This drug presents significant liver toxicity.

The aim of this work was to develop an automatic procedure based on flow analysis for the quantification of the drug nilutamide in pharmaceutical formulations, which could constitute a simpler alternative for routine quality control in industry relatively to chromatographic methods.

The proposed work involved the exploitation of the concept of multipumping flow analysis based on the use of multiple solenoid micro-pumps, which are the only active components in the system and that can be activated individually or in combination. The automatic control of these devices based on programming routines dependent on time or number of pulses, enables to implement versatile analytical methodologies, whose configuration parameters, such as flow rate, insertion of sample, sequence of the addition of reagents and selection of different sampling strategies, can be selected and adapted to each analytical situation.

A new methodology was developed based on the reaction of the drug with sodium hydroxide. The monitoring of the reaction products was performed at the wavelength of 432 nm by spectrophotometry. In this work, the potentialities of the pulsed flow in the multipumping approach were exploited taking into account the high viscosity of the sodium hydroxide solutions. The pulsed flow originates a chaotic movement of solutions inside the tubes thus promoting a high degree of mixing of solutions during transport of the reaction zone for detection.

Acknowledgements:

David S. M. Ribeiro thanks the “Fundação para a Ciência e Tecnologia” and FSE (Quadro Comunitário de Apoio) for the Ph.D. grant no. SFRH/BD/42571/2007. This work has been supported by “Fundação para a Ciência e a Tecnologia” through grant no. PEst-C/EQB/LA0006/2011.

# Strong Electrolyte Aqueous Solutions: Solute Concentration. Computer Simulation Development

C. Alves, C. Morais, J. Paiva, and M. Ribeiro da Silva

Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 687, P-4169-007, Porto, Portugal

Computer simulations are valuable resources in education, since they can contribute to improve students' interest and motivation, combining Chemistry to the new technologies pedagogic potential. Furthermore, the simulations using representative system models, may help to reduce the abstraction required to understand most of the chemical concepts.

The purpose of this project is to develop a computer simulation, “*Soluções aquosas de eletrólitos fortes: concentração do soluto*” - Strong electrolyte aqueous solution: solute concentration -, that addresses the way of preparing solutions (from a solid solute or by the dilution of a more concentrated solution) and the different ways of expressing the quantitative composition of these solutions. The application of this simulation as a didactic resource in different levels of education (10 to 14 years old, 15 to 18 years old and university level) is the aim of this project.

A primary version was developed where the user can mimic the preparation of strong electrolyte aqueous solution from a solid solute (available online at <http://nautilus.fis.uc.pt/cec/solaquosas/> (Fig. 1)).

After the preparation of the solution, the user can verify the mass concentration, the molar concentration and the corrected concentration for interaction effects - activity - of the prepared solution. The simulation contains a hypertext, with information on the solutions chemistry, which is available in three different scientific levels.

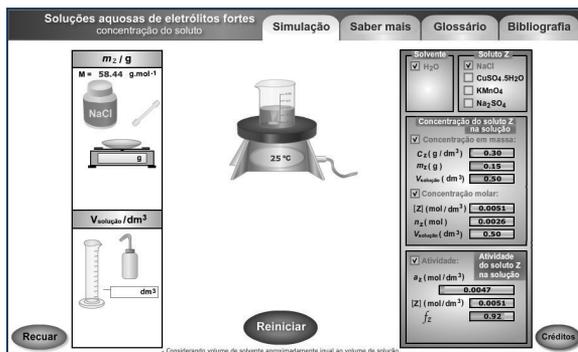


Fig. 2. Simulation interface print screen.

## Acknowledgment

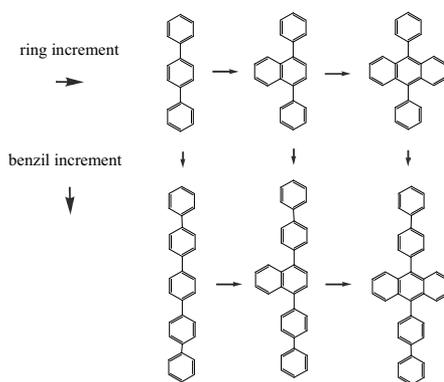
Thanks are due to Casa das Ciências (Fundação Calouste Gulbenkian) for the support to the project and for the award of a grant to C. A.

# Suzuki-Miyaura Synthesis of Some Linear Arylacenes

**C.F. Ribeiro, C.F.R.A.C. Lima and L.M.N.B.F. Santos**

Centro de Investigação em Química, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, Porto, Portugal

Arylnaphthalenes and its derivatives are promising organic materials with potential interest in electronic devices.<sup>[1,2]</sup> This work is focused on the synthesis and characterization of some linear conjugated aromatic compounds derived from the introduction of phenyl and 4-biphenyl substituents in benzene, naphthalene and anthracene. The studied compounds are illustrated in figure 1. The compounds were synthesized by the Suzuki-Miyaura cross-coupling reaction<sup>[3]</sup>, purified by conventional techniques, and characterized by <sup>1</sup>H and <sup>13</sup>C NMR, and UV-Vis spectroscopy.



**Figure 1.** Schematic representation of the studied compounds.

UV-Vis and DSC (Differential Scanning Calorimetry) was used in order to explore the influence of subtle structural changes at the molecular level on the: A) photophysical properties, which are related to the intrinsic electronic structure; B) relative solid/liquid phase stability, which is chiefly influenced by the strength of dispersive interactions and molecular shape.

## References:

- [1] Tang, C.W. and Van Slyke, S.A. (1987) *Applied Physics Letters*, 51, 913
- [2] Pei, Q., Yu, G., Zang, C., et al. (1995) *Science*, 269, 1086
- [3] N. Miyaura, A. Suzuki, *Chem. Rev.*, **1995**, 95, 2457.

# Synthesis and characterization of functionalized Fe- Au/core-shell magnetic nanoparticles for bioassay applications

**M. Freitas<sup>1</sup>, S. Viswanathan<sup>2</sup>, M.B.P. P. Oliveira<sup>1</sup>, Cristina Delerue-Matos<sup>2</sup>**

<sup>1</sup>REQUIMTE, Dep. Ciências Químicas, Faculdade Farmácia, Universidade do Porto, Portugal.

<sup>2</sup>Requimte, Instituto Superior de Engenharia do Porto, Portugal.

The development of novel materials with fascinating properties and enhanced performance is a consecutively expanding research field, which encompasses subjects ranging from chemistry, biology, and physics to material science.

Noble metal coated magnetic nanoparticles provide stable nanoparticles, which protects the core from oxidation and corrosion. Gold-coated magnetic nanoparticles have been used because of their facile and robust interaction with thiol and disulfide groups that enable them functionalization with various molecules. Surface modification with gold (Au) further helps to reduce particle agglomeration by steric or electronic repulsion and enhances biocompatibility [1]. Thus, magnetic nanocrystals covered with an Au-shell would provide all the characteristics of the Au element suitable for many applications and deliver magnetic properties for further manipulation. Significant progress has been made for the functionalization of gold nanoparticles using thiol chemistry, which facilitated the attachment of organic molecules using a variety of thiol linkers [2].

In this study, we report the chemical synthesis and functionalization of magnetic and gold-coated magnetic nanoparticles. The surface functionalization of Au and magnetic nanoparticles coated with gold shells (M/Au) by proteins labels for the creation of nanoprobe for use in electrochemical bioassays. Gold-iron nanoparticles are functionalized with mercaptopropionic acid, cystamine, or mercaptoethanol self assembled monolayers (SAMs) and characterized using dynamic light scattering, zeta potential, and electrochemical performance on magnetized electrode.

## References:

[1] Wang, L.Y. Luo, J. Fan, Q. Suzuki, M. Suzuki, I.S. Engelhard, M.H. Linv, Y.H. Kim, N. Wang, J.Q. Zhong, C.J. (2005) *Monodispersed Core-Shell Fe<sub>3</sub>O<sub>4</sub>@Au Nanoparticles*. Journal of Physical Chemistry B 109:21593

[2] Liu, H.L. Sonn, C.H. Wu, J.H. Lee, K.M. Kim, Y.K. (2008) *Synthesis of streptavidin-FITC-conjugated core-shell Fe<sub>3</sub>O<sub>4</sub>-Au nanocrystals and their application for the purification of CD4+ lymphocytes*. Biomaterials 29:4003

Acknowledgments: This work has been supported by Fundação para a Ciência e a Tecnologia through grant no. PEst-C/EQB/LA0006/2011.

# Synthesis and micellization properties of novel serine-based gemini surfactants: Assessing the role of the spacer linkage and spacer length

S. Goreti Silva, Cláudia M. Alves, Eduardo F. Marques, M. Luísa C. do Vale

CIQ (UP)/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Gemini surfactants have attracted increasing attention over the last years, owing to their excellent performance and enhanced physicochemical and biological properties when compared to those of conventional single-chained surfactants [1]. Over the last years, our research group has been engaged in the synthesis and assessment of the physicochemical and toxicological properties of ionic amino acid-based surfactants [2,3]. More recently, serine-based gemini surfactants with the spacer introduced at the amino group of the amino acid, mimetizing the conventional *bis-quats*, have been studied [4]. In order to evaluate the influence of the spacer linkage and the spacer chain length on the physicochemical profile of gemini surfactants, a series of cationic gemini surfactants derived from serine, with spacer linkages of varied size and chemical nature (amides, esters, see Fig.1) were synthesized and their basic micellization properties were determined. The surfactants were obtained from the corresponding *N*-alkyl amino acid derivatives by condensation with diamines (1) or dialcohols (2). The critical micelle concentration for the different amphiphiles was determined by surface tension measurements. Furthermore, evaluation of the cytotoxicity of the surfactants is currently under course.

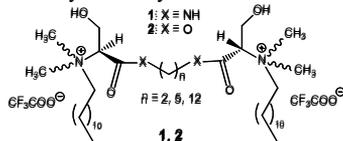


Figure 1: General structure of the gemini surfactants studied.

Acknowledgements: Thanks are due to FCT (CIQ; research project PTDC/QUI/115212/2009; grant SFRH/BD/61193/2009) and FEDER (re-equipment program REDE/151/RMN/2005) for financial support.

## References:

- [1] Menger, F. M.; Keiper, J. S.(2000), *Gemini Surfactants*, Angew. Chem. Int. Ed. (39), 1906-1920.
- [2] Silva, S. G.; Rodríguez-Borges, J. E.; Marques, E. F.; do Vale, M. L. C. (2009), *Towards novel efficient monomeric surfactants based on serine, tyrosine and 4-hydroxyproline: synthesis and micellization properties*, Tetrahedron (65), 4156-4164.
- [3] Brito, R. O.; Marques, E. F.; Silva, S. G.; do Vale, M. L. C.; Gomes, P.; Araújo, M. J.; Rodríguez-Borges, J. E.; Infante, M. R.; Garcia, M. T.; Ribosa, I.; Vinardell, M. P.; Mitjans, M.. (2009), *Physicochemical and toxicological properties of novel amino acid-based amphiphiles and their spontaneously formed catanionic vesicles*, Colloids Surf. B:Biointerfaces (72), 80-87.
- [4] Silva, S. G.; Fernandes, R. F.; Marques, E. F.; do Vale, M. L. C. (2012) *Serine-Based Bis-quat Gemini Surfactants: Synthesis and Micellization Properties*, Eur. J. Org. Chem. (2), 345-352.

# Synthesis and Structural Elucidation of New Chiral Xanthone Derivatives

**C. Carneiro**<sup>1,2</sup>, **S. Monteiro**<sup>1,2</sup>, **C. Fernandes**<sup>1,2</sup>, **M.E. Tiritan**<sup>1,3</sup>, **C. Afonso**<sup>1,2</sup> and **M. Pinto**

<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP)

<sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal

<sup>3</sup>Centro de Investigação em Ciências da Saúde, Instituto Superior de Ciências da Saúde-Norte, (CICS-ISCN), Gandra, Portugal.

The differences normally exhibited by the enantiomers in pharmacodynamics, pharmacokinetics and toxicity, makes the therapeutics with single enantiomers, comparing with racemates, of unquestionable advantages [1, 2]. Consequently, the development of efficient methodologies for synthesis of chiral compounds in high yields and with high enantiomeric purity is becoming one of the most important tasks in the field of Medicinal Chemistry.

The development of a library of chiral xanthone derivatives (CXDs) that will potentially reveal interesting biological activities is in progress in CEQUIMED-UP. In this context, using a carboxyxanthone derivative as building block and the *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) as coupling reagent, two new CXDs were obtained (**Fig. 1**). The structures of the synthesized compounds were elucidated by spectroscopic methods (IR, <sup>1</sup>H and <sup>13</sup>C NMR, MS).

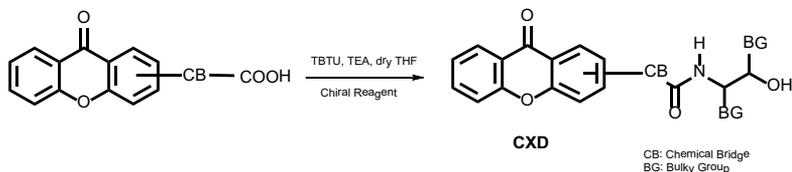


Fig. 1. General scheme for the synthesis of CXDs

The new CXDs will be evaluated for growth inhibition of human cancer cell lines and for anti-inflammatory activity by inhibition of enzymes involved in the inflammatory process, namely phospholipase A2 (PLA2) and cyclooxygenases (COX-1 and COX-2).

## References:

[1] Brocks, D.R. (2006), *Drug Disposition in Three Dimensions: An Update on Stereoselectivity in Pharmacokinetics*, Biopharm. Drug Dispos. 27 (387).

[2] Kasprzyk-Hordern, B. (2010), *Pharmacologically active compounds in the environment and their chirality*, Chem. Soc. Rev. 39 (4466).

Acknowledgments: CEQUIMED-UP (PEst-OE/SAU/UI4040/2011), FEDER, POCI, for financial support.

# Synthesis of 2'-hydroxy-3,4,4',5,6'-pentamethoxychalcone and analogues by MAOS

**S. Domingos**<sup>1,2</sup>, **A.C. Pereira**<sup>1,2</sup>, **D. Pereira**<sup>1,2</sup>, **S. Cravo**<sup>1,2</sup>, **M. Pinto**<sup>1,2</sup>, **H. Cidade**<sup>1,2</sup>

<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal

<sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal.

Chalcones are naturally occurring flavonoids composed by two aromatic rings connected by a three-carbon chain containing an  $\alpha,\beta$ -unsaturated carbonyl group. They are pharmacologically relevant because of their ability to exert a wide range of biological activities, namely as antitumor, antioxidant, anti-inflammatory and antimicrobial [1,2]. Having such a variety of biological activities, these natural compounds have attracted the attention of medicinal chemists and therefore several classic and non-classic methodologies, have been developed for their syntheses. One of these methodologies is microwave-assisted organic synthesis (MAOS), which offers considerable advantages concerning substantial enhancements of rate of heating and of the reactions, compared with conventional heating. On this basis, we used MAOS to synthesize three methoxylated chalcones (**1-3**) by a base-catalyzed aldol reaction of appropriated substituted acetophenones with benzaldehydes. Structures were established by IR, <sup>1</sup>H and <sup>13</sup>CNMR techniques.

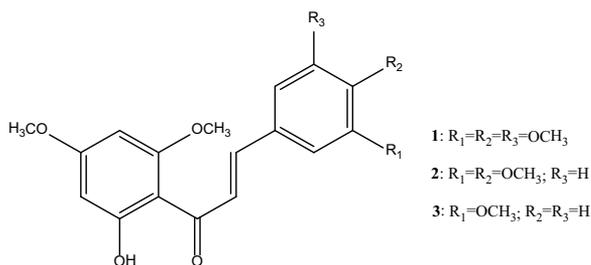


Fig. 1 – Structures of chalcones **1-3**.

## References:

[1] Go, M. L., Wu, X. and Liu, X. L. (2005), *Chalcones: An Update on Cytotoxic and Chemoprotective Properties*, *Curr. Med. Chem.*, 12, 483-499.

[2] Nowakowska, Z. (2007), *A Review of Anti-infective and Anti-inflammatory Chalcones*, *Eur. J. Med. Chem.*, 125-137.

## Acknowledgments:

This work is funded through national funds from FCT – Fundação para a Ciência e a Tecnologia under the project CEQUIMED – PESt-OE/SAU/UI4040/2011, FEDER funds and COMPETE program under the projects FCOMP-01-0124-FEDER-011057 and FCOMP-01-0124-FEDER-015752.

## Synthesis of bioactive xanthone derivatives

C. Sobral<sup>1,2</sup>, A.M. Paiva<sup>1,2</sup>, E. Sousa<sup>1,2</sup> and M. Pinto<sup>1,2</sup>

<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), <sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal

Xanthone derivatives are heterocyclic compounds with a dibenzo- $\gamma$ -pyrone scaffold. A large number of naturally occurring and synthetic xanthenes with interesting biological and pharmacological activities have been reported in the past few years, namely as antitumor agents [1]. 3,4-Dihydroxyxanthone (**1**, Fig. 1) was revealed as a cell growth inhibitor of several human tumor cell lines [2] and as an interesting model to pursue with the development of new antitumor xanthone derivatives [3].

In order to obtain enough quantity of derivatives for *in vivo* assays and as well as for further molecular modifications, the synthesis of 3,4-dihydroxyxanthone (**1**) was accomplished (Fig. 1):

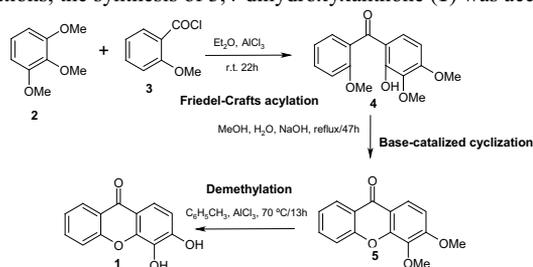


Fig. 1. Synthesis of compounds **1** and **2**.

The condensation of 1,2,3-trimethoxyphenol (**2**) with the appropriate substituted benzoyl chloride **3** afforded benzophenone **4** which gave 3,4-dimethoxyxanthone (**5**) by cyclization. Compound **5** was demethylated to furnish 3,4-dihydroxyxanthone (**1**).

Compound **1** will be used as a building block for the creation of a library of xanthonic derivatives to investigate their antitumor activity.

### References:

- [1] Pinto, M., Sousa, E. and Nascimento, M. S. J. (2005), *Xanthone derivatives: new insights in biological activities*, *Current Medicinal Chemistry*, 12, 2517-2538.
- [2] Pedro M., Cerqueira, F., Sousa, M.E., Nascimento, M.S.J. and Pinto, M. (2002), *Xanthenes as inhibitors of growth of human cancer cell lines and their effects on the proliferation of human lymphocytes in vitro*, *Bioorganic Medicinal Chemistry*, 10 (12), 3725-3730.
- [3] Palmeira A., Paiva A., Sousa E., Seca H., Almeida G. M., Lima R. T., Fernandes M. X., Pinto M. and Vasconcelos M. H. (2010), *Insights into the In Vitro Antitumor Mechanism of Action of a New Pyranoxanthone*, *Chemical Biology Drug Design*, 76, 43-58.

Acknowledgements: To FCT – Fundação para a Ciência e a Tecnologia under the project CEQUIMED - PEst-OE/SAU/UI4040/2011 and PTDC/SAU-FAR/110848/2009, FEDER through the COMPETE program under the projects FCOMP-01-0124-FEDER-011057 and FCOMP-01-0124-FEDER-015752; and to U.Porto/Santander Totta for financial support. A.M. Paiva thanks for a grant (PTDC/SAU-FCT/100930/2008) in the scope of the project.

# Synthesis of Prenylated Xanthenes Inhibitors of the Growth of Cancer Cell Lines

N. Machado<sup>1,2</sup>, E. Costa<sup>1,2</sup>, E. Sousa<sup>1,2</sup>, M. Pinto<sup>1,2</sup>

<sup>1</sup>Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), <sup>2</sup>Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal

Many naturally occurring and synthetic xanthenes have been found to possess interesting biological and pharmacological activities [1]. We have focused our attention on the synthesis of xanthone derivatives and their capacity to inhibit the *in vitro* growth of some tumor cell lines. The nature and/or position of the different prenyl substituents on the xanthonic scaffold were associated to the antitumor effect, being found that some of these derivatives were not only potent growth inhibitors but also selective for MCF-7 (breast adenocarcinoma) cells [2]. It was based on this premise that we proceeded with the prenylation of *trans*-kielcorin C (**1**), a growth inhibitor which has previously shown selectivity for MCF-7 [3] (Fig. 1).

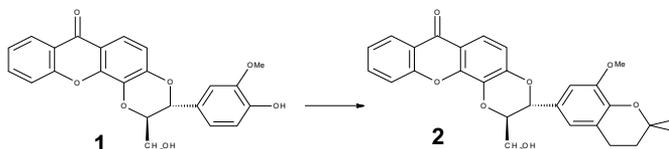


Fig. 1. Prenylation of *trans*-kielcorin C: prenyl bromide, K<sub>10</sub> clay, CHCl<sub>3</sub>, MW (200W/ 108°C/ 60 min).

The new prenylated derivative, the dihydropyrano **2**, was elucidated by <sup>1</sup>H, <sup>13</sup>C NMR and HR-MS data. The prenylation of the precursor of compound **1**, 3,4-dihydroxyxanthone, was also performed and the already known dihydropyrano derivative was elucidated based on <sup>1</sup>H NMR data. These compounds will be further investigated for their biological effects.

## References:

- [1] Pinto, M., Sousa, E. and Nascimento, M. S. J. (2005), *Xanthone derivatives: new insights in biological activities*, Current Medicinal Chemistry, 12, 2517-2538.
- [2] Castanheiro, R.A.P., Pinto, M.M., Silva, A.M.S., Cravo, S.M.M., Gales, L., Damas, A.M., Nazareth, N., Nascimento, M.S.J. and Eaton, G. (2007) *Dihydroxyxanthenes prenylated derivatives: synthesis, structure elucidation and growth inhibitory activity on human tumor cell lines with improvement of selectivity for MCF-7*. Bioorganic Medicinal Chemistry, 15, 6080-6088.
- [3] Sousa, E.P., Silva, A.M.S., Pinto, M.M.M., Pedro, M.M., Cerqueira, F.A.M. and Nascimento, M.S.J. (2002) *Isomeric Kielcorins and Dihydroxyxanthenes: Synthesis, Structure Elucidation and Inhibitory Activities of Growth of Human Cancer Cell Lines and on the Proliferation of Human Lymphocytes In Vitro*. Helvetica Chimica Acta, 85, 2862-2876.

Acknowledgements: To FCT – Fundação para a Ciência e a Tecnologia under the project CEQUIMED - PEst-OE/SAU/UI4040/2011 and PTDC/SAU-FAR/110848/2009, FEDER through the COMPETE program under the projects FCOMP-01-0124-FEDER-011057 and FCOMP-01-0124-FEDER-015752.

# Synthesis, characterization and properties of a rosamine-based potentiometric ammonia gas sensor

I. Cardoso<sup>1</sup>, C. Queirós<sup>2</sup>, S. C. Lopes<sup>1</sup>, P. Gameiro<sup>1</sup>, B. de Castro<sup>1</sup>, M. Rangel<sup>2</sup>,  
A. M. G. Silva<sup>1</sup>, G. González-Aguilar<sup>3,\*</sup>

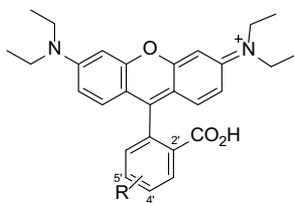
<sup>1</sup> REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal.

<sup>2</sup> REQUIMTE, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4099-003 Porto, Portugal.

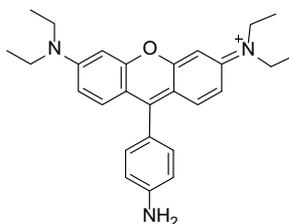
<sup>3</sup> INESC-Porto, Departamento de Física, Faculdade de Ciências, Universidade do Porto, Porto 4169-007, Portugal.

Rosamines are fluorophores, like rhodamine, fluorescein and eosin, which belong to the family of xanthene derivatives. In comparison with 4'- or 5'-functionalized rhodamines, 4'-substituted rosamines lack the carboxylic acid functional group in position 2', and for this reason they are less problematic to synthesize and to purify, being easily isolated as a single regioisomer.<sup>[1]</sup> Rosamines also exhibit interesting spectral properties namely high molar absorptivity, intense fluorescent spectrum in the visible region, which have enabled its successfully use for different optical applications, namely as bioimaging probes<sup>[2]</sup> and also as fluorescent chemosensors for metal ions detection.<sup>[3]</sup>

In the present work, a new rosamine bearing an amino functional group in position 4' (**rosNH<sub>2</sub>**) was synthesized and characterized. This compound was further covalently linked to a silica matrix through a sol-gel process. The potentiometric measurements of the prepared electrodes revealed the specificity of this compound as an ammonia sensor with a linear dependence for a concentration range between 29-120 µg/L of NH<sub>3</sub>.



4'- or 5'-substituted rhodamine



4'-amino substituted rosamine (**rosNH<sub>2</sub>**)

## References:

[1] Wu L and Burgess K. (2008), *Synthesis and spectroscopic properties of rosamines with cyclic amine substituents*. J. Org. Chem, 73, 8711-8718.

[2] Ahn Y.-H., Lee J.-S., Chang Y.-T. (2007), *Combinatorial rosamine library and application to in vivo glutathione probe*. J. Am. Chem. Soc., 129, 4510-4511.

[3] Iyoshi S, Taki M, and Yamamoto Y. (2008), *Rosamine-Based Fluorescent Chemosensor for Selective Detection of Silver(I) in an Aqueous Solution*. Inorg. Chem. 47, 3946-3948.

## The effect of dissolved organic matter on the degradation of UV filters in swimming pool water

Diana M.A. Crista, M.S. Miranda, J.C.G. Esteves da Silva

Centro de Investigação em Química (CIQ-UP), Department of Chemistry and Biochemistry, Faculty of Sciences of University of Porto, Portugal. Email: diana.crista4@gmail.com.

Ultraviolet (UV) absorbing compounds are increasingly used in personal-care products (e.g., sunscreens, shampoos and hair sprays) as a result of growing concern about exposure to sunlight causing skin cancer [1]. However, in chlorine disinfected water these compounds are unstable and they suffer degradation mainly through reaction with disinfectant products. With these processes of degradation, new compounds are originated which are usually highly toxic and harmful towards human health and the environment.

New analytical methodologies are being developed to determine and regulate the presence of these new compounds, both in environmental and commercial samples and/or products [1]. The objective of this study is identify the degradation percentages of the UV filter butyl methoxy dibenzoylmethane (BDM) in chlorinated waters containing some dissolved organic matter (DOM). The effect of DOM in the degradation percentages of UV filters is being done using response surface experimental design methodologies using the following variables: pH, temperature, chlorine concentration, solar irradiation exposure and dissolved organic matter concentration. Moreover, the formation of toxic disinfection by products is being investigated. Experimentally, the quantitative analysis is being made using a high performance liquid chromatography coupled with UV detection (HPLC-UV-DAD) methodology.

This communication will present the most important results about the effect of DOM on the degradation of BDM and on the type of disinfection by products that are generated by this UV filter.

### References:

[1] A. Joel M. Santos, Margarida S. Miranda and Joaquim C.G. Esteves da Silva (2011), *Chlorine disinfection by-products in swimming pools*, in *Chlorine: Properties, Applications and Health Effects*, (Book edited by: Roger Mangione and Dana Carlyle), Chapter 4. Nova Publishers, Series: Chemical Engineering Methods and Technology, Environmental Health - Physical, Chemical and Biological Factors.

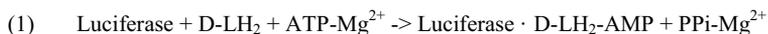
# Theoretical Study of the Effect Exerted by Inhibitory Compounds on Firefly Luciferase Bioluminescence

**J. Vieira, L. Pinto da Silva and J.C.G. Esteves da Silva**

Centro de Investigação em Química (CIQ-UP), Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal

Firefly luciferase, an oxidative enzyme, takes part as a catalyst in the bioluminescent phenomenon for which fireflies are commonly known.

The emission of light is the product of a two-step process [1], the first of which (see Eq. (1)) consists in the reaction of luciferin (LH<sub>2</sub>), the substrate of luciferase, with ATP bound to an Mg cation, forming inorganic pyrophosphate (PPi) and luciferil-adenylate (LH<sub>2</sub>-AMP) as an intermediate product.



Subsequently, as portrayed in Eq. (2), LH<sub>2</sub>-AMP undergoes oxidation and decarboxylation, culminating in its conversion to oxyluciferin (OxyLH<sub>2</sub>) and the production of carbon dioxide and AMP. This step results in the emission of light typically ranging from yellow to green (550-570 nm) [2].



Luciferase also catalyzes side-reactions that interfere with its activity [3]. Such reactions involve inhibitors, either as intermediates or substrate-related substances, which may hinder the emission of light. Research on the effect of these compounds is significant to the understanding of the main reaction itself.

This study makes use of semi-empirical and molecular mechanics methods, to shed more light on the interaction of a variety of inhibitors with *Luciola cruciata* luciferase [4], and their effect on the bioluminescence process. While the role of these compounds on bioluminescence flash-profile has been the subject of several investigations over the years, it remains a mystery for the most part. The obtained results can then be used to improve the luciferase system for its current applications.

## References:

- [1] Marques, S.M. and Esteves da Silva, J.C.G. (2009), *Firefly Bioluminescence: a Mechanistic Approach of Luciferase-Catalyzed Reactions*, IUBMB Life, 61 (1), 6-17.
- [2] Pinto da Silva, L. and Esteves da Silva, J.C.G. (2011), *Theoretical modulation of the color of light emitted by firefly oxyluciferin*, Journal of Computational Chemistry, 32 (12), 2654-2663.
- [3] Leitão, J.M.M. and Esteves da Silva, J.C.G. (2010), *Firefly luciferase inhibition*, Journal of Photochemistry and Photobiology B: Biology, 101(1), 1-8.
- [4] Nakatsu, T., Ichiyama, S., Hiratake, J., Saldanha, A., Kobashi, N., Sakata, K. and Kato, H. (2006), *Structural basis for the spectral difference in luciferase bioluminescence*, Nature, 440 (7082), 372-376.

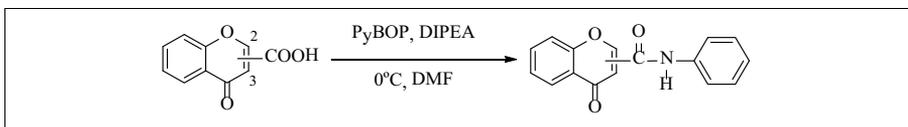
## Thermochemical study of a chromone 2-phenylcarboxamide

**Paulo Ferreira, J. Oliveira, A. Gaspar, F. Borges, M.D.M.C. Ribeiro da Silva**

Centro de Investigação em Química, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 687, P-4169-007, Porto, Portugal

Chromones (benzopyran-4-one) constitute one of the most abundant groups of heterocyclic compounds occurring naturally. Because of their structural features they occupy an important place in the realm of natural products and synthetic organic chemistry. In addition, remarkable antioxidant, anticancer and enzymatic inhibition properties have been ascribed to these systems.

Recently, chromone derivatives, with a carboxamide group located in positions 2- or 3- of the benzo- $\gamma$ -pyrone core, were synthesized, by a one-pot condensation reaction, using phosphonium salts as coupling reagents (Fig. 1). [1,2] The thermochemical characterization of these isomers is found to be relevant to get information on the energetics of their isomerisation and to correlate the energetic data with the structural characteristics and biologic activity of the molecules.



**Figure 1. General structure of the chromones under study**

The present work focuses the measurement of thermodynamic properties of a chromone 2-phenylcarboxamide (4-oxo-*N*-phenyl-4*H*-chromen-2-carboxamide) whose data are not available in the literature, in order to infer about the corresponding reactivity. Herein we will report the experimental results of the energy of combustion and enthalpy of sublimation of the mentioned chromone derivative obtained by static bomb calorimetry and high temperature Calvet microcalorimetry, respectively. These results allow the derivation of the standard molar enthalpy of formation, in the gaseous state, at  $T=298.15$  K.

- [1] Gaspar A, Reis J, Fonseca A, Milhazes N, Viña D, Uriarte E, Borges F. Chromone 3-phenylcarboxamides as potent and selective MAO-B inhibitors *Bioorg Med Chem Lett.* **2011**, 21, 707-709.
- [2] Gaspar, A.; Silva, T.; Yáñez, M.; Vina, D.; Orallo, F.; Ortuso, F.; Uriarte, E.; Alcaro, S.; Borges, F. Chromone, a privileged scaffold for the development of monoamine oxidase inhibitors. *J Med Chem* **2011**, 54, 5165-5173.

### Acknowledgment

A. G. and F. B. thank the financial support of the project PTDC/QUI-QUI/113687/2009 (FCT, FEDER, QREN and COMPETE)

## Use of cyclodextrins to tackle chromone's solubility

**J. Dias<sup>1</sup>, E. M. Garrido<sup>1,2</sup>, F. Borges<sup>1</sup>, M. J. Sottomayor<sup>1</sup> and J. Garrido<sup>1,2</sup>**

<sup>1</sup> CIQ/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal

<sup>2</sup> Department of Chemical Engineering, School of Engineering, ISEP, Polytechnic Institute of Porto, 4200-072 Porto, Portugal

Chromone is the central core of a number of active substances, of both synthetic and natural origin, with remarkable pharmacological significance. Recently, enzymatic inhibition properties were reported for this family of compounds, especially in relation to monoamine oxidase B (MAO-B), which is an isoenzyme that play an important role in neurotransmission metabolism, becoming a desirable target in neurodegenerative diseases such as Alzheimer's or Parkinson's [1]. One of the major drawbacks of the drug development process is connected with the low aqueous solubility of the chromone derivatives. In fact, solubility is one of the most important physicochemical properties in pharmaceutical industry since it could allow improving drug bioavailability and diverse pharmaceutical preformulation requirements.

One of the most recent and promising fields being explored by our group is chromone's incorporation in cyclodextrins. Cyclodextrins are cyclic oligosaccharides widely used in pharmaceutical industries because of their capability to interact with poor water soluble compounds increasing their solubility. Cyclodextrins have a unique shape that allows forming non-covalent dynamic complexes in solution, enhancing solubility and helping to control some unwanted physicochemical properties. In addition, the process can allow reducing the amount of compound needed to produce the desired effect, to mask undesirable properties and to lower toxic effects associated to a number of compounds.

Accordingly, the main goal of the present work is to evaluate the efficacy of incorporation of two chromone carboxamides isomers (Fig. 1) in cyclodextrins and the improvement of the water solubility of the compounds. The results obtained will be presented and discussed.

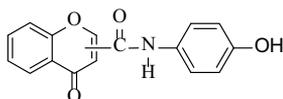


Figure 1 – Structure of the chromones carboxamides

### References:

[1] Gaspar, A., Silva, T., Yáñez, M., Vina, D., Orallo, F., Ortuso, F., Uriarte, E., Alcaro, S., Borges, F. (2011), *Chromone, a Privileged Scaffold for the Development of Monoamine Oxidase Inhibitors*, J. Med. Chem., 54 (14), 5165-5173.

### Acknowledgements:

Authors acknowledge financial support from the project PTDC/QUI-QUI/113687/2009 from FCT, FEDER, QREN and COMPETE

# Computational study of the catalytic mechanism of Human Renin protease

**A.R. Calixto, N.F. Brás, P.A. Fernandes and M.J. Ramos**

REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal.

The Renin-Angiotensin System (RAS) is one of the major and most intensively studied regulating systems of the arterial blood pressure in humans. It plays a primordial role in cardiovascular, renal and other metabolic diseases. The RAS consists of a two-step cascade. Firstly, the aspartic protease renin cleaves its only known substrate, angiotensinogen, in a rate limiting step, to the decapeptide angiotensin I (Ang I). Secondly, Ang I is transformed by angiotensin-converting enzyme (ACE) to produce the octapeptide angiotensin II (Ang II), which binds to angiotensin II subtype 1 receptors (AT<sub>1</sub> receptors), mediating cardiovascular events such as vasoconstriction [1,2].

Clinical intervention in the RAS was first achieved with the ACE inhibitors and latter the AT<sub>1</sub> receptor blockers were also introduced. Nowadays both classes of drug are widely used in the treatment of hypertension. However, blockade of the RAS at these levels leads to a feedback increase in renin secretion synthesis. Due to this rate-determining function and its high specificity for only one substrate, renin was identified as better target for antihypertensive drugs [3].

The aim of the present work is to investigate the catalytic mechanism of Human Renin protease using computational methods, to allow future studies of inhibition of this enzyme. In our calculations, to account for the effect caused by the presence of the enzyme we have used the hybrid ONIOM method (B3LYP:AMBER calculations), and the whole system was subdivided into two regions that were studied at different theoretical levels (Quantum Mechanics (Density Functional Theory - DFT) and Molecular Mechanics).

Although this work is under development, our data suggest that the molecular mechanism of Renin occurs by two steps and clearly shows the importance of the enzyme scaffold beyond the first shell of amino acids in the stabilization of the transition states.

## References:

- [1] Benzençon O., et al. (2009), *Design and Preparation of potent, nonpeptidic, bioavailable renin inhibitors*, Journal of Medicinal Chemistry, 52: 3689-3702.
- [2] Rahuel J., et al. (2000), *Structure-based drug design: the discovery of novel nonpeptide orally active inhibitors of human renin*, Chemistry and Biology, 7: 493-504.
- [3] Jensen C., Herold P., Brunner H., (2008), *Aliskiren: The first renin inhibitor for clinical treatment*, Nature Reviews: Drug Discovery, 7: 399-410.

## Tardigrades as bioindicators of air quality

**D. Santos<sup>1</sup>, P. Fontoura<sup>1,2</sup>, M. Almeida<sup>3</sup> and G. Ventura<sup>4</sup>**

<sup>1</sup> Department of Biology, Faculty of Science, University of Porto, Portugal.

<sup>2</sup> Eco-Ethology Research Unit (FCT 331/94)

<sup>3</sup> CIMAR/CIIMAR Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Portugal

<sup>4</sup> IDMEC, Institute of Mechanical Engineering, Faculty of Engineering, University of Porto, Portugal

Tardigrades are micrometazoans, closely related to Arthropods that can be found all over the world, including urban ecosystems. Contrary to other animals, the ubiquity, small size (about 500 µm) and association with cryptogams (mosses and lichens) of Tardigrades are suitable characteristics to use them as bioindicators. In addition, the value of these organisms for biomonitoring purposes can be enhanced by the fact that the cryptogams where Tardigrades live are rootless and the leaves are only one cell-thick layer, which makes negligible the contamination by the soil or other kind of substrate [1]. Nevertheless, during the last decades, only a few attempts to profit of these Tardigrade capabilities have been carried out, despite the promising results obtained, showing that the density and diversity of Tardigrade communities are affected by exposure to abnormal levels of sulfur dioxide, SO<sub>2</sub> [2].

In this communication a multidisciplinary project aiming to develop a cheap method to access the air quality in urban environments using Tardigrades as bioindicators is presented. The project comprises the analysis of some atmospheric pollutants (hydrocarbons: benzene, toluene and xylene), the determination of the concentration of metals (Cu, Cr and Pb) in cryptogams, and the study of Tardigrade communities at 8 sampling sites with expected different degrees of air pollution located in the urban area of Porto. The selection of the sampling sites was based on the results published daily by the Portuguese Environmental Agency (APA).

At present, only the preliminary results of the biological analysis performed in two sampling sites are available (station 1, Espinho, presumably with good air quality; and 2, Sr<sup>a</sup> da Hora, with bad air quality). There, Tardigrades were extracted from lichen and moss samples, counted and mounted in slides for identification under phase contrast microscope (x 1000). Tardigrades were more abundant in the more polluted station (6.9 ind/g in station 1, and 10.6 ind/g in station 2). However, the diversity was higher in the unpolluted station. Actually, 6 genera and a Shannon-Weaver diversity index of 2.08 were recorded in station 1 (3 genera and a diversity index of 1.51 in station 2). However, these promising but scarce results must be considered with caution. In this preliminary stage, a lot of environmental factors, such as the habitat, were not still taken into account. On the other hand, it is necessary to correlate the biological and the chemical data, which will be done in the near future.

### References:

[1] Vargha, B., Otvos, E. and Tuba, Z. (2002) Investigations on ecological effects of heavy metal pollution in Hungary by moss dwelling water bears (Tardigrada) as bioindicators. *Ann. Agric. Environ. Med.*, 9, 141-146.

[2] Leatham, J.W., McNary, T.J., Dodd, J.L. and Lauenroth, W.K. (1982) Response of soil Nematodes, Rotifers and Tardigrades to three levels of season long sulfur dioxide exposures. *Water and Soil Pollution*, 17, 343-356.

## The endocannabinoid 2-Arachidonoylglycerol (2-AG) in pregnancy: effects in viability of cytotrophoblast cells

V. Guimarães<sup>1</sup>, MACosta<sup>1,2</sup>, BM Fonseca<sup>1,2</sup>, NA Teixeira<sup>1,2</sup> and G. Correia-da-Silva<sup>1,2</sup>

<sup>1</sup> Laboratory of Biochemistry, Biological Sciences Department, Faculty of Pharmacy, University of Porto

<sup>2</sup> Institute for Molecular and Cell Biology (IBMC), University of Porto, Portugal

Normal placental development is a highly regulated event essential for fetal growth of eutherian mammals. It requires proliferation, differentiation and death of cytotrophoblast cells. Abnormalities during these processes are associated with impairments in pregnancy outcome such as fetal growth restriction and preeclampsia. It is known that endocannabinoids (ECs) play an important role in pregnancy and dysfunction of endocannabinoid system have been implicated in infertility and miscarriages [1]. We have previously demonstrated that low concentrations of the two major endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), (ECs), induced apoptosis in rat decidual cells suggesting a role for ECs in decidual remodelling [2, 3]. Although cannabinoid receptors have been identified in placenta, the role of ECs during placental development remains unknown and the number of studies about 2-AG effects is still reduced. Here, we used a placental cell line (BeWo choriocarcinoma cells) to evaluate the effects of 2-AG in trophoblast cell viability and proliferation by MTT assay, LDH release and <sup>3</sup>[H]-thymidine incorporation assay. Morphological alterations were evaluated by phase contrast microscopy, Giemsa and Hoechst staining. 2-AG was used in a range of concentrations between 0.01 and 50 µM and cells were treated for 24, 48 and 72h of treatment. The loss of cell viability increased significantly for concentrations higher than 15 µM accompanied by a decrease on DNA synthesis being these effects concentration and time-dependent. In LDH release experiments no alterations were observed in concentrations till 15 µM. The antiproliferative effect was accompanied by morphologic alterations, including chromatin condensation as showed by Giemsa staining and Hoechst. This data provides novel insights into the regulation of trophoblast cell proliferation and viability by 2-AG and suggests a role for endocannabinoids in regulation of trophoblast cells turnover.

[1] Karasu T., Marczylo T.H., Maccarrone M., Konje J.C. (2011), *The role of sex steroid hormones, cytokines and the endocannabinoid system in female fertility*. Hum Reprod Update, 17, pp. 347-61.

[2] Fonseca B.M., Correia-da-Silva G., Teixeira N.A. (2009), *Anandamide-induced cell death: dual effects in primary rat decidual cell cultures*. Placenta, 30, pp. 686-92.

[3] Fonseca B.M., Correia-da-Silva G., Taylor A.H., Lam P.M., Marczylo T.H., Bell S.C., et al. (2010), *The endocannabinoid 2-arachidonoylglycerol (2-AG) and metabolizing enzymes during rat fetoplacental development: a role in uterine remodelling*, Int J Biochem Cell Biol, 42, pp. 1884-92.

*The authors thank Fundação para a Ciência e Tecnologia (FCT) for grants of MA Costa (SFRH/BD/70721/2010) and BM Fonseca (SFRH/BPD/72958/2010).*

# The Magpie (*Pica pica*, L.) at the Vila Nova de Gaia coastal area

I. Tavares<sup>1</sup> & A. Valente<sup>1,2</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto

<sup>2</sup> Eco-Ethology Research Unity, I. & D. - FCT - N° 331/94  
Rua do Campo Alegre, Edifício FC4, 4169-007 PORTO, PORTUGAL

The Magpie (*Pica pica* L.) is common and widespread in Europe, Asia and western North America [1]. In Portugal they are more abundant in two main areas, the north coast and the interior Alto Alentejo; the factors responsible for such distribution are not yet fully understood [2,3]. It can be found in agricultural habitats but has recently expanded into suburban and urban areas [1]. Although its presence and reproduction is well established, little is known about several aspects of its biology in Portugal, namely the reproductive biology and feeding habits [2]. Our study is a first approach to the study of the distribution and behaviour of the Magpie in Vila Nova de Gaia coastal area.

Magpie activity and behaviour (movements, feeding activity, intraspecific and interspecific interactions) occurring at a selected 1 ha agricultural field and surrounding area in the parish of Madalena, Vila Nova de Gaia, are registered and recorded in video for further analysis. To establish the area broadly used by the Magpie population in the Vila Nova da Gaia coastal area we explore it driving through the municipality roads. During the breeding season that will soon begin, an effort will be directed to the species reproductive behaviour. For practical reasons the daylight period was considered divided in three equal duration periods and in each visit the observations were restricted to only one such periods. Visits are scheduled in approximately in a weekly basis and will be done until four complete days of observations (both for behaviour and distribution) will be completed.

In the study area Magpies are associated with agricultural fields of the landscape mosaic. They are generally seen in pairs but occasionally are found alone. Bigger groups (up to 10 birds) are often seen both in the fields and at tree branches. The main social behaviour observed is flocking, although group cohesion seems very loose; a few agonistic interactions (chasing, hover-flying) were also seen. The main behaviours observed for each bird are feeding activity, top-sitting flying and vocalizing (ex. Chattering). Reproductive activity was not recorded so far.

The collected observations allow us to conclude that magpies usually are found in pairs (couples?) and that they feed and sleep in small areas, but until now we have not seen any evidence of territorial behaviour. On the contrary, several pairs of birds seem to use the same feeding and sleeping areas. However the breeding season as not yet started so it might be still early to observe such interactions.

## References:

[1] Birkhead, T.R. (1991), *The Magpies – The ecology and behaviour of Black-billed and Yellow-billed Magpies*, T & AD Poyser.

[2] Catry, P., H. Costa, Elias, G., Matias, R. (2010). Aves de Portugal. Ornitologia do território nacional. Lisboa. 941 pp.

[3] Equipa Atlas Equipa\_Atlas (2008). Atlas das aves nidificantes em Portugal (1999-2005). I. d. C. d. N. e. d. Biodiversidade, S. P. p. o. E. d. Aves, P. N. d. Madeira and S. R. d. A. e. d. Mar. Lisboa, Assírio & Alvim: 590.

# The Red Fox (*Vulpes vulpes*) in Portugal: Distribution, morphology and age

M. Ribeiro<sup>1</sup>, and A. Valente<sup>1,2</sup>

<sup>1</sup>Department of Biology, Faculty of Sciences, University of Porto, Portugal

<sup>2</sup>Eco-Ethology Research Unity, I. & D. - FCT - N° 331/94  
Rua do Campo Alegre, Edifício FC4, 4169-007 PORTO

The Red Fox (*Vulpes vulpes*) has the widest geographical range of any Carnivora species, being established across the entire northern hemisphere (nearly 70 million Km<sup>2</sup>), so it is not surprising that they show geographical variation in size, related to latitude. Fox habitat is extremely diverse (from Arctic tundra to hot desert) so population density is highly variable, some populations showing an increase in number in parts of Europe [1, 2]. This carnivore can live up to nine years in the wild, but generally lives less than three years [1]. Red fox has been hunted since the 4<sup>th</sup> century B.C. [1, 2], and today hunting represents its major source of mortality but most countries have regulated closed seasons and hunting restrictions [1]. Comparing with other European countries the information on fox populations in Portugal is scarce.

Since the beginning of the hunting season hunter's associations and clubs are being contacted in order to estimate the distribution and abundance of the Red Fox. Fox carcasses offered were brought to the laboratory and body weight, height, length of body, tail, ear and hind foot [3] of each fox is registered, along with the head maximum perimeter and width and abdomen and chest perimeter. The canine teeth are extracted after maceration for age determination, using counting the incremental lines.

Until now only 15 foxes were obtained but the hunting season is not yet finished. The sex ratio is not at equilibrium because only 2 vixens were obtained. This could be explained because males are more likely to be shot due to their higher mobility [2]. Males are heavier and largest in all the external measurements (body, tail, ear, hind foot, height, perimeter of head, perimeter of chest, perimeter of abdomen and width of head). All foxes are adult animals because they present molar teeth. The canines are being prepared for incremental line counts to determinate the exact age.

The database that is being made with the collected foxes in Portugal will hopefully allow to determinate the distribution, abundance and morphology of the specie. Other aim of this study is the appraisal of the hunting pressure that the Red Foxes are submitted, as well as the increase of information about the Red Fox in the Mediterranean area.

## References:

- [1] Silero-Zubiri, C., M. Hoffman, et al. (2004). *Canids: Foxes, Wolves, Jackals and Dogs. Status survey and conservation action plan*. Gland, Switzerland and Cambridge UK.
- [2] Tryjanowski, P., T. H. Sparks, et al. (2009), *The relationship between hunting methods and sex, age and body weight in a non-trophy animal, the red fox*. *Wildlife Research* 36(2): 106-109.
- [3] Travaini, A. and M. Delibes (1995). *Weight and external measurements of Red Foxes (Vulpes vulpes) from SW Spain*. *Zeitschrift Fur Säugetierkunde-International Journal of Mammalian Biology* 60(2): 121-123.

## The role of Wnt signalling and anti-oxidant beverages in diabetic cardiomyopathy

**G. Conceição<sup>1</sup>, M. Machado<sup>3</sup>, C. Moura<sup>1</sup>, C. Morgado<sup>2</sup>, I. Tavares<sup>2</sup>, A. Leite-Moreira<sup>1</sup> and S. Pinho<sup>1</sup>**

<sup>1</sup> Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto, Portugal.

<sup>2</sup> Department of Experimental Biology, Faculty of Medicine, University of Porto, Portugal.

<sup>3</sup> Biomedical Engineering Master Course, Faculty of Engineering, University of Porto, Portugal.

Heart failure (HF) is one of the health problems with major social-economic impact in our society and can develop due to diabetes, among others. In diabetes, HF develops due to enhanced fatty acid (FA) metabolism, and reduced glucose and lactate metabolism. High FA metabolism augments production of Reactive Oxygen Species (ROS) leading to increased oxidative stress (OS). Increased OS can contribute to early senescence and apoptosis of cardiac cells, leading to the development of HF in diabetic patients. Increased OS induces the expression of Forkhead box O (FoxO) transcription factors, important mediators of OS signalling, which in turn induce the expression of genes actively involved in OS response, such as *catalase* and *superoxide dismutases* (SODs). Induction of these genes by the FoxOs requires the interaction of FoxO proteins with  $\beta$ -catenin, a transcription factor of the Wnt pathway. The role of Wnt signalling in heart disease, and most specifically in OS regulation in the heart, is still unknown. Taking this evidence, the goal of this project was to study the gene expression, by Real Time PCR, of factors involved in the response to oxidative stress, and effectors of the Wnt pathway in a Type I Diabetes rat model. Also, due to the fundamental role of OS in the progression of diabetic cardiomyopathy, we also analysed the expression of OS related genes in diabetic animals treated with Epigallocatechin gallate (EGCG), a potent green tea antioxidant.

The analyses were done at 2 time points upon diabetes induction: 4 and 10 weeks. At 4 weeks we observed increased expression of some Wnt ligands (*wnt2a* and *wnt11*), Fzd receptors (*fz3* and *fz5*) and Wnt co-receptor (*lrp6*) in heart tissue of diabetic animals. Also, and as expected, expression of *foxO1*, *foxO3* was highly upregulated at this stage. By 10 weeks, the expression of the Wnt effectors analyzed so far, *wnt2b* and *lrp6*, do not show statistically meaningful differences as observed at 4 weeks. However by this stage there was a pronounced difference in the expression of OS response genes – *catalase* and *sod2*, but not *sod1* - between control and diabetic group. Interestingly, in animals treated with green tea extract the expression of these genes was higher than in the diabetic animals.

With these results we conclude that at the early stages of diabetes we observe the upregulation of some Wnt pathway effectors and also OS related genes. Upregulation of the Wnt pathway may be important to induce stabilization of  $\beta$ -catenin, which will then be recruited by FoxO proteins to induce the expression of downstream targets. At later stages, the Wnt pathway seems not to be required anymore, although a response to OS seems to still be occurring. Treatment with EGCG seems to potentiate the OS response. Analyze of the effect of EGCG in the heart, at functional and morphological levels, is underway, to correlate an increase in the capacity of OS response to a possible amelioration of cardiac function.

## Toxicity of the heavy metal mercury to the marine microalgae *Tetraselmis chuii*

Mickaël Lima<sup>1,2</sup>, M. Miguel Oliveira<sup>1,2</sup>, Lúcia Guilhermino<sup>1,2</sup>

<sup>1</sup> ICBAS - Institute of Biomedical Sciences of Abel Salazar, University of Porto, Department of Populations Studies, Laboratory of Ecotoxicology, Lg. Prof. Abel Salazar, 2, 4099-003, Porto, Portugal.

<sup>2</sup> CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Laboratory of Ecotoxicology and Ecology, University of Porto, Rua dos Bragas, 289, 4050 Porto, Portugal.

Anthropogenic pollutants in several marine and estuarine ecosystems have significantly increased over the last few decades. Among these pollutants, heavy metals, which tend to accumulate in bottom sediment and release slowly into water bodies, have long been recognized as major marine pollutants [1] As primary producers, microalgae are most important to support marine food webs and to contribute to the overall production of oxygen. In addition, some microalgae are commercially important due to their wide use as food in the aquaculture industry. The marine microalgae *Tetraselmis chuii* has been as representative of marine producers in Ecotoxicology, being also an important species in aquaculture where it is used as a food source for other species.

The objective of the present study was to investigate the effects of mercury on *T. chuii* populations to increase the knowledge required for marine ecological risk assessment of metals.

Ninety six hours assays with algae cultures were carried out in laboratory conditions, in temperature ( $25 \pm 1^\circ\text{C}$ ) and photoperiod (12h light: 12 h dark) controlled rooms. Tests were conducted in Erlenmeyer beakers, in aseptic conditions, using 500 ml of test medium (f/2 Guillard medium) and following the OCDE guideline 201 for testing with microalgae [2]. Different concentrations of mercury and appropriate controls were tested without medium renewal. The effect criteria was the inhibition of culture growth.

Mercury induced toxic effects in the microalgae at high concentrations. Therefore, further studies on the long term exposure of this species to mercury should be carried out.

Acknowledgements: This study was carried out in the scope of the project “SIGNAL – Effects of pollution on estuarine ecological interactions zooplankton-zooplanktivorous fish in relation to climate changes” (FCT: PTDC/AAC-AMB/110331/2009).

### References:

[1] Mieiro, C.L., Duarte, A.C., Pereira, M.E., Pacheco, M. (2011), *Mercury accumulation patterns and biochemical endpoints in wildfish (Liza aurata): A multi-organ approach*, Ecotoxicology and Environmental Safety 74 (2011) 2225–2232.

[2] OCDE. (2011), *Guideline for testing the toxicity of chemicals n° 201. Freshwater Alga and Cyanobacteria, Growth Inhibition Test*. Organization for Economic Cooperation and development, Paris.

## Tracking antibiotic resistance along the Silk Road

**M. Mendes<sup>1</sup>, C. Novais<sup>3,5</sup>, P. Antunes<sup>3,4</sup>, J. Campos<sup>3</sup>,  
A. Freitas<sup>3</sup>, A. Abdukadir<sup>7</sup>, L. Peixe<sup>3</sup>, N. Monteiro<sup>2,5</sup> and S. Quinteira<sup>1,2,6</sup>**

<sup>1</sup> Department of Biology, Faculty of Sciences, University of Porto, Portugal.

<sup>2</sup> CIBIO - Research Center in Biodiversity and Genetic Resources. University of Porto, Portugal.

<sup>3</sup> REQUIMTE. Faculty of Pharmacy, University of Porto, Portugal.

<sup>4</sup> Faculty of Food Sciences and Nutrition, University of Porto, Portugal

<sup>5</sup> CEBIMED. Health Sciences Faculty. Fernando Pessoa University, Porto, Portugal

<sup>6</sup> CITS - Research Center in Health Technologies, Polytechnic Health Institute of the North (IPSN/CESPU), Famalicão, Portugal.

<sup>7</sup> Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China.

Information on the bacterial communities that inhabit wild-animal species, some of which facing extinction in the near future, as well as information on the spreading of antibiotic resistance into relatively pristine ecosystems, remains scarce. Accordingly, the goal of this study was to evaluate if wild or feral animals, inhabiting remote areas of the globe (from the Tibetan plateau to the Gobi desert), carry antibiotic resistant bacteria. During the SilkRoad2010 expedition (CIBIO and Chinese Academy of Sciences), faecal samples were collected from nine iconic mammal species, most of which endangered or in the verge of extinction. Faecal samples (0.1ml) were plated in different selective culture media (e.g. Slanetz-Bartley agar for *Enterococcus* spp., MacConkey agar for *Enterobacteriaceae* and non fermenter Gram negative bacteria, XLD and SS agar for *Salmonella*), with/without antibiotic supplements, before and after a pre-enrichment step. Different bacteria morphotypes were selected for further characterization. Susceptibility to several antibiotics was tested by disk diffusion method (CLSI). Two hundred and seventy bacterial isolates (97 Gram positive and 173 Gram negative) were collected from 22 faecal samples, from different animal species: Mongolian wild ass, Dhole, Mongolian gazelle, Przewalski horse, Gray marmot and Bactrian camel. Different antibiotic resistance rates were detected amongst *Enterococcus* spp: tetracycline-49,5% (48/97), erythromycin-36% (35/97), High Level of Resistance (HLR) to gentamicin -15,5% (15/97), ampicillin - 6% (6/97), and Quinupristin/dalfopristin -23,7% (23/97). Decreased susceptibility (n=10) and resistance (n=6) to vancomycin were observed. Among Gram negative lactose fermentors, resistance was observed for ampicillin - 39% (31/79), ampicillin+clavulanic acid - 15% (12/79) and cefotaxime -16,5% (13/79). Variable susceptibility to aminoglycosides was observed: gentamicin-19% (21/108); tobramycin-16% (20/123). Two isolates (obtained from Bactrian camel and Gray marmot) showed resistance to imipenem. Although preliminary, results emerging from this study show that even though inhabiting remote areas with extremely low human pressure, the critically endangered Silk Road fauna surprisingly harbours bacteria carrying antibiotic resistance. As so, drug resistance, far from limited to hospitals, may be spreading into the most remote areas of the globe.

Acknowledgments: This research was supported by Universidade do Porto/Santander Totta "Projectos Pluridisciplinares 2010".

# Understanding the effects of proline against Cu toxicity in *Solanum nigrum* L. plant growth

**Joana Leal, A. Santiago, J. Teixeira and F. Fidalgo**

Center for Biodiversity, Functional & Integrative Genomics (BioFIG)  
Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

What is the effect of exogenous proline (Pro) in plants under stress, specifically stress caused by the absorption of copper (Cu)? Will the effect of the Pro, help the plant to absorb more Cu without causing damage in the tissues, than in its absence, ie, the Pro has a protective effect against Cu stress?

Cu as an essential micronutrient is necessary for normal plant growth and development, but when in excess causes phytotoxicity interfering with many metabolic processes, causing several visible injuries, reduced photosynthetic rates, nutritional imbalances, growth and vitality decrease, and induces oxidative stress [1]. A common response of plants to Cu toxicity is the activation of the antioxidant defense system. The osmolyte Pro, now recognized as a potent non-enzymatic antioxidant, is considered as an indicator of environmental stress. This amino acid accumulates in many plants under several stress conditions, showing a protective action against heavy metal (HM) stress by up-regulating the antioxidant defense system [2]. Pro protects and stabilizes antioxidant enzymes and also activates alternative pathways of detoxification [2].

The aim of this work, was to assess the contribution of exogenous Pro in Cu plant tolerance, through the evaluation of growth parameters in *Solanum nigrum* L. plants, which is a pioneer species fast growing in polluted sites worldwide and capable of hyperaccumulate HM [3]. Seeds were surface sterilized and incubated in a sterile nutrient medium (Hoagland's solution at 50%). Plants were grown in a vermiculite:perlite (2:1) substrate and were watered with Hoagland's solution, during 4 weeks. Then they were divided into three groups: 0  $\mu\text{M}$  Cu (control- tray 1), 500  $\mu\text{M}$  Cu (tray 2) and 500  $\mu\text{M}$  of Cu +1 mM Pro (tray 3). At this time the nutrition at the tray 1 and 2 was not altered, but the plants at tray 3 were watered with 1 mM Pro, for 24 hours. After this period, the surplus of the solution was removed and plants at the trays 2 and 3 were watered with Hoagland's solution supplemented with 500  $\mu\text{M}$  Cu final concentration for a week to create a shock treatment. After these five weeks, at least 5 plants of each treatment were used for studying several biometric parameters: shoot height, root length and shoot and root fresh weight. It was possible to observe a decrease in shoot height and root length in plants treated with 500  $\mu\text{M}$  Cu compared to the control; root length of plants treated with 500  $\mu\text{M}$  decreased in comparison with those treated with 500  $\mu\text{M}$  Cu + 1 mM Pro. Regarding shoot and root fresh weight, there were no significant differences between the three groups of plants. Future studies will be performed to evaluate the degree of stress that plants were subjected when exposed to the shock treatment of 500  $\mu\text{M}$  Cu as well as to understanding the effects of exogenous Pro against Cu toxicity in *S. nigrum*.

[1] Marschner, P. (2012), *Marschner's mineral nutrition of higher plants* (3<sup>th</sup> ed). Elsevier, Ltd.

[2] Szabados, L. and Saviouré, A. (2009), *Proline: a multifunctional amino acid*. Trends in Plant Science, 15, 89-95.

[3] Marques, A.P.G.C., Oliveira, R.S., Rangel, A.O.S.S., Castro, P.M.L. (2006), *Zinc accumulation in Solanum nigrum is enhanced by different arbuscular mycorrhizal fungi*. Chemosphere, 6, 1256-1263.

## Volatile and semi-volatile profiling of wild mushrooms by GC-MS and their importance in species identification

**L. Carvalho<sup>1</sup>, F. Carvalho<sup>1,2</sup>, M. de Lourdes Bastos<sup>2</sup>, P. Baptista<sup>3</sup> and P. Guedes de Pinho<sup>1,2</sup>**

<sup>1</sup> Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

<sup>2</sup> REQUIMTE/Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

<sup>3</sup> CIMO/School of Agriculture, Polytechnic Institute of Bragança, Portugal

Nowadays the mushrooms are important in many contexts, but, in general, they are best known for their culinary value. However, in nature many species of mushrooms are toxic and therefore not edible, leading, in several cases, to death. Therefore, it is necessary to correctly identify the species, which is not always easy due to the high morphological similarity between certain species. Currently, chemotaxonomy is an additional method to mushroom identification, resorting to molecular analysis, essentially, DNA sequencing, but also to chemical analysis, including the study of amino acid (AA) and fatty acid (FA) profiles, as well as secondary metabolites such as volatile compounds. In the present work, the quantification of some AA, FA and sterols was performed, as well as the identification of the main volatile compounds present in seven species of wild mushrooms: *Amanita caesarea* (Scop.) – AC (edible); *Amanita muscaria* L. (Lam.) – AM (hallucinogenic); *Amanita vaginata* (Bull.) – AV (non-edible); *Boletus edulis* Bull. – BE (edible); *Lactarius controversus* (Pers.) Pers. – LC (non-edible); *Russula cyanoxantha* (Schaeff.) Fr. – RC (edible); and *Russula delica* Fr. – RD (edible). In the species AC and BE the differential analysis of these compounds in cap and stipe, was also carried.

The quantification of AA, FA and sterols was done by gas chromatography-mass spectrometry (GC-MS) after a previous derivatization procedure with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). The volatile fraction was analysed by GC-MS after a head space solid phase microextraction (HS-SPME).

The statistical treatment of data, by using ANOVA ( $p < 0.05$ ) and Principal Component Analysis - PCA (total variance for 3 principal Axes – 88.1%), showed that alanine was the AA present in higher levels and, this AA, as well as methionine, serine, threonine and L-tryptophan allows to distinguish the species BE from all the others. By comparing the mushrooms' cap and stipe, it appears that in the species AC, alanine, glycine and valine are mostly in the cap. For BE species there is no difference between the distribution of AA among cap and stipe. Palmitic and stearic acids were present in higher amounts in all mushrooms studied. Both species AC and LC can be distinguished from the others due to the higher contents in myristic and linoleic acids, respectively. Ergosterol was the only sterol identified, and this compound is in greater quantity in the species AV. The volatile analysis allowed the identification of the most common volatile compounds in mushrooms: 3-octanone, 3-octanol and 1-octen-3-ol, but further studies are needed to identify possible volatile compounds that can be used as markers of species/genus.

These preliminary results infer that primary metabolites (AA and FA) can be used as molecular markers and could help to a more accurate identification of the species/genus. We expect that secondary metabolites will be more promising as chemical markers, which will be explored in the course of this work.



# INDEX

## ORAL SESSIONS

#	'O, Sir, I did not look so low'. Dislocations in Shakespeare's The Comedy of Errors	133
A	A blogosfera como plataforma privilegiada para as notícias hiper-regionais: Associações desportivas de Vale de Sousa	319
	A casa do arquitecto Agostinho Ricca, Porto: 1963 / 1974	331
	A GIS predictive performance of habitat suitability model for Iberian red deer [Cervus elaphus hispanicus Hilzheimer, 1909]	373
	A methodology to assess fast-evolving domains on protein-coding genes	280
	A narrative of a Physical Education pre-service teacher about the class control	87
	A Rule Based System of Portuguese Verbal Forms	135
	A stellar test of the physics of unification	247
	A transitory complex abstraction	223
	Adenosine mediates a positive feedback mechanism facilitating ACh release from rat myenteric motoneurons through the cross-talk between A2A, M3 and NK1 receptors	344
	Aesthetics training for teachers	46
	An Investigation into the Use of Cement-based Materials as Alternative Printmaking Substrates	336
	Anaerobic Threshold assessment in young swimmers	363
	Analysis of Bisphenol A and B in canned tuna combining acetonitrile dispersion and dispersive liquid-liquid microextraction followed by gas chromatography	140
	Analytic Models for the Evolution of Semilocal String Networks	245
	Angiotensinogen concentration in patients with chronic heart failure	300
	Anthocyanin content and radical-scavenging activity of strawberries, jams and red fruit juices: influence of storage conditions	95
	Antioxidant activity in minimally processed Bimi® Broccoli under different sanitizing techniques throughout shelf-life	94
	Antioxidant activity mediated by non-steroidal anti-inflammatory drugs of the anthranilic acid chemical group	347
	Application of Lean Production Management to manufacturing modules of automotive industry parts	379
	Archaeology of drawing - mapped Rome	334
	Architectural Interactive Surfaces Modeled in Composed Materials: The Multi Sensorial Stimuli	335
	Assessment of the origin of the Iberian populations of Podarcis sicula using mitochondrial markers	235
B	Bacterial discrimination by ATR-FTIR spectroscopy: towards a faster diagnosis of infection and a more targeted antimicrobial therapy	284
	BETWEEN PUBLIC AND PRIVATE. The Domestic Architecture of Marques da Silva at Oporto	292
	Biodegradation of petroleum hydrocarbons in estuarine sediments: influence of metals	216
	Biofilm-production ability of widespread Escherichia coli clones	325
	Biogeography and conservation of the common snipe Gallinago gallinago: application of ecological niche models to different spatial scales during the breeding season	374
	BIOSWIM - Validation of Textile Electrodes for Measuring ECG	199
	Bologna Process and teaching and learning in UPorto: an analysis to institutional reports	41
	Bringing The Portuguese Primary Care Health Records to the Cloud using ASTM E2369-05	
C	Continuity of Care Record	117
	Can brewers yeast, a by-product of brewing industry, be used in fish diets?	79
	Canine Osteoarthritis and Stem Cell Therapy: A Case Study	256
	Casa Amarela; Collectivization and Resistance	314
	Changes in the purinergic modulation of high-affinity GABA and glutamate uptake in human epileptic brain	346
	Characterization of heart rate variability in critical situations	21
	Characterization of school food service of Portuguese Municipalities	328
	Characterization of soils in an area of prescribed fire	215
	Characterization of the oral fungal microbiota in smokers and non-smokers	324
	CHARACTERIZATION OF THE QUERCETIN AND THE EPIGALLOCATECHIN GALLATE INHIBITORY EFFECTS UPON GLUCOSE UPTAKE BY BREAST CANCER CELLS	253
	Characterizing male intimate partner offenders	265
	Charcoal Analysis in Monte Mozinho - Preliminary results from sector A	274
	Chiral xanthone derivatives: Synthesis, physicochemical properties and anti-inflammatory activity determination	73
	Chronic ingestion of high-fat diet and energy restriction modulates expression of VEGF, VEGR2, angiopoietins and Tie2 in aged rat myocardium	161

Civil Liability for contents uploaded and transmitted on the Internet:	
The user generated content and the web 2.0. paradigm.....	308
Clock synchronization using servo-clock.....	196
Coffee Silverskin nutritional profile: agro agricultural by-products valorisation.....	354
Comparative study of methodologies for assessing the hydromorphological quality of river systems.....	167
Constraints on the CMB temperature redshift dependence from SZ and distance measurement.....	246
Contribution of the Sport Education Model for the students involvement in learning of Acrobatic Gymnastics.....	88
Contributions from Community Psychology for the fulfilment of a culture of justice:	
Implementation and evaluation of a family empowerment project.....	125
Cooperation and Conflict(s). The fishing “companhas” in Mira’s coast in the first decades of the 19th century (1802 – 1830).....	275
Cortical Thymic Epithelial Cells are controlled by lympho-stromal interactions during thymic development and are regenerated upon genotoxic aggression in the adulthood.....	343
Cosmic String Evolution with a Conserved Charge.....	244
Current perspectives on the post-translational modulation of Prrx1 function.....	190
Daniel Faria and the Sísifo’s complex. Brief considerations about the cyclic process of poetry.....	130
<b>D</b> Data base for ethnological heritage.....	269
Deaf Space: education for which Urbanity? – Paths of investigation.....	121
Deidendera - doença-cura no corpo feminino, pleno de fluido e de memória.....	164
Designing Serendipity.....	224
Determination of copper in freshwaters based on reversed flow injection analysis using a liquid core waveguide capillary cell.....	172
Determination of the sodium content in vegetable soups served at public institutions’ canteens to different age groups.....	142
Development and characterization of lipid nanoparticles as delivery systems for anticellulite active compounds.....	207
Development of ibuprofen selective electrode for pharmaceutical formulations and surface waters.....	355
Development of innovative lipophilic hydroxycinnamic antioxidants: a solution to tackle mitochondrial oxidative damage.....	208
Development of Notch-based polymeric biomaterials as a platform to study Notch Pathway.....	188
Development of resistance to agricultural antifungal compounds - emergence of multidrug cross resistance among the human fungal pathogens <i>Candida</i> , <i>Cryptococcus</i> and <i>Aspergillus</i> .....	217
Diclofenac interactions with lipid membranes - insight into the NSAIDs-induced gastrointestinal toxicity.....	345
Differential expression of A1 receptors between sinoatrial node and cardiomyocytes does not account for adenosine chronoselectivity in the spontaneously beating rat atria.....	303
Driving Coach: a Smartphone Application to Evaluate and Teach Driving Efficient Patterns.....	149
Dynamic balance of surfers: The effect of sex and foot base position on board.....	361
<b>E</b> Ecological characterization of surface water bodies from the Hydrographic Regions 1 and 2.....	371
Economic Dispatch of Pumped Hydro Storage Interconnected with Thermal and Wind energy ? An Educational Tool.....	198
Educational Intervention Modes in Higher Education as a Response to Current Challenges: A Case Study.....	43
Effect of a program of hidrogymnastic on body composition and strength. Study performed with and without drag material.....	359
Effect of an all-out rowing ergometer test in the proprioceptive sensibility and manual dexterity in young rowers.....	362
Effect of body position on girths in older adults.....	162
Effect of fatty acids upon catecholamine handling by adrenal chromaffin cells.....	301
Effect of fresh and composted spent coffee grounds on lettuce photosynthetic capacity.....	93
Effect of intermittent hypobaric hypoxia and endurance training in cardiac mitochondrial function.....	23
Effect of the baking process on DNA degradation of conventional and transgenic maize bread.....	98
Effects of a metalaxyl photocatalytic-treated solution on biomarkers of oxidative stress in <i>Solanum nigrum</i> L. plants.....	218
Effects of exercise in the hepatic regeneration.....	22
Effects of glucosamine and chondroitin sulphate on human osteoclastogenesis.....	257
Efficacy of progressive muscle relaxation technique in chronic musculoskeletal pain and depression in institutionalized elderly adults.....	113
Elasto-static analysis using a meshless method.....	377
Endurance training and chronic intermittent hypobaric hypoxia modulate in vitro salicylate-induced hepatic mitochondrial dysfunction.....	24
Enzyme Selection for Bioethanol from Brewery Spent Grains.....	81
Epigallocatechin Gallate prevents spinal oxidative stress damage and neuropathic pain in STZ-diabetic rats.....	36

	Evaluation of the conditions for photoconversion and imaging of Kaede and mEosFP by laser scanning confocal microscopy in transformed plant cells	279
	Evolutionary Genomics of The Hedgehog Gene Family in Metazoans: Identification of The Desert Hedgehog Gene on Avian Genomes	233
	Expansion of IFC Data Model to Kinematic Sensors	381
<b>F</b>	Facebook Filter Plugin	151
	Fault Localization using Dynamic Code Coverage	147
	Fiber optical tweezers for single cell manipulation: numerical analysis and experimental implementation	211
	Fiscal Sustainability in an Overlapping Generations Model with Endogenous Growth and Public Debt	51
	Flavonoids profiles of <i>Bauhinia forficata</i> Link: authenticity and relation with biological potential	71
	For the practice of Dance Criticism as a form of Legacyis	317
	Functionalized silica nanoparticles with thermo- and photochromic dyes for textile application	104
<b>G</b>	Ghrelin decreases the IOP in an acute glaucoma model	160
	Glass and Print: Creation of alternative substrates and matrix for printmaking	28
	Glial Cell Development and Function in the <i>Drosophila</i> eye	191
	GPGPU N-Body Cosmological Particle-Particle Simulations	239
	Growth of common gobies <i>Pomatoschistus microps</i> : is growth similar for populations distant apart?	372
	GUITAR: Graphical User Interface Tool for Automata Representation	195
<b>H</b>	H2A Project: Heritage, History and Art	313
	Health Promotion Lifestyle Profile II Scale (HPLP-II) Portuguese version	114
	Housing and Extreme Poverty	293
	Housing cooperatives and the crisis - Which Answers?	181
	How do they defend themselves? Retailers perspective about shoplifting in two different contexts	261
	HPLC analysis from the crude extract and metabolites isolated from the marine fungus <i>Eurotium cristatum</i>	281
<b>I</b>	Identifying features of the teaching profession	90
	Image Filter: a semi-automatic image classifier	200
	Immobilization of commercial laccase on modified spent grain	106
	Immunofluorescent study of VEGF, angiopoietins, VEGFR1, VEGFR2 and Tie2 in the corpus cavernosum of diabetic rat after epigallocatechin gallate treatment	35
	Implementation of a Real-Time Database with Sockets	197
	Importance of Shame and Guilt in Moral Behavior: A systematic review	264
	In vitro assessment of NSAIDs-membrane interaction: the role of biophysics on drugs pharmacological and toxic effects	348
	Incorporation and management of ambivalent belongings: a comprehensive approach to the identifications with profession expressed by junior psychiatrists	126
	Influence of charcoal type on polycyclic aromatic hydrocarbon formation in barbequed muscle foods	139
	Insight on transcriptional network that controls <i>Prrx1</i> expression in nociceptive neurons	255
	Insights into South-American colonization through mtDNA analysis in Colombian populations	187
	INTERSUBJECTIVITY AND INTERPRETATIVE COOPERATION IN UMBERTO ECO AND STANLEY FISH	131
	Inventory of Pictorial Portraits of Bishops of Porto	316
	Isoxanthohumol inhibits in vivo vascular proliferation and stabilization	254
<b>J</b>	José Júlio de Souza Pinto na Bretanha	315
	José Marques da Silva: From the trip to the project	332
<b>L</b>	Land use/land cover changes and flooding surface estimation in Alqueva (Portugal) using 18 years of Landsat data	380
	Leonese dialects in Portugal: linguistic-genetic relationships through Y chromosome analysis	185
	Lightweight Approach to Automatic Error Detection Using Program Invariants	150
	Linear alkylbenzene sulfonate (LAS) removal present in commercial laundry wastewater in anaerobic fluidized bed reactor	169
<b>M</b>	Machado's story and the voices in the discursive representation of the social in nineteenth century	134
	Machinima: player-as-producer paradigm	225
	Macroalgae chemical profile versus cholinesterases activities	72
	Macroeconomic Fundamentals of Poverty and Deprivation: an empirical study for developed countries	50
	Making necklaces, weaving a network: indigenous women in Manaus	271
	Manual Dexterity and Functional Motor Asymmetry. Study in boys and girls without sports practice	364
	Methodology for assessing the state of disrepair of the building	291
	MNEMOSINE	226
	Modelling and simulation of a catalytic reactor for hydrogen production via methane autothermal reforming	101
	Modelling of the Fenton Process for the Degradation of Paraquat Pesticide in Water with Artificial Neural Networks	105
	Modulation of <i>Streptomyces natalensis</i> secondary metabolism: evidence of cross-talk between quorum-sensing and oxidative stress	229

	Molecular and phenotypic characteristics of the emerging <i>Salmonella enterica</i> serotype 4,5,12:i: - (Monophasic variant of <i>S. Typhimurium</i> ) from Portugal	326
	Molecular characterization of Bet v 1 - homologues and profilin from pollen of <i>Quercus</i> spp.	232
	Monitoring a Draught Beverage Machine	78
	Monitoring Drivers' Vital Signs for characterisation of Stress and Fatigue in Urban Traffic	299
	Minimization of wort and beer proteolysis by size exclusion and reverse phase liquid chromatography	80
	Motor performance and body composition of children aged seven and eight of the participating centers Municipal Sports and Recreation in Manaus	360
<b>N</b>	Nationalism and Extreme Right: The Portuguese Reality in Post April 25, 1974	309
	Neuregulin attenuates right ventricular hypertrophy and dysfunction in an experimental model of pulmonary hypertension	302
	New Biomolecular Force Field: lipid components	353
	New challenges in cyberjournalism Is there any business model?	61
<b>O</b>	Obesity: the role of melanocortins in rat adipose tissue	159
	Observation of the Douro River Plume Using Satellite Images	168
	On the development of reduced salt bread through the incorporation of aromatic herbs and spices	97
	On the Viability of Cooling Integrated Circuits using Magnetocaloric Materials	383
	On the voltammetry of chalcones	352
	Opinions of Caregivers concerning school meals	327
	Organising Capacity for Sustainable Urban Planning	52
	Painting and Printing Techniques: intersections and contaminations	27
<b>P</b>	PCA tools for probing dark energy with varying couplings	241
	Photography, design process reading by images	333
	Phytoremediation of a metalaxyl-photo-Fenton-treated solution by <i>Solanum nigrum</i> L. plants	369
	Polimerase chain reaction as a tool for diet studies of crustaceans	285
	Politics 2.0: the use of the social networks facebook and twitter in electoral campaigns and the 2011 Portuguese presidential election case study	60
	Porto and Vila Nova de Gaia, near the Douro City, identity and opportunity management of public spaces	179
	Prehistoric Spaces and Territories - metal analysis of Iron Age artifacts from Crasto de Palheiros - Murça (Vila Real - Portugal)	273
	Probing dark energy with ESPRESSO and CODEX	242
	Proposal of Rehabilitation and Maintenance for Copan Building's Façades	290
	Proteolytic activity of surplus yeast extracts	33
	Public Space in Oporto Social Quartes - A Conceptual Plan to Space and Social Rehabilitation	289
	Purification Methods of Biodiesel from Animal Fats	102
<b>Q</b>	Quality of life in urban context	178
	Quantification of cocaine and morphine in hair samples by a validated gas chromatographic ? mass spectrometry method	351
<b>R</b>	Radioluminescent fiber dosimeter for dosimetry in brachytherapy and external radiotherapy	212
	Real-time PCR technique to detect hazelnut as a potential hidden allergen in commercial chocolates	283
	"Representations of the East in "O Mundo Português" (1934-1947)"	270
	Residual stress determination using FBG sensors	378
	Resolution of chiral xanthenes by HPLC and docking studies	69
	"Revisiting strategies for class control through written reflection: the "look" of a pre-service teacher of Physical Education"	44
	Risk factors associated with one-year mortality of extremely premature newborns in Portugal	118
	RKIP and CD147 expression as prognostic factors in squamous cell lip carcinoma	258
	Role of border-like cells in defence against microorganisms in <i>Arabidopsis thaliana</i> roots	230
	Row Cemeteries and Tombs of Chiefs: the Franks and the Burial Archaeology in northern Gaul (V-VIII centuries)	272
	RZoltar: a plug-in Eclipse for Regression Testing	148
<b>S</b>	<i>Saccharomyces cerevisiae</i> brewing biomass as a promising source of nucleotides for flavour enhancers production	34
	Scaling Properties of Domain Wall Networks	243
	Sea-lamprey nutritional profile and reference intake values for human consumption during reproductive migration	141
	Seasonality in human diet: from production to nutritional status	144
	Self control or morality: what matters in antisocial behavior?	262
	Sequence diversity analysis of the human mtDNA control region: comparative analysis in two populations from the Portuguese-Spanish border	189
	Single and binary metal oxide catalysts for VOC abatement	170
	Sodium quantification in meals: from methodology to diagnosis	70
	<i>Solanum nigrum</i> L. as a tool to remediate Cr(VI)-polluted sites ? the contribution of its metallothioneins	219

	Species identification and authentication of hare ( <i>Lepus</i> ) meat by the use of the mitochondrial cytb gene.....	234
	Spectral Synthesis using the Metropolis-Hastings algorithm.....	240
	Standardization of antioxidant capacity assessment through microchemical methods using a novel kinetic matching approach.....	96
	Strain sensitivity enhancement in suspended core fiber tapers.....	382
	Strategies to optimize good behaviours in Physical Education classes: learning through reflections.....	86
	Students school performance and attention: presentation of a research project.....	85
	Students' autonomy and responsibility in the context of Physical Education classes: reflections of a Pre-service teacher.....	42
	Study of Planctomycetes pigments by a HPLC-DAD and LC-MS methodology.....	282
	Sunscreens: Photodegradation and stabilization.....	74
	SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPIONPs) ARE TOXIC TO HUMAN NEURONAL CELLS IN VITRO.....	210
	Susceptibility of European eel, <i>Anguilla anguilla</i> , and this parasite, <i>Anguillicoloides crassus</i> , to heavy metals concentrations in the International river Minho.....	370
	Susceptibility profile of deep-seated yeasts isolates from a university hospital in the northern region of Portugal.....	323
	Synthesis of Dual Inhibitors of Tumor Cell Growth and of P-Glycoprotein.....	209
T	TD-DFT/MM Study of the Firefly Multicolor Bioluminescence.....	356
	The (re)construction of Professional Identity in the practicum context. Main results of a research project.....	89
	The antecedents of burnout syndrome in a retail bank and its effects on the affective commitment.....	49
	The antioxidant treatment with EGCG prevents the diabetes-induced decrease of brainstem pain-related noradrenergic neurons.....	77
	"The assault on the "Liberty Fleet"" Comparative study of traditional and online press coverage of the Israeli attack on the boat pro-Palestinian in 2010".....	59
	The costs of law enforcement in the drivers' crimes: The case of the Tribunal de Pequena Instância de Oporto.....	307
	The effect of green spaces' (bio) structure on climatic parameters.....	175
	The effects of EGCG, a potent antioxidant present in green tea, on the serotonergic neuronal population of pain control centres of the brain in STZ-diabetic rats.....	37
	The historic center of cities in the dynamics: The historic centre of Oporto.....	180
	The impact of international technological-knowledge diffusion on economic growth.....	53
	The influence of athletes' motivational goals on the pre-game beliefs.....	124
	The Influence of Obesity and Gestational Weight Gain on the Newborn Weight in a Group of Woman with Gestational Diabetes.....	163
	The influence of physical and economical factors in conservation of <i>Caesalpinia echinata</i> , on Bahia South Region.....	220
	The internationalization profiles of Portuguese SMEs.....	54
	The Mathematics Behind Google - PageRank Algorithm.....	152
	The military intervention in the Libyan conflict: a comparative analysis of six international newspapers news content.....	58
	The New European Standard Set of Forensic Genetic Markers in the Portuguese Population.....	186
	The optimization of the spatial arrangement of the green spaces in the areas of urban expansion as a strategy for the design of a system of greenways.....	176
	The protection model of juvenile delinquency in Portugal: a study from a justice facility.....	263
	The Relationship between the Teacher and the Student: The Importance of Affectiveness in the Teaching-Learning Process.....	45
	The role of the plant cell wall in the regulation of protein sorting.....	231
	The Standardization of news on Twitter: Political Journalism.....	62
	The Tile Panels of S. Bento Train Station: history, context and iconography.....	29
	The transition to a management function: Experiences of socialization and occupational stress in new position.....	122
	The work-family articulation experienced by Portuguese women managers: a source of occupational stress?.....	123
	Tocar a Imagem.....	63
	Tourism and Leisure Activity in Nature, The Framework Programs in Finance.....	177
	Translation and cultural adaptation of the Portuguese version of Patient Assessment of Chronic Illness Care: PACIC-Portugal.....	115
	TRANSMUTATION OF BEING: Investigation into Image Construction as a time-based process within an installation-based context.....	30
	Treatment of textile effluents in a continuous packed-bed reactor by a Fenton-like process.....	103
	Treatment of wastewater resulting from washing of phytopharmaceutical plastic containers using combined biological and solar-driven advanced oxidation processes.....	171
U	United Nation's Intervention in Libya: media coverage.....	57

V	Valorisation of food private labels: exploring consumer perceptions.....	143
	Videogame practice and reaction time. Study in first and second grade school children.....	365
	Violência da Fruição entre Sade e Salò.....	132
	Visual Poetry in Portugal (1915-1977): Fields of Expression.....	129
W	What is the relevance of percutaneous endoscopic gastrostomy on the survival of patients with amyotrophic lateral sclerosis?.....	116
	When The Watchmen Take Your Watch: Corruption of Public Officials and its Causes, Consequences and Solutions.....	310
	Who is going to the movies in Grande Porto? A profile of the consumer.....	318

## POSTERS

#	"Acute toxicity of the antibiotic cephalixin to the freshwater cladoceran <i>Daphnia magna</i> ".....	477
	"B" TEAM: a stage on development process of young soccer players".....	562
	"Chemistry: A Science Without Borders" - Science Promotion Among Teenagers.....	589
A	A Adegas do Olho é Aqui: A Context of Rural-Urban Symbiosis.....	512
	A morphometric analysis of the Cholinergic fiber varicosities in the Retrosplenial Cortex of the Epileptic rat.....	415
	A reflection of the history of sports adapted.....	563
	A structural insight into Tenofovir biological action as a HIV-1 RT inhibitor.....	590
	Abdominal Circumference: Rate of risk factor in sedentary men and women starting with weight training.....	564
	Accelerated release pharmaceutical dosage forms: solid dispersions using the solvent evaporation method.....	416
	Activation of IL-27p28 gene transcription on antigen presenting cells infected with <i>Leishmania infantum</i> .....	455
	Adoption, identity and intimacy: An exploratory study of the adoptees' discourse.....	400
	Agar as a film forming agent for food packaging and edible coating.....	407
	Aged female diet anti-oxidant supplementation: effect on reproductive outcome.....	417
	An auscultation system using electronic stethoscope and Smartphones.....	418
	An electrochemical approach to the study of the phase solubility of MCPA - Methyl- $\beta$ -cyclodextrin inclusion complex.....	478
	An expert view on Perinatal Indicators.....	419
	An Intergenerational School-based Program of Physical Activity: An Exploratory Research Project.....	565
	Antimicrobial resistant bacteria in shrimp.....	420
	Antioxidant capacity and related parameters of commercial fruit smoothies.....	421
	Antioxidant enzymes in response to metalaxyl stress in <i>Solanum nigrum</i> L. cell suspension cultures.....	479
	Antioxidant potential of binary mixtures of phenolic compounds: Synergistic or antagonistic effects.....	422
	<i>Aplysia fasciata</i> Poirret: the potential of a sea slug.....	423
	Assesment of <i>Cynara cardunculus</i> L. ethanol extracts biological potential.....	424
	Assessing service quality in public transportation: Metro do Porto.....	389
	Assessing the Efficiency of Portuguese Banks using Data Envelopment Analysis.....	509
	Ausubel meaningful learning among Biology and Geology high-school students.....	396
	Automatic Segmentation of Diabetic Retinopathy Exudates in Retinal Images.....	581
B	Backstroke technical characterization of 11-13 years old swimmers.....	566
	Bacterial resistance to copper and antibiotics: two sides of the same problem in the animal production setting?.....	425
	Behaviour of a group of lions ( <i>Panthera leo</i> ) in captivity.....	456
	Bioassays to study the ecotoxicity of soils contaminated by pharmaceuticals.....	480
	Biological activities of <i>Echium plantagineum</i> L. pollen hydro-alcoholic extract.....	426
	Biological effects of exemestane metabolites in an estrogen-receptor-positive aromatase-overexpressing breast cancer cell line.....	427
	Biometric characterization of squid species available in the Portuguese market.....	591
	Bioremediation: possible application for the elimination of oil buried in beaches affected by oil spills.....	481
	Brow shrimp <i>Crangon crangon</i> feeding preferences: do multiple choice experiments confirm field opportunistic feeding?.....	457

	Brown macroalgae from Portuguese coast: a source of antioxidants.....	428
<b>C</b>	Can fungicide application in vineyards induce azole resistance in <i>Aspergillus</i> spp.?.....	430
	Cardiovascular and structural abnormalities induced by histone deacetylase inhibitors on zebrafish embryonic development.....	431
	Characterization of Macrogols phase transitions.....	432
	Characterization of the cholinesterases present in specific tissues of <i>Corbicula fluminea</i> (Mollusca: Bivalvia).....	482
	Characterization of the immune response to experimental calicivirus infection.....	433
	Characterization of the macromineral content of <i>Loligo reynaudi</i> .....	592
	CHARISMATIC LEADERSHIP: THE CASE OF JORGE NUNO PINTO DA COSTA.....	390
	Chemical oxidation of amoxicillin in contaminated water using potassium permanganate.....	483
	Chemical study from an Azorean macroalgae: <i>Gelidium microdon</i> .....	593
	Clinically relevant bacteria in bovine meat.....	434
	Co1-xMnxFe2O4 ferrite nanoparticles: preparation and characterization.....	594
	Communication between Planctomycetes.....	458
	Comparative study of the sun protection factor and water resistance of two silicone emulsions containing ethyl hexyl methoxycinnamate.....	435
	COMPARISON OF L-DOPA UPTAKE BETWEEN PROLIFERATIVE AND DOPAMINERGIC NEURONS LIKE SHSY5Y CELLS AS A CELLULAR MODEL FOR PARKINSON DISEASE STUDIES.....	436
	Comparison of manual and pedal time reaction between preferred and non- preferred swimming techniques.....	567
	Computational Study of Electronic and Structural Properties of Molecular Species in Confined Spaces.....	595
	Computational study of the catalytic mechanism of Human Renin protease.....	640
	Concept Analysis: Healthy Lifestyles, according to the traditional method of Walker and Avant (2005).....	437
	Concept maps and meaningful learning: a study with Biology and Geology contents.....	397
	Construction of a family of criteria to evaluate the sites invaded by the species <i>Corbicula fluminea</i> ? preliminary results.....	484
	Contribution for the discovery of a novel class monoamine oxidase B inhibitors based on chromone scaffold as a pharmacological approach to Parkinson's treatment.....	596
	Contribution of the plant/rhizosediment system to the phytoremediation of metals.....	485
	Contribution to the study of the relationship between occlusal contacts, head posture and center of gravity of the body, in patients with parafunctions and temporomandibular disorders.....	438
	Cultivation and reproduction of the marine sponge <i>Hymeniacidon perlevei</i> : Evidences of horizontal transmission of cyanobacteria.....	486
	Cultural competence: an analysis of the concept.....	439
	<i>Cystoseira tamariscifolia</i> (Hudson) Papenfuss (Phaeophyta): an alternative to plants?.....	440
<b>D</b>	Denoising of phonocardiograms.....	499
	Dental Polishing in Composite Resin Restorations: State of the Art Searching for Efficiency.....	441
	Design of new fluorescent chemical sensors using cleaner and more efficient methodologies.....	597
	Desirability-based Multi-criteria Quantitative Structure-Selectivity Relationships of Antimicrobial Peptidomimetics.....	598
	Determination of selenium in food supplements by HR-CS-ETAAS.....	408
	Development a GIS-Based online Database for Municipality Emergency Planning Service.....	513
	Development and optimisation of targeted drug delivery systems towards visceral leishmaniasis.....	442
	Development and validation of the Inventory «Articulation Person-Organization» (APO).....	402
	Development of a Cost-Effective Tool for Home-Based Rehabilitation of Stroke Patients.....	582
	Development of a sequential injection analysis system for the evaluation of aquatic pollutant's toxicity.....	487
	Development of an inhibition bioassay for the prediction of ionic liquid's human toxicity.....	459
	Development of biosilica nanostructures with immobilized proteases for incorporation in screening methodologies.....	599
	Development of novel dry multi-pin electrodes for electroencephalogram recording.....	583
	Differential distribution of ecto-NTPDases 1, 2 and 3 at the rat urinary bladder: Functional implications and drug targeting.....	443
	Disturbance of fine-tuning modulation of excitatory nicotinic ?3?2 and muscarinic M1 autoreceptors by ADA overactivity in rats with toxin-induced Myasthenia gravis.....	444
	Diversity and virulence profiles of <i>Escherichia coli</i> ST69, ST393 and ST405 clones.....	445
	Drug screening of bisnaphthalimidopropyl derivatives on <i>Trypanosoma brucei</i> .....	446
<b>E</b>	Ectoparasitic fauna of Atlantic mackerel, <i>Scomber scombrus</i> , from Matosinhos and Figueira da Foz.....	460
	Effect of doxycycline on the proliferation and differentiation of rat bone marrow-derived osteoblastic cells.....	447
	Effect of gallic acid and its alkyl esters on the oxidative stability of unsaturated long-chain fatty acids.....	600
	EFFECT OF MEDITERRANEAN LIFESTYLE IN REDUCING THE WEIGHT AND BLOOD PRESSURE.....	448
	Effect of the sport in pedal dexterity for young practitioners of basketball, swimming and skateboarding.....	568

	Effects of copper exposure on the expression of the metallothionein gene family of <i>Solanum nigrum</i> L. plants	461
	Effects of proton pump inhibitors on co-cultures of human osteoclasts and breast cancer cells	462
	Effects of uridine nucleotides on the proliferation and type I collagen synthesis in fibroblasts of the rat subcutaneous tissue: interplay between P2Y2 and P2Y6 receptors	449
	Efficiency Analysis of Portuguese Hospitals	391
	Electrochemical biosensors for carbamate pesticides detection	601
	Electrochemical nanosensor for simultaneous determination of MCPA and its metabolite 4-chloro-2-methylphenol	488
	Endocannabinoids and programmed cell death: Anandamide mediates p38 MAPK activation in rat decidual cells	450
	Energy Content of Two Flatfish from the Dutch Wadden Sea: the flounder <i>Platichthys flesus</i> and the plaice <i>Pleuronectes platessa</i>	463
	Energy reserves of the brown shrimp <i>Crangon crangon</i> (Linnaeus, 1758)	464
	Ethics in clinical studies: a systematic review on patients' consent for publication of case reports	451
	Evaluation of anti- <i>Leishmania</i> compound released from polymeric nanoparticles	602
	Evaluation of compressibility indicators of technological excipients of tablets	452
	Evaluation of oxidative stress markers in chronic heart failure	453
	Evaluation of partition coefficients and protein binding affinity of nimesulide incorporated in ionic liquid systems	465
	Exploiting bisnaphthalimidopropyl polyamine as an electroactive specie for ISE preparation	603
	Exploratory evaluation of nutritional information displayed on breakfast cereals package labels	409
	Exploring Argentine honey and propolis	454
	Extraction and HPLC-DAD determination of chlorogenic acid (CGA) from Lamb's lettuce	604
	Forest fire risk maps in a GIS Open Source environment for Norwest of Portugal	584
<b>F</b>	From Thermoelectric Materials to Thermoelectric Devices	500
	Functional and pharmacological studies of p53 family proteins: screening for selective small-molecule modulators	519
	Furanic compounds in espresso coffee capsules: validation of a HS-SPME-GC-MS methodology	605
	Furfural stability in model systems containing ascorbic and citric acids: effect of pH, time and temperature	606
<b>G</b>	Gas-diffusion microextraction (GDME): study of influencing parameters on the extraction of aldehydes	607
	Green synthesis of zero-valent iron nanoparticles using vine leaves	489
<b>H</b>	Heavy metal toxicity in Planctomycetes	466
	HELENA ALMEIDA - DRAWING AS AN APPROPRIATION ACT FOR A PERFORMATIVE	394
	Human osteoclastogenesis over microstructured and nanostructured hydroxyapatite surfaces	467
<b>I</b>	Identification of <i>Hypericum</i> species for herbal tea authentication	410
	Identification of the mycotoxin system patulin/producing mold in different fruits	411
	Impact of dietary protein sources on lipid content and fatty acid profile of Senegalese sole juveniles ( <i>Solea senegalensis</i> Kaup, 1858)	468
	Improving instruction in a pre-service teacher of Physical Education presentation of a research project	569
	IN THE RAT BRAIN CORTEX NMDA RECEPTOR-EVOKED NORADRENALINE RELEASE IS INHIBITED BY ADENOSINE A2A RECEPTORS: MECHANISMS INVOLVED	520
	In vitro radical scavenging potential of <i>Colocasia esculenta</i> (L.) Shott.	521
	Influence of calcium in staurosporine-induced cell death in <i>Neurospora crassa</i> .	469
	Influence of lidocaine hydrochloride and bovine serum albumin on physical characteristics and stability of thermo-responsive PLURONIC® F127 hydrogels	522
	Influence of porins OmpF and OmpC in the influx of sparfloxacin in <i>Escherichia coli</i>	470
	Initial Training Teachers in Science Education at the UFPR - Coast - Brazil: Possibilities of Renovation	398
	Interaction of 3,4-dihydroxyxanthone with double stranded DNA	608
	Interaction of Chromone with Double Stranded DNA	609
	Interactive maternal behaviours and intellectual ability of children at school age	401
	Intergenerational perspective of the adoption process: grandparents, parents and grandchildren	403
	Involvement of Planctomycetes in the nitrogen cycle	471
	Iron meteorites and the formation of the early Solar System	501
	Iron oxide nanoparticles as heterogeneous catalysts for the oxidation of methylene blue using Fenton reaction	610
	Is the presence of BRAF mutation a marker of aggressive Papillary Thyroid Carcinoma?	523
<b>J</b>	J. J. Forrester, knowledge and contribution in the nineteenth century cartography of the Douro region	514
	Jousting Neurodegenerative Diseases Using Novel Lipophilic Antioxidants based on Ferulic and Caffeic Acid	611
<b>K</b>	Keratin-based biodegradable films from bovine hair, human hair and chicken feathers	412
	Keynes e o Brasil de 1929	392

<b>L</b>	Laboratorial characterization of a Fe-Ni meteorite.....	502
	Laterality and Ballet. The effect of Crossed vs Ipsilateral Hand/Foot Preference on the Static Balance of Classical Dancers.....	570
	LC-MS detection of degradation products of Chimassorb 944 in UV-aged geotextiles.....	612
	Localization of inhibitory A1 and facilitatory A2A adenosine receptors on motor nerve terminals of rats with Experimental Autoimmune Myasthenia Gravis (EAMG) by confocal microscopy.....	472
	Magnetic ferrites in Nano-MEMS: from real-time synthesis control to automatic transport devices.....	503
	Magnetic susceptibility of soils close to a highway.....	490
	Manual dexterity and functional motor asymmetry in symmetric and asymmetric sports. A study in swimmers and tennis players.....	571
	Marine cyanobacteria toxicology: toxic effects on marine invertebrates, marine microalgae, bacteria and cytotoxic effects on human cells.....	491
	Marine sponge-associated fungus <i>Eurotium cristatum</i> : a great source of anthraquinone derivati.....	524
	Microwave Organic Reactions Applied to the Synthesis of Novel Monoamino Oxidase Inhibitors.....	613
	Mitochondrial metabolism and dynamics following exposure to desacetylase inhibitors in neurons.....	525
	Modeling users for interactive decision support systems for gastroenterology.....	429
	Monovacant polyoxometalates @ MIL-101: synthesis and heterogeneous catalytic studies.....	614
<b>N</b>	Necrosis induced by carbon tetrachloride in the liver of a model organism: the zebrafish ( <i>Danio rerio</i> ).....	526
	<i>Neosartorya spinosa</i> , a marine fungi isolated from the sponge <i>Rhizadernia</i> sp.....	527
	Neuregulin attenuates pulmonary endothelial dysfunction in an experimental model of pulmonary hypertension.....	528
	New methodology to obtain custom prosthesis for rehabilitation of external deformities.....	585
	New polymorphisms within the non-coding region of the OTC gene.....	473
	Nickel metallo-organic polymer as a new electrochromic material.....	615
	Novel functional nanostructured semiconductors for Dye-sensitized Solar Cells.....	586
	Nutritional Risk and Survival in Cancer Patients Undergoing Percutaneous Endoscopic Gastrostomy in the Instituto Português de Oncologia do Porto Francisco Gentil, Entidade Pública Empresarial.....	529
<b>O</b>	O MUSEU DA RESISTÊNCIA: MUSEU TRANSNACIONAL.....	506
	Opening a new avenue in neurodegenerative therapy: development of mitochondria-targeted antioxidants based on benzoic scaffold.....	616
	Opening a new avenue in neurodegenerative therapy: lead optimization of a mitochondria-targeted cinnamic antioxidant.....	617
	Optical detection based on the biocatalytic growth of gold nanoparticles.....	618
	Optimization of an analytical method for PAHs determination in estuarine sediments.....	619
	Optimization of an experimental protocol for chorionic villi culture in prenatal diagnosis.....	530
	Optimization of the covalent linkage between cyclodextrins and textile substrates by experimental design techniques.....	620
	Organizational metaphors in movies: an analysis of the archetypes in films that focus on interpersonal relationships present within corporations.....	395
<b>P</b>	P2Y6 RECEPTORS EXPRESSED BY ACTIVATED MICROGLIA INHIBIT ASTROGLIAL PROLIFERATION BY INDUCING NITRIC OXIDE MEDIATED APOPTOSIS.....	531
	Packaging contaminants: bisphenol A and B in mussels.....	413
	<i>Padina pavonica</i> (L.) Thivy: the secret story.....	532
	Parasitology of the Brown Shrimp <i>Crangon crangon</i> Linnaeus, 1758.....	551
	Pattern discovery in Gene expression.....	552
	Penetration of different components of dental amalgam and composite resin in the interface between restoration and dentin.....	533
	Perceptions of Physical Education pre-service teachers concerning the learning process during the practicum context.....	399
	Phosphomolybdates immobilized onto functionalized silica nanoparticles as new photochromic materials.....	621
	Phylogeny of the Genus <i>Crangon</i> Fabricius, 1798.....	553
	Physical Education as a contribute to the integration of a student with special educational needs.....	572
	Physiological and biochemical response of <i>Oriza sativa</i> L to toxic <i>Microcystis aeruginosa</i> extracts.....	492
	Polyphenolic content of grapevine leaves and stems: optimization of extraction parameters.....	414
	Portuguese Investor Profile.....	393
	Potential use of laccase for the detoxification of phenol pollutant.....	587
	Preliminary forensic science study of dispersion in sawed-off shotgun.....	510
	Prenylated Xanthonic Derivatives: Optimization of synthetic methodologies and evaluation of physicochemical and biological properties.....	534
	Preparation and characterization of Europium-sandwich polyoxometalate. Incorporation into Multilayer Films.....	622

	Preparation of metallic nanoparticles functionalized with peptides for applications in biosensors.....	623
	Privileged xanthonic structures as inspiration for synthesis of new inhibitors of growth of human tumor cell lines.....	535
	Production and purification of a nucleocapsid protein from a novel canine norovirus in <i>Saccharomyces cerevisiae</i> .....	536
	Progressive floods along the valley of the Arda river: implications on the land planning in the Arouca municipality (Portugal).....	515
R	Rapid simultaneous potentiometric determination of sodium and chloride in bread.....	624
	Relationship between grandparents and adopted grandchildren.....	404
	Revealing sea secrets: <i>Holothuria forskali</i> Chiaje.....	537
	Ribavirin is a poor inhibitor of norovirus replication in the murine model.....	538
	RNA profiling for the determination of post-mortem interval.....	511
	Road networks - contributes for a multi-risk analysis.....	516
	Roof nesting gulls ( <i>Larus michaellis</i> ) of downtown Porto: An eco-ethological approach.....	554
S	Satureja parvifolia: bioactive compounds and antioxidant activity.....	539
	Shift of the adenosine A1/A2A receptor balance regulating Ca <sup>2+</sup> influx in the hippocampus and neocortex of patients with mesial temporal lobe epilepsy (MTLE) towards the activation of excitatory A2A receptors.....	541
	Simultaneous quantification of cholesterol, vitamin E, total and fatty acids profile in Octopus: method validation.....	625
	Socio-cognitive development of adopted preschoolers:exploratory study.....	405
	Soil microorganism selection for amoxicillin biodegradation.....	493
	Solid lipid nanoparticles (SLN) for Miconazole encapsulation: preparation, in vitro release and long-term stability studies.....	542
	Spatial Ecology and Behaviour of Free-roaming Cats ( <i>Felis catus</i> ) in Urban Environment (Porto, Portugal).....	555
	Species-specific PCR detection and real-time PCR quantification for the authentication of cooked hams.....	556
	Specific IgE and IgG to recombinant <i>Aspergillus fumigatus</i> allergens in patients with allergic bronchopulmonary mycosis, asthma and cystic fibrosis. A pilot study.....	540
	Spectrophotometric determination of nitamide by multipumping.....	626
	Spiny sea-star ( <i>Marthasterias glacialis</i> Linnaeus): beyond what we see.....	543
	Strong Electrolyte Aqueous Solutions: Solute Concentration. Computer Simulation Development.....	627
	Structure and textured panels for modular architectural surfaces.....	508
	Studies on <i>Arundo donax</i> development for utilization in constructed wetlands for saline aquaculture effluent treatment.....	494
	Studies on DNA, fertility, proteins and allergenicity of <i>Platanus</i> spp. pollen following in vitro exposure to Ozone.....	557
	Studies on structure-dependent photodegradation of pesticides MCPA and 2,4-D.....	495
	Study of genetic polymorphisms implicated in sensitivity to taste in African populations.....	558
	Styles of Supervision in the context of a practicum group: a study with Cooperating Teachers of Physical Education.....	573
	Suzuki-Miyaura Synthesis of Some Linear Arylacenes.....	628
	Swimming Team Cohesion: differences between gender and competitive levels.....	574
	Synthesis and characterization of functionalized Fe- Au/core-shell magnetic nanoparticles for bioassay applications.....	629
	Synthesis and micellization properties of novel serine-based gemini surfactants: Assessing the role of the spacer linkage and spacer length.....	630
	Synthesis and Structural Elucidation of New Chiral Xanthonic Derivatives.....	631
	Synthesis of 2'-hydroxy-3,4,4',5,6'-pentamethoxychalcone and analogues by MAOS.....	632
	Synthesis of bioactive xanthonic derivatives.....	633
	Synthesis of Prenylated Xanthonic Inhibitors of the Growth of Cancer Cell Lines.....	634
	Synthesis, characterization and properties of a rosamine-based potentiometric ammonia gas sensor.....	635
T	Tardigrades as bioindicators of air quality.....	641
	Technologies for interactive decision support systems for gastroenterology.....	588
	Tectonic Geomorphology in the Forno Valley (Amarante) and some implications for planning.....	517
	The contribute of the school resident teachers to the learning process in the practicum context: a self-narrative of a Physical Education pre-service teacher.....	575
	The early stages of the Solar System Evolution: constraints from the small icy worlds.....	504
	The effect of dissolved organic matter on the degradation of UV filters in swimming pool water.....	636
	The effect of practice (futsal and athletics) on pedal reaction time and pedal dexterity in young athletes.....	576
	The endocannabinoid 2-Arachidonoylglycerol (2-AG) in pregnancy: effects in viability of cytotrophoblast cells.....	642
	The importance of the group experience for the (re)construction about the pre-service teacher understanding of being a teacher.....	577

The influence of benthic macroinvertebrates on the degradation of organic matter in river systems.....	496
The Living Book: the myth of the Ideal Perfect Library at Prosas Portuguesas of Rafael Bluteau.....	507
The Magpie (Pica pica, L.) at the Vila Nova de Gaia coastal area.....	643
The Red Fox in Portugal: Distribution, morphology and age.....	644
The role of anticipatory socialization and practicum experience to the (re)construction of Professional Identity: a study with Pre-service Teachers of Physical Education.....	578
The role of Wnt signalling and anti-oxidant beverages in diabetic cardiomyopathy.....	645
The Sandage-Loeb test for dynamical dark energy models.....	505
The Teacher's Active Control as a requirement of a good environment: A self-analysis of a Physical Education pre-service teacher.....	579
The transition to adulthood: The quality of parental relationship, career exploration and family-work perspectives on young people with different academic trajectories.....	406
Theoretical Study of the Effect Exerted by Inhibitory Compounds on Firefly Luciferase Bioluminescence.....	637
Thermochemical study of a chromone 2-phenylcarboxamide.....	638
Toxicity of the heavy metal mercury to the marine microalgae <i>Tetraselmis chuii</i> .....	646
Toxicity of the pesticide mancozedo to the Asian Clam ( <i>Corbicula fluminea</i> ).....	497
Trace elements in human brain: age-related changes and anatomic region specific differences.....	544
Tracing the emerging pathogen <i>Escherichia albertii</i> .....	545
Tracking antibiotic resistance along the Silk Road.....	647
Transit-Oriented Development ? a methodological test for the city of Porto.....	518
<b>U</b> Ubiquinone analogues and histone deacetylase inhibitors in an induced zebrafish model of mitochondrial dysfunction.....	546
Understanding the effects of proline against Cu toxicity in <i>Solanum nigrum</i> L. plant growth.....	648
Use of cyclodextrins to tackle chromone's solubility.....	639
Using yeast to study the role of ceramide pathway in the regulation of mammalian protein kinase C isoforms.....	547
UV Filter Encapsulation Using Aero-Dynamically Assisted Jet Extrusion.....	548
<b>V</b> Velocidade de Reação Manual em atletas de Basquetebol em Cadeira de Rodas.....	580
Volatile and semi-volatile profiling of wild mushrooms by GC-MS and their importance in species identification.....	649
<b>W</b> Waist-to-Height Ratio as a possible Health Index for Swedish Children.....	549
Whole blood manganese: Reference range and Mn levels in several diseases states.....	550
<b>Z</b> Zircons from metagreywacke of the ?Complexo Xisto-Grauváquico?: constraints from petrographic and lithogeochemical data.....	498



