



## Repositioning Bacterial Lysates: Inhalation as a Strategy in the Management of Respiratory Diseases

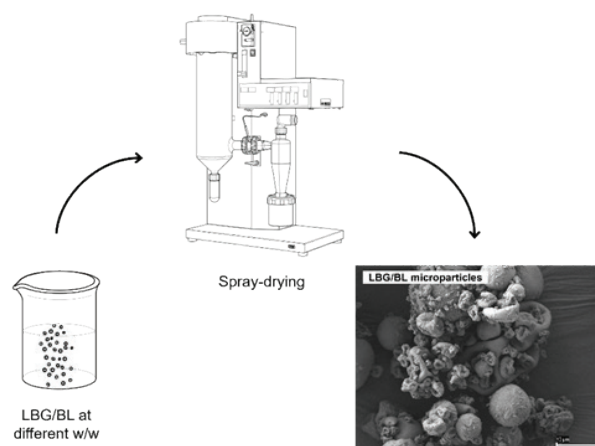
Pinto-da-Silva J.<sup>1\*</sup>, Berzosa M.<sup>2</sup>, Delgado-López A.<sup>3</sup>, Gamazo C.<sup>3</sup>, Grenha A.<sup>1</sup>

### ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** Respiratory infections have a significant impact worldwide, and vaccination plays a crucial role in reducing their frequency and severity. Bacterial lysates (BL) obtained from pathogenic bacteria are commonly employed as immunomodulators for the prevention of respiratory tract infections and are typically delivered orally. However, their effectiveness varies, often leading to weak mucosal responses and inadequate lung protection<sup>1</sup>. Because the lungs are the primary site of infection, there is potential for pulmonary administration via inhalation to enhance the immune response. This study investigated the application of BL as inhalable immunomodulators, for an application in respiratory diseases prevention.

**Methodology:** A commercial BL formulation was combined with locust bean gum (LBG) to create inhalable microparticles (MP) through spray-drying (Figure 1). LBG is expected to have a role in targeting antigen presenting cells<sup>2</sup>. LBG dispersion at 1% (w/v) was mixed with BL at different LBG:BL mass ratios, varying within 10:0.2 and 10:1.2. The yield of the process was determined by gravimetry. Microparticle characterization involved field emission scanning electron microscopy for morphology analysis, Bradford protein assay for determination of association efficiency (AE) and loading capacity, and cascade impaction for aerodynamic evaluation (Andersen cascade impactor). Immunochromatography and serum agglutination tests were used to assess the preservation of BL antigenicity. A metabolic test (MTT assay) was performed to evaluate cytotoxicity on alveolar epithelium (A549) cells.



**Figure 1.** Preparation of locust bean gum microparticles loaded with bacterial lysates. Scale bar on SEM image represents 10  $\mu$ m.

<sup>1</sup> Centro de Ciências do Mar, Universidade do Algarve, Faro, Portugal.

<sup>2</sup> Queen's University Belfast, Belfast, United Kingdom.

<sup>3</sup> Universidad de Navarra, Pamplona, España.

\*Corresponding author: Joana Pinto-da-Silva (a54138@ualg.pt).

**Results:** Spray-dried MP loaded with BL were successfully produced using different ratios of LBG:BL, with AE reaching 81%. The best formulation (LBG:BL = 10:0.2) exhibited mass median aerodynamic diameter (MMAD) below 5  $\mu\text{m}$ , indicating potential to effectively reach the lungs. Exposure of A549 cells to MP for 24 h resulted in minimal impact on cell metabolic activity, as indicated by the MTT assay, with cell viability generally exceeding 70%. Additionally, antigen diagnostic tests confirmed that the spray-drying process did not compromise the antigenicity of BL, as native antigens were preserved on the surface of BL-loaded microparticles.

**Discussion:** The current findings show promising outcomes in the development of an inhalable platform utilizing BL-loaded MP for the prevention of respiratory infections.

**Keywords:** inhalation, respiratory tract infections, spray drying.

**Acknowledgments:** Funding from the Portuguese Foundation for Science and Technology (UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020) and the PhD scholarship to Joana Pinto da Silva (2021.05769.BD) are acknowledged.

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## Insect Oil: a High Value Excipient for Lipid-Based Nanocarriers to Tackle Atopic Dermatitis'

Pereira-Leite C.<sup>1,2</sup>, Almeida C.<sup>1,3</sup>, Roque R.<sup>1</sup>, Júlio A.<sup>1</sup>, Saraiva N.<sup>1</sup>, Rosado C.<sup>1\*</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Atopic dermatitis (AD) is characterized by a compromised skin barrier due to changes in ceramides and fatty acids (FA) forming the lipidic layer of the stratum corneum<sup>1,2</sup>. The oil extracted from *Hermetia illucens* larvae biomass holds potential as a biomaterial due to its high concentration of saturated FA<sup>3</sup>. This lipid blend can be of added value in nanodelivery systems to target AD, both to enhance the safety and efficacy of glucocorticoids and to act as an emollient providing barrier recovery<sup>3</sup>.

This work aimed to develop nanocarriers to encapsulate dexamethasone based on the lipidic extract from *H. illucens* larvae. These formulations were characterized and assessed for stability. Cytotoxicity was studied using *in vitro* assays conducted in keratinocytes and safety and efficacy were established in human volunteers.

**Methodology:** The SLN formed by the larvae lipid extract and the surfactant were prepared by ultrasonication. The nanoformulations were characterized during storage at 25 °C for 60 days regarding particle size, polydispersity index (PDI), zeta potential (ZP), and pH. The encapsulation efficacy (EE) of dexamethasone and loading capacity (LC) of the nanocarriers were also evaluated. The MTT assay was used to assess cell viability upon HaCat cells exposure to dexamethasone, the lipid extract, unloaded and dexamethasone-loaded nanocarriers, at previously selected concentrations for 24h. The *in vivo* safety and efficacy study was approved by the local ethics committee (Parecer CE.ECTS P01-22) and compared skin properties before and after treatment with the nanoformulations without glucocorticoids (SC hydration, TEWL, and erythema). A dose of 5 mg/cm<sup>2</sup> was applied, forming a lipidic film on the skin surface. Additionally, the Plastic Occlusion Stress test was used to assess the impact of the nanoformulations on skin barrier function after treatment of the skin for 48h.

**Results and Discussion:** The nanocarriers loaded with dexamethasone displayed particle sizes under 185 nm, a PDI < 0.26, and promising ZP values (< -34 mV) during the 60 days. Suitable results were obtained in terms of EE (around 84%), LC (1.3%) and pH (compatible with the skin). The MTT assay provided favorable results. *In vivo* studies confirmed that the nanoparticles exhibited good compatibility with the skin and improved hydration and barrier.

**Keywords:** atopic dermatitis, nanodelivery systems, insect oil.

<sup>1</sup> CBIOS - Universidade Lusófona's Research Center for Biosciences & Health Technologies, Lisboa, Portugal.

<sup>2</sup> LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.

<sup>3</sup> Universidad de Alcalá - Departamento de Ciencias Biomédicas, Madrid, España.

\*Corresponding author: p1657@ulusofona.pt.

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## A Nanotechnological Strategy to Improve Nystatin's Potential in Antifungal Treatment

Sousa F.<sup>1,2\*</sup>, Ferreira D.<sup>1,2</sup>, Reis S.<sup>3</sup>, Costa P.<sup>1,2\*</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Nystatin is a versatile antifungal drug with untapped potential. Nanotechnology strategies have been devised to address its administration challenges (nephrotoxicity, instability and poor absorption). Nanoemulsions are a promise approach due to improved solubility, stability, bioavailability, drug delivery, permeation and enhanced antifungal activity. The goal of this work was to develop nystatin-loaded nanoemulsions aimed at incorporation in polymeric microparticles for inhalation therapy.

**Methodology:** Nanoemulsions were prepared under constant magnetic stirring by mixing all the excipients (surfactant, co-surfactant, water, preservative/stabiliser, oil). Afterwards, nystatin was dissolved in the mixture and stirred on Ultra-Turrax (7000 rpm for 15 minutes), followed by ultrasonication (80% Amplitude for 15 minutes) and immediate ice immersion. A Box-Behnken (BB) Design was employed to optimise the three factors identified as critical: surfactant ratio, stirring speed and sonication amplitude. The responses defined were particle size, polydispersity index, zeta potential and encapsulation efficiency. Likewise, thermodynamic stability tests were performed to evaluate stability and phase integrity under varying temperature conditions and centrifugal force. The model suggested by BB design confirmed the significance of several factors in the design. In vitro drug release study was performed using the dialysis bag method and phosphate-citrate buffer pH 5.5 as release medium. Thermal analysis was made by differential scanning calorimetry. Long-term stability was assessed by keeping the optimized nanoemulsions at 3 different temperatures (4, 25 and 40°C) for 6 months.

**Results and discussion:** Alveolar macrophages are the primary phagocytic cells in the lungs and are responsible for clearing fungal spores of intracellular pathogens, thus eliminating the infection. The optimized nanoemulsion achieved an ideal particle size ( $255.5 \text{ nm} \pm 17.39$ ) for effective delivery of nystatin to the alveolar macrophages, along with a suitable polydispersity index ( $0.317 \pm 0.03$ ) and high zeta potential values ( $-42.20 \text{ mV} \pm 0.25$ ). It exhibited a controlled release pattern, with 24.9% release over 48 hours following zero-order kinetics. Thermal analysis demonstrated successful encapsulation of nystatin, as evidenced by the disappearance of its characteristic endothermic peak around 150°C and the overlapping thermograms of blank and nystatin-loaded nanoemulsions. Stability testing over a 6-month period revealed no significant changes in the analysed parameters.

<sup>1</sup> UCIBIO, REQUIMTE, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira n° 228 4050-313 Porto, Portugal.

<sup>2</sup> Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

<sup>3</sup> LAQV, REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira n° 228 4050-313 Porto, Portugal.

\*Corresponding author: fsousa@ff.up.pt (FS) and pccosta@ff.up.pt (PC.).

**Conclusion:** Overall, this new nystatin nanoemulsion depicted improved physical and thermal properties, an ideal particle size for intracellular fungal diseases and improved stability, predicting long-term shelf-life. *In vitro* and *in vivo* assays are needed to confirm these positive results.

**Keywords:** nanoemulsions, delivery systems, fungal infections.

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## Phytosomes Loaded with Dragon Fruit (*Hylocereus Costaricensis*) Extract: *In vivo* Study of Anti-Hyperglycemic Activity

Sousa I.<sup>1\*</sup>, Antunes F.<sup>1</sup>, Rednak S.<sup>1,2</sup>, Gaspar M.M.<sup>3</sup>, Reis C.<sup>3</sup>, Direito R.<sup>3</sup>, Figueira M.E.<sup>3</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Dragon fruit (*Hylocereus costaricensis*) extracts have biologically active compounds that are beneficial to health. Phenolic compounds in general have poor bioavailability and, consequently, large intakes are required to feel the biological effects in the body. To increase the efficiency of these compounds in extracts, it is necessary to encapsulate them. The objective of this work is to develop encapsulations of dragon fruit extract, characterize the phytosomes, and evaluate the antihyperglycemic activity.

**Methodology:** Phytosomes were prepared by adding extract to phosphatidylcholine dissolved in 20 mL of ethanol (1:1, molar ratio). The mixture was heated to 25°C with a rotation of 300 rpm for 2 hours. 40 mL of 2% acetic acid was added, and the mixture remained in the same conditions for 24 hours. The encapsulation efficiency was estimated through the difference between the content of total phenolic compounds in the initial extract and the content of total phenolic compounds present in the supernatant. In the *in vivo* study of antihyperglycemic activity, the animals were divided into three groups: the free extract group (5 mg/kg); the extract-loaded phytosomes group (2.3 mg/kg); and control group which were administered with water. Oral administrations of the free extract and phytosomes were performed daily, during 14 days, by gastric gavage. After 14 days, glucose was administered in all groups (2 g/kg), and blood glucose was measured at 30 minutes, 60 minutes, and 120 minutes after administration.

**Results:** The encapsulation efficiency of the extract was measured to be 46%. Animals in the control group showed increases in glycemic levels of 25.81% and 39.45% at 30 minutes and 60 minutes respectively, and glycemia returned to baseline values at 120 minutes. Animals treated with 5 mg/kg of extract did not show lower results than the control group, and after 120 minutes they still had an average value of 17.57% ( $p < 0.01$ ) higher than the baseline values. The group treated with phytosomes (2.3 mg/kg) demonstrated lower glycemic levels than the group treated with extract at 60 and 120 minutes (38.72% and 13.92%, respectively).

**Discussion:** Phytosomes have a better effect compared to the free extract, showing a higher efficiency even when using a lower dose.

**Keywords:** *hylocereus costaricensis*, antihyperglycemic activity, phytosomes.

<sup>1</sup> Faculty of Pharmacy - University of Lisbon, Lisbon, Portugal.

<sup>2</sup> Faculty of Pharmacy - University of Ljubljana, Ljubljana, Slovenia.

<sup>3</sup> Laboratory of Systems Integration Pharmacology, Clinical and Regulatory Science - Research Institute for Medicines of the University of Lisbon, (iMed.Ulisboa), Lisbon, Portugal.

\*Corresponding author: isousa2@edu.ulisboa.pt.

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## Design and Fabrication of a Liposomal Gel System for Rheumatoid Arthritis

Weiss-Steider B.<sup>1</sup>, Romero-Altamirano I.S.<sup>1</sup>, Vázquez R.S.<sup>1</sup>, Valdés-Corona L.F.<sup>2</sup>, Vargas-Angeles C.A.<sup>1</sup>, Rangel-Corona R.<sup>1</sup>, Ortega M.T.C.<sup>1</sup>

### ORIGINAL ARTICLE

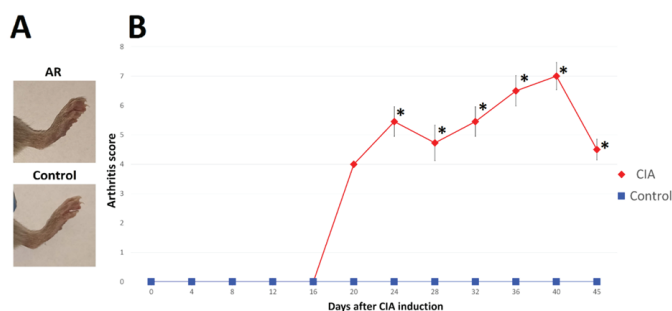
#### ABSTRACT

**Introduction:** Due to their characteristics, they are capable of vectorizing hydro- and lipophilic active compounds, modifying their pharmacokinetics. Additionally they can be designed for site-specific release of your content. In the present work we use penicillamine, an amino acid product of penicillin, encapsulated in cationic liposomes and formulated in gel to modify inflammation in an animal model of rheumatoid arthritis using mice DBA/1 strain, which have the characteristic of being prone to autoimmune diseases.

**Objectives:** Produce an anti-inflammatory gel formulation for topical application and detect its effect in a mouse model of rheumatoid arthritis.

**Methodology:** A liposomal system containing penicillamine was manufactured with nanometric dimensions ( $\approx 80$  nm), using the cationic lipid spermidine-cholesterol and phosphatidylcholine in a 1:1 molar ratio by sonication. A Noveon<sup>TM</sup> mucoadhesive gel was fabricated with different concentrations until one was found that supports the liposomal system without affecting its integrity. A rheumatoid arthritis-like inflammation model was implemented in DBA/1 strain mice<sup>1</sup> and the anti-inflammatory effect was tested by determining serum cytokines<sup>2,3</sup> using a Beckton Dickinson CBA<sup>TM</sup> Bead Kit and flow cytometry, spleen size and determination of inflammation in diarthrodial joints through photographic follow-up.

**Results:** The results showed that the induction of inflammation was successful, clearly showing in their joints, in addition to an increase in the size of their spleens and significant changes in the concentrations of inflammatory cytokines, especially IL-6 and IL-12, compared to control animals and untreated (Figure 1).



**Figure 1.** Arthritis score/days after CIA induction. (A) Representative animal from CIA group (AR n=11) and control group (n=4). (B) Arthritis score average by day. (\*) Statistical significant values ( $p < 0.05$ ).

<sup>1</sup> Facultad de Estudios Superiores Zaragoza. Universidad Nacional Autónoma de México (UNAM). Av. Guelatao no. 66, Colonia Ejército de Oriente, Iztapalapa C.P. 09230, Ciudad de México, México.

<sup>2</sup> Hospital Angeles Lomas. Vialidad de la Barranca s/n, Huixquilucan, México.

\*Corresponding author: tcvaldes@unam.mx, ORCID: 0000-0003-4448-9234.

**Conclusions:** It was possible to have a model of rheumatoid arthritis in mice and demonstrate the passage of the liposomal formulation through the skin, showing systemic effect on proinflammatory cytokines.

**Keywords:** penicillamine, rheumatoid arthritis, liposomal formulation.

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## Synthesis, Functionalization and Characterization of Polymersomes for the Treatment of Glioblastoma

Alves A.<sup>1,2,3\*</sup>, Nunes C.<sup>4</sup>, Reis S.<sup>4</sup>, Pinto M.<sup>2,5</sup>, Ferreira D.<sup>1,3</sup>, Costa P.<sup>1,3</sup>, Correia-da-Silva M.<sup>2,5</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Globally, about 189,000 people die every year because of any kind of brain cancer, being glioblastoma (GBM) the most common and aggressive form<sup>1</sup>. Chemotherapy is difficult due to the heterogeneity of brain cancer and great efficacy of Blood Brain Barrier (BBB) making drug absorption into brain very difficult<sup>2</sup>. The main objective of this study was to develop polymersomes (PMs) of PEG-PLA able to cross the BBB with the help of transferrin peptide (TRFp) and deliver a synthetic xanthone with proven antitumor activity, at the site of the tumor<sup>3</sup>.

**Methodology:** PEG-PLA PMs formulations were studied and functionalized with TRFp by click chemistry. PMs were prepared by solvent displacement method. TRFp-PEG-PLA PMs was periodically evaluated regarding mean diameter using dynamic laser scattering (DLS). The entrapment efficiency was analyzed by high performance liquid chromatography (HPLC).

**Results and Discussion:** The conjugation efficiency was 68%. Different amounts of TRFp were tested, 0%, 0.1%, 0.25%, 0.5% and 1% and the number of ligands was 0, 4, 13, 23, and 31 respectively. The mean diameter was around 100 nm. Transmission electron microscopy (TEM) images corroborate the mean diameter obtained in the DLS. The entrapment efficiency with XGAC was 65%. Viability assay showed that the different amounts tested of TRFp-PEG-PLA PMs were non-toxic. The binding/uptake showed that the 0.1% of TRFp-PEG-PLA PM formulation seemed to be the most absorbed. PMs showed a small size distribution. The empty formulations were not cytotoxic to bEnd3 cells. The functionalization of PMs with TRFp showed an increased binding/uptake by the bEnd3 cells, particularly the formulation with 0.1% (mol/mol) TRFp. On the other hand, the permeability study showed that the formulation with 0.25% TRFp-PEGPLA seemed to be the best formulation. Since the 0.1% TRFp-PEG-PLA PM and 0.25%TRFp-PEG-PLA PM formulations showed better results in binding/uptake and permeability, these were tested with XGAC incorporated into bEnd3 and U-373 cells. We found that the formulations showed no toxicity in bEnd3. Regarding U-373 MG, the empty formulations showed lower toxicity when compared to the formulations with XGAC incorporated.

<sup>1</sup> UCIBIO – Applied Molecular Biosciences Unit, MedTech-Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Rua Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

<sup>2</sup> Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

<sup>3</sup> Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal.

<sup>4</sup> LAQV/REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

<sup>5</sup> Interdisciplinary Center of Marine and Environment Research (CIIMAR), University of Porto, Terminal dos Cruzeiros do Porto de Leixões, Avenida General Norton de Matos 4450-208 Matosinhos, Portugal.

\*Corresponding author: Ana Alves (anadaniela92@hotmail.com).

**Keywords:** polymersomes, glioblastoma, synthesis.

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## Chemical and Bioactive Characterization of Phytosterol Esters-rich Fractions Derived from Sulphite Pulping with Potential Cosmeceutical Application

Evtyugin D.<sup>1</sup>, Pinho M.<sup>2</sup>, Neves B.<sup>3</sup>, Casal S.<sup>4</sup>, Domingues M.R.<sup>3\*</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** In recent studies, sterol-derivatives have been discovered in extracts obtained from by-product streams of sulphite pulping (alkaline extracts - AE), generated during bleaching processes. However, the detailed structure and bioactivity of these sterol-derivatives remains largely unknown. A comprehensive evaluation to determine their potential applications in cosmeceutical, or other industries, is required.

**Methods:** Sitosterol enriched fractions were obtained from AE-derived samples using nontoxic food grade solvents (hexane and ethanol) (Evtyugin et al., 2023). The lipidic components (Folch extraction) were analysed using thin layer chromatography (TLC) for the identification of major lipid pools. Gas chromatography mass spectrometry (GC-MS) was used to identify free and esterified fatty acids (as FAME). Electrospray mass spectrometry (ESI-MS/MS) was used to identify phytosterol esters (PE). In vitro anti-inflammatory and antidiabetic activities were evaluated using commercial inhibitory screening assay kits for cyclooxygenase-2 (COX-2) and  $\alpha$ -amylase and  $\alpha$ -glycosidase, respectively; and the antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

**Results:** Total lipids accounted for 88.3% (hexane) and 97.5% (ethanol) of the extracts. Three main fractions were identified in both extracts: free sterols (FS), free fatty acids (FFA) and PE. The analyses of PE by ESI-MS and MS/MS allowed the identification of 3 PE as  $[M+Li]^+$  ions, namely sitosteryl linoleate, sitosteryl oleate and sitostanyl linoleate, corroborating FA identification. PE were identified after total acid hydrolysis, silylation and GC-MS analysis. The lipid extract exhibited inhibitory activities for  $\alpha$ -amylase,  $\alpha$ -glycosidase and COX-2, being the highest for the latter, in the order of 95.1 (ethanol) to 96.8 % (hexane), and antioxidant activity was of  $24.1 \pm 8.3$  (ethanol) and  $18.2 \pm 0.7$  (hexane) trolox equivalents.

**Conclusions:** Lipid fractions enriched in PE from pulping by-products were characterized for the first time, allowing the identification of PEs, FS and FFA. The observed anti-inflammatory, anti-diabetic and antioxidant activities support their potential use in development of bioactive additives in cosmeceutical/other industries.

**Keywords:** phytosterols, bioactivity, cosmeceutical.

<sup>1</sup> CICECO, Department of Chemistry, University of Aveiro, Aveiro, Portugal.

<sup>2</sup> CESAM—Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Aveiro, Portugal.

<sup>3</sup> CESAM and Mass Spectrometry Centre, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal.

<sup>4</sup> LAQV-REQUIMTE, Faculty of Pharmacy, University of Porto, Porto, Portugal.

\*Corresponding author: mrd@ua.pt.

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## Efficacy of a 3D-Printed Personalized Hydrogel Patch to Prevent Skin Lesions

Graça A.<sup>1\*</sup>, Pinto P.<sup>1,2</sup>, Raposo S.<sup>3</sup>, Ribeiro H.M.<sup>1</sup>, Marto J.<sup>1</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** It is known that healthcare professionals (HCP) use Personal Protective Equipment (PPE), such as FFP2 and surgical masks, for long periods of time. Flare-up rosacea has been reported as one of the most common mask-related skin lesions, alongside “maskne” and dermatitis, due to the tension forces exerted by the mask<sup>1</sup>. A possible way to prevent this type of injury is by incorporating dressings between the face and the PPE, where pressure is more concentrated and suffers repeated rubbing, such as the upper edge of the nose bridge and cheekbones. HCPs already adopted this strategy. However, an innovative two-in-one treatment approach would be using the same dressing as a vehicle to incorporate active ingredients for treating PPE-related skin lesions. 3D Printing is a technology used to produce personalized hydrogel-based patches with different drugs, concentrations, patterns, and release rates<sup>2</sup>. This study aims to develop and test the efficacy of a personalized gelatin-based hydrogel patch containing metronidazole to treat rosacea and prevent further skin lesions<sup>3</sup>.

**Material and Methods:** The patch formulation containing gelatin, tannic acid and other sustainable ingredients was optimized using a 3-factor central composite design using a Quality by Design approach (QbD). The design of the patches (placebo and with metronidazole) were personalized using Allevi2 Online Slicer. Three designs were evaluated: an occlusive patch and two other patches with different patterns varying the infill type (Grid vs Triangular) and an infill distance of 1.3 mm. The efficacy of the occlusive hydrogel patch (placebo) as an effective skin-barrier protector was assessed by *in vivo* studies using 10 healthy volunteers while using FFP2 masks. Facial skin temperature, skin surface, transdermal water loss (TEWL), and red spots were evaluated using biometric equipment. The *in vitro* release assay (n = 6) was performed with metronidazole-containing patches using Franz diffusion cells, with an occlusive system using part of a surgical mask to simulate the moist environment provided by the prolonged and continuous use of masks and how it impacted the drug release. The concentration of metronidazole was determined at 315 nm using an HPLC.

**Results:** The QbD approach was useful to optimize the formula. The *in vivo* results showed an overall slight decrease in the facial temperature and TEWL, an increase in hydration ( $p < 0.05$ ), and the red spots decreased over 20%. Triangular-design and occlusive patches showed a similar release kinetic of  $55 \pm 3.38\%$  after 4 hours, while Grid-design showed a release of  $75.29 \pm 6.43\%$ .

<sup>1</sup> Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; angelicagrac@campus.ul.pt (A.G.); hribeiro@campus.ul.pt (H.M.R.); jmmarto@ff.ulisboa.pt (J.M.)

<sup>2</sup> PhD Trials, Avenida Maria Helena Vieira da Silva, n° 24 A – 1750-182, Lisboa, Portugal; geral@phdtrials.com (P.P.)

<sup>3</sup> Laboratório Edol – Produtos Farmacêuticos, S.A., Linda-a-Velha, Portugal; sraposo@edol.pt (S.R.)

\*Corresponding author: angelicagrac@campus.ul.pt.

**Conclusion:** The different release rates of different designs proving personalized treatment and the biological efficacy are good indicators that this device can prevent skin lesions and promote healthier skin during mask usage.

**Keywords:** 3D Printing, *in vivo* study, *in vitro* release.

**Acknowledgments:** Funded by the Fundação para a Ciência e Tecnologia, Portugal (UIDB/04138/2020 and UIDP/04138/2020 to iMed.U.Lisboa, CEECINST/00145/2018 to J. Marto and fellowship 2020.10138.BD to A. Graça).

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## From 3D Printing to the Delivery of Bioactives From Topical Patches: a Personalized Approach

Bom S.<sup>1\*</sup>, Santos C.<sup>1,2,3</sup>, Cláudio R.<sup>1,2</sup>, Pinto P.<sup>1,4</sup>, Ribeiro H.M.<sup>1</sup>, Marto J.<sup>1</sup>

### ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** The cosmetic industry is currently witnessing a surge in the personalized skincare trend. Simultaneously, 3D Printing (3DP) is emerging as a solution for creating customized skincare products that address individual skin needs by enabling the adjustment of bioactives release rates and dosages, and to print different designs and geometries<sup>1,2</sup>. This research aimed to study the influence of patches' internal design in releasing kinetics and penetration profiles of 3D-printed gelatin-based patches for topical delivery of niacinamide, using *in vitro* and *in vivo* approaches.

**Materials and Methods:** Patches containing 40% (w/w) gelatin type B (Acofarma, Madrid, Spain), 10% (w/w) sucrose (Fisher Scientific, Hampton, United States), 10% (w/w) glycerin (Lacrilar, Torres Vedras, Portugal), and 5% (w/w) RonaCare<sup>®</sup> Nicotinamide as an anti-aging cosmetic bioactive (INCI: Niacinamide, Merck KGaA, Darmstadt, Germany) were printed by an extrusion-based 3D-printer (Allevi2, Allevi, USA). Before printing, the design of the porous patches was personalized using the Allevi2 Online Slicer, employing a grid infill (line distance=1.3 mm). The *in vitro* release assay (n = 6) was performed using a Franz cell system mounted in a sandwich manner, mimicking a Plastic Occlusion Stress (POST) application technique<sup>3</sup>. The samples (200  $\mu$ L) were collected up to 24h with the volumes being replaced with fresh Phosphate-buffered saline (PBS) solution, and the absorbances were measured at 262 nm in a Fluostar Omega microplate reader (BMG Labtech, Germany). For *in vivo* penetration studies, occlusive and porous patches were applied to the ventral side of the forearm of healthy volunteers (n=10) using a POST technique. A Confocal Raman Spectroscopy (CRS) was used to quantify the niacinamide penetration (gen2-SCA, RiverD, Netherlands), recording at a maximum depth of 40  $\mu$ m.

**Results and Discussion:** *In vitro*, porosity influenced niacinamide release, with the porous-designed patches showing a 44.3% increased release rate compared to the occlusive ones, after 10h. The CRS *in vivo* data demonstrate that niacinamide penetrated up to 38  $\mu$ m (stratum corneum), showing a maximum penetration of 69.55 mg niacinamide/cm<sup>3</sup>. Other relevant studies are ongoing to explore: i) *in vivo* the influence of different internal designs; and, ii) understand the possibility of incorporating different concentrations of niacinamide or mash-up different internal designs within the same patch structure according to the skin location needs.

<sup>1</sup> Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal.

<sup>2</sup> EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Portugal.

<sup>3</sup> CQE Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1049-001, Lisboa, Portugal.

<sup>4</sup> PhD Trials, Avenida Maria Helena Vieira da Silva, n<sup>o</sup> 24 A – 1750-182, Lisboa, Portugal.

\*Corresponding author: sarabom@campus.ul.pt.

**Conclusion:** This work delivered insight into the practicality of using 3DP to personalize the bioactives' release by employing straightforward printing design strategies. In addition, new solutions for evaluating and adjusting cosmetic outcomes can be explored by quantifying such effects *in vivo*.

**Keywords:** 3D printing, topical patches' personalization, *in vivo* performance.

**Acknowledgments:** This research was funded by the Fundação para a Ciência e Tecnologia, Portugal (UIDB/04138/2020 and UIDP/04138/2020 to iMed.Ulisboa, CEECINST/00145/2018 to Marto J, CQE UIDB/QUI/00100/2020 to Santos C and fellowship UI/BD/153624/2022 to Bom S), and Instituto Politécnico de Setúbal (Project 3Dgelcomp/IPS).

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## Designing and Structural Analysis of an Innovative Pickering Emulsion for Skin Hydration and Photoprotection

Gomes M.<sup>1,2\*</sup>, Pezzini B.<sup>2</sup>, Gouveia L.<sup>1</sup>, Ribeiro H.<sup>1</sup>, Marto J.<sup>1</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Pickering emulsions are surfactant-free systems stabilized by solid particles, offering reduced toxicity, better skin compatibility and a more sustainable obtaining process. In this study, we used a quality by design approach to develop a Pickering emulsion with zinc oxide (ZnO) as the sunscreen, trihydroxystearin (THS) as a texture-improving thickener, and squalane (external phase) as an emollient that helps prevent moisture loss and restores suppleness to skin.

**Methodology:** A central composite design was performed to identify the critical variables of the composition (THS and water percents) and the process (time and stirring speed), affecting the droplet size distribution. The rheological and mechanical properties of the optimized formulation were investigated using a Kinexus Lab+ Rheometer on days 01 and 30 at 25 °C, and on day 30 at 40 °C.

**Results and Discussion:** The design space was established, being the optimal formulation defined as THS 13%, ZnO 20%, water 22% and squalane 45%, stirred for 10 min at 17,500 rpm to ensure high dispersion and small droplet size. The formulation showed a white-homogeneous appearance, pH ~5.5, and a monomodal size distribution with d(90) between 10 and 14  $\mu\text{m}$  (span <0.6). Under the microscope, ZnO particles were dispersed both in the internal and external phases. THS particles remained dispersed in the oil phase being responsible for the formation of the Pickering emulsion and acting as a thickener agent in the formulation. Enclosing ZnO particles in the dispersed phase contributed to acidic pH maintenance of the formulation. In the structural analysis, all samples behaved as non-Newtonian fluids (shear-thinning) with thixotropic hysteresis loops; all of them exhibited  $G'$  values higher than  $G''$  (elastic modulus predominant); and suffered deformation with recover of the former structure when removing the shear stress. Adhesiveness of the sample stored for 30 days at 40 °C was lower ( $p < 0.05$ ) compared to the unheated samples (days 1 and 30,  $p > 0.05$ ).

**Conclusion:** The use of THS and ZnO particles ensured small droplets and suitable rheological properties, resulting in a stable and innovative photoprotective Pickering emulsion for topical application. Further investigation will be required to explore the photoprotective efficacy, safety and water resistance of this formulation.

**Keywords:** cosmetic technology, pickering emulsion, sunscreens agents.

<sup>1</sup> Research Institute for Medicine (iMed.Ulisboa), Faculty of Pharmacy, University of Lisbon, 1649-003 Lisbon, Portugal.

<sup>2</sup> Department of Pharmaceutical Sciences, Federal University of Santa Catarina, Florianópolis, 88040-900, Santa Catarina, Brazil.

\*Corresponding author: marina.gomes@posgrad.ufsc.br.

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## Formulations of mPEG-PLGA Nanoparticles Dry Powder for Subsequent Antibody Loading Aimed at Lung Cancer Treatment by Inhalation

Viegas C.<sup>1,2,3,4\*</sup>, Duarte S.<sup>3,4</sup>, Lourenço J.<sup>5,6</sup>, Grenha A.<sup>2,6</sup>, Fonte P.<sup>2,3,4,6\*</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Lung cancer has a high mortality rate among all common cancers, estimated to be responsible for about 1 in 5 cancer deaths<sup>1</sup>. Conventional therapies are usually administered intravenously with low selectivity for tumor cells, requiring high doses to achieve the therapeutic effect, leading to potential side effects. Therapeutic proteins such as antibodies are useful in treatment due to their higher specificity and bioactivity, and lower toxicity compared to small molecule drugs. Antibody encapsulation into nanoparticles for pulmonary delivery is a promising strategy, which combines targeted and controlled drug delivery with the ability to protect antibody structure and bioactivity<sup>2</sup>. Therefore, the aim of this work was the development of mPEG-PLGA nanoparticles formulated into a dry powder by spray-drying aimed at localized lung cancer treatment.

**Methodology:** The optimization of nanoparticles followed a Design-of-Experiment (DoE) approach to target the desired features: small particle size and good colloidal stability. The polymer mass and surfactant concentration were considered as variables with a significant effect on nanoparticles properties, namely in particle size. The spray-drying optimization revealed that D-mannitol and L-leucine, used in combination, were the best matrix excipients to obtain particles (of micro scale).

**Results and discussion:** The optimized nanoparticles were produced with 150 mg mPEG-PLGA and 1% Tween<sup>®</sup>80, presenting the lowest particle size of  $\approx 300$  nm, polydispersity index of  $\approx 0.200$  (dynamic light scattering), and zeta potential of  $\approx -25$  mV (electrophoretic light scattering), considered to be suitable features for antibody encapsulation. In spray-drying optimization, D-mannitol and L-leucine used at concentrations of 2% and 1% (w/v), respectively, allowed an increase in the yield up to  $\approx 60\%$  and reduction in powder adhesion to the apparatus walls due to leucine ability as dispersibility enhancer. L-leucine also seems to reduce particle agglomeration after spray-drying due to its crystallization, surrounding the droplet with an outer shell. On the other hand, lactose, and D-trehalose were also tested as alternatives to mannitol, but resulted in hygroscopic powders with a sticky appearance. Further studies will focus on antibody loading, being bevacizumab the model, aimed at establishing an inhalable lung cancer therapy based on spray-dried microencapsulated nanoparticles<sup>3</sup>. Antibody-matrix interactions, its structure, and bioactivity maintenance will be also evaluated.

**Keywords:** antibody encapsulation, inhalation, lung cancer, nanoparticle, spray-drying.

<sup>1</sup> Faculty of Medicine and Biomedical Sciences (FMCB), Universidade do Algarve, Gambelas Campus, 8005-139 Faro, Portugal.

<sup>2</sup> Center for Marine Sciences (CCMar), Universidade do Algarve, Gambelas Campus, 8005-139 Faro, Portugal.

<sup>3</sup> IBB—Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, 1049-001 Lisboa, Portugal.

<sup>4</sup> Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001 Lisboa, Portugal.

<sup>5</sup> Centro de Química Estrutural (CQE), Instituto Superior Técnico, University of Lisbon, 1049-001 Lisboa, Portugal.

<sup>6</sup> Department of Chemistry and Pharmacy, Faculty of Sciences and Technology, Universidade do Algarve, Gambelas Campus, 8005-139 Faro, Portugal.

\*Corresponding authors: viegas.claudiasofia@gmail.com, prfonte@ualg.pt.

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## Sprawling Oral Cancer Treatment Using Mixed Pluronic®-Based Nanosystems

Domingues C.<sup>1,2,3\*</sup>, Jarak I.<sup>1,4</sup>, Veiga F.<sup>1,2</sup>, Dourado M.<sup>3</sup>, Vitorino C.<sup>1,5</sup>, Figueiras A.<sup>1,2</sup>

### ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** Oral squamous cell carcinoma (OSCC) is the most common type of mouth neoplasm, presenting high morbidity and poor 5-year survival<sup>1</sup>. To circumvent these issues the use of nanomedicine has emerged. Pluronics®, tri-block co-polymers, with “smart” properties have been investigated for the co-delivery of different therapeutic agents, including genetic material or imaging cargos<sup>2</sup>. Thus, the main goal of this work is to covalently link Pluronics® with polyethyleneimine (PEI) for gene/active compound co-delivery to boost OSCC treatment.

**Methods:** A two-step synthesis was performed to conjugate Pluronics® with PEI<sup>3</sup>. Structural characterization was performed using FTIR and <sup>1</sup>H-NMR spectroscopy. Physicochemical characterization was performed using Dynamic/Electrophoretic Light Scattering (DLS/ELS). A scrambled siRNA-Cy5 was used to assess the cellular uptake by flow cytometry and confocal microscopy. Gel electrophoresis was employed to estimate the polymers’ capabilities to complex genetic material. A phenolic acid was used as an active therapeutic compound model. The SCC-9 and HSC-3 OSCC cell lines were used for *in vitro* studies.

**Results:** Pluronics®-PEI synthesis was supported by characteristic peaks in FTIR and NMR spectra. All the synthesized polymers have demonstrated the ability to complex genetic material, with improved cellular uptake of siRNA-Cy5 for the coded modified polymer PP, which appears to perform similarly to Lipofectamine in OSCC cells. Following the aim of dual therapy, a phenolic acid was used as an active compound and Pluronic®F127 was chosen as a “smart” co-polymer to improve its solubility. Mixing the modified polymer, PP, and Pluronic®F127 in a 1:3 molar ratio has resulted in enhanced aqueous solubility from 5 to 36 mg/mL. The hydrodynamic diameter decreases in the presence of the active compound from around 150 to 50 nm, with a polydispersity index between 0.1-0.4, and a positive zeta potential, which is advantageous for the electrostatic interactions with genetic material, and the cellular uptake. The different tested nanosystems have decreased metabolic activity in a dose, time and cell type-dependent manner<sup>3</sup>.

**Discussion:** These data suggest that the synthesized co-polymer mixed with Pluronic®F127 may constitute an advantageous dual therapeutic strategy to co-delivery genetic/active compounds to OSCC cells.

**Keywords:** Genetic material; mouth neoplasm; nanoparticles; phenolic acid.

<sup>1</sup> Univ Coimbra, Faculty of Pharmacy, Drug Development and Technologies Laboratory, Coimbra, Portugal.

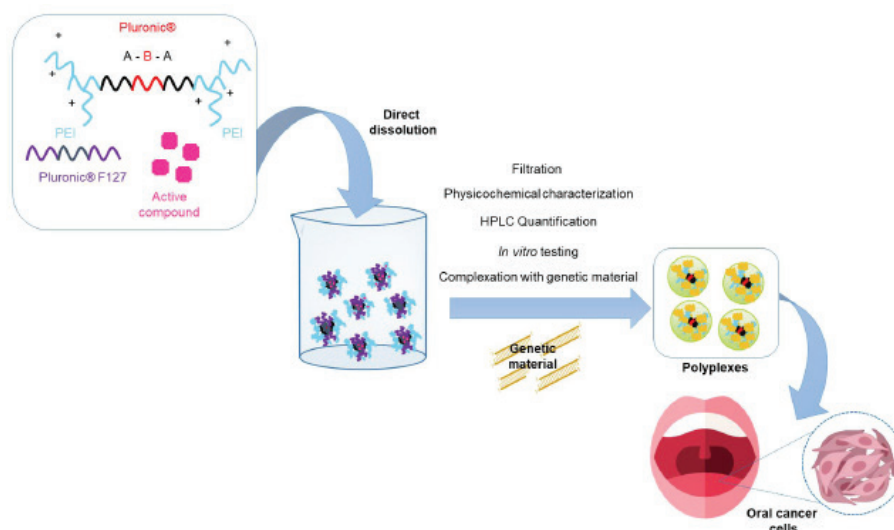
<sup>2</sup> REQUIMTE/LAQV, Drug Development and Technologies Laboratory, Faculty of Pharmacy, University of Coimbra, Portugal.

<sup>3</sup> Univ Coimbra, Institute for Clinical and Biomedical Research (iCBR) area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, Coimbra, Portugal.

<sup>4</sup> Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal.

<sup>5</sup> Univ Coimbra, Coimbra Chemistry Centre, Department of Chemistry, Faculty of Sciences and Technology, Coimbra, Portugal.

\*Corresponding author: cdomingues@ff.uc.pt.



**Figure 1.** Schematic representation of the production of polymeric micelles for gene/drug co-delivery intended for the treatment of oral cancer.

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## Toll-like Receptors as Oral Vaccine Adjuvants: Immunomodulatory Effects against SARS-CoV-2

Félix P.<sup>1\*</sup>, Costa J.P.<sup>1,2</sup>, Colaço M.<sup>1,2</sup>, Borges O.<sup>1,2</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Toll-like receptors (TLRs) are pattern recognition receptors responsible for detecting invading pathogens. CL097, a TLR7/8 agonist, and Poly (I:C), a TLR3 agonist, are TLR ligands that have demonstrated immunomodulatory properties<sup>1,2</sup>.  $\beta$ -glucan is another compound that has also exhibited immunomodulatory potential. This polymer can activate immune cells through receptors such as TLR2/6, dectin-1, and CR3<sup>3</sup>. Glucan particles (GPs) derived from baker's yeast (*Saccharomyces cerevisiae*) are a promising delivery system capable of encapsulating a variety of compounds. The objective of this work was to develop a glucan-based system with CL097 or Poly (I:C) encapsulated within GPs and evaluate their potential as immune response stimulants and vaccine adjuvants.

**Materials and Methods:** GPs were obtained by a series of alkaline and acidic extractions and both TLRs ligands (CL097 and Poly (I:C)) were encapsulated through osmotic gradient. Particle size and zeta potential were measured. Reactive oxygen species (ROS) and nitric oxide (NO) production were evaluated in the murine macrophage cell line, RAW 264.7. ROS production was also evaluated in human neutrophils isolated from buffy coat. A proliferation assay was performed on PBMC's isolated from human buffy coat. Additionally, cytokine quantification was performed by ELISA in C57BL/6 mice spleen cells' supernatant, RAW 264.7 and human neutrophils. SARS-CoV-2 antigen was incorporated into the GPs-CL097 formulation and tested as an oral vaccine in C57BL/6 mice, immunized subcutaneously on day 0, followed by two oral immunizations on day 14 and 28.

**Results:** GPs presented a size of approximately 5  $\mu$ m and a neutral surface charge. CL097 and Poly (I:C) loaded GPs showed concentration-dependent and synergistic effects on ROS and NO production in RAW 264.7 cells. Only free CL097 induce ROS production in human neutrophils. Proliferation assays showed concentration-dependent response with GPs. CL097 and Poly (I:C) loaded GPs promoted TNF- $\alpha$  and IL-6 production in mice spleen cells and RAW 264.7 cells. For the *in vivo* study it was observed a balance Th1, Th2 and Th17 immune response.

**Discussion:** The combination of different TLRs ligands with GPs demonstrated proinflammatory and immunomodulatory activity, highlighting their potential as vaccine adjuvants.

**Keywords:** vaccine adjuvants, Toll-like receptors, SARS-CoV-2 vaccine.

<sup>1</sup> Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

<sup>2</sup> Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.

\*Corresponding author: paulo.f.miguel0273@gmail.com.

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## Otimização da Entrega de Fármacos com Transportadores Lipídicos Nanoestruturados no Glioblastoma

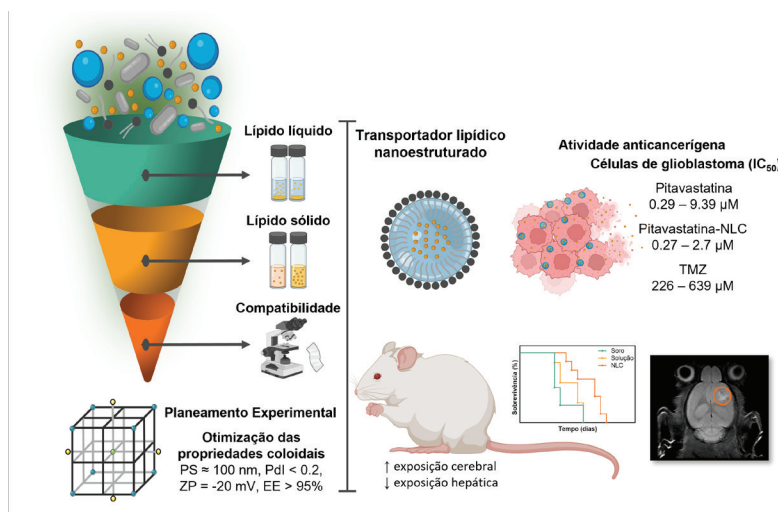
*Breaking Barriers: optimizing drug delivery in glioblastoma with nanostructured lipid carriers*

Basso J.<sup>1,2</sup>, Sousa J.<sup>1,2</sup>, Vitorino R.<sup>3,4,5\*</sup>, Fortuna A.<sup>1,6</sup>, Vitorino C.<sup>1,2</sup>

### ORIGINAL ARTICLE

### RESUMO

**Introdução:** O glioblastoma (GB) é o tumor primário cerebral mais frequente e letal, reconhecido pela sua heterogeneidade e falta de estratégias terapêuticas eficazes. Notavelmente, as estatinas demonstram, de forma consistente, um efeito anticancerígeno mais pronunciado do que a terapêutica clínica (temozolomida, TMZ)<sup>1</sup>. No entanto, o acesso das estatinas ao cérebro é limitado pela barreira hematoencefálica, pelo que possuem uma baixa disponibilidade no tecido tumoral<sup>2</sup>. Desta forma, foram desenvolvidos transportadores lipídicos nanoestruturados (NLCs, do inglês *nanostructured lipid carriers*) para a encapsulação de pitavastatina, através de um método com transposição facilitada de escala e sem recurso a solventes orgânicos (Figura 1).



**Figura 1.** Desenvolvimento e otimização de transportadores lipídicos nanoestruturados para a encapsulação de pitavastatina como nova abordagem terapêutica contra o glioblastoma.

<sup>1</sup> Faculdade de Farmácia, Universidade de Coimbra, Coimbra, Portugal.

<sup>2</sup> Centro de Química de Coimbra, Instituto de Ciências Moleculares, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Coimbra, Portugal.

<sup>3</sup> iBiMED, Departamento de Ciências Médicas, Instituto de Biomedicina-, Universidade de Aveiro, Aveiro, Portugal.

<sup>4</sup> UnIC@RISE, Departamento de Cirurgia e Fisiologia, Faculdade de Medicina, Universidade do Porto, Porto, Portugal.

<sup>5</sup> LAQV/REQUIMTE, Departamento de Química, Universidade de Aveiro, Aveiro, Portugal

<sup>6</sup> CIBIT, Centro de Imagem Biomédica e Investigação Translacional, Universidade de Coimbra, Coimbra, Portugal.

\*Autor correspondente: Rui Vitorino, csvitorino@ff.uc.pt.

**Metodologia:** Os NLCs foram produzidos por homogeneização a elevada pressão, a quente e otimizados segundo uma abordagem sistemática Quality by Design<sup>3</sup>. A atividade do nanossistema foi avaliada em diferentes linhas celulares de GB, e comparada com a pitavastatina e TMZ. Estudos *in vivo* de biodistribuição foram realizados em murganho após injeção intravenosa (15 mg/Kg), sendo o fármaco quantificado em seis matrizes diferentes, por HPLC com deteção por fluorescência. A eficácia foi avaliada num modelo ortotópico, através da medição periódica do volume tumoral por ressonância magnética nuclear<sup>4</sup>.

**Resultados:** Os NLCs possuem um tamanho nanométrico ( $\approx 100$  nm), são monodispersos ( $PdI < 0.2$ ), apresentam um potencial zeta negativo ( $\approx -20$  mV), e uma eficiência de encapsulação superior a 95%. Dados *in vitro* mostram uma libertação controlada da pitavastatina, bem como uma forte redução na viabilidade celular ( $IC_{50}=0.29-9.39 \mu M$  vs  $226 - 639 \mu M$  TMZ). Os resultados de biodistribuição destacam a elevada e reduzida exposição cerebral e hepática, respetivamente, bem como a elevada eficiência de vectorização (DTE = 175%). É evidenciado um crescimento tumoral mais lento associado a um prolongamento da sobrevida dos animais, quando comparado com o fármaco em solução e o grupo controlo (soro fisiológico). O tratamento mostrou ser seguro, como confirmado pela monitorização de vários parâmetros bioquímicos.

**Discussão:** A encapsulação da pitavastatina nos NLCs, como estratégia tecnológica para melhorar a sua solubilidade, permitiu a obtenção de uma formulação coloidal estável e com atividade antitumoral superior, comparativamente ao fármaco não encapsulado. A administração da formulação em murganhos promove uma maior exposição do cérebro ao fármaco, refletindo-se num crescimento mais lento do tumor, bem como num aumento da sobrevida dos animais.

**Palavras-chave:** glioblastoma, nanopartículas, pitavastatina.

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## Poly(D,L-lactic acid) Scaffolds as an Innovative Approach to the Treatment of Mixed *S. Aureus*-*C. Albicans* Biofilms

Zegre M.<sup>1,2\*</sup>, Barros J.<sup>3,4</sup>, Ribeiro I.A.C.<sup>1</sup>, Santos C.<sup>5,6</sup>, Caetano L.<sup>1,2</sup>, Gonçalves L.<sup>1</sup>, Monteiro F.J.<sup>3,4,7</sup>, Ferraz M.P.<sup>3,4,7</sup>, Bettencourt A.<sup>1</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** The treatment of bacterial joint and bone infections in patients after multiple revision arthroplasties is very challenging. An expanding number of studies report the co-isolation of fungal and bacterial species (such as *Candida albicans* and *Staphylococcus aureus*) from polymicrobial biofilm associated with infections related to bone infections<sup>1</sup>. Current investigations establish that local-specific drug delivery scaffolds with low toxicity and increased efficiency to specific sites, when compared to oral and systemic administration approaches, can considerably lower the number of viable microorganisms in polymicrobial biofilms, preventing simultaneously the progression of infection in bone disorder<sup>2</sup>. Notably, the development of co-delivery systems of at least two antimicrobials is yet a neglected approach, while it may be a critical strategy for the treatment of infections associated with polymicrobial biofilms. Simultaneously, it is recommended to assess the contribution of each microbial population within biofilm in order to select the best therapy to treat polymicrobial infections. Among different biomaterials used in scaffolds as drug-delivery carriers, poly (lactic acid) (PLA) based polymers are being widely studied due to their versatility, low toxicity and tailored biodegradability having the US Food and Drug Administration approval for clinical use. The adequate osteoconductive and anti-*S. aureus* effects of a collagen-functionalized poly(D,L-lactic acid) (PDLLA) porous scaffold loaded with minocycline (a tetracycline antibiotic) have been previously demonstrated<sup>3</sup>. In the present study, we focus on the problem of mixed bacterial-fungal biofilm infections and the joining of two antimicrobials in the PDLLA scaffold. Minocycline and voriconazole (an antifungal triazole) were the chosen model drugs, since minocycline may represent a promising drug that can be administered in combination with azoles (namely voriconazole) to treat infections caused by pathogenic *Candida* species. Morphological and chemical properties of the co-delivery PDLLA scaffolds, as well as drug release profiles, were examined. The antibiofilm activity of these drug delivery systems was tested against single- and dual-species biofilms of *S. aureus* and *C. albicans*. The formation of dual-species *S. aureus* – *C. albicans* biofilms was studied over time to understand the relationship between both microorganisms during in vitro biofilm formation. Cytocompatibility and osteoconductive tests were also conducted using MG-63 osteoblasts to assess the biocompatibility of the PDLLA scaffolds.

**Methods:** Using the solvent casting/particulate leaching method, PDLLA scaffolds loaded with minocycline (PDLLA-Min), voriconazole (PDLLA-Vor), and ultimately with both drugs (PDLLA-Min-Vor), were produced. Scanning electron microscopy (SEM) was used to evaluate the surface morphology, while Fourier transform infrared spectroscopy-attenuated

<sup>1</sup> Research Institute for Medicines (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal.

<sup>2</sup> H&TRC - Centro de Investigação em Saúde e Tecnologia, ESTeSL - Escola Superior de Tecnologia da Saúde de Lisboa, IPL - Instituto Politécnico de Lisboa, Lisboa, Portugal.

<sup>3</sup> i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal.

<sup>4</sup> INEB - Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Porto, Portugal.

<sup>5</sup> CQE - Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.

<sup>6</sup> EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Setúbal, Portugal.

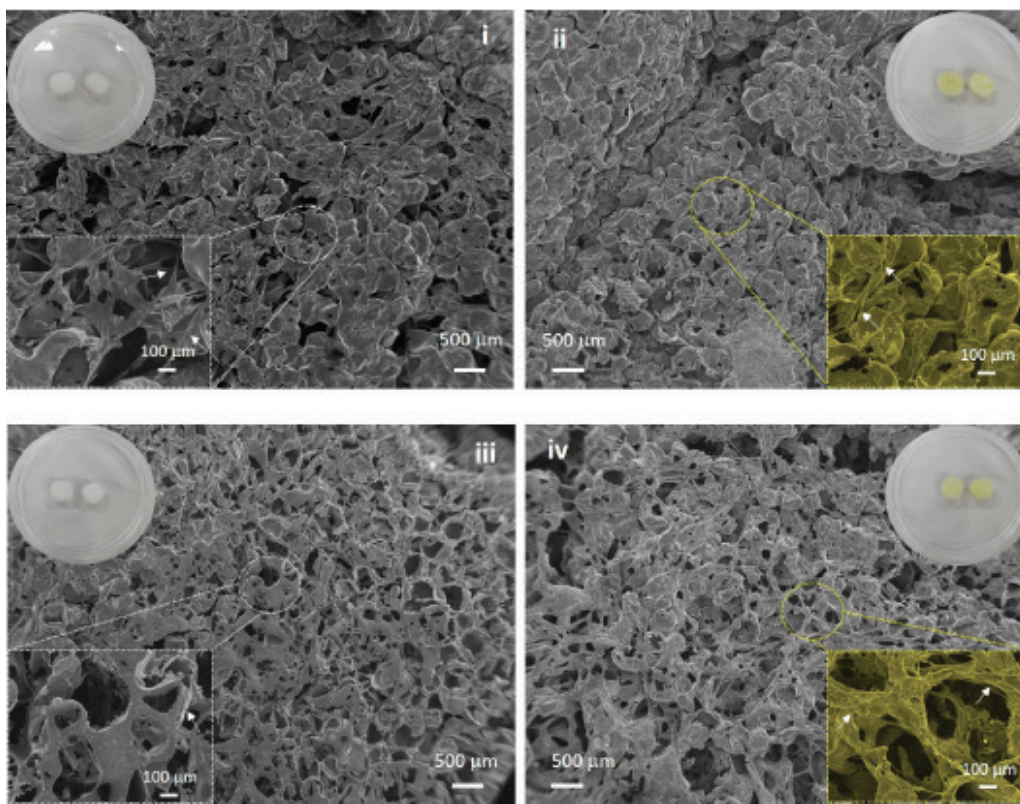
<sup>7</sup> FEUP/DEMM - Departamento de Engenharia Metalúrgica e de Materiais, Faculdade de Engenharia da Universidade do Porto, Porto, Portugal.

\*Corresponding author: miguel.zegre@estesl.ipl.pt.

total reflection (FTIR-ATR) enabled the investigation of chemical composition. Drug release profiles were investigated at 37°C with HEPES buffer, collecting aliquots of the supernatant at fixed time points and analyzing them in triplicate. In order to quantify the antimicrobials, for the antibiotic (minocycline) UV-spectrophotometry was selected ( $\lambda = 350$  nm), while for the antifungal (voriconazole), high performance liquid chromatography (HPLC) was preferred. The structure and morphology of both single and dual-species biofilms were analyzed, as the antibiofilm activity of the PDLLA scaffolds. At last, cytocompatibility studies were assessed *in vitro*, namely cell proliferation (AlamarBlue® assay), alkaline phosphatase (ALP) activity and matrix mineralization (Alizarin Red assay).

**Results and discussion:** Optical and SEM images of the four groups of scaffolds (PDLLA, PDLLA-Min, PDLLA-Vor, and PDLLA-Min-Vor) revealed high porosity and a sponge-like appearance. In general, all samples presented an open and well-structured porous network composed of interconnected spherical macropores, with micropores in the macropore pore walls that can support cell proliferation and nutrition. This type of pore architecture is typical of polymeric scaffolds prepared by the solvent casting/particulate leaching method. In the FTIR-ATR spectra of pristine and drug-loaded scaffolds, the characteristic absorption bands of PDLLA were observed, indicating that drugs did not induce structural changes on the PDLLA. Moreover, this result indicates that the drugs were physically adsorbed on the scaffolds, which can be an advantage for the release process. The scaffolds showed moderate capacity to be loaded with the selected drugs, which probably reflect the hydrophobic nature of the PDLLA polymer and its relative low capacity for water adsorption. With respect to drug release profiles (Figural1), immediately after placing the scaffolds in the release media, an initial large bolus of drug was released. This phenomenon is typically referred to as the “burst phase” leading to a higher initial drug delivery. In addition, a significant amount of the drug was released within the first 24 h, which may have a positive impact on infection control on the first day after implantation. The cumulative release profile of minocycline presents a significant release in the first 6 h period ( $6.7 \pm 2.8 \mu\text{g mL}^{-1}$ ) and another substantial release up to 48 h ( $13.0 \pm 2.8 \mu\text{g mL}^{-1}$ ), a phase sometimes referred to as the “second burst”. Voriconazole was delivered from all scaffolds at cumulative concentrations above the MIC90 value of *C. albicans* ( $0.125 \mu\text{g mL}^{-1}$ ), right after the initial 15 min period of release. In the co-delivery system, voriconazole cumulative release profile also presented an initial burst release in the first 6 h ( $14.1 \pm 3.2 \mu\text{g mL}^{-1}$ ). Further, in the co-delivery system, the voriconazole release rate till 6 h was higher ( $2.3 \pm 0.5 \mu\text{g mL}^{-1} \text{ h}^{-1}$ ) compared to the single delivery system ( $1.2 \pm 0.1 \mu\text{g mL}^{-1} \text{ h}^{-1}$ ). These results propose that both drugs were adsorbed by the polymer, even in the presence of the other one. In this study, a dual-species biofilm *in vitro* model including *S. aureus* and *C. albicans* was developed to represent part of the diversity observed in orthopedic infection and for comparison purposes, single-species biofilms were also developed. SEM analysis was used to study the structure/morphology and interactions between bacterial and fungal cells in single-and dual-species biofilms. Looking at the structure and morphology of single-species biofilms, it is noticed that *S. aureus* cells were scattered almost completely on the coverslips surfaces, either as single cells or/and clusters arrangements, forming thereby a three-dimensional (3D) biofilm with mushrooms- and pillar-like structures. The single-*C. albicans* biofilm revealed a 3D structure over time, consisting of a dense mat of hyphal and yeast forms clustered in mono and multilayers. Concerning dual-species biofilm, it was shown that *S. aureus* cells were attached to the surface and overhead to the fungal structures, for either hyphal and yeast forms. A typical structure of a mature dual-species biofilm was observed, with the structural arrangement of multiple layers of bacterial and fungi cells

embedded in extracellular polymeric substance. These results are in line with several authors who have shown that the co-culture of both microorganisms results in the formation of more rigid biofilms, where *S. aureus* is located mainly in the upper layers attached to and interspersed throughout the dense network of *C. albicans* (found mostly in the lower layers) hyphal growth. These findings show that these two microbes have a synergistic relationship co-operating with each other for biofilm formation, with the fungus *C. albicans* serving as the base substrate for bacterium *S. aureus* colonization. Regarding the single-species *S. aureus* biofilm, either PDLLA-Min or PDLLA-Min-Vor scaffolds were able to avoid biofilm formation, destroying the *S. aureus*-adherence biofilm entirely. PDLLA-Vor scaffolds, on the other hand, had no effect on *S. aureus* biofilm formation, a result expected since voriconazole was used as an antifungal agent. Concerning single-species *C. albicans* biofilm, PDLLA-Vor and PDLLA-Min-Vor scaffolds reduced fungal cell densities by 97% (1.5 Logs reduction) and 96% (1.4 Logs of reduction) compared to the control PDLLA scaffolds, respectively. In a dual-species *S. aureus*-*C. albicans* biofilm, the total number of sessile cells was not affected by PDLLA-Vor scaffolds, while PDLLA-Min and PDLLA-Min-Vor scaffolds reduced cell densities by 1.7 (97.8% reduction) and 2.8 Logs (99.8% reduction), respectively, when compared to PDLLA scaffolds (control). Overall, the obtained results emphasize that the biofilm architecture and composition can interfere with antimicrobial agents' activity. Data also suggest that the use of a co-delivery system combining antibiotic and antifungal agents could be beneficial in the control and treatment of polymicrobial-biofilm-associated infections. When designing a novel drug loaded scaffold, the optimal concentrations for antimicrobial activity can be at the same time toxic to the bone cells and/or interfere with their differentiation and/or proliferation, therefore it is important to evaluate the *in vitro* cytocompatibility of the PDLLA drug-loaded scaffolds. From the results of the Alamar Blue assay, it was shown that released minocycline and voriconazole did not impair cellular proliferation, as no statistical difference was found in relation to the PDLLA control group ( $p > 0.05$ ). As alkaline phosphatase (ALP) is an early marker of osteoblast differentiation, and increased level of ALP refers to active osteoblast differentiation/proliferation, in this study the ALP activity was evaluated qualitatively (stained cells with a blue-violet color) and quantitatively during osteoblast differentiation. ALP quantitative evaluation showed that there was no significant difference ( $p > 0.05$ ) in the osteogenic differentiation of the cells exposed to the drug loaded scaffolds and the PDLLA control group (scaffolds without the drugs). The Alizarin Red assay was used to evaluate calcium-rich deposits by cells in culture indicating cellular matrix mineralization (another indicator of osteoblast differentiation/proliferation), and in a comparable fashion to ALP activity, there was no toxic effect of the antimicrobials in the matrix mineralization process of the osteoblasts. Overall, the absence of statistically significant differences ( $p > 0.05$ ) between the control (scaffolds without drugs) and the other groups (loaded drug scaffolds) is a clear indicator that the presence of antimicrobials in the tested concentrations does not affect the viability or the differentiation/proliferation of the osteoblasts. These results are in line with other authors. The developed scaffolds displayed physicochemical, and pharmaceutical properties suitable for localized antimicrobial release. Although the scaffolds' loading capacity was low, it enabled the achievement of clinically/microbiologically relevant concentrations. The PDLLA scaffolds loaded with both drugs depicted high activity against single and dual biofilm species. Supporting evidence from alkaline phosphatase expression and matrix maturation of osteoblastic cells suggests *in vitro* osteoblast differentiation/proliferation. Altogether, our *in vitro* first stage study may open paths for future works mainly dedicated to PDLLA scaffolds as a co-delivery platform for the *S. aureus* - *C. albicans* mixed bone infections management.



**Keywords:** biofilms, drug delivery systems, tissue scaffolds.

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## Is 3D-printing a useful Tool for the Development of Biosurfactant-Chitosan Coatings Intended for Medical Devices?

Cardoso S.<sup>1\*</sup>, Narciso F.<sup>1,2</sup>, Monge N.<sup>3</sup>, Gonçalves M.<sup>1</sup>, Martin V.<sup>4,5</sup>, Duarte N.<sup>1</sup>, Santos C.<sup>6,7</sup>, Gomes P.<sup>4,5</sup>, Bettencourt A.<sup>1</sup>, Ribeiro I.<sup>1</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Under healthcare settings, infections associated with medical devices represent one of the most common complications that can be life threatening. Currently, preventing and treating these infections has become challenging due to the rising microbial resistance. To fight this issue, it is essential to explore advanced approaches, such as the utilization of antimicrobial polymeric coatings.

**Objectives:** To produce 3D-printed antibacterial mesh-based coatings using a chitosan-biosurfactant hydrogel capable of incorporating active components into the mesh. Moreover, this coating was designed to prevent infections in PDMS (polydimethylsiloxane)-based medical devices.

**Methodology:** Two biosurfactants, surfactin and sophorolipids (SLs), were produced and characterized (HPLC-MS). Chitosan hydrogels were synthesized at various concentrations (1-3%), followed by rheological assessment and antibiofilm activity tests. These hydrogels were utilized to fabricate square-shaped coatings (10x10 or 20x20 mm) with a pore size of 1x1 mm. Furthermore, an optimization study was conducted using the chosen hydrogel-ink formulation to determine the optimal 3D-printing conditions. Different setups for flow speed (ranging from 0.15 to 1 mm/s), layer height (from 0.25 mm to 0.5 mm), and number of layers (2, 3, and 5 layers) were tested. Various coatings were produced and subsequently characterized (wettability, FTIR-ATR analysis, antimicrobial activity, and biocompatibility).

**Results:** SLs were produced in higher yield and exhibited stronger antimicrobial activity. The chitosan hydrogels at 2 and 2.5% concentrations demonstrated the most suitable viscosity for printing. Furthermore, the resulting mesh structure enabled the incorporation of other active components, which could be advantageous for customizing therapies. Incorporating SLs into the 2.5% chitosan hydrogel led to a higher antibiofilm activity. This Chitosan-SLs coating showed the highest antimicrobial effect (61% of growth inhibition) and the most effective antibiofilm activity (2 log units reduction) when compared to controls. Additionally, in biocompatibility tests with human dermal fibroblasts, the 3D-chitosan-SLs coatings showed cytocompatibility.

**Conclusions:** The developed 3D-printed coatings have shown potential to be used as antibacterial coatings for PDMS-based medical devices and were suitable for incorporating other components into their structure, paving the way to be used for customized therapeutics.

**Keywords:** polymer-coating, PDMS, additive manufacturing.

<sup>1</sup> Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal.

<sup>2</sup> Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Lisboa, Portugal.

<sup>3</sup> Centro Interdisciplinar de Estudos Educacionais (CIED), Escola Superior de Educação de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal.

<sup>4</sup> LAQV/REQUIMTE, U. Porto, Porto, Portugal.

<sup>5</sup> Laboratory for Bone Metabolism and Regeneration, Faculty of Dental Medicine, U. Porto, Porto, Portugal.

<sup>6</sup> CQE Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.

<sup>7</sup> EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Setúbal, Portugal.

\*Corresponding author: saracardoso@campus.ul.pt.

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## Emulsions containing *Endopleura uchi* Standardized Extract and *Bertholletia Excelsa* Oil Prepared by Hot Melt Extrusion Technique

Alves P.P.<sup>1</sup>, Garcia Junior N.L.<sup>1</sup>, Kluczkovski A.M.<sup>2</sup>, Amado<sup>3</sup>, Nunomura, R.C.<sup>4</sup>, Souza, T.P.<sup>1\*</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** *Endopleura uchi* is an Amazon rainforest native plant that is used in traditional medicine in the treatment of genitourinary tract inflammation. It also has an antifungal activity. *Bertholletia excelsa* oil due to the high content of lipids, vitamins and minerals shows technological capabilities and sensory properties that could be used in several pharmaceutical formulations. Emulsions are widely studied as carriers of plants bioactive, however herbal extracts are complex biological matrices and is not easy to incorporate into a chemically unstable such as emulsions. The hot melt extrusion is used by the pharmaceutical industry as a simple, continuous, and fast method, capable of producing different pharmaceutical forms. The aim of this study was to develop emulsions containing *E. uchi* standardized extract and *B. excelsa* oil, using the hot melt extrusion technique and compare with the classic method.

**Methodologies:** The formulations were prepared by the conventional emulsification method and by mixing the lipid excipients in hot melt extrusion. The emulsions obtained were evaluated according to appearance, resistance to centrifugation, and electrical conductivity.

**Results:** By the classic method, the base formulation presented homogeneous appearance, however it was not possible to solubilize the extract of *E. uchi* with the lipid excipients, consequently in the centrifugation resistance test, both formulations, phase separation occurred. In the electrical conductivity test all the analyzed emulsions presented values above the value of distilled water, indicating that they were O/W emulsion. Emulsions prepared by hot melt extrusion showed better technological characteristics than the emulsions obtained by the conventional method indicating that the hot melt extrusion was more effective to the formation of droplets in the internal phase (oil) with better incorporation of lipid excipients and *E. uchi* extract.

**Discussion:** Therefore, the results demonstrate that the emulsions presented better characteristics when developed by hot melt extrusion than by the conventional method, with success in the incorporation of the extract in the oil phase. These data were promising and an important step to determine the compatibility of the *E. uchi* extract with the lipophilic internal portion of an emulsion, helping in future studies of a continuous production of this of formulation.

**Keywords:** *Bertholletia excelsa*, *Endopleura uchi*, emulsion, hot melt extrusion, lipid homogenization.

<sup>1</sup> Innovation and Development in Pharmaceutical Technology Laboratory, Pharmaceutical Innovation Program (PPGIF) - Pharmaceutical Sciences College, Federal University of Amazonas, Manaus, Brazil.

<sup>2</sup> Food Composition and Toxicology studies nucleus, Pharmaceutical Innovation Program (PPGIF) - Federal University of Amazonas, Manaus, Brazil.

<sup>3</sup> Health Sciences Program, Federal University of Grande Dourado, Mato Grosso do Sul, Brazil.

<sup>4</sup> Chemistry College, Federal University of Amazonas, Manaus, Brazil.

\*Corresponding author: tpsouza@ufam.edu.br.

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## Desarrollo de Extractos Secos Estandarizados de *Justicia pectoralis* Jacq. (Chambá)

Picanço K.M.S.<sup>1</sup>, Pimentel F.S.<sup>1</sup>, Alves P.P.<sup>1</sup>, Amado J.R.R.<sup>2</sup>, Chaves F.C.<sup>3</sup>, Pereira A.M.S.<sup>4</sup>, Souza T.P.<sup>1\*</sup>

### ORIGINAL ARTICLE

### RESUMEN

**Introducción:** La especie *Justicia pectoralis*, de la familia *Acanthaceae*, llamada chambá en Brasil, es una planta utilizada en la medicina popular para el tratamiento de enfermedades respiratorias. Su principal metabolito activo es la cumarina. Esta planta ha demostrado actividad antioxidante, antiinflamatoria, antinociceptiva y relajante muscular. Los extractos secos de plantas medicinales han despertado un gran interés en la industria farmacéutica debido a su precisión de dosificación, facilidad de manejo y mayor estabilidad fisicoquímica, lo que los convierte en una forma segura y eficaz de desarrollar un medicamento. El objetivo de este trabajo fue desarrollar un extracto seco estandarizado a partir de un extracto líquido acuoso e hidroalcohólico de *J. pectoralis*.

**Metodologías:** Para eso, se caracterizó fisicoquímica la materia prima vegetal (MPV), y se prepararon extractos líquidos acuosos (ESA) y extracto hidroalcohólico (ESHA). A continuación se realizó el secado por secado por aspersión (spray drying). Los extractos secos (ESA y ESHA) se caracterizaron mediante la cuantificación de polifenoles totales (espectrofotometría) y cumarina y ubeliferona (HPLC), así como sus propiedades reológicas (densidad y flujo). Adicionalmente se realizaron ensayos de viabilidad celular y estabilidad microbiológica. Resultados: Se encontraron diferencias estadísticas significativas en el contenido de extractos y cuantificación química entre las dos soluciones extractivas. El extracto seco obtenido a partir de la ESHA presentó un mayor contenido de polifenoles totales (14%, m:m), cumarina (3,97 µg/mg) y ubeliferona (0,41 µg/mg). El rendimiento operacional de secado fue del 78,47 y 85,80% para ESHA y ESA, respectivamente. Ambos extractos secos mostraron buena estabilidad microbiológica y flujo pobre. El ESHA mostró partículas de superficie lisa y tamaño uniforme, mientras que el ESA presentó partículas corrugadas y mayor dispersión granulométrica. A análisis por espectroscopia infrarroja con transformada de Fourier de los dos productos secos (ESHA y ESA) mostró que ambos tenían una composición química similar y mantenían la estabilidad después del secado por spray drying. Ambos extractos secos mostraron ausencia de citotoxicidad.

**Discusión:** se puede concluir que el tipo de solvente utilizado influyó cuantitativamente en el contenido de las sustancias activas, pero el método de secado no alteró la composición química, lo que favoreció la estabilidad del extracto.

**Palabras claves:** hierbas medicinales, extractos vegetales, secado por aspersión, *Justicia pectoralis*.

<sup>1</sup> Laboratorio de Innovación y Desarrollo en Tecnología Farmacéutica; Programa de Postgraduación en Ciencias Farmacéuticas; Facultad de Farmacia; Universidad Federal de Amazonas; Manaus-Brasil.

<sup>2</sup> Programa de Postgraduación en de Ciencias de la Salud; Universidade Federal da Grande Dourados; Mato Grosso do Sul – Brasil.

<sup>3</sup> Empresa Brasileira de Investigación Agropecuaria, Amazonia Occidental; Manaus-Brasil.

<sup>4</sup> Universidad de Ribeirão Preto, Ribeirão Preto-Brasil.

\*Autor correspondiente: tpsouza@ufam.edu.br.

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## Repurposing old Drugs for new Targets: Ketoprofen Intranasal Nanoemulgel Development for the Treatment of Glioma

Pires, P.C.<sup>1,2,3\*</sup>, Mafalda C.<sup>1</sup>, Veiga F.<sup>1,2</sup>, Paiva-Santos A.C.<sup>1,2\*</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Ketoprofen is an anti-inflammatory drug that has shown promising results in the field of drug repurposing for cancer therapy, including in the treatment of glioma<sup>1-3</sup>. Nevertheless, already developed formulations have achieved low drug strength and have been developed for intravenous administration only<sup>1,2</sup>. To tackle these limitations, this work aimed to develop a non-invasive maximized drug strength oil-in-water ketoprofen-loaded nanoemulgel, for intranasal delivery.

**Methodology:** The composition of developed formulations included Capryol® 90 (hydrophobic surfactant), Tween® 80 (hydrophilic surfactant), Transcutol® (co-solvent and permeation enhancer), and Pluronic® F-127 (surfactant and gelling agent). The preparations were characterized in what concerned their droplet size, polydispersity index (PDI), zeta potential, pH, osmolality, viscosity, accelerated stability and *in vitro* drug release.

**Results:** Results showed that the addition of Pluronic to lead nanoemulsions led to a significant droplet size and PDI reduction, from approximately 176 to 22 nm, and from around 0.3 to 0.1, respectively. Final formulations had a drug strength of 4 mg/mL, which is more than 50 times higher than the reported ketoprofen aqueous solubility. The optimized formulations revealed a very low viscosity at refrigeration temperatures (4 °C), high viscosity at room temperature (20 °C), and very high viscosity at nasal mean temperature (32 °C), making them ideal for nasal instillation after storage under refrigeration. Additionally, mean gelling temperatures varied from 14 to 22 °C, revealing that at mean nasal temperature all formulations are in true gel form, possibly leading to a longer retention in the nasal mucosa after administration, and, consequently, enhanced drug absorption. The developed formulations also appeared to have high stability (instability indexes between 0.130 and 0.265), and high cumulative drug release percentage after 24h (between 78 to 93%), in a controlled release manner, fitting a Korsmeyer-Peppas kinetic model (low AIC, 43.84 - 54.67 and high R<sup>2</sup>, 0.9725 - 0.9971).

**Discussion:** Thus, in the current study high drug strength, stability and drug release ketoprofen-loaded nanoemulgels were successfully prepared. Future studies will include *in vitro* cytotoxicity evaluation in glioma cells in order to assess the true potential of these formulations for brain cancer treatment.

**Keywords:** glioma, intranasal, nanoemulgel.

<sup>1</sup> Department of Pharmaceutical Technology, Faculty of Pharmacy of the University of Coimbra, University of Coimbra, Azinhaga Sta. Comba, 3000-548 Coimbra, Portugal.

<sup>2</sup> LAQV, REQUIMTE, Department of Pharmaceutical Technology, Faculty of Pharmacy of the University of Coimbra, University of Coimbra, Azinhaga Sta. Comba, 3000-548 Coimbra, Portugal.

<sup>3</sup> Health Sciences Research Centre (CICS-UBI), University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.

\*Corresponding authors: PCP - patriciapires@ff.uc.pt; ACP-S - acsantos@ff.uc.pt.

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## Miconazole Nanoemulsions for the Topical Treatment of Melanoma: Formulation Development and Characterization

Amado J.<sup>1</sup>, Veiga F.<sup>1,2</sup>, Paiva-Santos A.C.<sup>1,2\*</sup>, Pires P.C.<sup>1,2,3\*</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Melanoma is one of the most lethal skin cancers, with a high mortality rate and incidence. This has led to a huge demand for new more effective forms of treatment<sup>1</sup>. Miconazole is a hydrophobic drug that has been shown to reduce both the proliferation of melanoma cells and the potential of these cells to metastasize into distant organs<sup>2,3</sup>. On the other hand, nanoemulsions are widely used for topical drug delivery as a promising alternative to increase the solubility, skin permeation, and retention of hydrophobic drugs<sup>1</sup>. Hence, the purpose of this work was to incorporate miconazole in an oil-in-water nanoemulsion for topical administration for the treatment of melanoma.

**Methodologies:** Seventeen nanoemulsions were prepared by spontaneous emulsification. Formulation composition included Plurol® Diisostearique as oil and hydrophobic surfactant, Kolliphor® RH40 as hydrophilic surfactant, Transcutol® HP as cosolvent, and made of Milli-Q® water. Droplet size and polydispersity index (PDI) were analyzed using dynamic light scattering (Zetasizer Nano ZS apparatus).

**Results:** Nanoemulsions with a PDI below 0.3 and droplet size between 100 – 200 nm were selected for drug incorporation. After choosing the drug concentration that maintained the desired characteristics, the zeta potential and pH were measured. Accelerated stability studies, real-time stability studies and the *in vitro* release profile were also conducted. After drug incorporation (9 mg/mL) only one of the seventeen nanoemulsions maintained characteristics within the intended parameters. This formulation was selected and characterized: the zeta potential was -4.71 mV (neutral, expected due to used components), and the pH was between 6 and 7 (well tolerated for topical application). The instability index of the drug-free nanoemulsion was 0.522 and 0.542 for the drug-loaded formulation, demonstrating that drug incorporation did not affect the formulation's stability. Moreover, real-time stability studies confirmed that the developed preparation was stable up to 6 months. *In vitro* drug release studies showed that the nanoemulsion exhibited controlled drug release.

**Discussion:** Hence, the incorporation of miconazole into nanoemulsions allowed to greatly increase its solubility (up to 11795 times, compared to its water solubility), while also producing stable formulations for future studies (viscosity, *in vitro* drug permeation, cytotoxicity in melanoma cells).

**Keywords:** drug repurposing, melanoma, nanoemulsion.

<sup>1</sup> Faculty of Pharmacy (FFUC-UC), University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.

<sup>2</sup> REQUIMTE/LAQV, Group of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

<sup>3</sup> Health Sciences Research Centre (CICS-UBI), University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.

\*Corresponding authors: acsantos@ff.uc.pt; patriciapires@ff.uc.pt.

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## Niosomes Development and Characterization for Topical Delivery of Hydrophobic compounds

Tavares M.<sup>1</sup>, Dutka M.<sup>1\*</sup>, Teixeira A.<sup>1</sup>, Cunha S.C.<sup>1</sup>, Fernandes J.O.<sup>1</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Niosomes are multilamellar vesicles with highly desirable properties for topical delivery, since they have high stability, the ability to encapsulate both hydrophilic and lipophilic molecules, and can provide a controlled active compound release, while also being non-toxic, biodegradable, and biocompatible<sup>1</sup>. Hence, the main purpose of this study was to develop niosomes encapsulating a hydrophobic compound, propolis, with suitable for cosmetic application.

**Methodologies:** The vesicles were produced using the thin-layer evaporation method. Different temperatures (40-60 °C) and non-ionic surfactants (Tween 20, Tween 80, Kolliphor RH40, Span 80 or Imwitor 988) were tested. The lipid component was cetyl alcohol, with or without the addition of cholesterol. Lipids-to-surfactant ratio was fixed at 2:3.5. In order to obtain a smaller and more homogeneous particle size, extrusion was used (11-51 cycles, 200 nm pore membranes). Particle size, polydispersity index (PDI), encapsulation efficiency (EE%) and *in vitro* release were determined in selected formulations. Statistical analysis was done through two-way ANOVA testing, using GraphPad Prism<sup>®</sup> software.

**Results:** Results showed that niosomes' particle size drastically reduced with the use of extrusion, from around 2000 to 140 nm, making them suitable for cosmetic application (100-200 nm), with increased potential to penetrate the stratum corneum<sup>2</sup>. The PDI also improved with extrusion, to values below 0.3, and many times even below 0.2, proving that the developed nanosystems had high homogeneity. Temperature and number of extrusion cycles' variation didn't have significant effect in the evaluated parameters. The formulations that gave optimal particle size and PDI values were either made of Tween 20 + cetyl alcohol, Kolliphor RH40 + cetyl alcohol, or Kolliphor RH40 + cetyl alcohol + cholesterol. The propolis EE% was high, being between 78 and 86%. The propolis *in vitro* release was found to be between 10 and 40%, depicting a controlled release profile, with the formulations containing cholesterol achieving higher cumulative release. This could be due to the fact that cholesterol can influence niosome membrane rigidity<sup>3</sup>.

**Discussion:** Future studies will assess the true potential of the developed vesicles in *in vitro* cell assays.

**Keywords:** cosmetic, niosome, topical delivery.

<sup>1</sup> Laboratory of Pharmaceutical Technology, Faculty of Pharmacy of the University of Coimbra, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.

<sup>2</sup> REQUIMTE/LAQV, Group of Pharmaceutical Technology, Faculty of Pharmacy, Faculty of Pharmacy of the University of Coimbra, Coimbra, Portugal.

<sup>3</sup> Health Sciences Research Centre (CICS-UBI), University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.

\*Corresponding author: mykhaylodutka@gmail.com.

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## Evaluación de Materiales Absorbentes como Fases Selectivas para la Extracción de Benzodiazepinas en Medio Acuoso

Carro A.M.<sup>1\*</sup>, Outón-Gil M.<sup>2</sup>, Ares-Fuentes A.M.<sup>3</sup>, Alvarez-Lorenzo C.<sup>4</sup>

### ORIGINAL ARTICLE

#### RESUMEN

**Introducción:** Los productos farmacéuticos, de cuidado personal o las drogas de abuso se consideran actualmente contaminantes emergentes presentes en las aguas residuales. Los tratamientos convencionales para su eliminación son insuficientes y se investiga en nuevos materiales para la extracción selectiva de estos compuestos en ambientes acuáticos.

**Objetivos:** Se ha evaluado la capacidad extractiva de diferentes fases basadas en papeles de celulosa, telas de algodón e hidrogeles.

**Metodología:** El proceso de la funcionalización con  $\beta$ -ciclodextrina y N-hidroximetilacrilamida de las fases de celulosa (papeles y telas) se realizó siguiendo el método Fenton, adaptando el método de E. Vismara. Los hidrogeles a base de Hidroxietilmetacrilato (HEMA) se sintetizaron en dimetilsulfóxido, utilizando azobisisobutironitrilo como termoiniciador para la polimerización radicalaria y dimetacrilato de etilenglicol como agente reticulante. Se han utilizado 2 disoluciones de diferente composición de monómeros Bencil-metacrilato y Ácido metacrílico.

**Resultados:** Se presentan los procesos de captación-liberación de benzodiazepinas en medio acuoso utilizando las diferentes fases. Las medidas se realizaron en un espectrofotómetro UV-Vis y en un cromatógrafo HPLC-DAD, en ambos casos a 230 nm. Se ha comprobado que las fases de papel de celulosa y las telas de algodón estaban correctamente funcionalizadas. Se ha evaluado un proceso de mercerización en las telas para mejorar sus condiciones de captación al maximizar el número de sitios de unión disponibles para las ciclodextrinas.

**Conclusiones:** Se han obtenido buenos resultados de captación de benzodiazepina con los hidrogeles (35-40%) y de elución (90-100%). Las fases de celulosa (papeles y telas) no han demostrado niveles significativos de captación con las benzodiazepinas estudiadas. Sin embargo, resultaron eficaces para otro compuesto como es el diclofenaco.

**Palabras clave:** celulosa funcionalizada, hidrogeles de HEMA, extracción selectiva.

<sup>1</sup> Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química. Health Research Institute of Santiago de Compostela (IDIS), Spain.

<sup>2</sup> Instituto de Materiais (iMATUS). Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain. e-mail: tuchi.carro@usc.es

<sup>3</sup> Center for Applied Chemistry and Biotechnology (CQAB). University of Alcalá. 28805- Alcalá de Henares, Spain.

<sup>4</sup> Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, and Health Research Institute of Santiago de Compostela (IDIS) Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

\*Author correspondiente: tuchi.carro@usc.es.

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## Diseño, Desarrollo y Caracterización de Nanopartículas de Ciprofloxacino

Sabroso C.M.<sup>1,2</sup>, Bustos W.A.<sup>1</sup>, Castellanos J.M.<sup>1</sup>, Suárez A.I.<sup>1,2\*</sup>, Sánchez A.I.<sup>1,2</sup>

### ORIGINAL ARTICLE

#### RESUMEN

**Introducción:** El Ciprofloxacino es una fluoroquinolona de segunda generación, utilizado para infecciones contra las bacterias bacilos gramnegativas y algunas bacterias grampositivas. Actúa bloqueando la replicación del ADN bacteriano al inhibir la ADN-girasa y topoisomerasa IV<sup>1</sup>. La aparición de resistencias complica la obtención de una terapia microbiológicamente eficaz y está asociada con graves resultados adversos sobre la salud y la economía<sup>2</sup>. El objetivo del presente trabajo es desarrollar nanopartículas poliméricas de ciprofloxacino por el método de doble emulsión (W/O/W) extracción-evaporación del solvente con el fin de obtener mejores características antimicrobianas y mejores perfiles farmacocinéticos<sup>3</sup>.

**Metodología:** Para el desarrollo y optimización de las formulaciones se ha empleado el diseño experimental mediante el programa estadístico Minitab® 19 (Pennsylvania-USA). Se ha utilizado un diseño factorial completo (L12) de dos niveles que evalúa y determina el grado de influencia de parámetros del proceso de elaboración en las características de las nanopartículas. Previo a la evaluación del perfil de liberación del ciprofloxacino en las formulaciones seleccionadas se realiza un estudio de solubilidad del fármaco en tampón fosfato a distintos valores de pH.

**Resultados y discusión:** El diseño experimental determinó que la hidrofobicidad del polímero y la cantidad de ciprofloxacino fueron los únicos factores que mostraron una influencia estadísticamente significativa ( $p$ -valor  $<0.05$ ) en el tamaño de partícula y carga de antibiótico de las nanopartículas, respectivamente. El estudio de solubilidad demostró era necesario emplear como medio de cesión el tampón fosfato a pH 5.5 para garantizar las condiciones sink en el ensayo de cesión. La formulación elaborada con PLGA-502, 15mg de ciprofloxacino y NaCl en la fase externa acuosa, se seleccionó como la formulación más adecuada, mostrando un tamaño de partícula de 307nm, un PDI de 0.28, una eficacia de encapsulación de 62.46% y una liberación controlada del ciprofloxacino durante al menos 7 días.

**Palabras clave:** diseño de experimentos, ciprofloxacino, nanopartículas.

<sup>1</sup> Dpto. de Farmacia Galénica y Tecnología Alimentaria, Universidad Complutense de Madrid, España.

<sup>2</sup> Instituto de Farmacia Industrial y Galénica. Universidad Complutense de Madrid, España.

\* Autor correspondiente: galaaaa@farm.ucm.es.

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## New Dry Powder Aerosolization Device Designed for In Vitro Applications - Validation Tests

Pinto-da-Silva J.<sup>1\*</sup>, Braz L.<sup>1,2</sup>, Grenha A.<sup>1</sup>

### ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** Lung delivery is receiving increased attention, emphasizing inhalation as the preferred route for treating lung diseases<sup>1</sup>. Formulation development faces difficulties regarding the simulation of lung conditions in in vitro assays, limiting conclusions<sup>2</sup>. Our research group has recently developed a prototype to disperse dry powders over surfaces such as well plates<sup>3</sup>, useful in cell-based studies and release studies, among others. The device is now being tested and validated. The objective of this work was to evaluate the performance of the dry powder aerosolization device, testing dry powder composition, weighed dose, device features and operators as variables.

**Methodology:** The prototype for dry powder aerosolization (Figure 1) was tested using spray-dried microparticles (MP) previously developed by the team for lung applications, composed of locust bean gum, konjac glucomannan and mannitol. Two device bodies with different inner entry diameters were compared ( $\varnothing = 1.5$  or  $2.5$  mm). A Petri dish suitable for cell culture was weighed, as well as the dry powders (doses of 1, 5 and 10 mg). The device was assembled and actuated for powder aerosolization ( $n=10$ ). The assays were performed by two different operators replicating the testing conditions, providing information on operator effect in device actuation. The evaluated response was in all cases the yield of aerosolization, which was calculated as follows (Figure 1).

$$\text{Yield of aerosolization(\%)} = \frac{\text{Amount of powder deposited on the Petri dish}}{\text{Total amount of powder weighed}} \times 100$$



Figure 1. Dry powder aerosolization device.

<sup>1</sup> Centro de Ciências do Mar, Universidade do Algarve, Faro, Portugal.

<sup>2</sup> CESUALg - Centro de Estudos e Desenvolvimento em Saúde, Universidade do Algarve, Faro, Portugal.

\*Corresponding author: Joana Pinto-da-Silva (a54138@ualg.pt).

**Results:** The performed tests indicated that MP composition affects the yield of aerosolization, which varied roughly between 20 and 50%, particularly when higher amounts of powder are dosed. Different inner entry diameters of the device also revealed an effect in some cases, although there was no consistent pattern. Changing the operator had a mild impact, the observed differences mostly occurring when the device with higher inner entry diameter was used.

**Discussion:** The aerosolization device successfully disperses the powders. Its performance was influenced by the MP composition, the tested dose and, to a certain degree, the device inner entry diameter and operator. Higher amounts of LBG MP provided the best efficiency of the aerosolization process.

**Keywords:** aerosols, dry powder inhalers, *in vitro* techniques.

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## Development and Characterization of Mesoporous Silica Nanoparticles for Potential Incorporation of 5-Aminosalicylic Acid

Junior A.G.<sup>1\*</sup>, Meneguim A.B.<sup>1</sup>, Sábio R.M.<sup>1</sup>, Chorilli M.<sup>1</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** 5-Aminosalicylic acid (5-ASA), is a non-steroidal anti-inflammatory drug, considered the first-line treatment for mild to moderate stages of inflammatory bowel disease. Despite its effectiveness, very high doses of 5-ASA are usually required for this treatment due to rapid and almost complete systemic absorption in the upper intestinal portion, making it difficult to reach local therapeutic levels of the drug in the inflamed colonic mucosa<sup>1</sup>. In this context, mesoporous silica nanoparticles (MSNs) stand out as inorganic, robust, non-toxic, and stable nanoreservoirs, which allow the incorporation of 5-ASA inside the mesopores, in addition to a reduced size<sup>2</sup>.

**Methodology:** In this study, mesoporous silica nanoparticles are used to load 5-ASA (MSNs@5ASA) and protect against drug degradation and leaching. The exact amount of incorporated drug was determined by ultraviolet-visible spectrophotometry. For this, an analytical method for the quantification of 5-ASA was developed and validated, and the drug impregnated in MSNs was evaluated, followed by the differential scanning calorimetry (DSC) technique to confirm drug incorporation.

**Results:** In addition, the hydrodynamic diameter of the nanosystems was evaluated through nanoparticle tracking analysis (NTA). An ultraviolet spectrophotometric method ( $\lambda=300$  nm) was duly validated for the range of 10–55  $\mu\text{g/mL}$  and proved to be adequate for the quantification of 5-ASA, making it possible to evaluate and detect a high encapsulation efficiency value of 5-ASA (EE%,  $93.18 \pm 0.001$ ). DSC measurements suggest an improvement in the stability of the impregnated drug. According to the results obtained by NTA, the hydrodynamic diameter of MSNs ( $142.2 \pm 1.5$  nm) was slightly smaller than MSNs@5ASA ( $148.1 \pm 9.4$  nm).

**Discussion:** Overall, the developed method is suitable for testing porous materials loaded with 5-ASA to allow use in therapeutic applications.

**Keywords:** 5-Aminosalicylic acid, mesoporous silica nanoparticles, inflammatory bowel disease.

<sup>1</sup> Department of Drugs and Medicines, School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, Brazil.

\*Corresponding author: albertojunior.ap@gmail.com.

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## Sutezolid Liposomal Formulations to Tackle Mycobacterial Infection

Silva M.<sup>1</sup>, Granja A.<sup>2</sup>, Reis S.<sup>2</sup>, Borges M.<sup>1,3\*</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Mycobacterial infections are complex diseases that lead to millions of new cases each year. Although antimycobacterial treatment regimens are effective, they can become cumbersome and associated with toxicity and inconsistent compliance, which leads to increased failure rates, relapse, or reinfection, especially in drug-resistant strains<sup>1</sup>. Drug development has emerged towards the use of nano-delivery systems to overcome the challenges in delivery of antimicrobials<sup>2</sup>. Our work encompasses the development of sutezolid (STZ)-loaded liposomes (Figure 1) to reduce mycobacterial burden *in vitro*. Resulting nanoformulations were evaluated chemically and biologically in *M. avium*-infected bone marrow-derived macrophages (BMM) and bone marrow-derived dendritic cells (BMDC).

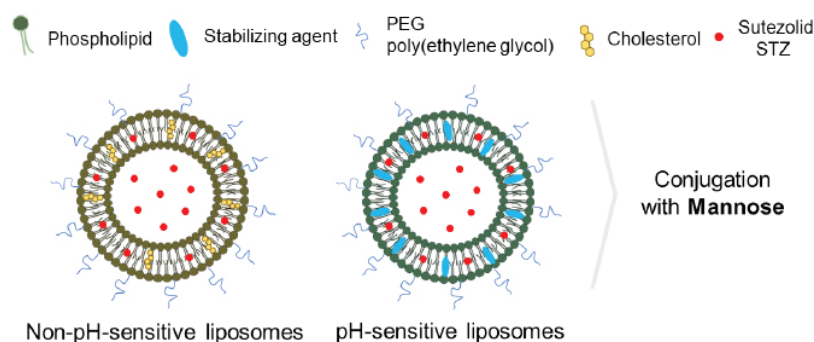


Figure 1. Schematic representation of liposome designs.

**Methods:** Mannose-coated non-pH-sensitive and pH-sensitive formulations were prepared. Liposome size, polydispersity, zeta potential, and entrapment efficiency were evaluated. Drug release assay was performed at pH 7.4 and 5.0 at several timepoints for 24h. Cellular uptake was analyzed by confocal laser scanning microscopy using fluorescent probe-loaded liposomes added for 15-, 30-, 60-, and 120-minutes. Cytotoxicity was evaluated by thiazolyl blue tetrazolium bromide assay in BMM and BMDC. Antimycobacterial activity was assessed using a well-studied *in vitro* *M. avium* 2447 infection model<sup>3</sup>. STZ and liposomal STZ were tested at equivalent drug concentrations.

<sup>1</sup> UCIBIO/REQUIMTE, Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313, Porto, Portugal.

<sup>2</sup> LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313, Porto, Portugal.

<sup>3</sup> Associate Laboratory i4HB-Institute for Health and Bioeconomy Faculdade de Farmácia, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313, Porto, Portugal.

\*Corresponding author: mborges@ff.up.pt.

**Results:** Both formulations were able to efficiently encapsulate STZ (above 79%) and promote a controlled release of the drug at physiological conditions (pH=7.4). pH-sensitive liposomes showed a significant increase in STZ release in acidic environment (pH=5.0), in comparison with the same formulation and the non-pH-sensitive liposomes at physiological pH. Cellular uptake studies showed internalization of liposomes at early timepoints with no effect on mannose. Cytotoxicity assays showed reduced toxicity to bone marrow-derived macrophages and bone marrow-derived dendritic cells. Infection assays showed a significant *in vitro* antimycobacterial effect at 4- and 7-days post-infection for both formulations at 25 and 50  $\mu$ M, in comparison to the untreated controls.

**Discussion:** These results demonstrate the ability of both formulations to effectively entrap STZ, be internalized by host cells, and deliver the drug to restrict intracellular mycobacterial growth at 4 and 7 dpi in both BMM and BMDC, without cytotoxicity. This work highlights a promising and effective targeted therapeutic nano-approach that should be further pursued *in vivo*, to ultimately improve a rather dire scenario of mycobacterial infections.

**Keywords:** mycobacteria, nanoparticle, infection.

**Acknowledgments:** This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/ 2020 and HEALTH-UNORTE I&D&I project (NORTE-01-0145-FEDER- 000039) co-financed by European Regional Development Fund (ERDF), through the NORTE 2020 (Programa Operacional Regional do Norte 2014/2020).

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# Impact of Formulation of Drug-Containing Blends on 3D Printing by Fused Deposition Modelling

Fernandes A.I.<sup>1\*</sup>, Figueiredo S.<sup>2,3</sup>, Carvalho F.G.<sup>3</sup>, Pinto J.F.<sup>2</sup>

## ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** Fused Deposition Modelling (FDM) is paving the way for the digitally-enabled on-demand production of personalized medicines. The thermoplastic polymer matrix determines the mechanical and rheological properties of HME filaments<sup>1</sup>, and ultimately the quality and performance of the 3D printed dosage forms<sup>2</sup>. An inappropriate combination of drug and excipients affects these properties and can preclude successful HME-FDM integration<sup>3</sup>. The impact of the physicochemical properties of drug (e.g. melting point, m.p.), and use of excipients, was investigated in this work.

**Materials & Methods:** Hydroxypropylcellulose (HPC) was used as matrix; the model drugs considered (covering a wide m.p. range) were paracetamol (PCT), paroxetine (PAR), and theophylline (TEO), at 30% w/w drug load. The lowest temperature settings required for HME-FDM coupling were determined and the need for processing aids evaluated. Extrusion and printing temperatures were maintained below drug degradation point. Extrudability and printability were evaluated respectively through the ability to produce (A) drug-loaded filaments with adequate dimensions and rheology and (B) tablets (batches of 10-20 units) with simple and more complex geometries, meeting Eur. Pharm. requirements for mass and content uniformity. For printability, each formulation required the addition of plasticizers to lower the melting temperature (e.g. Soluplus, 7.5-15% or triethylcitrate, 5%) and lubricants (e.g. magnesium stearate, 1-3%) to achieve adequate feeding of the filaments to the printer.

**Results & Discussion:** The work failed to establish a direct relationship between drug melting point and temperature required for filament production and printing (Table 1), indicating the influence of other factors, such as solubility, hydrophilicity, or ability to establish drug/matrix molecular interactions. Over-plasticization, by water, of HME filaments exposed to ambient relative humidity, affected rheological properties and prevented subsequent printing. Although TEO (highest m.p.) required more drastic conditions, PRX (lowest m.p.) was more demanding than PCT (medium m.p.). However, the temperature ranking followed a similar pattern for HME and FDM. The addition of excipients in the fraction range considered did not impact the temperature required for HME-FDM. The double application of heat resulted in solid-state interactions and amorphization of drugs. It appears that processing settings need to be adjusted on a case-by-case basis but further studies with other drugs are required to substantiate these findings.

**Table 1.** Characteristics of the model drugs and minimum processing conditions required for integrated HME-FDM

| Drug | MW (Da) | Melting point (°C) | Water solubility at 25°C (mg/mL) | HME Temp. (°C) | FDM Temp. (°C) |
|------|---------|--------------------|----------------------------------|----------------|----------------|
| PRX  | 374.84  | 120-138            | 1.13                             | 120            | 200            |
| PCT  | 151.16  | 168-172            | 14.00                            | 100            | 180            |
| TEO  | 180.17  | 270-274            | 7.36                             | 130            | 220            |

**Keywords:** 3D Printing, hot-melt extrusion, fused deposition modelling, extrudability, printability.

<sup>1</sup> Egas Moniz Center for Interdisciplinary Research (CiiEM); Egas Moniz School of Health & Science, Almada, Portugal.

<sup>2</sup> iMed.Ulisboa – Research Institute for Medicines, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal.

<sup>3</sup> LEF-Infosaúde, Laboratório de Estudos Farmacêuticos, Barcarena, Portugal.

\*Corresponding author: aifernandes@egasmoniz.edu.pt.

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## Avaliação do Desempenho de Carreadores Lipídicos Nanoestruturados contendo Curcumina contra *Helicobacter pylori*

Camargo B.<sup>1,2,3\*</sup>, Dutra J.<sup>1</sup>, Santos M.<sup>1</sup>, Chitas R.<sup>2,3</sup>, Bauab T.<sup>1</sup>, Parreira P.<sup>2,3</sup>, Martins C.<sup>2,3,4</sup>, S. Chorilli M.<sup>1</sup>

### ORIGINAL ARTICLE

#### RESUMO

**Introdução:** A bactéria *Helicobacter pylori* coloniza o estômago de mais de 50% da população mundial, provocando diversas patologias gástricas, entre as quais 90% dos casos de câncer gástrico. Devido à elevada resistência aos antibióticos, a Organização Mundial de Saúde classificou esta bactéria como tendo prioridade alta no desenvolvimento de novos antibióticos/tratamentos<sup>1</sup>. A curcumina (CUR) demonstrou ter atividade contra a *H. pylori* *in vitro* mas a sua baixa solubilidade aquosa é uma limitação ao seu uso *in vivo*<sup>2</sup>.

**Metodologia:** Os carreadores lipídicos nanoestruturados (CLN) foram desenvolvidos por emulsificação-sonicação, utilizando óleo de rícino, cera de abelha, Tween®80 e Span®60 e CUR (0,5 mg/mL; CLN-CUR). As CLN e as CLN-CUR foram caracterizadas por microscopia eletrônica de transmissão, dispersão de luz dinâmica, análise de rastreamento de nanopartículas e eficiência de encapsulação (EE). A atividade antibacteriana de CLN, CLN-CUR e CUR foi testada contra *H. pylori* J99. A citotoxicidade destes sistemas foi testada na linha celular GES-1 (epitélio normal da mucosa gástrica), determinando o IC<sub>50</sub> e o índice de seletividade (IS).

**Resultados:** Foram observadas partículas esféricas, com diâmetro hidrodinâmico médio (<100 nm), potencial zeta (-22,90 (CLN) e -23,90 mV (CLN-CUR)), e partículas monodispersas. CLN apresentaram concentração de  $8,6 \times 10^{14}$  e CLN-CUR de  $1,07 \times 10^{14}$  e a EE foi de 88,97%. A estabilidade de CLN e CLN-CUR foi avaliada durante 90 dias e não foram verificadas alterações. A concentração inibitória mínima e bactericida mínima da CUR foi de 15,62 µg/mL, determinada por contagem de unidades formadoras de colônias (UFC) (redução >3 log UFC/mL). CLN e CLN-CUR na concentração de  $10^{14}$  (0,5 µg/mL de CUR) erradicaram a bactéria (0 UFC/mL). O IC<sub>50</sub> foi de 34,15 (CUR),  $5,50 \times 10^{13}$  (CLN) e  $2,53 \times 10^{13}$  (CLN-CUR) e o IS demonstrou que CUR é mais seletiva para GES-1 (IS=2,19), e os nanossistemas são mais seletivos para *H. pylori* (CLN IS =550,10; CLN-CUR IS=252,91).

**Discussão:** Foram desenvolvidos nanossistemas (CLN e CLN-CUR) com sucesso e alta EE de CUR (CLN-CUR). Ademais, as CLN-CUR potenciam a atividade da CUR, sendo que se obtém erradicação com 30x menos CUR comparativamente com CUR livre. Os nanossistemas desenvolvidos são biocompatíveis e promissores para desenvolvimento de novos tratamentos para *H. pylori*.

**Palavras chave:** carreadores lipídicos nanoestruturados, novas terapias, curcumina, *Helicobacter pylori*.

<sup>1</sup> Faculdade de Ciências Farmacêuticas de Araraquara, Universidade Estadual Paulista Júlio de Mesquita Filho, Araraquara, São Paulo, Brasil.

<sup>2</sup> INEB-Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal.

<sup>3</sup> i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

<sup>4</sup> ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4050-313 Porto, Portugal.

\*Autor correspondente: bruna.furquim@unesp.br.

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## Optimization and Validation of an HPLC Method for Quantification of Docetaxel Encapsulated in Lipid Nanoparticles

Marques A.C.<sup>1\*</sup>, Costa P.C.<sup>1</sup>, Velho S.V.<sup>2</sup>, Amaral M.H.<sup>1</sup>

ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** Docetaxel (DTX) is a semisynthetic taxane with remarkable activity against several types of cancer<sup>1</sup>. Nevertheless, knowing that low solubility in water and systemic toxicity have been hampering its clinical use, drug delivery systems based on lipid nanoparticles, such as Nanostructured Lipid Carriers (NLC), pose a promising alternative to the commercial formulations of DTX<sup>2</sup>. For a complete characterization of nanoparticles, it is pivotal to quantify the amount of incorporated drug. This work aimed to develop and validate a high performance liquid chromatography (HPLC) method for quantifying DTX. Ultimately, the goal was to directly estimate the entrapment efficiency (EE) and loading capacity (LC) of the developed NLC containing DTX.

**Methodology:** Isocratic chromatography was performed on a C18 column and analyzed using Chromeleon software after UV detection at 231 nm. The optimized mobile phase consisted of acetonitrile and water (43:57, v/v), with a flow rate of 1 mL/min and short run time (5 min). The following characteristics were considered for validation: specificity, linearity, detection and quantitation limits, and precision. For drug quantification, DTX loaded NLC (0.1% w/w) were prepared in triplicate by sonication, diluted with ethanol, and centrifuged (4000 rpm, 20 min) using ultrafiltration centrifugal filter units (cut-off: 50 kDa). An aliquot of each filtered solution was finally diluted with acetonitrile/water (70:30, v/v) before injection.

**Results:** According to the ICH guideline, the method was found to be specific for DTX and linear over the concentration range of 1-12  $\mu\text{g/mL}$ , with a good correlation coefficient ( $r = 0.9997$ ). Based on the calibration curve, the method's detection and quantitation limits were 1.35  $\mu\text{g/mL}$  and 4.09  $\mu\text{g/mL}$ , respectively. For intra-assay and intermediate precision, the coefficient of variation was less than 6%. Finally, the EE and LC of the developed NLC were  $97.7 \pm 0.56\%$  and  $1.3 \pm 0.01\%$  (mean  $\pm$  standard deviation,  $n=3$ ), respectively.

**Discussion:** The proposed method proved to be simple, fast, and suitable for quantifying DTX in lipid nanoparticles. The mean values of EE and LC obtained suggest that the developed NLC are appropriate for DTX entrapment.

**Keywords:** high-performance liquid chromatography, docetaxel, nanoparticles.

<sup>1</sup> UCIBIO—Applied Molecular Biosciences Unit, MEDTECH, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; Associate Laboratory i4HB—Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal.

<sup>2</sup> i3S—Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal; IPATIMUP—Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal.

\*Corresponding author: amarques@ff.up.pt.

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## Enhancing Osteosarcoma Treatment: Promising Methotrexate Delivery with Pluronic® P105-PEI Nanosystems

Silva I.<sup>1\*</sup>, Jarak I.<sup>1,2</sup>, Domingues C.<sup>1,3,4</sup>, Correia D.<sup>5</sup>, Valoppi M.<sup>6</sup>, Cerqueira R.<sup>1</sup>, Veiga F.<sup>1,3</sup>, Figueiras A.<sup>1,3</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Osteosarcoma (OS) is an aggressive bone neoplasm mainly affecting young adults. Despite therapies, OS has a low survival rate, often metastasizes, and relapses frequently<sup>1</sup>. Amphiphilic block copolymers like Pluronics are studied for drug delivery due to their structure: a hydrophobic core and hydrophilic shell. Micelleplexes, formed by cationic amphiphilic copolymers, show promise as carriers, condensing nucleic acids and encapsulating hydrophobic drugs<sup>2,3</sup>. The aim of this study is to develop a nanosystem composed of Pluronic P105 conjugated with branched bPEI2k and load it with methotrexate (MTX) and therapeutic miRNA.

**Methods:** The nanosystem P105PEI was loaded with MTX by the thin-film method and direct dissolution methods followed by quantification of Mtx by HPLC. *In vitro* assays were performed to evaluate the release of the nanosystem compared to the free drug at pH 5.6 and 7.4 by dialysis. Polyplexes with different N/P ratios were prepared to evaluate cellular uptake by flow cytometry and to assess cell viability in 2UOS cells by the resazurin method.

**Results:** Nanoparticles from the thin-film method exhibited an average size of 22 nm, zeta potential (ZP) around 7 mV, encapsulation efficiency (EE%) of 15.1%, and loading capacity (LC%) of 0.5%. Direct dissolution notably improved efficacy to 84% EE and 2.7% LC. Efficacy comparisons between P105PEI (3% w/w) Mtx complexation and mixed micellar systems P105PEI/P105 (1:1, 6% w/w), and P105PEI/F127 (10:1, 3.3% w/w) revealed composition and drug amount influence on EE% and LC%. P105PEI/F127 nanosystem, with 2 mg of Mtx, achieved highest EE (89.6%) and LC (4.98%). The optimized nanosystem (P105PEI/F127) demonstrated controlled *in vitro* Mtx release at pH 5.6 and 7.4, peaking at 60% and 73% respectively, compared to free drug at 92%. Cellular uptake of Cy5-labeled siRNA was comparable between P105PEI and P105PEI/F127 nanosystems at 80nM (25%) and 50nM (40%). Pre-microRNA-143 mimic complexation in U2OS cells exhibited inconclusive results, necessitating further experimentation.

**Discussion:** The P105PEI:F127 nanosystem (10:1, 3.3% w/w) exhibited optimal attributes for OS therapeutic nanocarrier containing Mtx and miRNA.

**Keywords:** osteosarcoma, Pluronic® P105, micelleplexes.

<sup>1</sup> Laboratory of Drug Development and Technologies, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>2</sup> Instituto de Investigação e Inovação em Saúde, University of Porto, 4200-135 Porto, Portugal.

<sup>3</sup> REQUIMTE/LAQV, Group of Pharmaceutical Technology, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>4</sup> Institute for Clinical and Biomedical Research (iCBR) Area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>5</sup> Chemistry Department, Faculty of Sciences and Technology, University of Coimbra, 3000-056 Coimbra, Portugal.

<sup>6</sup> Department of Pharmacy and Biotechnology, University of Bologna, 40138 Bologna, Italy.

\*Corresponding author: nesilva00@gmail.com.

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## New therapeutic approach Pluronic®-PEI nanomicellar System for Drug-Delivery in the Colorectal Cancer Treatment

Valoppi M.<sup>1,2</sup>, Jarak I.<sup>1,3\*</sup>, Domingues C.<sup>1,4,5</sup>, Correia D.<sup>1,6</sup>, Silva I.<sup>1</sup>, Cerqueira R.<sup>1</sup>, Veiga F.<sup>1,5</sup>, Figueiras A.<sup>1,5</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** The current global cancer burden is characterized by increasing number of morbidity and mortality<sup>1</sup>. Colorectal cancer (CRC) is one of the most common malignant tumors and is characterized by a high rate of local recurrence and metastasis. Application of nanoscale drug delivery systems could overcome the main drawbacks of the chemotherapeutics, such as poor solubility, stability, low targetability, and narrow therapeutic window. Additionally, nanomicelles could be used for co-delivery of both conventional chemotherapeutic drugs and nucleic acids, enabling more efficient and safer therapeutic outcomes<sup>2</sup>. The objective of this study was to develop nanomicelles composed of Pluronics® F127 and P123 for improved 7-ethyl-10-hydroxycamptothecin (SN-38) delivery and to evaluate the addition of amphiphilic block co-polymer composed of Pluronic P105® and cationic PEI on drug loading. Optimization of SN-38 loading and characterization of optimized micelles was also studied.

**Methods:** Micelles were prepared at different F127:P123 ratios, either with the addition of cationic P105-PEI or without, and were loaded with SN-38. Micelles were prepared by either the thin-film or the direct dissolution method and at either physiological (pH: 7) or acidic (pH: 3) conditions. Encapsulation efficiency (EE%) and loading capacity (LC%) of SN-38 were determined by HPLC, while DLS was used to evaluate size, PDI, and stability of drug-loaded micelles<sup>3</sup>; ELS was used to determine the Z potential of drug-loaded micelles (at 25°C).

**Results:** Different conditions did not affect either the size or the Z potential of the micelles (22 nm and 0.5 mV on average). The encapsulation efficiency (EE%) was very low on average: in the samples prepared by the thin-film method and by the direct dissolution method at pH 3, the EE% was <1%. The highest EE% was obtained by preparing the micelles with the thin-film method at pH 7: the presence of P105-PEI increased the EE% at both F127:P123 studied ratios (1:2 and 1:8, w/w) to 22.5% and 19.2% against 0.2% and 0.3%, respectively, in the absence of P105-PEI.

**Discussion:** Addition of cationic co-polymer significantly improved the ability of Pluronic micelles to incorporate SN-38. Prepared stable micelles present solid foundation for developed of micelleplexes for co-delivery of SN-38 and an RNAi.

**Keywords:** micelleplexes, Pluronic, colorectal cancer, SN-38, RNAi.

<sup>1</sup> Laboratory of Drug Development and Technologies, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>2</sup> Department of Pharmacy and Biotechnology, University of Bologna, 40138 Bologna, Italy.

<sup>3</sup> Instituto de Investigação e Inovação em Saúde, University of Porto, 4200-135 Porto, Portugal.

<sup>4</sup> REQUIMTE/LAQV, Group of Pharmaceutical Technology, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>5</sup> Institute for Clinical and Biomedical Research (iCBR) Area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>6</sup> Chemistry Department, Faculty of Sciences and Technology, University of Coimbra, 3000-056 Coimbra, Portugal.

\*Corresponding author: jarak.ivana@gmail.com.

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## Multifunctional Polymeric Micelles for Combination Therapy of Colorectal Cancer

Correia D.<sup>1,2\*</sup>, Jarak I.<sup>1,3</sup>, Domingues C.<sup>1,4,5</sup>, Silva I.<sup>1</sup>, Valoppi M.<sup>1,6</sup>, Cerqueira R.<sup>1</sup>, Veiga F.<sup>1,3</sup>, Figueiras A.<sup>1,3</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Colorectal cancer (CRC) is considered the most frequent malignant cancer in the gastrointestinal tract<sup>1</sup>. The available antitumor drugs used in CRC treatment often suffer from chemoresistance and serious side-effects. The addition of chemosensitizer lonidamine can enhance the antitumor effects of chemotherapy without exacerbation the side-effects<sup>2</sup>. Additionally, the use of nanotechnology can enhance the overall therapeutic effects by enabling site-specific targeting and improving solubility/stability of hydrophobic drugs<sup>3</sup>. The primary objective was to optimize the composition of multifunctional nanomicelles based on amphiphilic copolymers Pluronic<sup>®</sup> for co-delivery of chemotherapeutic SN-38 in combination with a glycolytic inhibitor lonidamine (LND).

**Methods:** Mixed Pluronic<sup>®</sup> micelles (P123 and F127) were prepared by thin-film method and loaded with SN-38 and lonidamine (LND) at different drug ratios (1:0; 0.8:0.2; 0.5:0.5; 0.2:0.8; 0:1 mg/mL). Polymeric micelles containing encapsulated and free drugs were characterized by DLS. The encapsulation efficiency (EE%), loading capacity (LC%), and drug release profile were measured by HPLC. Cytotoxic effects of optimized nanomicelles used were carried out in a human colon adenocarcinoma cell line (LoVo). One-way ANOVA was used for multiple comparisons.

**Results:** Stable polymeric micelles with a CMC value of 0.019 mg/mL (P123:F127 8:1 w/w, 30 mg/ml). The optimized drug-loaded micelle (SN-38:LND, 0.5:0.5 mg/mL) displayed an average size of 19 nm and a polydispersity index of 0.113. The presence of the second drug significantly improved the loading parameters for both drugs, and they were the highest for drug ratio of 0.5:0.5 mg/ml. While for LND high drug loading was observed (97.6% EE and 2% LC), low encapsulation of SN-38 was observed (0.2% EE and 0.01% LC). This micellar system showed a controlled release profile, with 55% of LND released in 30h, in contrast to the free drug, which released 97% over that period. Preliminary tests were conducted on LoVo cell line and it was determined that at 20nM SN38 (IC<sub>50</sub>), nanoSN-38 and free SN-38 exhibit comparable impacts on cell viability. Concentration of encapsulated LND in the formulation optimized for maximum EE and DL of both drugs is not sufficient to contribute to in vitro efficiency under given experimental conditions.

**Discussion:** Although mixed Pluronic micelles optimized for maximum loading of two drugs demonstrate comparable effects on cell viability as the free SN-38, further optimization is needed to achieve improved therapeutic effects of combination therapy.

**Keywords:** colorectal cancer (crc), polymeric micelles, combined therapy.

<sup>1</sup> Laboratory of Drug Development and Technologies, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>2</sup> Chemistry Department, Faculty of Sciences and Technology, University of Coimbra, 3000-056 Coimbra, Portugal.

<sup>3</sup> Instituto de Investigação e Inovação em Saúde, University of Porto, 4200-135 Porto, Portugal.

<sup>4</sup> REQUIMTE/LAQV, Group of Pharmaceutical Technology, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>5</sup> Institute for Clinical and Biomedical Research (iCBR) Area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>6</sup> Department of Pharmacy and Biotechnology, University of Bologna, 40138 Bologna, Italy.

\*Corresponding author: dianapinheirocorreia@gmail.com.

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## Development and Characterization of Curcumin-loaded TPGS/F127/P123 Polymeric Micelles as a Potential Therapy for Osteosarcoma

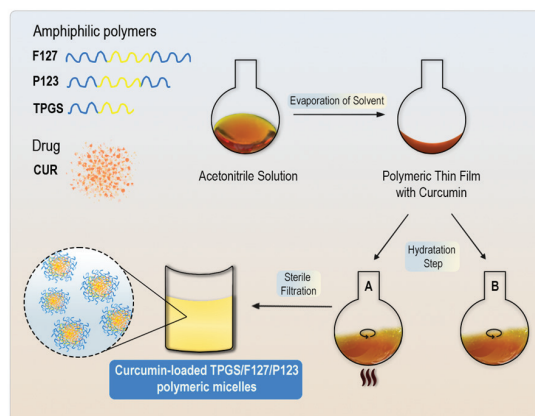
Cerqueira R.<sup>1\*</sup>, Domingues C.<sup>1,2,4,5</sup>, Jarak I.<sup>1,3</sup>, Silva I.<sup>1</sup>, Correia D.<sup>1</sup>, Valoppi M.<sup>1,6</sup>, Veiga F.<sup>1,2</sup>, Figueiras A.<sup>1,2</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Osteosarcoma (OS) is the third most common form of cancer in children<sup>1</sup>. The resistance to conventional treatments and the reported side effects have limited the success of conventional treatments<sup>2</sup>. Curcumin (CUR) is a natural compound with promising therapeutic potential and significant anticancer activity in vitro. However, its clinical translation has been hampered by poor solubility in aqueous medium and low bioavailability in humans. The use of Pluronic® mixed polymeric micelles with Tocopheryl polyethylene glycol 1000 succinate (TPGS) has been studied to overcome these issues<sup>3</sup>. To develop and characterize mixed polymeric micelles based on TPGS/F127/P123 to load curcumin as a novel therapeutic strategy for osteosarcoma.

**Methods:** Polymeric micelles with (PFT\_CUR) or without CUR (PFT) were fabricated using P123, F127, and TPGS by the thin film method (Figure 1). Two slightly different approaches were employed during the hydration step. Precisely, the hydration step in group A (n = 3) of curcumin-loaded micelles was performed under magnetic stirring in a 60°C bath, and in group B (n = 3) at room temperature. Both groups were subjected to a 60-minute stirring period. In parallel, an equivalent experiment was conducted with empty micelles. Physicochemical properties were evaluated by Dynamic and Electrophoretic light scattering. The Drug Loading (DL%) and Encapsulation Efficiency (EE%) were assessed by UV/Vis using a calibration curve for CUR and the 430 nm wavelength. Stability was evaluated 30 days after production.



**Figure 1.** Schematic representation of the production of curcumin-loaded TPGS/F127/P123 polymeric micelles.

<sup>1</sup> Laboratory of Drug Development and Technologies, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>2</sup> REQUIMTE/LAQV, Group of Pharmaceutical Technology, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>3</sup> Instituto de Investigação e Inovação em Saúde, University of Porto, 4200-135 Porto, Portugal.

<sup>4</sup> Institute for Clinical and Biomedical Research (iCBR) Area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, 3000-548 Coimbra, Portugal.

<sup>5</sup> Chemistry Department, Faculty of Sciences and Technology, University of Coimbra, 3000-056 Coimbra, Portugal.

<sup>6</sup> Department of Pharmacy and Biotechnology, University of Bologna, 40138 Bologna, Italy.

\*Corresponding author: ritacerqueira2000@gmail.com.

**Results:** The average diameter of PFT\_CUR prepared by condition A (PFT\_CUR\_A) was  $22,9 \pm 2,4$  nm and with condition B (PFT\_CUR\_B) was  $20,2 \pm 3,6$  nm. The surface charge was ca. -2,416 mV in PFT\_CUR\_A and -1,355 mV in PFT\_CUR\_B. The nanosystem produced by method B is characterized by higher EE% and DL%: 17.3 and 1.6 vs 31.9 and 2.9 for A and B, respectively. Increasing size and PDI were registered after storage for one month at 25°C, while the EE% and DL% remain seemingly unaffected.

**Discussion:** The exhibited results suggest that CUR-loaded TPGS/F127/P123 mixed polymeric micelles prepared under condition B present more desirable characteristics for future therapeutic applications, intended for osteosarcoma treatment.

**Keywords:** curcumin, polymeric micelles, osteosarcoma, novel therapeutic systems.

**Acknowledgments:** The authors gratefully acknowledge the financial support of the “Fundação para a Ciência e Tecnologia” (FCT - Portugal) through the research project PTDC/NAN-MAT/1431/2021 and the Ph.D. grant 2021.08095.BD.

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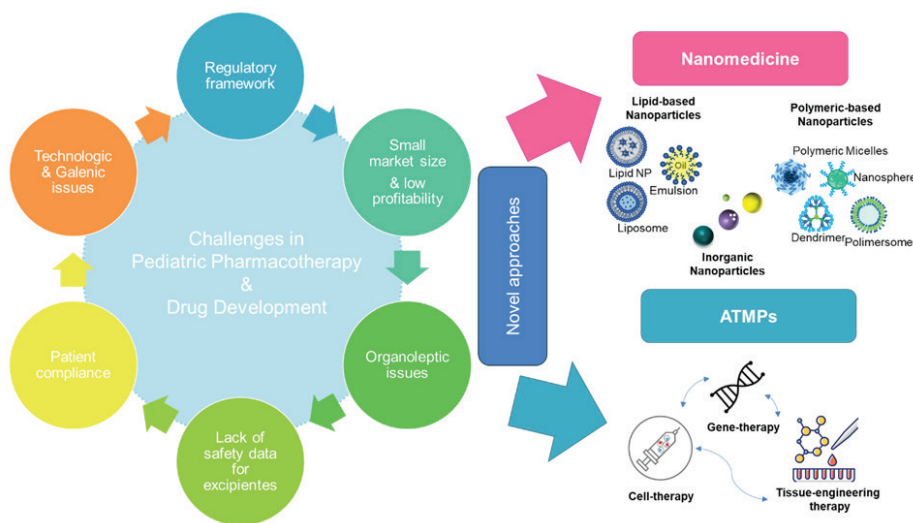
## Reviewing Pediatric Drug Formulation - on the Trail of Nanomedicine and Advanced Therapies

Domingues C.<sup>1,2,3\*</sup>, Jarak I.<sup>1,4</sup>, Veiga F.<sup>1,2</sup>, Dourado M.<sup>3</sup>, Figueiras A.<sup>1,2</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Pediatric drug formulation continues to be a hot and challenging topic. The “off-label” prescription of adult medicines or the use of extemporaneous preparation for pediatric patients has led to adverse reactions and toxic effects that demand age-appropriate formulation<sup>1</sup>. Therefore, taking the opportunity of the networking of the “X Congresso Iberoamericano de Ciências Farmacêuticas”, this work aims to reinforce the need for age-specific formulations for pediatrics by reviewing issues in pediatric drug formulation and tackling prospects of innovative approaches, such as nanomedicine and Advanced Therapy Medicinal Products (ATMPs) (figure 1).



**Figure 1.** Schematic summary of the factors that can affect pediatric pharmacotherapy and drug development and the use of innovative therapeutic approaches like nanomedicine and advanced therapy medicinal products (ATMPs) to help tackling some of these challenges.

<sup>1</sup> Univ. Coimbra, Faculty of Pharmacy, Drug Development and Technologies Laboratory, Coimbra, Portugal.

<sup>2</sup> REQUIMTE/LAQV, Drug Development and Technologies Laboratory, Faculty of Pharmacy, University of Coimbra, Portugal.

<sup>3</sup> Univ. Coimbra, Institute for Clinical and Biomedical Research (iCBR) area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, Coimbra, Portugal.

<sup>4</sup> Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal.

\*Corresponding author: cdomingues@ff.uc.pt.

**Methods:** A revision of literature in different databases, such as Pubmed, Web of Sciences, and ScienceDirect, using the following MeSH terms: Pediatrics, Nanoparticles, Gene therapy, Cell-and Tissue-Based Therapy, was performed. Other core databases were assessed, including [ema.europa.eu/en](http://ema.europa.eu/en), [fda.gov](http://fda.gov), [clinicaltrials.gov](http://clinicaltrials.gov), and [clinicaltrialsregister.eu](http://clinicaltrialsregister.eu), among others.

**Results:** Based on the performed literature search, pediatric pharmacotherapy faces old challenges that claim the need for novel technological and galenic requirements. Nanomedicine takes advantage of the unique bio and physicochemical properties of materials at the nanoscale. A workforce joining pharmaceutical developers and physicians has been proposed to perform a standardized framework for pediatric drug development. The advent of ATMPs has also brought the possibility of curing pediatric pathologies with complete remission of the disease, like hematologic pathologies<sup>2</sup>.

**Discussion:** Despite the incentives from the regulatory agencies promoting pediatric research, a considerable rift remains<sup>3</sup>. Some challenging questions regarding the safety and efficacy of nanomedicines and ATMPs, and the reported related immunogenic adverse effects seem to hamper their use in pediatrics, notably in how they can impact the healthcare system as summarized in the “four As”: authorization, availability, assessment and affordability.

**Keywords:** pediatric, nanoparticles, gene therapy, cell-and tissue-based therapy.

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## Brain-Targeted Liposomes Loading Differentiation-Inducing Drug to Enhance Neuronal Differentiation of iPSC-derived Neuroepithelial Stem Cells

Moreira R.<sup>1,2,3\*</sup>, Henriques D.<sup>1,2,4</sup>, Nóbrega C.<sup>5</sup>, Pereira de Almeida L.<sup>1,2,3</sup>, Mendonça L.<sup>1,2,4,6</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Machado-Joseph disease is a neurodegenerative disorder leading to significant cerebellar neurons loss<sup>1</sup>. Consequently, cell-replacement therapy has emerged as a promising therapeutic strategy<sup>2</sup>. In our lab, human iPSC-derived Neuroepithelial Stem cells (NESC) triggered encouraging results after brain transplantation in mice. However, we also observed limited cell migration and differentiation in the host brain. Therefore, we speculate that differentiation- and migration-inducing drugs may improve cell therapy outcomes. Nonetheless, brain drug delivery is hampered by the blood-brain barrier and drug accumulation in peripheral organs. Hence, drug delivery in brain-targeted liposomes might increase their bioavailability<sup>3,4</sup>.

**Methodology:** PEGylated and pH-sensitive liposomes, conjugated with a brain-targeting ligand, encapsulating a stem cell differentiation-inducing drug, were developed and characterized. Moreover, liposomes' ability to enhance neuronal differentiation was evaluated *in vitro*. Accordingly, the brain-targeted liposomes were evaluated for size by Nanoparticle Tracking Analysis. Drug encapsulation efficiency, ligand post-insertion efficiency, and the liposomal pH-sensitivity assessed by rhodamine release at pH 7.4 versus pH 5.5, were also evaluated. Finally, the impact of the liposomes on NESC differentiation was evaluated by immunocytochemistry analysis of the increase in neuronal markers ( $\beta$ 3-Tubulin and MAP2) and quantification of NeuN-positive neurons.

**Results:** the developed brain-targeted liposomes exhibited a size of  $128.2 \pm 9.6$  nm, a drug encapsulation efficiency of  $83.6 \pm 16.6\%$ , and a post-insertion efficiency of the brain-targeting ligand of 32.8%. At pH 5.5, the rhodamine-loaded liposomes presented a significant increase in rhodamine release of 46%, compared to pH 7.4. Confocal fluorescence microscopy revealed that rhodamine-loaded liposomes are internalized by NESC at 37°C, as opposed to incubation at 4°C. Our preliminary data demonstrated an increase in  $\beta$ 3-Tubulin- and MAP2-positive neurons and a 15% tendency increase in NeuN-positive cells after NESC incubation with liposomes loaded with the differentiation drug, compared to controls.

**Discussion:** data revealed a successful formulation of PEGylated, pH-sensitive, brain-targeted liposomes encapsulating a cell differentiation-inducing drug that is able to induce NESC differentiation into neurons *in vitro*. Next, we plan to test these liposomes *in vivo* for their ability to specifically deliver the differentiation-inducing drug to the brain and enhance NESC differentiation into neurons after transplantation.

**Keywords:** Machado-Joseph disease, liposomes, brain-targeting.

<sup>1</sup> Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.

<sup>2</sup> Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal.

<sup>3</sup> Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

<sup>4</sup> Institute of Interdisciplinary Research, University of Coimbra, Coimbra, Portugal.

<sup>5</sup> Algarve Biomedical Center Research Institute, University of Algarve, Faro, Portugal.

\*Corresponding author: Ricardo Moreira (ricardo.gomoreira@gmail.com).

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## Optimization of a Nanovaccine for Toxoplasmosis

Brito C.<sup>1</sup>, Nunes C.<sup>2</sup>, Borges M.<sup>1,3\*</sup>, Reis S.<sup>2</sup>

ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** *Toxoplasma gondii* is an obligate intracellular parasite that affects about one third of world's population and may cause toxoplasmosis. This infection can lead to encephalitis and death of immunocompromised individuals. In pregnant women, primo infection or re-infection with highly virulent strains can lead to abortion or congenital toxoplasmosis. Current therapies decrease the risk of developing disease, but do not complete eliminate the latent parasitic form responsible for chronic infection and, exhibit toxic side effects. At present there are several studies aimed at developing a vaccine for toxoplasmosis and this seems to be the best option compared to treatment with anti-toxoplasma drugs. In fact, the development of nanoparticles in preventive vaccine formulations, not only used as immunostimulatory adjuvants but also as a vehicle for immunogen delivery to target immune cells are promising<sup>1</sup>. The goal of this work was to optimize a delivery nano system containing an antigen extract of *T. gondii* for intranasal administration, previously shown to induce a partial protection against *T. gondii* infection (data unpublished).

**Methodology:** The synthesis of the formulation was done by single emulsion method and the physical-chemical parameters were evaluated by nanoparticle tracking analysis and dynamic light scattering<sup>2</sup>. Encapsulation efficiency was determined by Lowry assay and UV-Vis. Biological assays were performed to assess the cytotoxicity activity of the nano formulation using mouse and human cell lines. The transwell assay was used for evaluating nanoparticle nasal epithelium permeability.

**Results/Discussion:** This work allowed the achievement of nanoparticles with a size of  $208 \pm 2\text{nm}$  and  $0.28 \pm 0.02$  of polydispersity, adequate physical-chemical properties known to induce an effective immune response<sup>3</sup>. The use of the formulation at 7.5 mg/mL has shown to penetrate nasal epithelial cells with no cytotoxicity activity in 24h of exposure. Current work is being undertaken to find out immunomodulatory properties of the nanoparticles using primary cell cultures. These *in vitro* data are essential to pursue experiments aiming to determine if the nanoformulation developed will be able to induce effective protection using a mice model of congenital *T. gondii* infection.

**Keywords:** nanoparticle, nanovaccine, toxoplasmosis.

<sup>1</sup> UCIBIO – REQUIMTE, Laboratory of Biochemistry, Department of Biological Sciences, Faculty of Pharmacy, Porto Portugal.

<sup>2</sup> LAQV - REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, Porto, Portugal.

<sup>3</sup> Associate Laboratory i4HB-Institute for Health and Bioeconomy, Porto, Portugal.

\*Corresponding author: Margarida Borges; mborges@ff.up.pt.

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## Influence of the Organic Phase on the Stability of Polymeric Nanoparticle Suspensions under Various Storage Conditions

Sias-Fonseca L.<sup>1,2\*</sup>, Poletto F.S.<sup>3</sup>, Saraiva L.<sup>4</sup>, Costa P.C.<sup>1,2</sup>, Amaral M.H.<sup>1,2</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Polymeric nanoparticles (NP) have been extensively investigated as drug carriers due to several advantages, such as enhancing the bioavailability of lipophilic agents. However, the stability of these systems remains a challenge. An important factor, in addition to the polymer itself, is the organic phase, as this medium is where hydrophobic agents are incorporated in quantities dependent on their solubility. This study aimed to assess the influence of different organic phases on the stability of NP under varying storage conditions.

**Methodology:** For this purpose, suspensions of poly(lactic-co-glycolic acid) (PLGA) nanoparticles were prepared, using medium-chain triglycerides (MCT) (2 formulations) or monoolein (Mono) (2 formulations) as the organic phase, through the preformed polymer nanoprecipitation method<sup>1</sup>. These NP were subjected to stability studies at room temperature (~25 °C), in an incubator (~37°C) and in a freezer (~-3 °C) for 60 days. Periodically, the NP were evaluated (in triplicate) for average particle size and polydispersity index, zeta potential and pH.

**Results and discussion:** No variations in pH were observed, with values remaining close to neutrality. Upon preparation, NP with MCT exhibited an average size of 145 nm, while NP with Mono had an average size of 130 nm. These sizes remained without significant differences over 60 days ( $p > 0.05$ ), except for the Mono-based NP stored in the incubator ( $p = 0.003$ ), which showed increased values from the fourth day as depicted in figure 1 (a). This outcome likely stems from aggregate formation, coinciding with increased polydispersity ( $p = 0.002$ ), as shown in figure 1 (b). The remaining polydispersity results all stayed below 0.2, indicating good stability, albeit MCT formulation results tended to be smaller than those of the Mono-based NP. In figure 1 (c), can be seen that zeta potential results were around -40 mV for both types of NP under all conditions, suggesting a good stability<sup>2</sup>.

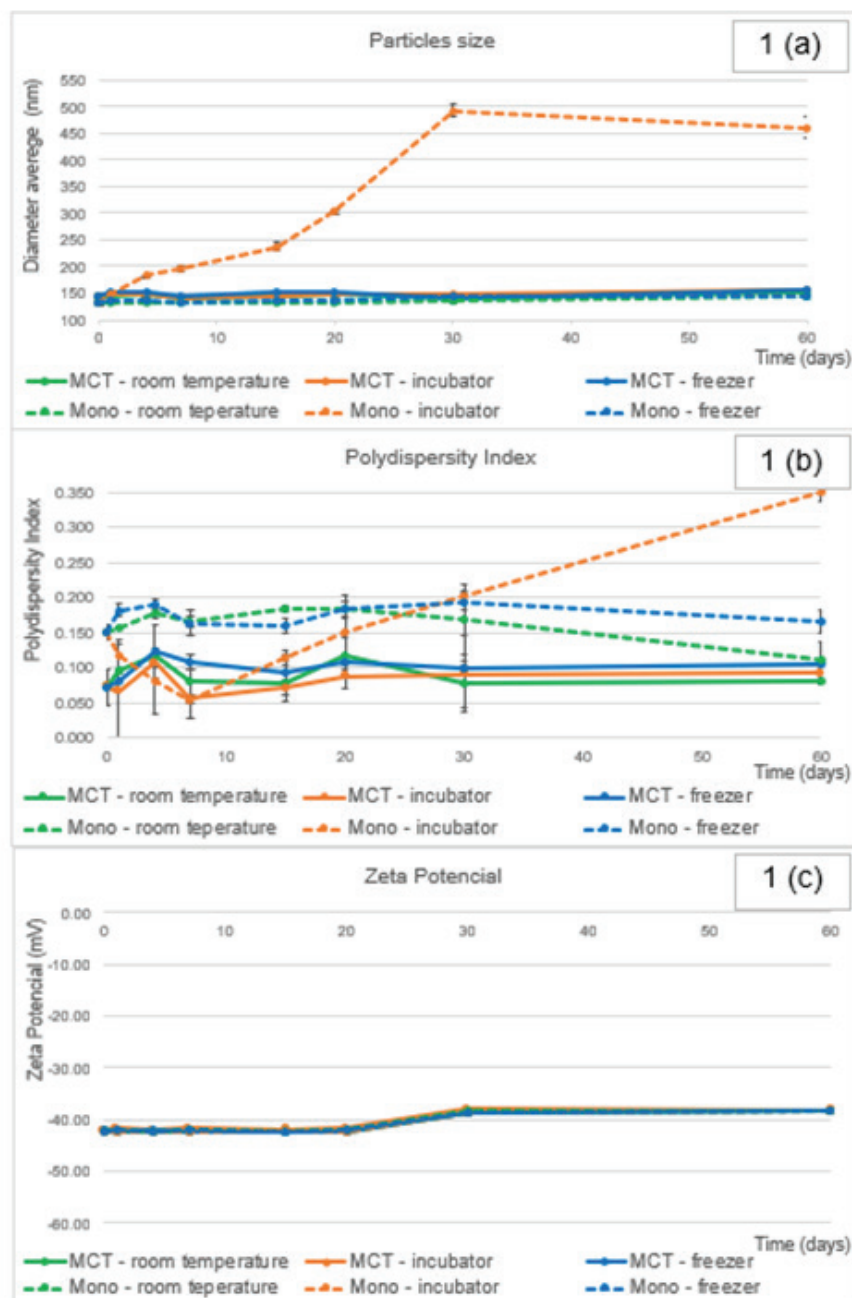
<sup>1</sup> UCIBIO - Applied Molecular Biosciences Unit, MedTech - Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Porto, Portugal.

<sup>2</sup> Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal.

<sup>3</sup> Laboratory of Supramolecular Chemistry for Biomedical Applications, Institute of Chemistry, University of Rio Grande do Sul, Porto Alegre, Brazil.

<sup>4</sup> LAQV/REQUIMTE, Microbiology Laboratory, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.

\*Corresponding author: Leticia Sias-Fonseca (sias.leticia@gmail.com).



**Figure 1.** Results of stability study (a) nanoparticle sizes, (b) polydispersity index and (c) Zeta Potential.

**Conclusion:** In conclusion, NP prepared with MCT exhibited better stability when stored at a higher temperature comparing with NP prepared with Mono, confirming that the organic phase can indeed influence the stability of polymeric nanoparticles.

**Keywords:** polymeric nanoparticles, stability, organic phase.

**Acknowledgments:** This research was funded by Fundação para a Ciência e Tecnologia (FCT), Portugal (grant reference: 2020.04785.BD). This work was also supported by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and UIDB/50006/2020 (LAQV/REQUIMTE).

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## Desenvolvimento e Caracterização de Comprimidos Matriciais de Dupla Camada Contendo um AINE

Dolovitsch L.<sup>1</sup>, Marques A.C.<sup>1\*</sup>, Costa P.C.<sup>1</sup>, Amaral M.H.<sup>1</sup>

ORIGINAL ARTICLE

### RESUMO

**Introdução:** No caso de certos fármacos, como os anti-inflamatórios, uma fração da dose terapêutica deve atingir a corrente sanguínea num curto período para o alívio rápido da dor e da inflamação, enquanto a restante fração deve prolongar o efeito terapêutico por mais algum tempo. O ibuprofeno (IBU) é um anti inflamatório não esteroide (AINE), bastante utilizado devido também à sua ação analgésica e antipirética<sup>1</sup>. Os comprimidos matriciais de libertação prolongada permitem manter a concentração plasmática do fármaco em níveis terapêuticos durante um longo período. O objetivo deste trabalho foi desenvolver e caracterizar comprimidos matriciais de dupla camada constituídos por uma camada de libertação rápida contendo Ibuprofeno DC<sup>®</sup>85 (constituído por IBU e croscarmelose sódica), e uma camada de libertação lenta, contendo o mesmo AINE e um agente formador de matriz inerte (etilcelulose).

**Metodologia:** Inicialmente, comprimidos monocamada constituídos por IBU e etilcelulose, em diferentes proporções (40:60; 50:50 e 60:40) e 0,5% de lubrificante (Aerosil<sup>®</sup> 200), foram produzidos por compressão direta e caracterizados quanto à uniformidade de massa, dureza e perfil de dissolução do IBU. Posteriormente, foram produzidos os comprimidos matriciais de dupla camada, tendo estes sido também caracterizados quanto à uniformidade de massa, dureza e perfil de dissolução.

**Resultados:** O perfil de dissolução dos comprimidos monocamada contendo IBU e etilcelulose na proporção 50:50 permitiu obter uma libertação de IBU nem demasiado rápida (como a obtida com os comprimidos 60:40), nem demasiado lenta (como a dos comprimidos 40:60), tendo sido a proporção utilizada na camada de libertação lenta dos comprimidos matriciais de dupla camada. Nenhum dos comprimidos de dupla camada apresentou uma massa fora dos limites de  $\pm 5\%$  em relação à massa média (Farmacopeia Portuguesa 9) e todos apresentaram valores de dureza entre 60 100 N.

**Discussão:** Os comprimidos de dupla camada produzidos proporcionaram uma rápida libertação do IBU a partir da camada constituída por ibuprofeno DC<sup>®</sup>85, prolongando se a libertação por mais de 8 horas a partir da camada de libertação lenta.

**Palavras-chave:** comprimidos matriciais, ibuprofeno, libertação prolongada.

<sup>1</sup> UCIBIO—Unidade de Ciências Biomoleculares Aplicadas, MEDTECH, Laboratório de Tecnologia Farmacêutica, Departamento de Ciências do Medicamento, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal; Laboratório Associado i4HB—Instituto para a Saúde e a Bioeconomia, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal.

\*Autor correspondente: amarques@ff.up.pt.

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## Optimizing Fisetin Encapsulation in Polycaprolactone Nanoparticles Suspensions

Sias-Fonseca L.<sup>1,2\*</sup>, Poletto F.S.<sup>3</sup>, Hyppolito L.<sup>3</sup>, Paese K.<sup>4</sup>, Souza P.<sup>4</sup>, Gündel S.S.<sup>4</sup>, Saraiva L.<sup>5</sup>, Costa P.C.<sup>1,2</sup>, Amaral M.L.<sup>1,2</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Fisetin, an abundant flavonoid, exhibits a repertoire of promising pharmacological features, making it an excellent anticancer agent<sup>1</sup>. However, this compound exhibits low water solubility which difficult its bioavailability and targeting, hindering its use *in vivo*<sup>2</sup>. To overcome the unfavourable fisetin physicochemical characteristics, its encapsulation in polymeric nanoparticles (NP) have been studied. This work focused on optimizing the encapsulation efficiency of fisetin within PCL (polycaprolactone) nanoparticles. In this pursuit, the primary objective was to determine the upper limit of fisetin encapsulation while maintaining suitable physical characteristics.

**Methodology:** The encapsulation of fisetin was achieved through a process involving the preparation of NP by a controlled technique<sup>3</sup>, using medium-chain triglycerides (MCT), polysorbate 80, sorbitan monostearate and fisetin in the following percentages: 0% (control), 0.4%, 4.0%, 5.0% and 8.0%. The encapsulation process was adjusted to strike a balance between maximizing the drug loading while preserving the structural integrity of the NP. The particle sizes and polydispersion were analyzed by three different techniques, laser diffraction, dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA), in addition to the zeta potential evaluation (always in triplicate). The formulation containing the highest possible amount of fisetin and which maintained the best physical characteristics, was submitted to a fisetin encapsulation efficiency study through an indirect method, using ultrafiltration tubes with a 50,000 Dalton molecular weight membrane and analysed in HPLC.

**Results and discussion:** The results obtained through the three mentioned techniques were coherent and provided information that was examined to verify the presence of agglomerates. The results presented in Table 1 unveil that the maximum of fisetin encapsulation efficiency was attained in the case PCL NP containing 4.0% of this drug. This optimal percentage of fisetin represents the equilibrium between loading capacity and suitable physical characteristics of NP. Beyond this threshold could potentially lead to compromised stability. The encapsulation efficiency of fisetin in this formulation was 90.9%. This result demonstrated a high amount of fisetin incorporated in the nucleus of the NP.

<sup>1</sup> UCIBIO - Applied Molecular Biosciences Unit, MedTech - Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Porto, Portugal.

<sup>2</sup> Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal.

<sup>3</sup> Laboratory of Supramolecular Chemistry for Biomedical Applications, Institute of Chemistry, University of Rio Grande do Sul, Porto Alegre, Brazil.

<sup>4</sup> Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Rio Grande do Sul, Porto Alegre, Brazil.

<sup>5</sup> LAQV/REQUIMTE, Microbiology Laboratory, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.

\*Corresponding author: Letícia Sias-Fonseca (sias.leticia@gmail.com).

**Table 1.** Results of average size, span value and presence of agglomerates of PCL nanoparticles with different percentages of fisetin, analysed by laser diffratometry.

| Fisetin (%) | DLS       |       |                | Mastersizer |       | NTA       | Presence of agglomerated/cristals |
|-------------|-----------|-------|----------------|-------------|-------|-----------|-----------------------------------|
|             | size (nm) | PDI   | Potencial Zeta | d(4.3) (um) | Span  | size (nm) |                                   |
| 0           | 264.2     | 0.214 | -13.2          | 0.42        | 2.79  | 223.6     | no                                |
| 0.4         | 223.8     | 0.173 | -14.2          | 0.31        | 1.62  | 233.6     | no                                |
| 4.0         | 200.6     | 0.096 | -12.1          | 0.25        | 1.77  | 211.0     | no                                |
| 5.0         | 225.0     | 0.154 | -13.3          | 1.27        | 2.07  | 227.6     | yes                               |
| 8.0         | 306.7     | 0.293 | -9.3           | 2.53        | 13.79 | 299.3     | yes                               |

**Conclusion:** This study led to the conclusion that the incorporation of 4.0% of fisetin allowed to obtain NP with adequate physical characteristics and a high encapsulation efficiency.

**Keywords:** polymeric nanoparticles, fisetin, encapsulation, polycaprolactone.

**Acknowledgments:** Acknowledgments: This research was funded by Fundação para a Ciência e Tecnologia (FCT), Portugal (grant reference: 2020.04785.BD). This work was also supported by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and UIDB/50006/2020 (LAQV/REQUIMTE).

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## Bio-enabling Technologies for Amorphous Solid Dispersions

Figueiredo J.<sup>1\*</sup>, Mendes M.<sup>1,2</sup>, Sousa J.J.<sup>1,2</sup>, Vitorino C.<sup>1,2</sup>

ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** The proportion of active pharmaceutical ingredients with poor aqueous solubility has increased over the years and dominates the early drug development portfolio, posing a significant obstacle in pharmaceutical development<sup>1</sup>. Considering this, enabling formulation approaches must be implemented to overcome these limitations in hopes of enhancing solubility and dissolution. Amorphous solid dispersions (ASDs) are a promising formulation technique widely employed for drugs designated as Biopharmaceutics Classification System class II and IV compounds<sup>2</sup>. The fundamental idea behind employing ASDs is to make the most out of the solubility advantage of the amorphous form of an active compound. To combat the stability concern associated with ASDs, polymers are used as a matrix to form a stable homogeneous amorphous system<sup>3</sup>.

**Methodology:** The present work aims at designing ASD-based formulations under the umbrella of quality by design principles for the improvement of oral drug bioavailability, as well as take advantage of the dispersion created for oral delivery to investigate its effects on the transdermal route of administration. For the oral administration route, solubility tests were carried out to assess the solubilization capacity of different polymers for Celecoxib as model drug. ASDs were prepared from selected polymers, using different manufacturing techniques: high shear homogenization, high pressure homogenization, microfluidics and spray drying. The obtained dispersions were further optimized resorting to a 32 full factorial design. The resulting formulations were evaluated in terms of analytical centrifugation and the influence of the different polymers on the intrinsic dissolution rate of ASDs. The optimal formulation was assessed in regards of characterization and *in vitro* performance. For transdermal administration, a transdermal drug delivery system based on the optimal dispersion obtained for the previous stage of this work was developed. Different adhesives and permeation enhancers were studied in order to understand the release profiles and permeation behavior of the transdermal patches.

**Results:** A dispersion in the nanometric scale was obtained by means of microfluidization and structural characterization confirmed the amorphous status of the formulation. Its *in vitro* performance was assessed, yielding an increment of 30-fold in terms of intrinsic dissolution rate and the conversion of this formulation to capsules is in line with the enhanced performance trend. A drug in adhesive patch was successfully formulated, containing PEG 400 that herein serves the dual function of plasticizer and permeation enhancer.

<sup>1</sup> Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

<sup>2</sup> Coimbra Chemistry Centre, Department of Chemistry, University of Coimbra, Coimbra, Portugal.

\*Corresponding author: joanatsfigueiredo@gmail.com.

**Discussion:** Overall, the incorporation of bio-enabling technologies is seen with great promise, potentially paving the way for future advancements in pharmaceutical development.

**Keywords:** amorphous solid dispersions, polymers, *in vitro* performance.

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## Hybrid Nanoparticles as a Multifunctional Platform for Brain Tumor Therapy

Rasteiro P.<sup>1\*</sup>, Mendes M.<sup>1,2</sup>, Sousa J.J.<sup>1,2</sup>, Vitorino C.<sup>1,2</sup>

ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** Glioblastoma (GBM), the most aggressive primary brain cancer, presents significant challenges in treatment due to its resistance to conventional therapies, high recurrence rates, and complex tumor heterogeneity. In recent years, nanotechnology has emerged as a promising strategy for developing innovative strategies to overcome treatment resistance and improve drug delivery<sup>1</sup>. This study focuses on the development of targeted hybrid nanoparticles (HNPs) as a novel approach to enhance GBM treatment. The HNPs comprise celecoxib (CXB)-loaded lipid nanoparticles as a chemotherapeutic strategy and iron oxide nanoparticles (IONPs), which served as a mediator for chemodynamic therapy (CDT) and magnetic targeting. Additionally, the covalent attachment of Apolipoprotein E (ApoE) peptide is hypothesized to enhance therapeutic efficacy and to impart specific targeting properties to breach the blood-brain-barrier (BBB)<sup>2</sup>.

**Methodology:** The optimization process of HNPs was achieved by a systematic experimental design where the optimized formulation was prepared by the ultrasonication technique and composed of a lipid phase (7.5% w/w) of Precirol® ATO 5: oleic acid (75:25), CXB (3% w/w, in relation to lipid content) and IONPs (20% v/v) and an aqueous phase of Tween® 80 (4.2% w/w). After achieved a balance between colloidal properties and magnetic properties, the HNPs were further functionalized by covalently linkage of ApoE peptide (designated as HNPsApoE). HNPs were also characterized through *in vitro* studies in U87- MG cells (cytotoxicity studies, reactive oxygen species production and cellular uptake) and *in vivo* studies (pharmacokinetics studies).

**Results:** HNPsApoE are characterized by their small particle size (107±4 nm) and narrow size distribution demonstrate efficient encapsulation of CXB and IONPs. Surface functionalized nanoparticles evidenced higher cytotoxicity than unmodified HNPs. Moreover, the catalytic properties of HNPs trigger the conversion of endogenous hydrogen peroxide into highly reactive hydroxyl radicals, inducing oxidative stress within U87-MG cells. HNPsApoE showed selectively higher cytotoxicity in U87-MG cells. In turn, the cellular uptake was not significantly different over the time in comparison to non-targeted nanoparticles. In the *in vivo* biodistribution studies, HNPs-CXBApoE revealed its favourable selectivity for the brain (DSI = 2.12). The application of an external magnetic field into mice head led to a 1.23-fold increase in drug accumulation in the brain.

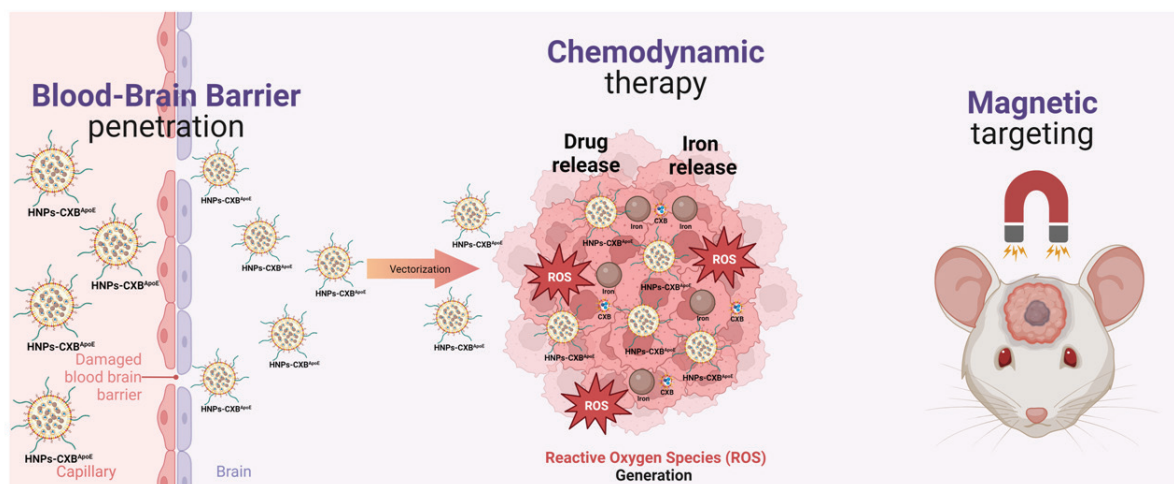
**Discussion:** These findings highlight the multifunctional strategy of HNPsApoE harnessing the potential of CDT through the reactive oxygen species (ROS) production, and the magnetic potential to reach the brain via an external field.

**Keywords:** hybrid nanoparticles, chemodynamic therapy, magnetic targeting.

<sup>1</sup> Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

<sup>2</sup> Coimbra Chemistry Centre, Department of Chemistry, University of Coimbra, Coimbra, Portugal.

\*Corresponding author: patriciarasteiro14@gmail.com.



Graphical abstract

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## Microencapsulation of Liposomes in Retrograded Starch/pectin Microparticles as a Potential Strategy for the Treatment of Colorectal Cancer

Santos A.<sup>1\*</sup>, Carvalho S.<sup>1</sup>, Chorilli M.<sup>1</sup>

ORIGINAL ARTICLE

### ABSTRACT

**Introduction:** Colorectal cancer (CRC) is the third most common malignant tumor in the world, and liposomes offer a promising approach to enhance the drug accumulation at tumor sites. Its encapsulation in colon-specific microparticles should offer significant protection against the degradative regions of the upper gastrointestinal tract (GIT), in addition to allowing targeted delivery of anticancer drugs to the colon under oral administration. Microparticles based on retrograded starch and pectin (RS/P) are a rational strategy to achieve the colon specific drug delivery, due to resistance to proteases and amylases. The aim of this work was to develop and characterize liposomes incorporated into retrograded starch and pectin microparticles for colon-specific delivery of 5-fluorouracil (5-FU).

**Methods:** Liposomes were obtained by the thin lipid film hydration method, and were characterized by size, polydispersity index (PDI), zeta potential and 5-FU loading efficiency. These liposomes were further encapsulated into RS/P microparticles using ionotropic gelation technique and characterized by size, yield and morphology.

**Results and discussion:** DLS measurements revealed that the liposomes had an average size between 96.4 to 127.5 nm, with PDI 0.15-0.18, and zeta potential values between + 47.6 to 45.4 mV, indicating the potential stability of liposomes. The 5-FU loading efficiency was 53% for liposomes. The liposomes-loaded RS/P microparticles showed an average size of 908  $\mu\text{m}$  with yield of 88%, indicating that the ionotropic gelation technique was efficient for microencapsulation of liposomes. The microscopy (FEG-SEM) showed successfully incorporation of liposomes into these microparticles, with spherical shape and smooth surface. These results indicate that RS/P microparticles are promising carriers for delivering drug-loaded liposomes to the colon, and multifunctional systems, such as the one presented here, offer a powerful means to address challenges in the treatment of CRC.

**Keywords:** microparticle, liposomes, colorectal cancer.

<sup>1</sup> São Paulo State University (UNESP), School of Pharmaceutical Sciences, Araraquara, SP, 14800-903, Brazil.

\*Corresponding author: aline.santos@unesp.br.

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## Enfermedad de Menkes: seguimiento de un Paciente Tratado con Histidinato de Cobre Inyectable de Elaboración Magistral

Guzmán M.L.<sup>1</sup>, Barbieri F.<sup>1</sup>, Luciani-Giacobbe L.C.<sup>1</sup>, Fierro C.B.<sup>2</sup>, Gavelli M.E.<sup>2</sup>, Apas A.<sup>1</sup>, Alovero F.<sup>1</sup>, Olivera M.E.<sup>1\*</sup>

### ORIGINAL ARTICLE

### RESUMEN

**Introducción:** Previamente se desarrolló un protocolo de elaboración magistral de histidinato de cobre inyectable (Hi-Cu/iny) de estabilidad mejorada. Esta formulación es de alto riesgo microbiológico ya que no admite autoclavado ni el uso de conservantes antimicrobianos. El objetivo de este trabajo fue determinar si la presencia de cobre reduce el riesgo microbiológico de la formulación y documentar el desempeño de la formulación en un paciente con diagnóstico de Enfermedad de Menkes (EM).

**Metodología:** La formulación de Hi-Cu/iny fue elaborada según protocolo y sometida a ensayo de eficacia antimicrobiana de acuerdo a Farmacopea Argentina (FA)<sup>1</sup>. Se utilizaron cultivos de *Pseudomonas aeruginosa*, *Staphylococcus aureus* y *Escherichia coli* de 108 UFC/mL. Se tomaron muestras a 7, 14 y 28 días y se determinó el número de microorganismos viables por método de recuento en placa. Complementariamente se utilizó la formulación para tratar a un paciente (masculino, 16 meses) con diagnóstico de EM que presentaba convulsiones, hipotonía global sin control cefálico, crecimiento dental retardado, ictiosis, pili torti en cejas y ausencia de pelo en cabeza, con valores de cobre y ceruloplasmina de 27 ug/dl y 4,7mg/dl. Se le indicó Hi-Cu/iny 50 ug/kg/día y se valoró cobre y ceruloplasmina séricos a 1, 4, 10 y 15 meses.

**Resultados y discusión:** El recuento de *Pseudomonas aeruginosa* y *Escherichia coli* de la formulación tras el ensayo de eficacia antimicrobiana arrojó una variación de + (aumento) 0.35 y +0.45 de unidades logarítmicas a los 7 días, de +0.10 y – (reducción) 0.56 a 14 días y de -0.16 y +0.58 a los 28 días respecto del recuento inicial. Para *Staphylococcus aureus* se observó una reducción de -1.7, -3.8 y -2.53 en los tres tiempos. De acuerdo a las especificaciones de FA, la presencia de cobre no confiere capacidad de autoconservación, posiblemente por su acomplejamiento con histidina. Por otra parte, tras el inicio del tratamiento, el paciente logró valores de cobre y ceruloplasmina normales en todas las mediciones. Su evaluación clínica mostró signos favorables: síndrome convulsivo mejorado, mejorías en el tono y control cefálico, erupción dentaria normal, piel sin ictiosis y crecimiento de cabello.

**Conclusión:** La presencia de cobre en la formulación no redujo su riesgo microbiológico, lo cual sigue siendo un aspecto crítico en su diseño. Por otra parte, la formulación estabilizada cuyo protocolo fue desarrollado previamente permitió brindar tratamiento al paciente con impactos significativos en su calidad de vida.

**Palabras clave:** enfermedad Menkes, histidinato de cobre.

<sup>1</sup> Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA), CONICET y Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, X5000 HUA, Córdoba, Argentina.

<sup>2</sup> Farmacia Central del Hospital Nacional de Clínicas, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Santa Rosa 1564, X5000 ETF, Córdoba, Argentina.

\*Autor correspondiente: eugenia.olivera@unc.edu.ar.

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## Exploring the Pharmacological Potential of an Amazonian by-Product

Mazzola P.G.<sup>1\*</sup>, Coco J.C.<sup>1</sup>, Silvério L.A.L.<sup>1</sup>, Sueiro A.C.<sup>1</sup>, Ataíde J.A.<sup>1</sup>, Santos E.M.<sup>1</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Euterpe oleraceae Mart., also known as açaí, is a plant native to the Amazon region. The commercially used part of açaí is the fruit pulp, yet 85% of its weight is comprised of the seed, which is mostly discarded. It is estimated that 1,000,000 tons of seed are generated annually in the Amazon region. This seed could be more effectively utilized due to its pharmacological properties, such as antioxidant, anti-inflammatory, and antimicrobial effects. Therefore, the objective of this study was to investigate the antioxidant activity and compounds present in açaí seed.

**Methodology:** To extract the sample, it was macerated using 70% ethanol, followed by rotary evaporation and freeze-drying. Antioxidant activity was determined through DPPH and FRAP analyses. In addition, the presence of total phenolics, tannins and flavonoids were also characterized.

**Results:** The obtained sample exhibited a substantial antioxidant effectiveness of  $99.17 \pm 2.21\%$  and an EC<sub>50</sub> of 9.32 mg/mL in the DPPH assay. Similarly, in the FRAP assay, the sample displayed an antioxidant capacity of  $84.52 \pm 4.49$  mg GAE/g and an EC<sub>50</sub> of 7.80 mg/mL. The total phenolics content was quantified at  $158.82 \pm 24.10$  mg GAE/g, whereas the tannin content and flavonoid content were determined to be  $351.01 \pm 0.68$  mg TAE/L and  $312.62 \pm 12.13$  mg QE/g, respectively. In other studies, açaí seeds displayed an AA% of 86.8%, while the total phenolics content was measured at  $37.08 \pm 8.56$  g GAE/100g of the sample.

**Discussion:** This study presented promising results compared to other studies and given that açaí seed is a byproduct with substantial antioxidant activity and bioactive compounds, this extract can be explored in the future for incorporation into pharmaceutical or cosmetic formulations.

**Keywords:** acai, antioxidant, flavonoid.

<sup>1</sup> Faculty of Pharmaceutical Sciences, University of Campinas (Unicamp), Campinas, Brazil.

\*Corresponding author: pmazzola@fcf.unicamp.br.

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## Exploring a Brazilian Native by-product as an Antioxidant Adjuvant in Liposomal Drug Delivery Systems

Mazzola P.G.<sup>1\*</sup>, Dias C.G.<sup>1</sup>, Silvério L.A.L.<sup>1</sup>, Sueiro A.C.<sup>1</sup>, Ataíde J.A.<sup>1</sup>, Santos E.M.<sup>1</sup>, Coco J.C.<sup>1</sup>

### ORIGINAL ARTICLE

#### ABSTRACT

**Introduction:** Baru nuts (*Dipteryx alata* Vog.) are a native species from Brazilian Cerrado, popular for consumption and oil production. The baru oil production generates a residue that may have a valuable antioxidant composition. Liposomes are promising nanostructures, but often unstable due to their phospholipidic bilayers being prone to oxidation, leading to drug leakage. Our goal was to investigate the antioxidant potential of baru by-product and whether it could be used as an adjuvant in liposomal drug delivery of an antifungal model drug, fluconazole.

**Methodology:** Baru nuts were cold pressed, and the residue was macerated with ethanol 70% (v/v) for 3 days. Baru by-product extract was investigated regarding phenolic content and antioxidant activity by DPPH radical inhibition and ferric reducing antioxidant power (FRAP) assay. Liposomes with soy lecithin and baru by-product extract were produced by direct sonication and evaluated regarding morphology by cryo-electron microscopy. Stability of physicochemical parameters of liposomes were evaluated for 15 days, regarding mean diameter, polydispersity index and zeta potential in the Zetasizer. Fluconazole encapsulation efficiency and release by diffusion cell apparatus were also evaluated. Finally, liposomes were induced to peroxidation by AAPH, an azo compound that generates free radicals.

**Results:** Baru by-product extract presented promising phenolic content (27mg GAE/g) and antioxidant activity (DPPH IC<sub>50</sub> of 1.4mg/mL; FRAP EC<sub>50</sub> of 2.6mg/mL). Control and baru by-product liposomes presented sizes of 170 nm, zeta potential of -41 mV, were monodisperse (PDI <0.28), spheric, and unilamellar.

**Discussion:** Baru extract improved the physicochemical stability of liposomes for 15 days and effectively reduced the oxidation process of liposomes by 97% in the AAPH-induced peroxidation assay after 5h. Fluconazole encapsulation efficiency (38%) and release (46% after 72h) were not modified by the presence of extract. In conclusion, baru by-product extract presented antioxidant activity, and enhanced the stability of liposomes without affecting the encapsulation or release of fluconazole, with potential to be further explored as an antioxidant adjuvant in drug delivery.

**Keywords:** baru, liposome, adjuvant.

<sup>1</sup> Faculty of Pharmaceutical Sciences, University of Campinas (Unicamp), Campinas, Brazil.

\*Corresponding author: pmazzola@fcf.unicamp.br.

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## Producción de Xantano para Formulaciones Farmacéuticas a partir de Melaza

del Moral A.<sup>1\*</sup>, Páez R.<sup>1</sup>, Martínez-Checa F.<sup>1</sup>

### ORIGINAL ARTICLE

### RESUMEN

**Introducción:** La goma xantana es un polisacárido natural producido por *Xanthomonas campestris*. Se utiliza en la formulación de productos farmacéuticos, así como en una variedad de industrias, incluyendo alimentos, cosméticos y productos de cuidado personal. Sus aplicaciones en la industria farmacéutica son numerosas: agente espesante y estabilizador en medicamentos líquidos, para geles y ungüentos tópicos, proporciona una textura adecuada y distribución uniforme de ingredientes activos en formulaciones de liberación controlada, incluso tiene aplicación en preparaciones oftálmicas<sup>1</sup>. El xantano es generalmente considerado seguro para su uso en productos farmacéuticos, siempre y cuando se utilice en concentraciones adecuadas y bajo las directrices de formulación establecidas. Debido a que cada vez es mayor la demanda mundial en el mercado de este interesante hidrocoloide, en el presente trabajo se ha planteado la posibilidad de obtener xantano, empleando un sustrato barato como es la melaza de remolacha residuo procedente de la industria azucarera.

**Metodología:** El medio para la producción de xantano se preparó disolviendo la melaza en agua destilada a diferentes concentraciones: 1; 2; 5; 7,5 y 10% (p/v). En todos los casos el pH se ajustó a 7,1. Los medios se inocularon en proporción 2% (p/v) y se incubaron en agitador orbital a 30°C y 200 rpm. Se realizaron determinaciones de producción de xantano y características reológicas del mismo.

**Resultados:** La mayor producción de xantano (12,24 g/l) se obtuvo en el medio con 10% de melaza (p/v). Sin embargo, al analizar las propiedades físicas y composición química del producto se observó una menor viscosidad de las soluciones acuosas. El xantano obtenido de melaza al 10% presentó una viscosidad de 0,07 Pa frente a 0,236 de la melaza al 2,5%. Por otra parte, el xantano obtenido de melaza al 10% tenía menor contenido de carbohidratos.

**Discusión:** Si bien los ensayos realizados demuestran que la melaza de remolacha puede ser un sustrato industrial adecuado y una alternativa barata para la producción de este hidrocoloide de uso farmacéutico, son necesarios estudios adicionales para ajustar las condiciones que proporcionen el producto de mejores propiedades reológicas.

**Palabras clave:** exopolisacárido, xantano, melaza.

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<sup>1</sup> Facultad de Farmacia. Universidad de Granada. España.

\*Autor correspondiente: admoral@ugr.es.

## Caracterização e Citotoxicidade *in vitro* frente a DU-145 e PNT-2 da Curcumina Encapsulada em Nanopartículas Poliméricas

Araújo J.<sup>1\*</sup>, Junior A.T.<sup>1</sup>, Dutra J.<sup>1</sup>, Santos A.<sup>1</sup>, Chorilli M.<sup>1</sup>

ORIGINAL ARTICLE

### RESUMO

**Introdução:** A curcumina (CUR) é um polifenol obtido da *Curcuma longa* que possui atividade contra diferentes linhagens celulares de câncer de próstata. Contudo, apresenta alta hidrofobicidade, o que limita sua utilização. Logo, sua incorporação em nanopartículas poliméricas contendo succinato de D- $\alpha$ -tocoferil polietilenoglicol 1000 (TPGS), podem viabilizar sua utilização. O TPGS é um tensoativo que auxilia no processo de internalização celular e indução de apoptose.

**Metodologia:** As nanopartículas contendo TPGS e curcumina (NC) e as nanopartículas branca (NB) foram desenvolvidas pelo método de nanoprecipitação, variando a concentração de TPGS de 0,05 a 0,5% (p/v). Foram caracterizadas quanto ao tamanho de partícula (TP) e distribuição por dois métodos (DLS e NTA), além de potencial zeta (PZ), concentração de partículas/mL (NTA) e eficiência de encapsulação. A estabilidade foi avaliada durante 90 dias e a citotoxicidade *in vitro* frente as células de câncer de próstata (DU-145) e epitélio normal prostático (PNT-2) foi determinada utilizando o indicador de viabilidade resazurina após 48 horas de exposição na faixa de 1 a 20  $\mu\text{g/mL}$ .

**Resultados:** A melhor concentração de TPGS foi de 0,05%, onde a NC e a NB apresentaram TP de 176,7 e 186,4 nm (DLS), 155,5 e 159,0 nm (NTA), distribuição de 0,164 e 0,228 (DLS) e 0,92 e 0,89 (NTA), PZ de -30,1 e -26,1 mV e concentração de  $1,34 \times 10^{16}$  e  $1,03 \times 10^{16}$  partículas/mL, respectivamente. Além disso, a NC apresentou 87,5% de eficiência de encapsulação de curcumina. Ambas as nanopartículas se apresentaram visualmente estáveis, sem a percepção de partículas precipitadas ou aglomerados durante 90 dias. Entretanto, houve aumento de 20 nm no dia 90, discreto aumento na distribuição de 0,164 para 0,221 e no PZ de -30,1 para -33,5 mV para a NC. A NB apresentou apenas aumento no PZ entre os dias 1 e 90 de -26,1 para -38,0 mV. Frente a linhagem de câncer de próstata DU-145, na menor concentração apenas a CUR reduziu 3,3% da viabilidade celular. A partir de 3  $\mu\text{g/mL}$  houve redução da viabilidade celular de 4,2%, 4,1% e 6,8% e em 20  $\mu\text{g/mL}$  com 82,6%, 94,4% e 84,9%, para CUR, NC e NB, respectivamente, com concentração inibitória mínima (CI<sub>50</sub>) de 10,36, 6,24 e 7,12  $\mu\text{g/mL}$ , respectivamente. Na linhagem celular PNT-2, apenas na maior concentração houve redução da viabilidade celular, sendo 6,1%, 0,7% e 30,1% para CUR, NC e NB, respectivamente, sem determinação da CI<sub>50</sub>.

**Discussão:** Assim, o processo de encapsulação da CUR foi obtido com propriedades físico-químicas satisfatórias, apresentando-se estáveis durante 90 dias, com alta eficiência de encapsulação, além de promover a potencialização do efeito citotóxico frente a células de câncer de próstata, DU-145, e baixa citotoxicidade frente a células saudáveis, PNT-2.

**Palavras chave:** açafrão, câncer de próstata, nanopartículas poliméricas.

<sup>1</sup> Faculdade de Ciências Farmacêuticas de Araraquara, Universidade Estadual Paulista Júlio de Mesquita Filho, Araraquara, São Paulo, Brasil.

\*Autor correspondente: jennifer.thayanne@gmail.com.

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