

Development of Differential Optrodes for Highly Sensitive and Reliable Chemical Sensing

João Pedro Sampaio Mendes

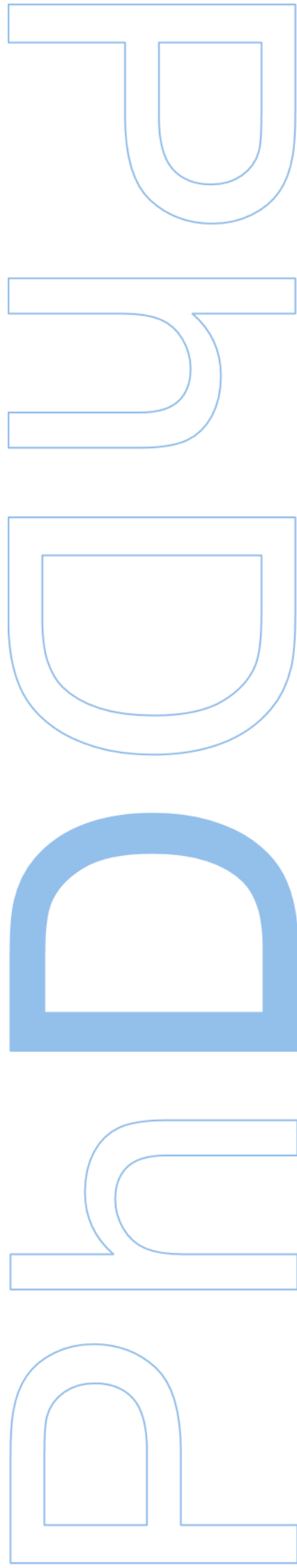
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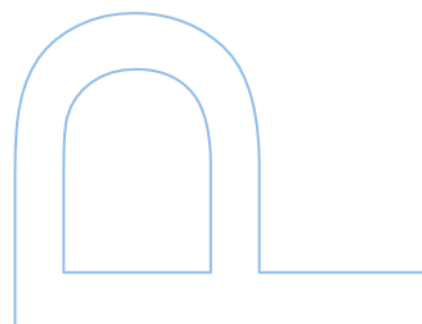
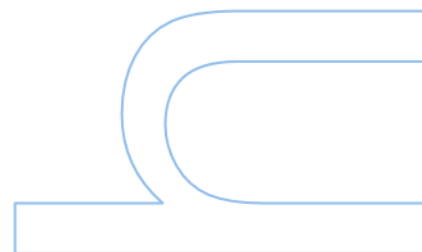
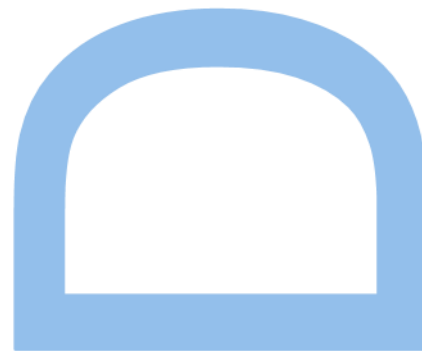
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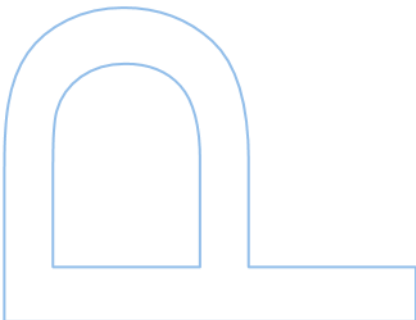
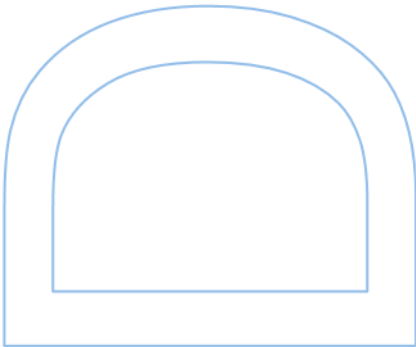


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Resumo

A tese aqui exibida, apresenta os resultados experimentais obtidos a partir da pesquisa e desenvolvimento de estruturas em fibra ótica sustentadas em diferentes esquemas de transdução, envolvendo materiais químicos ou bioquímicos distintos com a finalidade de desenvolver sensores em fibra ótica associados à deteção de espécies químicas ou biológicas.

É apresentada uma visão geral e detalhada sobre as arquiteturas em fibra ótica mais utilizadas para o desenvolvimento de sensores, utilizando a fibra ótica como parte integrante de um dispositivo sensor ou sendo a fibra o próprio elemento de transdução. Além disso, são expressas e discutidas as principais técnicas utilizadas para a fabricação de estruturas sensíveis em fibra ótica (tais como redes de período longo), assim como técnicas para a realização de diferentes tipos de revestimentos em torno da sua geometria cilíndrica (tais como revestimentos metálicos), para a imobilização de elementos sensíveis (tais como anticorpos, aptâmeros ou recetores poliméricos) na sua superfície, assim como para a análise química dos elementos sintetizados e imobilizados.

Adicionalmente, são descritos e discutidos os princípios de funcionamento de alguns dos elementos sensíveis para deteção colorimétrica, fluorométrica e biológica, assim como são também referidos exemplos de aplicações dirigidas à deteção de diferentes espécies, utilizando as diferentes configurações em fibra ótica abordadas.

Os estudos experimentais incluem a investigação da dinâmica do dióxido de carbono dissolvido em ecossistemas próximos aos observados em instalações de aquacultura. O estudo experimental foi executado a partir da aplicação de uma membrana com propriedades colorimétricas num sistema em fibra ótica. São discutidos os resultados obtidos durante toda a investigação, incluindo os estudos efetuados para a obtenção da melhor combinação de materiais sensíveis para a construção do sensor. Após a sua conceção, o sensor foi calibrado em água doce e salina, revelando limites de deteção de 1,23 e 1,05 mg/L, respetivamente. É também demonstrada e comparada a performance do sensor aqui desenvolvido com a performance de um sensor comercialmente disponível, em situações muito próximas do real ambiente observado em aquacultura.

Além disso, o desempenho de dois sensores refratométricos, sustentados em redes de período longo, que se baseiam em medidas de diferencial entre o sensor de medida e um sensor de referência, foram também investigados.

A primeira plataforma visa estudar os efeitos provocados por ligações específicas anticorpo/antigénio (IgG/anti-IgG), usando uma rede de período longo funcionalizada com nano-esferas de sílica, cuja superfície foi previamente modificada pela imobilização dos elementos recetores (anticorpos IgG). O resultados experimentais obtidos com a deteção do analito alvo (anticorpos anti-IgG) através do sistema diferencial, composto pela fibra funcionalizada e uma rede de período longo não modificada, mostram uma melhor performance (comparativamente aos resultados obtidos apenas pela fibra modificada), exibindo uma sensibilidade e um limite de deteção de 72 nm.mL/ μ g e 0,12 μ g/mL, respetivamente.

Uma outra plataforma refratométrica apoiada em redes de período longo e impressão molecular foi utilizada para estudar a cinética de ligação entre cavidades molecularmente impressas e anticorpos IgG. A avaliação experimental foi executada por um sistema composto por uma rede de período longo funcionalizada com o filme polimérico molecularmente impresso e uma outra rede de período longo funcionalizada por um filme não impresso (ou seja, sem cavidades), revelando uma notória melhoria relativamente ao sistema sem a fibra de referência. O sistema diferencial mostrou uma sensibilidade e um limite de deteção de 0,057 nm.L/nmol e 0,25 nmol/L, respetivamente.

Foi também desenvolvida e investigada uma nova abordagem para o fabrico de uma nova configuração sensível apoiada em plasmões de superfície numa fibra ótica multimodo. O princípio de funcionamento desta nova configuração, está fortemente dependente do processo de redução do núcleo, proporcionando que a luz que escapa pela região reduzida possa interagir, a partir do exterior, com as nanopartículas imobilizadas na superfície do filme metálico. Dessa interação, resulta o re-acoplamento da luz incidente, através do meio efetivo criado pelas nanopartículas, de volta para o interior do núcleo da fibra. Dessa forma, da interação entre a luz acoplada e o filme metálico resulta a formação de plasmões de superfície cujo campo eletromagnético é amplificado pela presença do meio efetivo formado pelas nanopartículas e o meio externo.

O comportamento da nova configuração foi estudado variando o índice de refração externo, bem como o tamanho das nanopartículas usadas na sua conceção. A investigação mostrou sensibilidades ao índice de refração superiores a 4400 nm/RIU

numa gama de trabalho de entre 1.333 e 1.380. A sua aplicabilidade para aplicações na área biológica é também demonstrada, utilizando como modelo biológico um complexo aptâmero/trombina. O sensor revelou uma sensibilidade e um limite de deteção de 9 nm.L/nmol e 0.04 nmol/L, respetivamente.

Os resultados apresentados oferecem importantes avanços no desenvolvimento de novas metodologias para a deteção de elementos químicos e/ou biológicos através de dispositivos óticos, recorrendo a técnicas de diferenciação a fim de aumentar a precisão e exatidão dos sistemas sensores.

Palavras-chave: Fibra ótica, sensores químicos, sensores biológicos, técnicas de diferenciação, redes de período longo, plasmões de superfície, nanopartículas, impressão molecular, recetores biológicos.

Abstract

This thesis presents the experimental results achieved from the research and development of fiber optic structures based on different transduction schemes comprising distinct chemical or biochemical materials for sensing applications associated with chemical or biological entities detection.

A detailed overview about the extrinsic fiber optic configurations used for chemical or biological sensing is presented. Additionally, it is included a brief discussion about the working principles and some of the sensing elements for colorimetric and fluorometric detection. Examples of different applications targeting different entities are also addressed.

Moreover, are outlined and discussed the main techniques used in the thesis to fabricate in-fiber structures in bare fibers, to perform distinct types of coatings around the cylindrical fiber geometry, to immobilize sensitive elements on the fiber surface, and to perform the chemical analysis of the synthesized elements. Different investigations considering those type of fiber optic structures, targeting chemical or biological entities are also addressed.

The experimental studies include the investigation of the dissolved carbon dioxide dynamics in environments close to aquaculture ecosystems obtained from the application of a fiber optic colorimetric sensor. The results obtained from the performed experiments are addressed and discussed to attain the best sensitive materials combination to build the fiber optic sensor. Experimental calibrations of the colorimetric fiber optic sensor in fresh and saline water, using carbon dioxide standard solutions, are covered exhibiting limits of detection of 1.23 and 1.05 mg/L respectively. The comparison of the performances of the developed sensor and a commercial sensor is also demonstrated.

The performance of two distinct differential refractometric sensors-based on long period fiber gratings (LPFG) is investigated using self-compensated LPFG systems. The antibody/antigen (IgG/anti-IgG) specific bindings are evaluated by an optical system composed by a LPFG modified with IgG-modified silica nanoparticles and a bare LPFG to correct fluctuations caused by undesired events. The experimental results show a sensitivity and a limit of detection of 72 nm.mL/ μ g and 0.12 μ g/mL, respectively.

The binding kinetics between a molecular imprinted polymer (MIP) layer and IgG antibodies is investigated including a non-imprinted polymer (NIP) using a self-compensated MIP/NIP system in a working range from 1 to 100 nmol/L. The MIP layer formation is monitored in real-time by following the LPFG resonance wavelength band. The LPFG/MIP/NIP system revealed a sensitivity and a limit of detection of 0.057 nm.L/nmol and 0.25 nmol/L, respectively.

A new methodology to fabricate fiber optic plasmonic sensors, with enhanced sensitivity and FOM, was conducted by coupling plasmonic thin films with plasmonic nanostructures. This new hybrid plasmonic configuration was characterized by varying the external refractive index as well as the size of nanoparticles revealing sensitivities ranging from 4100 to 4400 nm/RIU in a refractive index working range from 1.333 to 1.380. The applicability of the developed sensor for applications in the biological field, using as biological model an aptamer/thrombin complex, is demonstrated. The sensor revealed a sensitivity and a limit of detection of 9 nm.L/nmol and 0.04 nmol/L, respectively, in a working range from 0.01 to 100 nmol/L.

The presented results provide important advances in the development of new methodologies for the monitoring of chemical and/or biological elements through optical devices using differentiation techniques to increase the reliability of sensor systems.

Keywords: Fiber optic technology, chemical sensors, biosensors, differential technique, long period fiber gratings, surface plasmon resonance, nanoparticles, molecular imprinting, bioreceptors.

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Acronyms and Abbreviations

AChE - Acetylcholinesterase	FT - Fourier Transformation
APTES - (3-aminopropyl) triethoxysilane	FTIR - Fourier-transform Infrared
AuNp - Gold Nanoparticles	FW - Framework
AuNS - Gold Nanospheres	FWHM - Full Width at the Half Maximum
BCAT - p-tert-butylcalix[4]arene-tetrakis	gCO ₂ - Gaseous Carbon Dioxide
BCP - Bromocresol Purple	GO/CA - Graphene Oxide/Cellulose Acetone
BSA - Bovine Serum Albumin	HD4 - Hydromed D4 Hydrogel
CDRs - Complementary Determining Regions	HTR - Human Transferrin
CEMUP - Materials Centre of the University of Porto	Ig - Immunoglobulins
CFPI - Composite Fabry-Pérot Interferometer	IgG - Immunoglobulin G
ChitPAA - Chitosan/poly Acrylic Acid	IR - Infrared
CK17 - Cytokeratins 17 Proteins	IR - Infrared
CVD - Chemical Vapor Deposition	ITO - Indium Tin Oxide
dCO ₂ - Dissolved Carbon Dioxide	LbL - Layer-by-Layer
E. -coli - Escherichia coli	LPFG - Long Period Fiber Gratings
EFOBS - Enzyme Fiber optic Biosensors	LSPR - Localized Surface Plasmon Resonance
EMA - Effective Medium Approximations	MDM - Metal-Dielectric-Metal
ERI - External Refractive Index	MG - Maxwell Garnett
ESA - Electrostatic Self-assembly	MI - Michelson Interferometers
FBG - Fiber Bragg Gratings	MIPs - Molecularly Imprinted Polymers
FOCS - Fiber Optic Chemical Sensors	MMF - Multimode Optical Fiber
FOEW - Fiber optic Evanescent Wave	MOF - Microstructured Optical Fibers
FOM - Figure of Merit	MTES - Methylethoxysilane
FOS - Fiber optic Sensors	MUA - 11-mercaptoundecanoic Acid
FP - Fabry-Pérot	MZI - Mach-Zehnder Interferometer
FPI - Fabry-Perot interferometers	nanoMIPs - Nanoscaled Molecularly Imprinted Polymers

NM-OFs - Nanoporous Metal-Organic Frameworks	SPA - Staphylococcal Protein A
OSA - Optical Spectrum Analyzer	SPP - Surface Plasmon Polaritons
PAA - Poly(acrylic acid)	SPR - Surface Plasmon Resonance
PAH - Poly(allylamine hydrochloride)	SPS - Solid-phase Synthesis
PBS - Phosphate Buffered Saline	SPW - Surface Plasmon Wave
PCF - Photonic Crystal Fibers	TBA - Thrombin Binding Aptamer
PCSF - Plastic-clad Silica-core Fiber	TEOS - Tetraethoxysilane
PDDA - Poly diallyldimethyl Ammonium Chloride	TESPSA - 3-(Triethoxysilyl) Propylsuccinic Anhydride
PE - Polyelectrolyte	TFBG - Tilted Fiber Bragg Gratings
PEG - Poly(ethylene glycol)	THR - Thrombin
PEI - Polyethyleneimine	TMA-OH - Tetramethylammonium Hydroxide
PLA - Polylactide	TP - Turning Point
PLL - Poly-L-Lysine	TPU - Thermoplastic Polyurethane
PMMA - Poly(methyl methacrylate)	UV - Ultraviolet
POF - Plastic Optical Fiber	VA - Vancomycin
PVA - Poly(vinyl alcohol)	Vh - Variable Heavy Chain
PVD - Physical Vapor Deposition	Vi - Variable Light Chain
RH - Relative Humidity	VOCs - Volatile Organic Compounds
RI - Refractive Index	
S2 PEG ₆ COOH - Dithiolalkane aromatic PEG ₆ -COOH	
SAM - Self-assembled Monolayers	
SC - Spin-coating	
SELEX - Systematic Evolution of Ligands by Exponential Enrichment	
SERS - Surface Enhanced Raman Spectroscopy	
SI-LOV - Lab-on-Valve	
SMF - Single Mode Fibers	

Chapter 1 – Introduction

1.1. Motivation

Optical fiber technology emerged to book its position in the history of telecommunications technology by opening new perspectives and possibilities in the way in which information is carried. Since those achievements, the evolution of optical fiber technology led to the development of new sensing devices, not just using the fiber as a carrier of information but also for optical processing entirely in fiber, taking the advantages linked to optical fibers such as small size, long distance covering, electromagnetic immunity, and intrinsic multiplexing capabilities [1]. Although the development of fiber optic sensors, for diverse applications, has been increasing exponentially in the past 20 years, there is still a wide margin to grow and acquire the knowledge necessary for the widespread application of this technology for multi- and reliable sensing.

In this perspective, the work herein presented was proposed considering the competences in optical fiber technology and optical fiber sensing of the Center of Applied Photonics of INESC TEC, and the knowledge in chemistry of the Chemistry Research Unit of the University of Porto (CIQUP). Moreover, the experimental work related to this PhD was developed by taking the advantage of in-house technologies to establish the know-how and technological competencies in the field of microfabrication and thin film-based optical fiber sensing, and to acquire the expertise to develop new sensing materials to be implemented on fiber optic architectures.

In recent years, optical fiber technology enabled the development of diverse types of sensors, comprising a wide variety of transducing schemes such as fluorescence, colorimetry, fiber gratings and/or plasmonic-based fiber structures, for diverse applications, including the sensing of chemical (e.g., gases, metals, or other contaminants) or biological entities (e.g., viruses, proteins, antibodies, among others) in several areas of interest (e.g., environmental monitoring, aquaculture, clinical diagnoses, etc).

In addition, the research was conducted on the development of different fiber optic architectures targeting distinct analytes of interest. The fiber optic configurations were realized by using different types of fibers (single- and multimode) and transductions schemes, leading to the development of an extrinsic fiber optic sensor based on colorimetric measurements, and intrinsic fiber optic sensors based on long-period gratings or the plasmonic resonance phenomena. Moreover, the investigation also includes referencing schemes to improve the performance of the fabricated fiber optic sensors, thus resulting in reliable measurements, mainly reducing the impact of “false positive” results.

The relevance of optical sensing in the future of a highly technological society, the role of fiber optic sensing devices, and the demand for the highest reliability, mainly for *in situ* and real-time measurements, were the main features that motivated me to outline this PhD program along the optical fiber sensing domain. The outcome of this effort is exposed in the chapters of this thesis.

1.2. Scientific Contributions

The investigation on sensing structures based on optical fibers combining new sensing materials and new fiber architectures, resulted in a number of scientific contributions here summarized:

- The impact of polymerization on the performance of a pH indicator was studied, revealing an increased sensitivity to pH changes, in particular improving the pH working range and the acidity constant (pKa) of the material allowing to differentiate small pH changes by measuring the light absorbance.
- In addition, the changes in absorption (sensitivity) of the produced materials to different gaseous carbon dioxide (gCO₂) concentrations at specific wavelengths was assessed, exposing a better performance for the polymerized material immobilized in a gel matrix instead of a covalent approach.

- Moreover, the polymerized material exhibited a remarkable performance in the ratiometric detection of low concentrations of dissolved CO₂ (dCO₂), demonstrating its suitability for the evaluation of small changes in the gas concentration in different waters (fresh and saline).
- The applicability of the developed sensor for *in situ* and real-time reading of the dCO₂ levels in aquaculture systems was confirmed by studying the performance of this optical dCO₂ sensor in quasi-real situations, revealing a better performance in comparison with a commonly used commercial sensor.
- Furthermore, was developed a sensing system comprising long-period fiber gratings (LPFGs) and molecular imprinted receptors to detect IgG antibodies, in which a second LPFG carrying a non-imprinted layer was used for real-time correction. Was observed that the NIP-coated LPFG can be applied to correct temperature or strain variations as well as to avoid other common undesired effects such as the "bulk effect" and improves the limit of detection (LOD) of the entire system.
- Moreover, both correct systems exhibited a remarkable impact in the response of the sensors in the presence of interferent/similar species, by reducing the response to them in, at least, 75%, revealing highest reliability and potential to be used in point-of-care applications.
- Surface plasmon resonance and localized plasmon resonance (SPR and LSPR, respectively) coupled effects on fiber optic structures based on chemical etching were also researched, resulting in the development of novel plasmonic fiber optic sensors with increased sensitivity.
- Additionally, was observed that the resonance band position (in wavelength) strongly depends on the size (diameter) of the gold nanospheres (AuNS) used to create the localized plasmons, allowing to tune the resonance band in a wavelength range from the visible to the near-infrared regions.
- Moreover, the novel plasmonic structure was used as a biosensor, revealing a low LOD (picomolar) and in a wide working range (from picomolar to nanomolar).

1.3. Structure of the Thesis

This thesis focuses on the study of fiber optic sensors comprising different transducing systems and targeting different analytes of interest. It is structured in such a way that each section includes a brief description about the state-of-the-art related with the subjected dealt with in the chapter. Once that the investigation reported here had

resulted in different types of sensors, the thesis is divided in two main sections: Extrinsic Fiber Optic Sensors and Intrinsic Fiber Optic Sensors.

The document is divided in 9 chapters. Chapter 1 defines the motivation that led me to carry out investigation on different approaches to implement fiber optic sensors, explain about their potential according with their sensing methodologies, by summarizing the main scientific contributions resulted from the research performed.

Due to the different methods used to fabricate extrinsic and intrinsic fiber optic sensors, chapter 2 addresses a brief introduction about the optical fiber technology, which types of fibers are currently available, and the sensing architectures commonly used to perform the different types of fiber optic sensors.

Chapter 3 presents an overview about the state-of-the-art of the mostly used configurations to develop extrinsic fiber optic sensors for chemical or biological sensing, describing the working principles and sensing elements.

The state-of-the-art of the most common architectures used to fabricate intrinsic fiber optic sensors is addressed in chapter 4, where are described different transducing approaches such as fiber gratings, tapered fibers, or plasmonic effects. Are also referred the main coating techniques used to enhance optical features of fiber structures or to develop layers as sensing elements, and the most used biological receptors for the development of fiber optic biosensors as well as their characteristics according with the required application.

Chapter 5 describes the methods and the materials used to fabricate the fiber optic sensors studied in this research.

Chapters 6, 7, and 8 describes the experimental investigation as well as the experimental achievements resulted from this research. Moreover, the information presented in chapters 6 and 7 was already published in international journals.

In chapter 6, a set of experimental tests aiming the detection of carbon dioxide is presented. The sensor is based on colorimetric measurements by evaluating the absorption of light (at specific wavelengths) by a sensitive layer fabricated from a pH indicator (*p*-nitrophenol). In order to increase the sensitivity of the material, polymerization processes were conducted. Different approaches to turn the material sensitive to carbon dioxide (gaseous and dissolved) are studied as well as the applicability of the developed sensor to detect small variations of the gas in order to be used in aquaculture applications.

Chapter 7 exhibits two different methodologies to build LPFG-based biosensors. In a first approach is described the immobilization of biological receptors onto spherical silica nanoparticles to fabricate a number of nanosensors, using LPFGs as a transducer to evaluate the binding events between the nanosensors and the target. The second approach studies the combination of LPFGs with molecularly imprinted layers using an *in situ* polymerization process. The impact of introduction of additional LPFGs working as reference schemes on the performance of the proposed sensors is also investigated.

Chapter 8 deals with the fabrication of plasmonic sensors on optical fibers. A set of experimental studies, resulting in the development of a novel plasmonic platform are described. Additionally, is also exhibited the performance of the novel structure in biosensing assays. For this purpose, studies on the kinetics of the binding events between thrombin and an aptamer-based probe are used as a model system for sensor validation.

Finally, the main conclusions resulting from the R&D activity are presented in chapter 9, highlighting the scientific achievements and where future work is also discussed.

1.4. List of Publications

1.4.1. Papers published in National and International Journals

1. Mendes, João P., Coelho, L. C., Jorge, P. A., & Pereira, C. M., Differential Refractometric Biosensor for Reliable Human IgG Detection: Proof of Concept. Biosensors, 2022, 12.7: 515. <http://doi.org/10.3390/bios12070515>.
2. J.P. Mendes; L. Coelho; C.M. Pereira; P.A.S. Jorge. 2020, Colorimetry-based System for Gaseous Carbon Dioxide Detection: Membrane Optimization. U.Porto Journal of Engineering, 6:2 (2020) 59-69; https://doi.org/10.24840/2183-6493_006.002_0006.
3. J.P. Mendes; L. Coelho; B. Kovacs; J.M.M.M. de Almeida; C.M. Pereira; P.A.S. Jorge; M.T. Borges. 2019. Dissolved Carbon Dioxide Sensing Platform for Freshwater and Saline Water Applications: Characterization and Validation in Aquaculture Environments. Sensors. <https://doi.org/10.3390/s19245513>.

1.4.2. Papers Published in National and International Conferences

1. J. P. Mendes, L. C. C. Coelho, C. M. Pereira, and P. A. S. Jorge, Differential Refractometric Platform for Reliable Biosensing based on Long-period Gratings and Molecular Imprinting, in 27th International Conference on Optical Fiber Sensors, Technical Digest Series (Optica Publishing Group, 2022), paper Th4.72. <https://doi.org/10.1364/OFS.2022.Th4.72>.
2. Mendes, J.P.; Coelho, L.C.C.; Pereira, V.P.; Azenha, M.A.; Jorge, P.A.S.; Pereira, C.M. Label-Free Anti-Human IgG Biosensor Based on Chemical Modification of a Long Period Fiber Grating Surface. Chem. Proc. 2021, 5, 48. <https://doi.org/10.3390/CSAC2021-10454>
3. Mendes, João; Coelho, Luís; Rocha, André; Pereira, Carlos; Kovacs, Barna; Jorge, Pedro; Borges, Maria Teresa. 2019. "Colorimetric Fiber Optic Based Probe for Real-Time Monitoring of Dissolved CO₂ in Aquaculture Systems". Proceedings 15 (1): 5. <http://dx.doi.org/10.3390/proceedings2019015005>.

1.4.3. List of other Publications

During my PhD, I was invited to participate in parallel research which resulted in several publications. The following list presents the publications (in national/international journals or conferences) resulted from those contributions (the experimental results are not present in this thesis).

1. Paulo S. S. dos Santos, João Mendes, B. Dias, I. Pastoriza-Santos, J. M. M. M. de Almeida and Luís C. C. Coelho, Strongly coupled plasmonic systems on optical fiber sensors: A study on nanomaterial properties, 2022, J. Phys.: Conf. Ser. 2407 012052. <http://doi.org/10.1088/1742-6596/2407/1/012052>.
2. P. M. da Silva, J. P. Mendes, L. C. C. Coelho and J. M. M. M. de Almeida, Optical fiber sensors for monitoring cement paste carbonation, 2022, J. Phys.: Conf. Ser. 2407 012038. <http://doi.org/10.1088/1742-6596/2407/1/012038>.

3. Helena Vasconcelos, Ana Matias, João Mendes, João Araújo, Bernardo Dias, Pedro A.S. Jorge, Cristina Saraiva, José M.M. M. de Almeida, Luís C.C. Coelho, Compact biosensor system for the quantification of hydrogen peroxide in milk, *Talanta*, Volume 253, 2023, 124062, ISSN 0039-9140, <https://doi.org/10.1016/j.talanta.2022.124062>.

4. Dias, B., Mendes, J. P. S., de Almeida, J. M. M. M., & Coelho, L. C. C., Simple Optical Fiber Interferometer for Dynamic Measurement of Refractive Index and Thickness of Polymer Films. *IEEE Sensors Journal*, 2022, 22.12: 11732-11739, <http://doi.org/10.1109/JSEN.2022.3171046>.

5. Arcadio, F., Seggio, M., Del Prete, D., Buonanno, G., Mendes, J., Coelho, L. C., ... & Cennamo, N., A Plasmonic Biosensor Based on Light-Diffusing Fibers Functionalized with Molecularly Imprinted Nanoparticles for Ultralow Sensing of Proteins. *Nanomaterials*, 2022, 12.9: 1400, <https://doi.org/10.3390/nano12091400>.

6. Dias, B.; Carvalho, J.; Mendes, J.P.; Almeida, J.M.M.M.; Coelho, L.C.C. Analysis of the Relative Humidity Response of Hydrophilic Polymers for Optical Fiber Sensing. *Polymers* 2022, 14, 439. <https://doi.org/10.3390/polym14030439>.

7. Vasconcelos, H.; Matias, A.; Jorge, P.; Saraiva, C.; Mendes, J.; Araújo, J.; Dias, B.; Santos, P.; Almeida, J.M.M.M.; Coelho, L.C.C. Optical Biosensor for the Detection of Hydrogen Peroxide in Milk. *Chem. Proc.* 2021, 5, 55. <https://doi.org/10.3390/CSAC2021-10466>.

8. Dias, B.; Mendes, J.P.; de Almeida, J.M.M.M.; Coelho, L.C.C. Characterization and Comparison of the Relative Humidity Response of Hydromorphic Polymers in Long-Period Fiber Grating Structures. *Chem. Proc.* 2021, 5, 42. <https://doi.org/10.3390/CSAC2021-10461>.

Chapter 2 – Optical Fibers for Sensors: A Brief Introduction

In the last decades, optical fibers came to transform the telecommunications technology by allowing the signal transmission over glass filaments at long distances. Optical fibers are typically made of a cylindrical core surrounded by a cladding layer and both can be of pure silica or polymeric materials and has been explored for different applications [2]. It is a technology that operates using light as carrier and its body as a vehicle to sense and transmit external signals, offering high stability, high integration, long-distance transmission with low attenuation and without electromagnetic interference [3, 4]. The light propagates along the optical fiber based on the “total internal reflection” phenomenon, and the angle at the total reflection occurs is known as critical angle of incidence, that is established by the Snell’s law. At angles greater than the critical angle, the light is totally reflected into the glass medium [5]. Considering the modes in which the light propagates, optical fibers can be divided into two main groups (Figure 2.1a): single mode fibers (SMF) and multimode fibers (MMF). Looking for the refractive index (RI) profile, they can be divided into step index fibers and gradient index fibers (also known as graded index fibers). Very briefly, step index fibers have a constant RI over the whole cross section, while gradient index fibers have a nonlinear RI profile, which drops off from the center of the fiber to outwards (Figure 2.1b) [6].

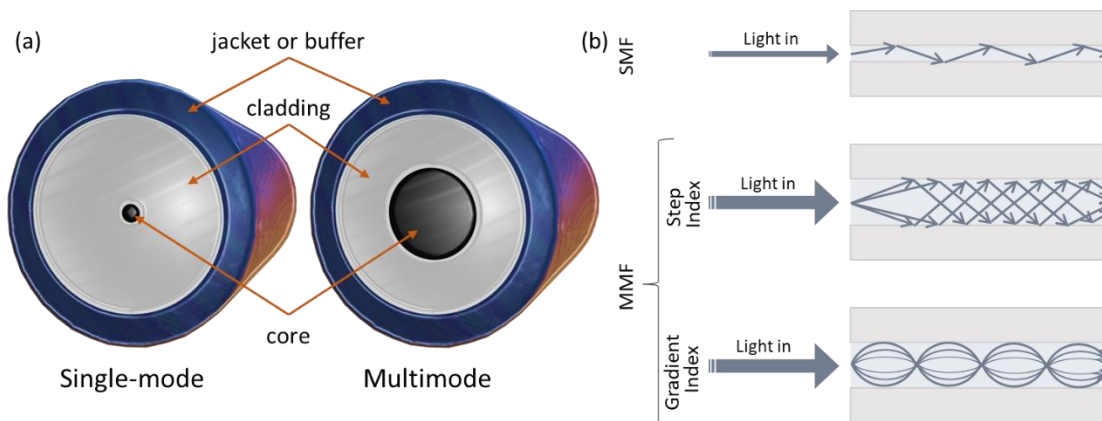


Figure 2. 1 (a) Different types of optical fibers; and (b) longitudinal cross-section displaying the light propagating in the core according to the refractive index type of the core.

Besides their magnificent performance in communications and networking (in particular the germanium-doped silica glass SMF), optical fibers revealed to be useful to develop small size fiber optic sensors (FOS) with high sensitivities [7-9]. The massive research and development of FOS configurations has extended their applications to a wide range of scientific fields, such as medical, chemical, and environmental technologies. FOS have started to be applied to evaluate physical parameters such as temperature, electric and magnetic fields, tension, torsion, pressure, vibration, and many other properties [2]. Quickly several investigations suggested the suitability of FOS for the detection and/or quantification of chemical or biological entities relies mainly on the use of fiber optic chemical sensors (FOCS) and fiber optic biosensors (FOBS). FOBS are devices used to evaluate a target analyte combining biological receptors or biomimetic components with physicochemical transducers. Differently from FOBS, fiber optic chemical sensors do not use biological elements for specific recognition. FOCS are based on chemical reactions that produce a signal, such as color change or fluorescence, and the employed transducer translates the physicochemical event into an analytical signal. Such devices are also known as “Optodes” or “Optrodes” [10]. In general, the FOS devices are composed by an optical source (laser, LED, ...), optical fibers (single mode, multimode, or fiber bundles), sensing elements (chemical or biological), and an optical detector. The optical signals can be converted to analytical signals by applying optical modulation methods [10, 11]. Figure 2.2 illustrates these components in more detail.

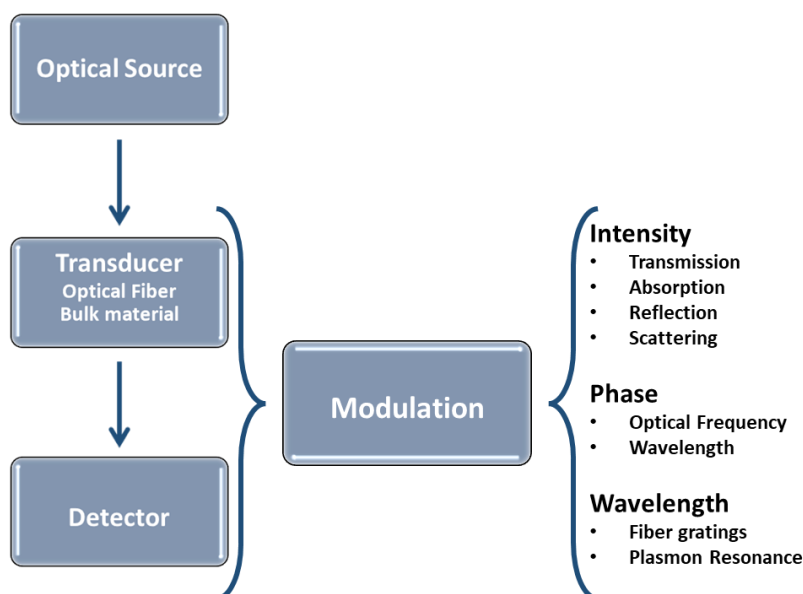


Figure 2. 2. Schematic of a basic structure of a FOS, exemplifying the transducers system and the possible modulation approaches.

In the intensity-modulated sensors, the variation in light intensity is detected according to the induced perturbations by a measurand on a transducer. Materials with reflective, transmissive, absorptive, fluorescent, or scattering properties can be incorporated into the fiber or can be employed as an independent part on a sensing platform (for instance, a sensing material that is placed between two align fibers separated by a certain distance) [12]. The amount of light that reaches the second fiber depends on the absorption properties of the sensing substance, occurring a lost in intensity comparing with the incident radiation, thus allowing an intensity modulation by measuring the absorption of light by the sensing element [12, 13]. Another example are the sensors based on luminescence [12]. Usually, these systems comprise a fiber bundle to illuminate the luminescent material and to recover the emitted light. Fiber bundles are very useful tools to transmit and collect light using a single probe. Usually, they assume the “Y” form (Figure 2.3a) and are performed by two or more multimode optical fibers (Figure 2.3b) [12]. In both configurations, the analytical data is obtained by measuring the intensity amplitude of the emitted signal [14].

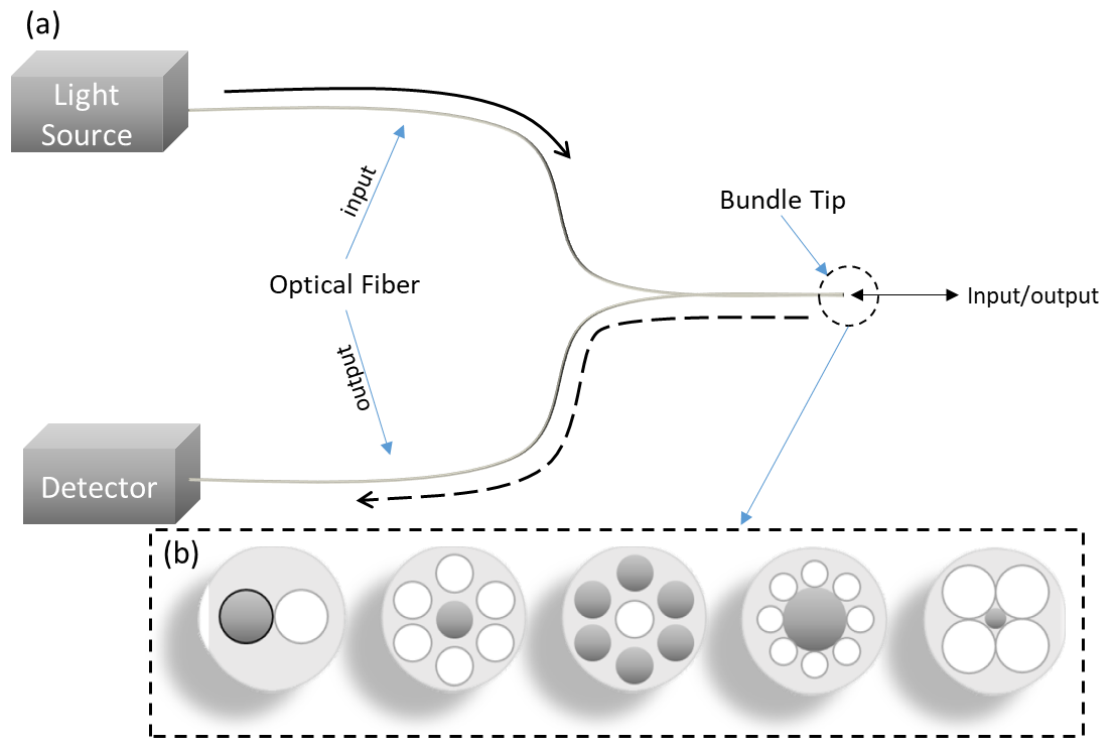


Figure 2. 3. Schematic figure of: (a) a fiber bundles in which the light is transmitted by one fiber arm and collected by a second fiber arm; and (b) examples of possible variations of a fiber bundle cross section in which the illuminating and collecting fibers are represented by the filled circles and by the empty circles, respectively.

Optical phase modulation offers accurate and highly sensitive sensors, mainly when compared with the intensity-modulated sensors. Phase-modulated sensors are based in interferometric layouts, such as the Mach-Zehnder, Michaelson, or Fabry-Pérot interferometers. In general, the interferometric systems are based in two fibers by where the light is injected and split. Generally, one the arms is used as a sensor and the other arm is used as reference. When a perturbation occurs in the sensing fiber, a phase shift arises between them. The transmitted spectra can be used to evaluate such perturbation by analyzing the intensity variation or exploring the wavelength position of the spectral bands [15].

Wavelength-modulated sensors are related with optical fiber gratings fabricated on single mode fibers, such as short-period gratings and long period gratings. The inscribed microstructures create a small modulation in the effective refractive index of the core with a certain periodicity. The introduced modulation produces unique spectral characteristics in the transmission spectra, whose wavelength position strongly depends on the period of the grating [16]. Other configurations based on wavelength modulation are related with plasmon resonance. Surface plasmon polaritons are induced at the interface between a metallic thin film (typically Ag and Au) and a dielectric (sensing membrane or external

medium) due to the interaction of the evanescent field of the guided core mode. When the incident light reaches the metallic film at a certain angle, part of the light is coupled through the metal, exciting the free electrons at the layer surface. The absorbance spectra display a well-defined absorption band, whose wavelength position strongly depends on the type of the coating material and the thickness of the film [17]. Both transmitted and absorbance spectra are used to develop very sensitive FOS, by modulating its wavelength position as response to the external perturbations. Moreover, the optical fiber surface or the metal coating are suitable for chemical and biological modifications, leading to the development of powerful biosensors.

Further chapters will describe in detail the main fiber optic configurations using these modulation approaches. The fiber optic sensors will be classified into two main domains: Intrinsic and Extrinsic FOS.

Chapter 3 – Extrinsic Fiber Optic Sensors

This chapter aims to present an overview about the state of the art of the mostly used configurations to develop intensity-modulated extrinsic FOS for chemical or biological sensing. Brief discussion about the working principles and sensing elements. Examples of extrinsic FOS targeting different entities are also addressed.

3.1. Introduction

Extrinsic FOS have been applied over the years with very success. In this type of FOS, the optical fiber is used to guide the light to and from a transducer element, which is external to the optical fiber. Usually, the transducer element is based on small components with optical properties, specially designed to modulate the light in response to changes produced by physicochemical perturbations of a measurand of interest. Typically, extrinsic FOS configurations include an optical fiber to transmit the optical energy to the sensing element (or sensing head) and a second fiber to drive the modulated light to a detector [11]. Depending on the properties of the sensing scheme (transmission, absorption, reflection, or scattering), the sensing configuration can assume different formats. In particular, for optical chemical sensing, the main exploited phenomena are absorption and fluorescence [11]. Usually, extrinsic FOS architectures are performed by using multimode optical fibers due to their large core which is an

advantage to transmit a certain amount of light, being also a benefit to receive the modulated light.

The main configurations and working principles of extrinsic FOS, based on absorption and fluorescence spectroscopy, will be described in the next sections for chemical or biological sensing according to properties of the employed transducer.

3.2. Principles and Configurations

Extrinsic FOS can assume different structural types in respect to their working principle. The basic configuration comprises an optical fiber to guide the light to the location where the transduction event occurs and other one to drive the modulated energy to a detector in order to transform the optical output into a measurable signal (Figure 3.1) [11]. The modulation approach will depend on the optical configuration and can be established through external modulators. The modulation of color/wavelength of the light is a very effective approach and it can be done by moving masks or through absorptive chemical indicators [18]. To summarize, for absorbance, luminescence, or reflectance-based devices, usually is used the intensity modulation approach. Moreover, to achieve high measurement accuracy, different reference schemes, such as dual-wavelength approaches, are used to correct source drifts or external interferent actions during measurements [19].

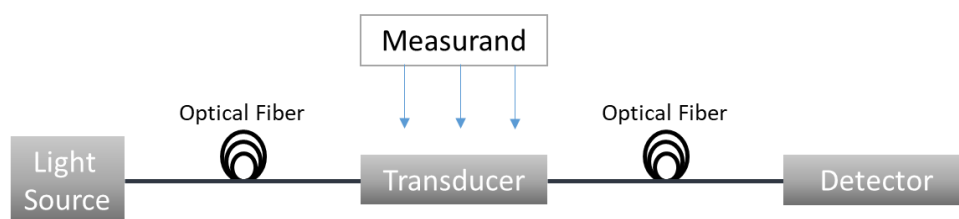


Figure 3. 1. Basic configuration of an extrinsic FOS.

Intensity-modulated FOS offers simplicity of construction as main advantage. Linked to extrinsic fiber optic sensors, they can assume a wide selection of architectures. For instance, the displacement of one fiber relative to the other, *i.e.*, measuring the attenuation of light between two aligned fibers or collection of modulated light reflected from a target exposed to a measurand. In these types of sensors is important to assure the compensation for any vibration/fluctuation in the intensity of the light source, on the optical fibers, and/or any interference that can occur on the traveling light path when it leaves the fiber and starts to travel in an external medium. Figure 3.2 shows a basic configuration of two types of intensity-modulation extrinsic FOS: from A to B, and from A

to C. In the former, is presented an example of a measurand-induced variable attenuation of transmitted light across an input fiber and an output fiber (e.g., absorbance). The latter describes a generic example of a measurand-induced modulation either through reflection/scattering or through photoluminescence from a chemical material or solution excited by the incident light.

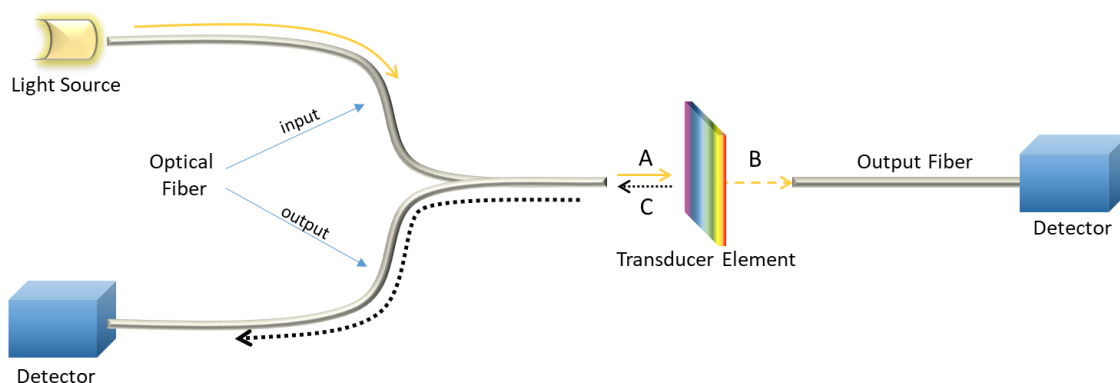


Figure 3. 2. Generic illustration of an intensity-modulated extrinsic FOS using two aligned optical fibers (A-B path) or a Y fiber coupler configuration (A-C path) where: from A to B is the mechanism for attenuation measurements of a transmitted light modulated by a measurand with absorptive properties; and from A to C is the mechanism to measure reflected/scattered light or to measure the emission by a fluorescent material.

In its simplest form (see Figure 3.2, A-B path), the intensity-modulation extrinsic FOS includes a transducer placed between two aligned fibers that modulates the incident light due to its absorptive characteristics at specific wavelengths. In that way it is possible to evaluate the changes induced in the transmitted light by the transducing material that is usually affected by physiochemical perturbations. A different configuration is used for measurements related with the emission of light, such as by a fluorescent material. It is necessary an optical fiber to guide the incident light that excites the fluorescent material and another fiber (or multiple fibers) to receive the light resulting from the excitation and guide it until a detector. Usually, a Y fiber optic coupler fiber optic or a Y fiber optic bundle are used. In the first, two optical fibers are combined in a single optical fiber while in the second two multimode fibers are glued along the same portion of their length. The light is injected through one fiber arm and the light resulted from the excitation is received by the other fiber arm (see Figure 3.2, A-C path). The fluorescent material is sensitive to specific physicochemical perturbations, allowing to obtain qualitative and/or quantitative information about the presence of a measurand of interest. Furthermore, such materials can be used to coat the optical fiber tip (Figure 3.3) [20-22]. Those configurations work with the same principles. The light reaches the transducer that modulate the light according to their optical properties (absorbance or photoluminescence) and is collected by a second fiber (or multiple fibers), driving the light until the detector. Particularly, in

the case of the absorbance based extrinsic FOS, it is necessary to employ a reflective material (e.g., mirror) to reflect the light back to be collected by the fiber and guide it to the detector (Figure 3.3b).

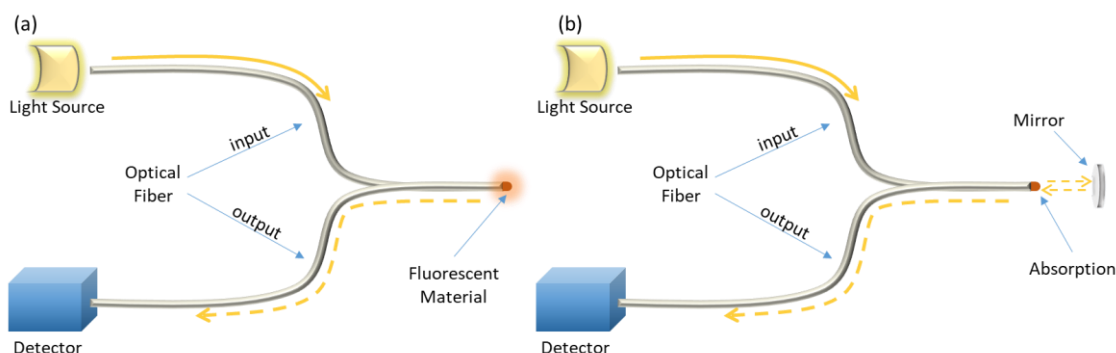


Figure 3. 3. Basic configurations of: (a) extrinsic fiber optic tip sensor based on fluorescence phenomenon; and (b) extrinsic fiber optic tip sensor based on absorbance phenomenon comprising a reflective surface (mirror) to reflect the light back to fiber to be collected and driven to the detector.

This type of configuration can also be used in applications where the light is reflected by a reflective material. In those cases, the optical platform intends to monitor the intensity of the back-reflected light from a certain surface in relation to the transmitted light intensity. Depending on the absorption properties of such surface and the amount of scattered light, it is possible to monitor quantitatively the amount of light that is reflected by the surface, being then collected by a receiver optical fiber. This approach is a very useful mechanism to displacement measurements, to assess the characteristics and quality of materials surfaces, among other applications (Figure 3.4) [23-26].

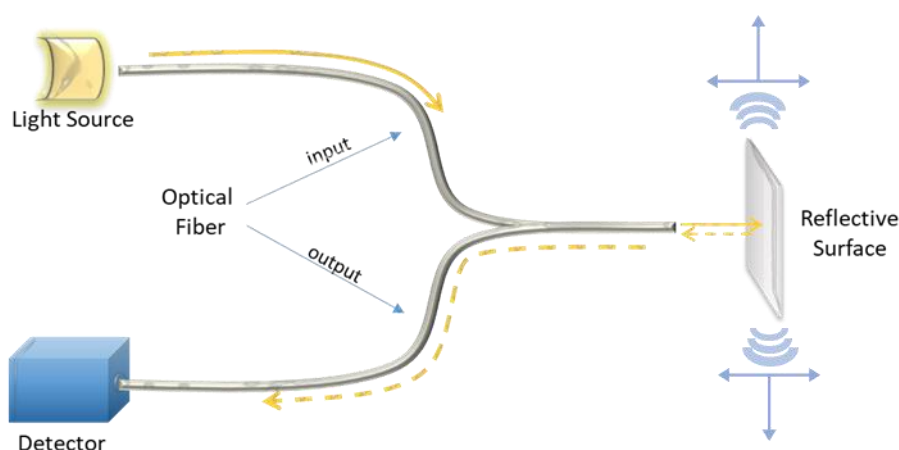


Figure 3. 4. Schematic illustration of a reflective extrinsic FOS where a fiber optic transmits the light from a source, and it is reflected to another fiber optic that drives the light to a detector, measuring the reflectivity from the surface.

The main spectroscopic sensing principles based on intensity modulation approach used in extrinsic FOS are discussed in the next subsections.

3.2.1. Absorption-based Spectroscopy

Absorbance-based extrinsic FOS are usually connected to colorimetric sensors or to the natural spectroscopic characteristics of the target. The formers are based upon recognition of a measurand-induced color change in the sensor material (the transducer). The latter relies on the detection of the target analyte by probing its intrinsic molecular absorption. The optical energy absorption results from transitions in the electronic, vibrational, and/or rotational energy states of the atoms and molecules. The electronic excitation is induced by visible and ultraviolet (UV) radiations, whereas the vibrational excitations and the rotational transitions are caused by infrared and microwave radiations, respectively [12]. The phenomenon arises if the difference in the energy states involved matches the energy of the exciting photons. The absorption leads to a loss of the incident radiation as it passes through the target material or solution. When the incident light reaches the absorbing species, the initial intensity, I_0 , is transmitted by the sample with a reduced intensity, I . Those species, absorb the incident radiation at specific frequencies or wavelengths, transmitting the other frequencies without any reduction on the incident light intensity. The light intensity attenuation at specific wavelengths is determined by the concentration, c , of the absorbing species in solution or immobilized on a substrate through the Bouguer-Beer-Lambert equation [27]:

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon lc \quad (3.1),$$

where A is the absorbance, l is the length of the light path, and ε is the molar absorptivity. According to Equation 3.1, the absorbance increases linearly as the concentration of the absorbing species also increases. However, at very high concentrations deviations from the linear behavior are observed, often connected to saturation effects leading to the aggregation of the absorbing species. This phenomenon may result in light scattering or in the modification of the absorption properties of the absorbing species [12].

Absorbance based fiber optic chemical sensors can be designed using one of the fiber optic architectures described above, allowing the guided light in the fiber to interact with a target material (e.g., colorimetric indicator) that acts as a transducer by changing its color as result of a chemical reaction caused by an analyte of interest. Generally,

colorimetry can be defined by a quantitative measurement of UV-visible (UV-VIS) absorbance or reflectance, being one of the oldest analytical method used in optical sensing [28]. Traditionally, colorimetric sensors make use of three-channel visible range (wavelength ranges including red, green, and blue; RGB) or by selecting highest peaks in the UV-VIS spectral range.

3.2.2. Luminescence-based Spectroscopy

The phenomenon known as luminescence arises when species, electronically excited by specific radiations, are deactivated by emitting radiation ranging from UV to near-infrared (NIR) [12]. The absorption of that radiation by the molecules in solution induces an excitation state. When the excited molecule returns to the fundamental state, emits energy in form of light at specific frequencies. Moreover, if the exciting energy photons come from UV to blue radiation, the emitted energy by the excited species is called photoluminescence (chemiluminescence occurs when the emission results from a chemical excitation). Nowadays, luminescent events are well-understood, being the basis for several powerful analytical methods. In particular, photoluminescence is one of the most investigated techniques, being widely applied in many well-established sensing methods [12]. The phenomenon happens in a large spectral window ranging from UV to NIR (usually from 200 to 1000 nm). After absorbing high-energy photons, the excited molecule arises with a wide variety of decay mechanisms by which it can back to its fundamental state. Moreover, during the decay event, the emission of energy is not always in the form of photons. There are nonradiative events releasing the energy in the form of heat. In other cases, some molecules undergo chemical reactions, disabling their fluorescence ability. This phenomenon is known as photodegradation [12].

Photoluminescence phenomena is classified according with the electronic transitions involved in the process. Fluorescent transitions usually happen between the excited singlet state of lowest energy (S_1) and the ground singlet state (S_0). These events are spin-allowed transitions (*i.e.*, the electron can keep its spin in the transition between states) and are characterized by high transition rates and lifetimes in the nanosecond scale. Differently from fluorescence, phosphorescence transitions involve levels with electrons having the same spin orientation, known as triplet states (T_1 and T_2). Therefore, the electrons involved in the transition must change their spin, resulting in low transition rates and lifetimes ranging from a few microseconds to milliseconds or even longer [29]. Figure 3.5 displays the electronic transitions of the excited electrons from the ground

singlet state to higher electronic states and the respective decay (absorbance and fluorescence), and from triplet to singlet states (phosphorescence).

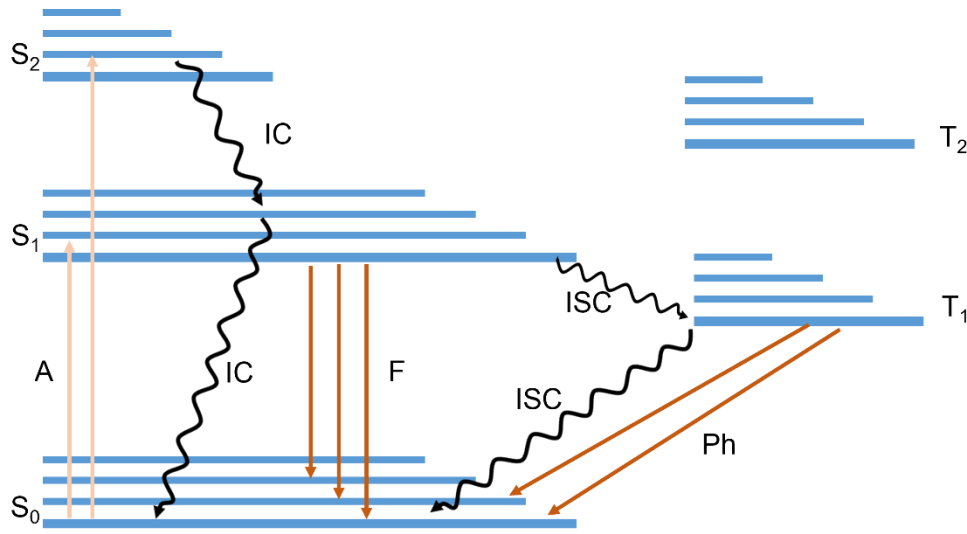


Figure 3. 5. Diagram of the electronic transitions, where *A* is the absorption, *F* is the fluorescence; *Ph* is the phosphorescence; *IC* correspond to internal conversion; and *ISC* correspond to intersystem crossing. Adapted from reference [30] under CCBY license. ©Springer Nature Copyrights.

The fluorescence lifetime is defined as the average time that the molecule stays in the excited state before returning to the fundamental state. Although the emission probability for different molecules is the same for a certain interval, not all molecules emit at same instant. It depends on the rate constants related with the radiative and nonradiative mechanisms involved. The fluorescence lifetime can be described through Equation 3.2, where k_r^S is the radiative rate constant of fluorescence (related with the $S_1 \rightarrow S_0$ transition), k_{nr}^S represents the nonradiative rates associated to the internal conversion (IC) processes, k_{IC}^S , and intersystem crossing (ISC) processes, k_{ISC}^S [31].

$$\tau_0 = \frac{1}{k_r^S + k_{nr}^S} \quad (3.2).$$

Thus, when a fluorescent specie is excited by a pulse of light, a maximum concentration of excited molecules is reached, and decreases at a rate of $1/\tau_0$. The intensity of the luminescent signal, I , is proportional to the concentration of the excited molecules. The luminescent intensity, I_0 , that arises after excitation, decreases exponentially with a time constant given by τ_0 (Equation 3.3) [12].

$$I(t) = I_0 e^{-t/\tau_0} \quad (3.3).$$

Considering the Equation 3.3 where $I(t)$ is the impulse response of the system, the time required for the fluorescent intensity to decrease by $1/e$ of its initial value, can be defined by τ_0 . If ISC is favored, phosphorescence arises instead of fluorescence [32].

Usually, the luminescent-based systems are associated to high-cost bulky laboratory equipment. In the particular case of the optoelectronic field, cost-effective and simple architectures can be performed by placing together low-cost blue and/or UV lasers and LEDs, miniaturized CCD spectrometers, and high-sensitive photodiodes. In this context, fiber optic cables are a very interesting tool to develop luminescence-based fiber optic probes, widening the field of applications for these types of sensors.

3.3. Sensitive Dyes: From Colorimetry to Fluorescence

Optical chemical sensors based on colorimetric or fluorescent changes relies on intermolecular interactions between a chromophore or a fluorophore and an analyte of interest [33]. Sensors based on chemoresponsive colorants (e.g., dyes) evaluate the chemical reactivity of the analytes, instead of their physical properties, offering high sensitivity, remarkable distinction among similar targets, and fingerprinting of very similar mixtures over a large range of analyte categories in both gaseous and liquid phases. As reported, the optical platforms are endowed by UV, visible, or infrared light sources, always considering the nature of the transduction mechanisms on which are based (absorbance or fluorescence), and optical waveguides to transport the light from the source and, after the proper modulation, to a photodetector. In this particular context, optical fibers have contributed to the rapid growth of the number of efficient and integrated optical sensors [34].

The transducing materials to be incorporated in this type of optical sensors need to obey to two fundamental requirements: the ability to interact selectively with analytes, and the capacity to report or provide feedback on such interactions through chromophores or fluorophores. Such materials may be semi-selective (i.e., cross-reactive with multiple analytes) or highly selective (specific for a single analyte or unique class of analytes). In this context, dyes are often chemoresponsive materials, in which their colorimetric or fluorescence properties are determined by their chemical environment (e.g., pH indicators) [28]. Although chemical dyes and fluorophores fulfil the requirements for

semi-selective sensors, there are other materials more indicated for selective sensors development. However, these materials (e.g., supramolecular receptors) do not respond via their optical properties. It is necessary to modify them by attaching chromophores or fluorophores on their macrostructures [35].

The following subsections addresses the basis of molecular interactions that allow these types of materials change their optical properties for further colorimetric or fluorometric sensing.

3.3.1. Intermolecular Interactions

For colorimetric or fluorometric sensing, the selection of the adequate sensing dyes is of extremely importance for the construction of the sensor. For this selection is important to consider the analyte to be detected as well as the intermolecular interactions that the transducer will perform with the measurand. There is a wide range of molecular interactions between molecules that can generate a continuum strength of interaction, from the weakest (van der Waals forces) to the strongest (covalent or ionic bonds) interactions. Depending on that, chemoresponsive dyes can be classified, according with their cross-reactive, into: Brønsted acidic or basic dyes (pH indicators); Lewis acid/base dyes (metal complexes or metal-ion containing chromogens); redox indicator dyes; colorants with permanent dipoles (vapochromic or solvatochromic dyes) detecting local polarity or hydrogen bonding; and chromogenic aggregative materials [28].

3.3.1.1. Brønsted Acid/base Dyes

The colorimetric compounds of Brønsted acids or bases are pH dependent, *i.e.*, the protonation or deprotonation induces pH changes and, consequently, change the UV-VIS absorption spectra. There are a wide variety of pH indicators available. Some of them can be naturally obtained from plants (e.g., anthocyanin dyes), and others can be synthetically produced. For instance, the production of nanoprobe using pH indicators immobilized on polymer hydrogels for intracellular pH sensing exploring the fluorescence properties of the used indicators have been investigated [36]. Other organic chromophores (e.g., azo dyes, triphenylmethane dyes, nitrophenols, phthaleins, among others) have been investigated to evaluate the pH of aqueous solutions or biological usage [37, 38]. The pK_a values of these indicators in aqueous solution ranges from below 0 to above 14 and depends how easily the molecule is protonated or deprotonated. Table 3.1 exhibits some examples of pH indicators, their nature and color/pH range.

Table 3. 1. Some examples of pH indicators, their pH working range and colors, and source.

Indicator/Pigment	pH Color	Source	Ref.
Anthocyanin	3 – 10 	Red Cabbage	[39]
	2 – 12 	Blueberry	
	2 – 11 	Black Carrot	[40]
Bromocresol Green	3.8 – 6.4 	Synthetic	[41]
Bromothymol Blue	6.0 – 7.6 		
Bromocresol Purple	5.2 – 6.8 		[42]
Thymol Blue	1.2 – 2.8 / 8.0 – 9.6 		[43]

3.3.1.2. Lewis Acid/Base Dyes

There are numerous compounds that are connoted as Lewis’s acids or bases. Considering the acidic dyes, metalloporphyrins are a common choice for the detection of metal-ligating volatiles due to the open axial coordination sites and the large color changes from both wavelength and intensity shifts of strong $\pi - \pi^*$ absorbances resulted from ligand binding. Moreover, there are some examples of metalloporphyrins as strong Lewis’s acid indicators in daily life. For instance, the difference in color of the red arterial blood *versus* the purple of venous blood, is a case of colorimetric detection of O_2 using porphyrins. Additionally, porphyrins present excellent solvatochromic properties, leading to a perceptible colorimetric change before and after interactions even with analytes that lack strong ligating functionalities, such as arenes, halocarbons, or ketones. So, metalloporphyrins and their derivatives result in a very interesting group of dyes for colorimetric and fluorometric detection [44, 45]. Another important application of Lewis acid indicators is the anion detection. In recent years simple anions such as Cl^- , F^- , NO_3^- , PO_4^{3-} , and complex anions such as ATP, lipid anions, nucleic acids, and others phosphorylated biomolecular anions, have attracted much greater interest due to their

biological, medical, and environmental significance [28]. Anion receptors can be neutral or positively charged and the interactions induced by them are dominated by electrostatic forces or hydrogen bonding. Lewis's acids can be used directly if have intrinsic fluorescent capabilities or can be combined with a chromogenic or fluorescent reporter and a specific chelating receptor. Dye displacement assays, indicators with urea, thiourea, or naphthylamide, and metal ion containing chromogens, have been highly investigated for colorimetric or fluorescent detection of ions [46-48].

On the other hand, Lewis bases involve mainly chelating and macrocyclic ligands that may have high affinity to Lewis's acids. The semi-specific chelating Lewis acidic dyes or so-called complexometric indicators, have chelating sites capable to induce strong coordination with metal ions, causing a rapid color change often with significant selectivity among possible metal ions. The level of specificity of these ionochromic dyes depend on their chemical structure. For instance, Eriochrome Black T is commonly used to detect Ca^{2+} , Mg^{2+} , and Al^{3+} ; calcein is often used to detect Ca^{2+} ; hematoxylin for Cu^{2+} ; murexide is used for Ca^{2+} , Ni^{2+} , and rare earth ions; xylene orange can be used to detect Ga^{3+} , In^{3+} , and Sc^{3+} [49-52]. Classical complexometric titrations are based on displacement reactions. The metal ions bound to the indicator that is replaced by the addition of EDTA. The free dye molecule acts as the end of point indicator. However, as the selectivity of the indicators is limited and the chelators have, usually, multiple pK_a values, the pH needs to be carefully controlled during titration [28].

3.3.1.3. Redox Indicator Dyes

Redox indicators dyes, *i.e.*, indicators that undergo reduction or oxidation processes, are colorimetric compounds that offer a characteristic color change at specific potentials. Boron-dipyrromethene (BODIPY), *o*-dianisidine, diphenylamine, eriogreen, Nile blue, methylene blue, and 2,2'-bipyridine, are some examples of organic dyes revealing reversible redox reactions [53]. The working principle involves the participation of protons in the electron transfer process and this type of dyes can be classified as pH dependent or pH independent indicators. Examples of optical sensors based on reduction/oxidation indicator dyes are the enzymatic-based optical sensors. There are in literature several examples of sensors involving enzymatic redox reactions for various targets, comprising reversible redox indicators dyes immobilized or linked at different transduction schemes and using different fabrication techniques [54-58].

3.3.1.4. Solvatochromic and Vapochromic Dyes

Solvatochromic and vapochromic dyes experience color changes caused by changes in the polarity of the local environment. The mechanism comes from the differential stabilization between the solvation energy and the excited state of the chromophores in different solvents. If the dipole moment of the excited state is larger than the dipole moment of the grounded state, it will be more stabilized in a more polar environment and vice-versa (positive solvatochromism). Generally, the most used solvatochromic dyes belong to classes of chemical compounds including the merocyanines, azobenzenes, thiazines, oxazones, nitro-amino-substituted polythiophenes, among others. This type of compounds has “push-pull” structures with a large conjugated π system with strong electron donor groups at one end, and strong electron extracting groups at the other [28]. Additionally, those structures can be chemically modified to enhance their optical properties, creating new classes of solvatochromic materials. For instance, designs including phenol-linked imidazole derivatives exhibit extraordinary color change *via* proton tautomerism, revealing high potential as the next generation of solvatochromic dyes in the fabrication of environmental stimuli-responsive materials [59]. There are several examples in literature about the usage of solvatochromic dyes to fabricate optical probes detecting a wide range of analytes including biomolecules (nucleic acids, proteins, biomembranes, etc.) [60-62].

Vapochromic and vapoluminescent compounds are other class of solid-state materials that offer color change as response, in this case, to solvent vapors. Generally, vapochromic pigments are based on organometallic or coordination complexes materials, mainly obtained from square planar Pt (II) and Au (I) compounds. The concept (vapochromism) includes the intercalation of solvent molecules into the crystals, causing color or luminescent changes that occurs from relatively weak interactions within the solids involving π - π stacking, coordination of solvent molecules to the metal binding sites, hydrogen bonding and other non-specific interactions [63]. Although there are several examples of optical probes, based on vapochromic dyes, targeting different type of analytes described in literature, these materials present very slow response time due to the slow diffusion of vapor molecules into the solid materials, and the weak interactions involved in the detection process restrict their selectivity and sensitivity [28, 64-66]. Those drawbacks can be overcome by developing nanoscale and porous coordination complexes in order to reduce the diffusion time and expand the surface contact area, thus enhancing the interactions at the solid-gas interfaces.

3.3.1.5. Chromogenic and Fluorogenic Aggregative Indicators

The synthesis of chromogenic or fluorogenic indicators, whose color or fluorescence is induced by particle aggregation is an area in a massive growth. Metal salts precipitation, nanoclusters formation upon sulfide reactions, metallic or metal oxides, carbon-based nanotubes, graphene variants, carbon or quantum dots, are examples of materials that have been used as chromogenic or fluorogenic aggregative probes covering applications such as biosensing or immunosensing [67-70]. The concept includes the aggregation, dispersion, or formation of colloidal materials that produces changes in color or fluorescence through multiple optical mechanisms (absorption, scattering, plasmonic absorption, aggregation-caused quenching, or aggregation-induced emission luminescence). The change in color or fluorescence is induced by the analyte binding to the chromogenic or fluorogenic material, caused by the dispersion of the agglomerates or the release of fluorophores in response to the binding event.

A different class of materials, such as the synthetic materials, can be used to increase the selectivity against potential interferents using the molecularly imprinted approaches (detailed in Chapter 4). Once their production method comprises the presence of the target analyte (template molecule), the resulted particle is, in theory, highly selective to that specific analyte. Doping the imprinted particles with fluorescent materials, the binding event will cause the release of the fluorophores, offering the possibility to produce high-selective fluorescent probes [71].

3.4. Applications

The advantages of optical fibers as a light driver have attracted several investigators to use them to perform sensors for a wide range of applications and using different approaches for light modulation.

A colorimetric methodology was developed and studied by *Xiong et al.* for free chlorine detection [72]. The optical platform comprises a light source (LED emitting at 520 nm) and two multimode silica fibers (600 μm , core $\varnothing$) as a light transmission element (from the source and to the detecting device). The optical sensor was integrated with a sequential injection lab-on-valve (SI-LOV) where the sample and other desired reagents were injected in order to pass between the two aligned fibers (Figure 3.6). The colorimetric measurements were based on the reaction the chromogenic indicator *N,N*-diethyl-*p*-phenylenediamine and free chlorine, resulting in the production of a chromophore with a maximum absorbance at 511 and 551 nm. The authors have

evaluated the total chlorine concentration by employing the Beer-Lambert law (often referred as Beer's law), presenting a linear behavior ranging from 10 to 400 $\mu\text{g/L}$, a limit of detection of 3.5 $\mu\text{g/L}$, and a response time of 6.85 seconds. The authors also reported that the fiber optic/SI-LOV sensor was successfully applied to on-line and real-time detection the target analyte with good selectivity, exhibiting better performances than the traditional spectrophotometry and titrate determination.

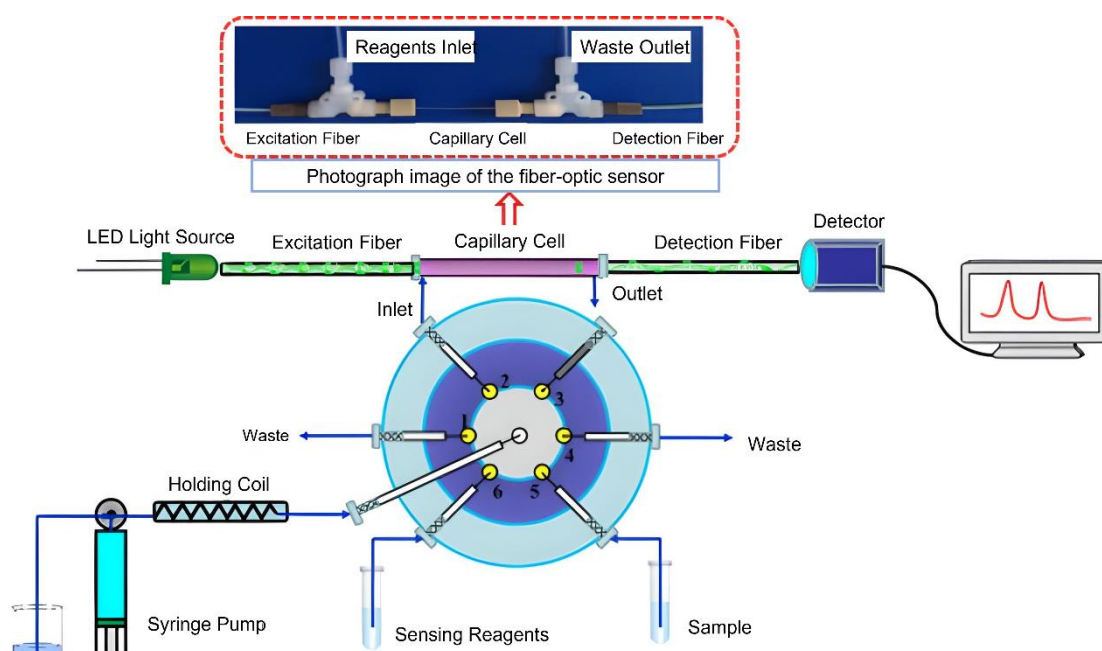


Figure 3. 6. Diagram of the whole fiber optic/SI-LOV system. Inset image is the picture of the constructed fiber optic sensor. Reproduced under CC-BY license from reference [72]. ©Elsevier Copyrights.

Another colorimetric approach was used by *Matejčec et al.*, by developing an extrinsic FOS for saliva pH detection [73]. The optical platform is based on an inlet and outlet silica fibers (300 μm and 200 μm , $\varnothing$, respectively), transmitting light from a halogen lamp to the sample and collecting the transmitted light from the sample to a diode-array spectrometer. The optical sensor was calibrated using a pH meter and Sorensen buffer solutions with bromothymol blue pH indicator. The saliva samples were collected from a healthy person at different times of the day (from the early morning to a couple of hours after lunch) and were mixed with the pH indicator before sampling. The saliva samples were tested by measuring the ratiometric signal between the referenced intensity (bromothymol blue in water) and the transmitted intensity after light passes through the saliva/bromothymol blue sample. The authors reported that the sensor successfully measured the pH values in saliva samples with this simple method, exhibiting a resolution of 0.3 pH values.

A different approach was revealed by *Batinić et al.*, to determine the color of a printed sample by electrophotography [74]. The technique was based on the estimation of the spectrum of the electromagnetic radiation in the visible range. The system consists of a set of six LED sources (with wavelength emissions at 400, 517, 572, 632, and 700 nm) transmitting through six multimode plastic fiber optic cables. On the receiver side was placed another multimode plastic fiber to collect the reflected light and guided it to a microcontroller unit. The colorimetric measurement is based on the change in intensity of the transmitted light when it is reflected from the measuring point. Depending on the color of that point, some wavelengths are absorbed, transmitting the others. The emission and collection of light was performed through an optical probe where the emitting six fibers were placed around the collecting seventh fiber. To optimize the response time and to reduce the noise in each measurement, the LEDs were programmed to emit in groups of three during the same period. So, in the first half of the period, three LED sources were simultaneously triggered at different frequencies, being the other three triggered in the second half of the period. The authors concluded that this methodology resulted in a low-cost approach, also reducing the dispersion elements (optical lenses and mirrors) which are often used in traditional methods.

Liu et al. presented a colorimetric methodology based on optical fibers and a pH indicator for carbon dioxide detection, by performing a thin film on the distal end of the fiber [75]. The colorimetric thin film is based on the well-known pH indicator thymol blue and the phase transfer agent tetramethylammonium hydroxide (TMA-OH). The chemical combination of these two compounds results in an ion pair formation, when the OH^- ion of the TMA-OH deprotonates the thymol blue. The detection of the gas occurs when the carbon dioxide reacts with the water molecules present in the ion pair conjugation, releasing H^+ protons that will protonate the thymol blue, leading to a color change (blue to yellow). To hold the thin film on the end of the optical fiber, the thymol blue/TMA-OH mixture was encapsulated into a sol-gel network. The gas diffuses through the mesoporous structure and interact with the transducer as described. The sensing platform was obtained by dip coating a 3dB multimode optical fiber coupler tip (62.5 μm , core $\varnothing$), in which one arm was used to transmit the light from the light source and the other was used to collect the reflected modulated light until a CCD spectrometer in order to evaluate the light absorption by the gas-sensitive film. The reversible sensor demonstrated to be sensitive to carbon dioxide in a range of 0-6%. The authors also reported that the sensing platform is also sensitive to relative humidity (0.19 nm/%RH; 0 – 90 %RH) due to the formation of a Fabry-Pérot cavity between the sol-gel film and the fiber tip. However, the authors concluded that the cross-sensitivity is negligible, and

the sensor can be used to measure, simultaneously, carbon dioxide and relative humidity with a percent error of 3.1% and 2.2%, respectively, when compared with a commercial datalogger.

A fluorescence method for pH determination using fiber optic cables was demonstrated by *Werner et al.*, by coating the tip of a 400 μm silica core optical fiber coupler with a pH-responsive indicator dye [76]. The probe was fabricated by covalently binding a fluorescent monomer (fluorescein *O*-methacrylate) exhibiting a strong luminescent signal for a pH range of 5.0 – 8.5. The indicator dye was mixed with a set of monomers, including a photo-initiator, and the polymerization process carried out under a power UV LED radiation (emitting at 365 nm) with the fiber tip dipped into the pre-polymeric solution. To calibrate the fiber optic sensor, the polymerized film was excited by a LED emitting at 470 nm using one of the ends of the fiber coupler, displaying maximum fluorescence at 520 nm that was collected by the other fiber coupler end. The performance was evaluated by using a set of pH solutions ranging from pH 5 to pH 8.5, measuring the deviations on the intensity of the fluorescence peak. The sensor showed fast response time ($t_{90\%} < 5$ s) and a resolution of around 0.04 pH units.

A fiber optic sensor for the detection of Fe (III) ions was investigated by *Zhou et al.* by using fluorescent CdTe quantum dots doped hydrogel [77]. The methodology is based on two types of quantum dots: the green emissive thioglycolic acid capped quantum dots (gQDs) and red emissive *N*-Acetyl-L-cystein capped quantum dots (rQDs). Whereas rQDs can be selectively quenched by the Fe^{3+} ion, the gQDs are immune to such ions, exhibiting stable fluorescence emission. The configuration allows the ratiometric detection of the target analyte by measuring the fluorescence intensity of each group of quantum dots, reducing the impact of interferent species. The optical platform was developed using a 3dB silica multimode fiber coupler (200 μm , core $\varnothing$) pigtailed to the quantum dots/hydrogel matrix, by employing a UV lamp to photo-crosslink the precursors and extend the MMF tip length. The excitation of both types of quantum dots carried out by transmitting a laser beam at 405 nm through one of the arms of the fiber coupler, while the other arm was used to receive the emitted radiation and guide it to a spectrometer. The authors reported a selective and quantitative detection Fe (III) ions with a linear behavior ranging from 0 to 3.5 $\mu\text{mol/L}$, with a limit of detection of 14 nmol/L.

A different approach for the development of a fluorescence-based fiber optic sensor targeting metal ions was studied and demonstrated by *Nguyen* and his colleagues [78]. The reported sensor is based on a turn-on fluorescence methodology for the real-time detection of Hg (II). For the development of the fluorescent probe, the fluorophore was

covalently immobilized onto a chemically modified multimode optical fiber tip using the ion imprinting technique, by mixing the monomers, the template molecule (HgCl_2), and the polymeric initiator. After complete polymerization on the fiber tip, the template was removed by immersing the fiber into a tris-EDTA buffer solution. The resulted fluorescent polymeric material is based on a derivative of coumarin (fluorophore) and an azathia crown ether moiety (ion receptor). The fluorescence phenomenon occurs through the photoinduced electron transfer (PET) mechanism, by employing the design principle of chromophore-spacer-receptor architecture. In this way, the empty receptor can quench the fluorescence of the linked fluorophore by the PET mechanism. However, when the binding event occurs in the presence of the target ion, the receptor loses the ability of quenching the fluorescence of the fluorophore (Figure 3.7a). The excitation was induced by a 3 mW light emitting diode, emitting at 375 nm, coupled to a 2 x 1 multimode silica fiber coupler (Figure 3.7b). Upon $\text{Hg}(\text{II})$ binding to the receptor, the irradiated fluorophore emits a portion of light that is collected by the fiber tip and is guided through the other end of the fiber coupler to a spectrometer. The authors reported that the sensor was successfully calibrated over a concentration range of 0 – 28 $\mu\text{mol/L}$, revealing a response time of 11 minutes (recovery time was around 30 minutes in tris-EDTA buffer solution) and a limit of detection of 0.15 $\mu\text{mol/L}$.

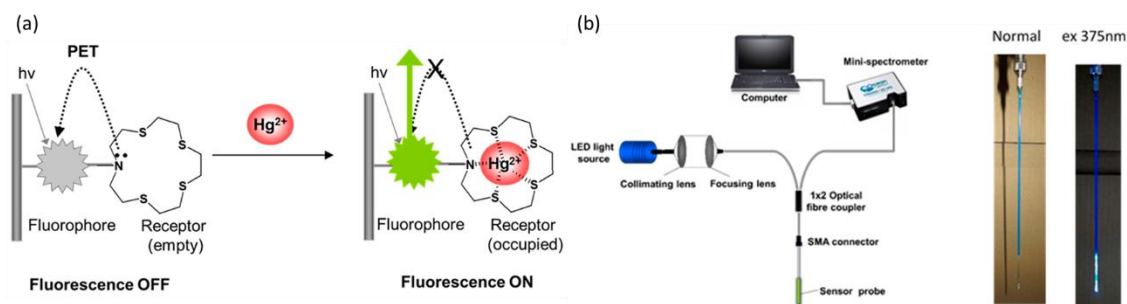


Figure 3. 7. Illustration of: a) fluorescence switching on caused by the binding of the mercury ion (II) *via* PET mechanism; and b) experimental setup including the light source, the optical fiber coupler and the spectrometer, and the normal and excited fluorometric probe on the fiber end. Reproduced under CC-BY license from reference [78]. ©MDPI Copyrights.

Ashokkumar *et al.* showed an analytical platform for sugar sensing in beverages using boronic acid (a Lewis acid) coupled with a fluorescent dye: the BODIPY [79]. In the described work, the researchers developed a boronic acid-functionalized BODIPY probe which binds selectively to fructose's adjacent diols, forming boronated esters. The probe was designed by placing a tertiary amino group adjacent to a boronic acid receptor to create a basic environment. This procedure results in the enhancement of the fluorescent responses through modulation of an intermolecular charge transfer process, increasing

considerably the pH range (pH 6 – 10; while the traditional methods work preferentially at pH >8). The platform comprises a PDMS microfluidic system where the samples are injected and excited by an external blue light emitting diode, whereas the emission radiation is collected in real-time by an optical fiber bundle that guides the light until a miniaturized spectrometer. The interferences coming from LED fluctuations were avoided by monitoring the back-scattered light, resulted from the excitation process, using the remain fiber of the bundle. The microfluidic sensor revealed good linearity in range of Fructose concentrations between 0 – 0.5 mmol/L, and a limit of detection of 7.6 $\mu\text{mol/L}$.

Another optical chemical sensor using intensity fluorescence signals targeting pH changes was reported by *Frankaer* and his co-workers [80]. The sensing probe was developed by mixing a pH-responsive fluorophore (diazaoxy-triangulenium; DAOTA) with a pH-responsive phenol group and a siloxane-functionalized linker, allowing the covalent immobilization of the dye. After full polymerization (a couple of weeks later), the resulted sol-gel matrix was spread onto a micropillar structured polycarbonate by the hemiwicking process and cured with temperature. To perform ratiometric measurements, a referencing dye exhibiting fluorescence in different a wavelength was immobilized in polystyrene, being deposited on the back of the polycarbonate substrate. The optical platform comprises a blueish green LED emitting at 505 nm, acting as excitation light that is transmitted through a set of 6 multimode fibers of a 6-1 optical fibers bundle. The emitted light (at 585 nm for the sensing dye, and 667 nm for the reference dye) resulted from the fluorescence properties of the dyes is collected by the seventh fiber, localized in the center of the fiber bundle. The authors demonstrated the operability of the sensor in a pH range of 4.6 – 7.6, exhibiting a response time of $t_{90} < 90\text{s}$ and a precision of 0.03 pH units.

Yang et al. proposed an optical fiber bundle sensor based on the fluorescence phenomenon, for the detection of the trace triacetone triperoxide (TATP) vapor [81]. The optical platform consists of an optical fiber bundle coated with a fluorescent probe based on an azetidine derivative of naphthalene diimides (DNNDI) fluorescent film. Basically, when the light travelling in the fiber used for excitation hits the film, it fluoresces and emits light that is collected by the receiving fiber located at the fiber bundle end, guided it to the spectrometer. The excited DNNDI molecules react with the TATP molecules, inducing a charge transfer between them. The electron transfer occurs between the DNNDI molecules in the excited state and the TATP molecules, causing the fluorescence suppression of the DNNDI when it backs to the low-energy state. Comparing the

fluorescence signal intensity before and after fluorescence quenching, the concentration of the target analyte can be estimated. The authors also evaluated the influence of the film thickness, 12.85 and 16.11 μm , in the sensitivity of the optical sensor and concluded that the thinnest film provided a better sensitivity, 446.50 1/ppb and 405.50 1/ppb respectively. The limit of detection was also assessed, exhibiting 19.40 ppb for the thinnest film and 21.0 ppb for the thicker film.

Besides sensors based on chromophores and pH indicators dyes, the optical fiber cables can be used to develop different extrinsic FOS only using the reflected light, measuring, for instance, displacement, reflectance, among other applications. Table 3.2 shows some examples.

Table 3. 2. Examples of applications of fiber optic sensors for measure displacement and reflectance measurements.

Type of Sensor	Platform Design	Purpose	Main Achievements	Ref.
Displacement	He-Ne laser (red light at 633 nm); OF bundle (1:1); reflecting mirror; PD detector	Measure the amplitude and frequency of heart rate signals	Working range: 50 – 300 bpm Sensitivity = 0.002 mV/ μ m Linearity: >99 %	[82]
	Light source; independent MMF to transmit and collect the reflected light; reflective surface; OP meter	Improve the amount of the reflected light collection by studying different architectures of the collecting fiber tip	BLOF showed improvements of 10.5% compared with the only-cleaved MMF tip; BLOF cleaved MMF showed an increment of 33%	[13]
	Light source (at 1310 or 1550 nm); SMF/patch cord/OF coil/SMF/OW design;	Real-time monitoring the stability of sides, berm of quarries	Working distance: 30 - 50 km Sensitivity: 0.2134 dB/N	[83]
Reflectance	Laser source (at 1310 nm); 2 x 1 MMF coupler spliced with a rounded tip NCF; PO	Study the intensity variation of the reflected light caused by changing the salt concentration in water	Working range: 0 – 20 % Sensitivity: 0.773 dB/%	[84]
	UV LED (at 370 nm); OF bundle (1:1; 200 μ m, $\varnothing$); MMF (600 μ m, $\varnothing$) embedded in the cement paste; micro spectrometer.	Monitor the curing process of the cement paste by assessing the capillarity coefficient (CC) at different water-to-cement ratios (w/c)	CC obtained for the different w/c, agree with the gravimetric measurements of sorptivity, proving the applicability of the method to measure the entire life-cycle of cement	[85]
	White light source; 1:6 (emitting:receiving) OF cable with various fiber diameters; spectrometer	On-line monitorization of different types of <i>Aspergillus</i> biofilm growth on cultural paper relics	Absorption sensitivity: - <i>A. niger</i> : 0.000385/ μ m - <i>A. flavus</i> : 0.000408/ μ m - <i>A.tamarii</i> : 0.000463/ μ m LOD: 10 μ m	[86]

OF: optical fiber; PD: photodiode; MMF: multimode fiber; OP: optical power; BLOF: ball-lensed optical fibers; SMF: single mode fiber; OW: optical wattmeter; NCF: no core fiber; LOD: limit of detection.

3.5. Conclusion

Fiber optic sensors based on intensity modulation caused by external transducers or by the reflectance properties of several types of surfaces have been extensively used over the years, being directed to a wide range of applications, covering the detection of distinct type of chemical analytes or physical parameters. The progressive investigations on this field have led to the development of different sensor designs ranging from to the simple measurement of the displacement between a fiber and a moving material or two fibers monitoring the distance between each other caused by slipping, to the development of highly sensitive colorimetric and fluorescence sensors, targeting chemical or biological entities.

Sensitive materials changing color or emitting light, due to their fluorescence properties, have demonstrated their applicability on the detection of various important analytes with a significant impact on the development and improvement of peripherals research fields. Their combination with the advantages adjacent to optical fibers, allows the researchers to develop sensors for *in situ* applications, able to operate in hard-to-reach locations and harsh environments. Numerous investigations have published different approaches of detection by applying distinct fiber architectures, such as in-line emitting/receiving fibers, optical fiber couplers, or bundles of optical fibers varying number of fibers emitting and receiving light, combined with simple dyes offering changes of color caused by the interaction with a measurand, or by monitoring the intensity of the fluorescence of fluorescent materials excited by specific light frequencies/wavelengths. The efforts of researchers on the field led to the development of very interesting detection schemes where the change in color or fluorescence is induced by high-selective receptors after a recognition event, resulting in highly sensitive and selective fiber optic sensors.

However, it is still necessary to improve the incorporation of these sensitive schemes in miniaturized or easy to transport integrated systems improving their robustness and ease of operation. Besides, outside of the well-controlled laboratorial environments, several interferences will affect the sensors performance. Thus, mainly in systems using external illumination or distinct optical fiber to transmit and collect light, it is necessary to overcome those issues by applying a system to correct/monitor the influence of external physical or chemical interferences to assure a reliable detection.

Considering those aspects, the next chapter addresses different approaches to fabricate fiber optic sensors where the own fiber is part of the transducing system, enabling the development of sensors using the optical fiber as single component to carry the light as well as the elements used for specific recognition of the target analytes.

Chapter 4 – Intrinsic Fiber optic Sensors

This chapter aims to detail the state of the art of the most used configurations to develop intrinsic FOS. Brief discussion about the coating materials and techniques used to modify the fiber surface. Examples of intrinsic FOS targeting chemical or biological entities are also addressed.

4.1. Introduction

Differently from extrinsic FOS, in intrinsic FOS one or more physical properties of the fiber undergo a change. The qualitative and/or quantitative response results from the light perturbation during its path along the optical fiber, at a single or multipoint series, that can be detected with an adequate electronic device (Figure 4.1) [87].

The trend in the research of intrinsic FOS follows the need to develop, at the same time, miniaturized, easy to install, and highly sensitive sensors. Nowadays, the efforts made by researchers in the field have resulted in various configurations, presenting distinct working principles, and using different signal modulation approaches.

Optical fibers are an attractive option to overcome those demands, mainly for real-time monitoring, emphasizing the advantages presented by optical fibers and characteristics such as fast detection and easy installation for *in situ* measurements [9]. In the last decade, optical fibers have been widely used to create several types of IFOS suitable to

be applied in different fields such as medicine [88, 89], general industry [90, 91] and environmental monitoring [9, 92], tracking chemical or and biologic entities.

Another important advantage, is the good (bio)chemistry compatibility of the fiber optic surfaces, making them suitable for several types of metal or polymeric coatings, and/or for chemical modifications, introducing chemical or biological specific receptors.

In the following sections of this chapter are described and discussed the main principles and configurations used to develop IFOS, the different coating techniques and materials used to enhance the optical properties of the optical structures, and the different options for chemical and biological sensing and their main characteristics. Some examples of applications employing intrinsic FOS for a diversified types of targets detections are also addressed.

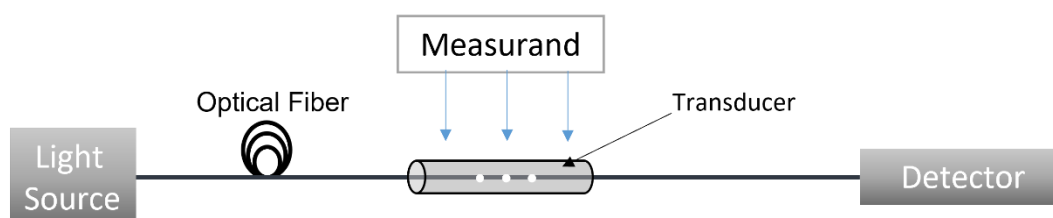


Figure 4. 1. Basic configuration of an intrinsic FOS.

4.2. Principles and Configurations

In this section are presented, described, and discussed the main configurations used to develop intrinsic FOS, including the respective optical principles such as the based on the evanescent field concept and plasmonic effects.

4.2.1. Evanescent Field

Evanescent wave FOS have been extensively used for chemical, biochemical, and environmental research [9]. The qualitative and/or quantitative results are given by the interaction of an analyte with an evanescent field of light crossing the optical fiber. As the penetration depth of the evanescent field acts at most a few hundred nanometers from the optical fiber, the measurements are not affected by the bulk solution [93]. In general, the light travels through the fiber core following the attenuated total reflection principle, $\theta_c = \sin^{-1} n_1/n_2$ for $n_2 > n_1$, where θ_c is the critical angle, and n_1 and n_2 are the refractive indices (RI) of the cladding and the core, respectively. Nevertheless, some

vibrations in the cladding occur in such a way that Maxwell's equations are not violated [94]. Those vibrations are called evanescent waves. Therefore, optical fibers sensors based on unclad optical fibers, side-polished and tapered fibers, fiber Bragg gratings (FBGs), tilted fiber Bragg gratings (TFBGs), and long period fiber gratings (LPFGs) come up with success in the last years for the detection of several analytes [95-98]. Along with the specific modifications it is possible to monitor physical (e.g., pH, temperature) chemical (e.g., oxygen, carbon dioxide), biological (e.g., virus, bacteria, protein), or biochemical (e.g., drugs/pharmaceuticals) species [94, 99-102].

4.2.1.1. Unclad Optical Fibers

In the unclad optical fibers, the attenuation of evanescent wave occurs at the interface between the measurand and the optical fiber's core. After cladding exclusion, if the evanescent field is higher enough to interact with the external medium, the resulted optical response can be used as a fingerprint to evaluate that interaction [9]. This interaction causes a change of the output intensity as a function of the optical properties of the absorbing medium. In this case if the incident angle, θ , is greater than the critical angle for total reflection at the core-analyte interface (θ_c), there are perfect conditions for the exponentially decaying field that is the capacity to breach the medium located in contact with the fiber and the penetration depth (P_D) can be expressed as showed in equation 4.1:

$$P_D = \frac{\lambda}{2\pi(n_2^2 \sin^2 \theta - n_1^2)^{1/2}} \quad (4.1),$$

where λ symbolizes the wavelength of incident light, and n_1 and n_2 are the refractive indices of the clad and the core, respectively [103]. Currently, the most studied structure based on unclad fibers is, probably, the so-called multimode plastic-clad silica-core fiber (PCSF).

4.2.1.2. Side-Polished and Tapered Optical Fibers

Other technique used to reach the fiber core is by side polishing. Commonly, the fiber is fixed in a suitable support and is, mechanically, polished in one side. When the cladding is close to the core, the evanescent field can strongly interact with the external medium. Other possible situation relies in the possibility to deposit a thin film over the polished section, creating a sensing surface by interacting with the evanescent field [104]. Reduce uniformly the thickness of the fiber, producing a taper, is also an alternative. Tapered

fibers can be produced by applying a longitudinal tension during heating process or applying an electric-arc discharge [105]. This process, results in the extension of the fused region until the required length and waist are achieved. The sensitivity of the tapered fiber-based sensors depends on the length as well as the waist diameter of the tapered region (Figure 4.2) [94]. In the tapered fibers, the strength of the evanescent wave is increased in the cladding region due to the penetration depth of the evanescent field at the fiber-measurand system is increased. Initially, the fundamental mode propagates as a core mode until reach the tapered region, being then distributed out into the cladding region. From here, the cladding acts as a waveguide [9].

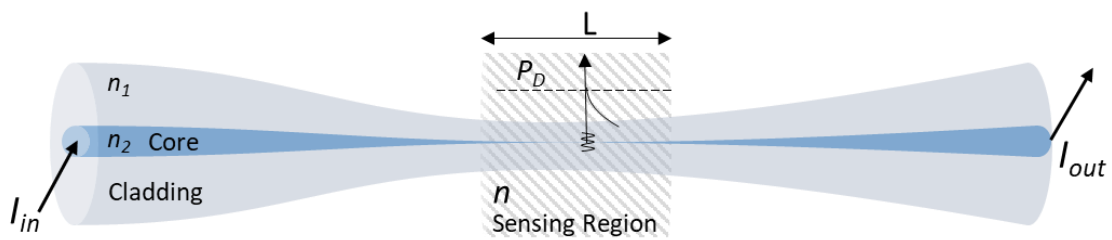


Figure 4. 2. Illustration of a tapered optical fiber where n , n_1 , and n_2 are the refractive index of surrounding medium, cladding, and core, respectively ($n_2 > n_1$). L is the length of the tapered fiber.

Another technique to reduce the fiber diameter is to submit the required portion of the fiber to a wet etching. Usually, the fiber is submersed in a known concentration of a hydrofluoric acid (HF) solution during a certain time until the expected diameter is achieved. The exposition time and the concentration of the HF solution are crucial parameters to control the waist of the tapered region. Contrary to what happens in the heating and pulling process, the dimensions of the core does not change [106].

Multimode optical fibers, namely the PCSF, can also be used to perform side-polished and tapered fiber sensors. After the mechanical or chemical exclusion of the plastic cladding, the polish, heat and pull or etching process is used to reduce the core diameter (e.g., D-shaped fibers) and/or fabricate tapered fiber tips [107].

4.2.1.3. Microstructured Optical Fibers

Another relevant structure using the evanescent field effect for sensing are the microstructured optical fibers (MOF). These structures involve the presence of air holes along the entire length of the fiber, resulting in a microstructured cladding region that is used to manipulate the propagation of the core and leaky cladding modes. The core can include doped region allowing the inscription of gratings. The resulting hybrid

waveguides are interesting configurations for chemical and biological sensing [108]. Microstructured optical fibers can assume several architectures and some of them are presented in Figure 4.3.

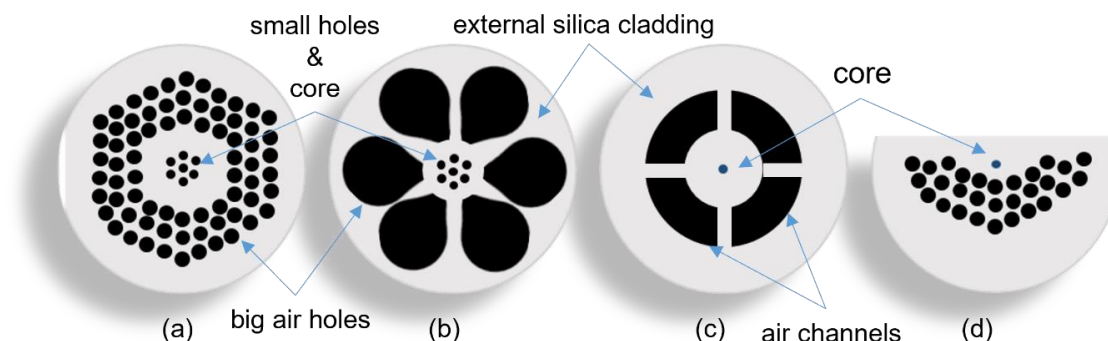


Figure 4. 3. Illustration of different architectures of microstructured optical fibers: (a) Photonic Crystal Fiber; (b) “grapefruit” MOF; (c) air-clad MOF; and (d) D-shaped MOF. Adapted from [109]. MDPI Copyrights.

Specific architectures such as is illustrated in Figure 4.3a are also known as photonic crystal fibers (PCFs). PCFs are considered a distinct class of optical fibers, distinguished by a periodical arrangement of air-filled microcapillaries in the fiber cladding around a solid or hollow core [110]. In the solid-core PCFs, the patterned air holes or channels surrounding the core offer cladding/air insulation to the guided waveform, thus trapping the light in the core, and minimizing its loss during transmission. PCFs offers a unique performance in a wide range of applications such as large-mode-area MMFs, dispersion compensation, nonlinear applications, and single polarization and high birefringence. The air structures in the PCF cladding, and often in the core, deals with the possibility of intrusion of fluids that can enhance their properties [110].

4.2.1.4. Fiber Optic Gratings

Fiber optic gratings are usually manufactured in single mode fibers, by periodically inscribing a small modulation (perpendicular to the fiber axis) in the effective refractive index of the core. Depending on the grating period (Λ), these types of structures can be divided into short period fiber gratings (Λ less than 1 μm), where are included the FBGs and the TFBGs, and LPFGs (Λ in a range of tens to hundreds of μm) [111].

Short-Period Fiber Bragg Gratings

FBGs are an example of a short period Bragg grating and acts as selective mirror for the light propagating in the fiber core, reflecting part of that light in a specific wavelength. In

general, the most common methods used to fabricate Bragg gratings are the interferometer technique, phase mask technology, and femtosecond laser inscription (point-by-point or line-by-line) [96]. The wavelength position of the reflected light is in accordance with the phase-match condition, and can be given by [9]:

$$\lambda_{Bragg} = 2 \times n_{eff} \Lambda \quad (4. 2),$$

where, λ_{Bragg} is the Bragg wavelength, n_{eff} is the effective refractive index of the core and Λ is the periodicity of the grating. The devices comprising a FBG can monitor parameters such as temperature or strain. As the fundamental mode of a FBG is independent of the external RI, the wavelength Bragg position is not affected by the refractive index of the measurand (Equation 4.3) [112].

$$\frac{\Delta \lambda_{Bragg}}{\lambda_{Bragg}} = \left(\frac{1}{n_{eff}} \times \frac{\Delta n_{eff}}{\Delta T} + \alpha \right) \Delta T \quad (4. 3),$$

where α is the thermal expansion coefficient of the fiber, and ΔT is the temperature variation of the measurand. However, in FBGs microfabricated in thinned cladding fibers (e.g., tapered fibers), the external RI can affect the FBG fundamental mode and such devices can be used to monitor changes of the external RI (or the measurand RI) (ERI) (Equation 4.4) [112].

$$\frac{\Delta \lambda_{Bragg}}{\lambda_{Bragg}} = \left(\frac{1}{n_{eff}} \times \frac{\Delta n_{eff}}{\Delta T} + \alpha \right) \Delta T + \left(\frac{\Delta n_{eff}}{n_{eff}} \right)_{ERI} \quad (4. 4).$$

Furthermore, for an accurate and simultaneous evaluation of the ERI (e.g., chemical, or biological entities) and the temperature of the medium, two FBGs (or two sensing regions FBG) are crucial.

Differently from FBGs, TFBGs are suitable for external RI assessment. The fundamental difference between them is the direction of the inscribed modulation, that, in case of TFBGs, forms a certain angle with the fiber axis. In this type of gratings, the transmitted light is coupled from the core to the fiber cladding due to the inclination in the modulation direction. Considering the Equation 4.5, the wavelength peaks, referred to the cladding modes, are affected by the surrounded RI and by the temperature variations [9]:

$$\lambda_{(CO-CL)} = (n_{co}^{eff} - n_{cl,m}^{eff})\Lambda/\sin\theta \quad (4.5),$$

where $\lambda_{(CO-CL)}$ is the resonance wavelength; n_{co}^{eff} and $n_{cl,m}^{eff}$ are the effective RI of the fundamental core mode and the m order cladding mode, respectively; Λ is the period of the grating and θ is the angle between the grating and the fiber axis. Both FBG and TFBG illustrations are presented in Figure 4.4.

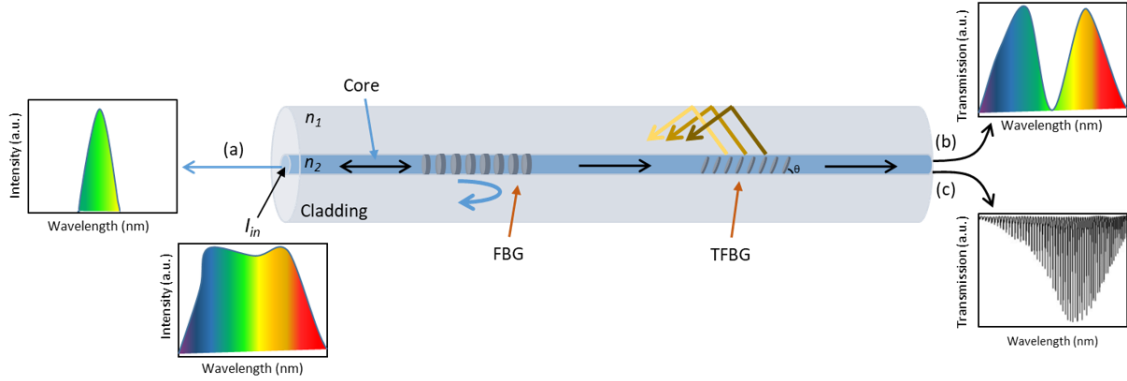


Figure 4. 4. Illustration and working principle of an FBG: (a) resulted spectrum of the reflected light by the fiber Bragg grating, (b) resulted spectrum of the transmitted light (minus the light that was reflected); and of a TFBG: (c) resulted spectrum of the transmitted signal after TFBG, where are visible the several cladding modes resulting from the coupling of the light to the cladding.

Long Period Fiber Gratings

A long period fiber grating is an optical component attained by inducing a periodic modulation in the RI of a fiber (usually SMF) with a typical period ranging from 100 μm to 1 mm. These structures are performed by well controlled microfabricated processes such as UV laser-based techniques, CO_2 laser, IR femtosecond laser, and electric arc discharges [16, 113-116]. The induced perturbation produces a resonant coupling between the fundamental mode propagating in the core and the co-propagating cladding modes (Figure 4.5). That coupling event works as a selective wavelength filter and the fiber transmission spectrum presents several attenuation bands associated to the different cladding modes. According to the phase-matching condition, the resonance wavelengths are given by:

$$\lambda_{LPG} = (n_{co}^{eff} - n_{cl,m}^{eff})\Lambda \quad (4.6),$$

where n^{eff} is the effective refractive index of the core (co) and cladding (cl), respectively. The penetration depth of the attenuation bands strongly depends on the product of $k_m \times L$, where k_m is the coupling coefficient between the core and the m order cladding mode, and L is the length of the grating [117].

Considering the figure 4.5, as the external RI (n) increases, the sensitivity of the LPFG enhances monotonically to a maximum value that is determined by the RI of the cladding mode (n_1). When $n = n_1$ the cladding mode becomes unguided. Therefore, if the RI of the core mode (n_2) is not changed by n , the effect of the external RI around the cladding can be expressed by:

$$\left(\frac{d\lambda}{dn}\right)_m = \left(\frac{d\lambda}{dn^{eff}}\right) \times \left(\frac{dn_{1,m}^{eff}}{dn}\right) \quad (4.7),$$

where λ and $n_{1,m}^{eff}$ are the transmitted spectra and the effective refractive index of the m order cladding mode. Attending to Equation 4.7, it is clearly perceptible that the resonance wavelength position is affected by the external RI, making them a very interesting components to integrate optical sensing devices, tracking chemical or biological entities [118].

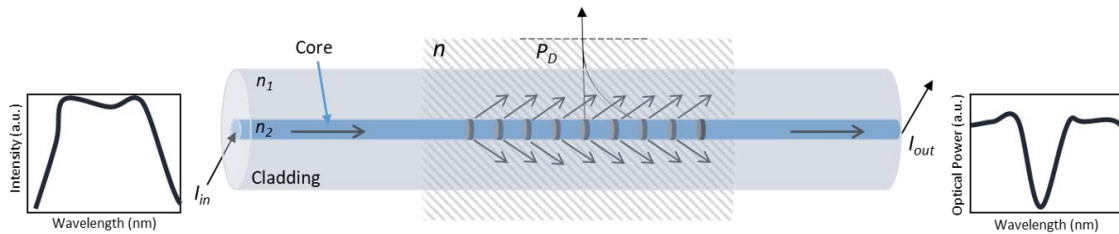


Figure 4. 5. Illustration and working principle of a long period fiber grating.

Several configurations can be used to perform sensors using LPFGs, such as LPFGs fabricated near to the turning point (TP) of the phase-matching curves. This concept offers the formation of two resonance wavelengths associated to the coupling with the same cladding mode. TP is the condition that defines the maximum sensitivity of each cladding mode of a LPFG, and it occurs at longer wavelengths for lower order modes, shifting to shorter wavelengths as the mode order increases. Used as a sensor, the separation of the dual resonance peaks tends to increase as the external RI increases, increasing the sensitivity of the device [119, 120]. Figure 4.6 shows the typical spectrum of a normal LPFG and its optical response changing the externa RI (4.5-a) as well as the

typical spectrum of a LPFG near the TP and its optical response changing from air to water (4.6b). From the figure 4.6a, it is also perceptible that full width at the half maximum (FWHM) also increases as the RI increases.

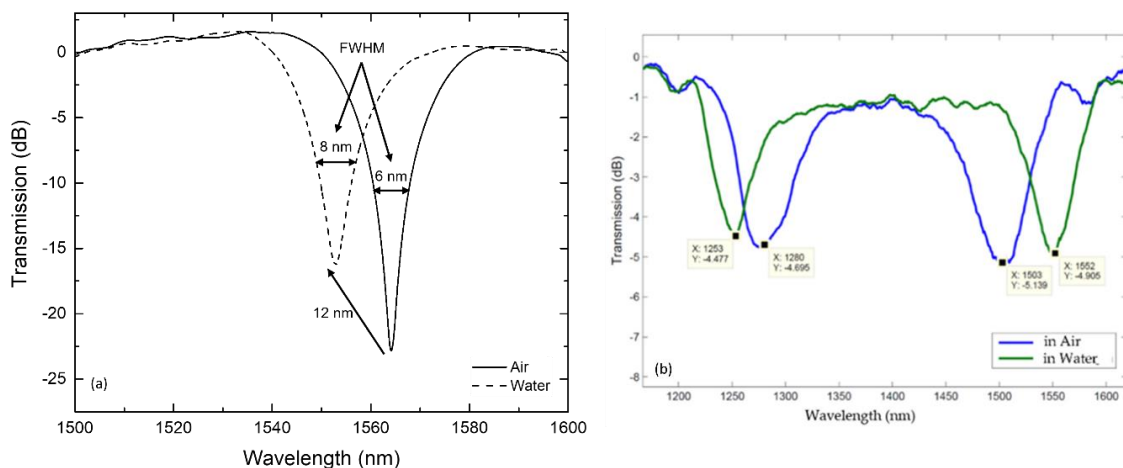


Figure 4. 6. (a) Typical LPFG transmitted spectrum in air (solid black line) and the consequent shift to lower wavelengths (dashed black line; and (b) typical near TP-LPFG transmitted spectrum (solid blue line) and the consequent bands separation in response to the RI changes. Figure (b) was reproduced from [16], MDPI Copyrights.

Furthermore, to increase the evanescent field absorption and the resonance wavelength shift, a normal LPFG can be thinned. As consequence, the modal redistributions in the LPFG are intensified as well as the evanescent-field/analyte interaction, thus improving the sensitivity [121]. Other techniques to enhance the sensitivity relies to the use of metal oxides coatings. These thin films on the LPFG, induces a re-distribution of the co-propagating cladding modes, leading to a wavelength and amplitude shift [122].

Moreover, as was described for the FBGs, sensors employing LPFGs also deals with the interference of other factors (e.g., torsion, strain, and temperature), besides the RI changes of the target. Thus, to obtain an accurate quantity of the measurand, a set of LPFGs needs to be used, or a LPFG/FBG combination is also recommended.

4.2.2. Plasmonic Effects

The plasmonic effect was firstly observed in the middle of the 20th Century with the discovery of the surface plasmon polaritons (SPP), suffering an impulse in the 1970s by the emergence of the surface enhanced Raman spectroscopy (SERS) [123]. Rapidly, becomes a developing field, combining fundamental research and applications in a wide range of scientific areas ranging from physics to engineering, chemistry, biology, food sciences, and environmental sciences [17, 124]. Due to its optical properties and

sensitivity to the surrounded medium, surface plasmons (SP) had a very significant impact in the last decades in the development of several sensing schemes used for various optical applications [124]. In that context, optical fibers have been proved to be an ideal platform form to accomplish plasmon sensors. Their mechanical stability and flexibility combined with the possibility to transmit at remote distances, makes them an outstanding basis for chemical and biological sensors [125].

4.2.2.1. Surface Plasmon Resonance in Optical fibers

Surface plasmon resonance (SPR), is generated by the excitation of SPPs, resulted from the combination of free electron density oscillations and electromagnetic waves on a metal-dielectric interface. SPR events belongs to transverse magnetic or p-polarized light wave, resulting in a vector wave perpendicular to the incident plane and propagates along the top of the metal surface with a propagation constant that can be expressed as [126]:

$$\beta_{SP} = \frac{\omega}{c} \sqrt{\frac{\epsilon_d \epsilon_m}{\epsilon_d + \epsilon_m}} \quad (4. 8),$$

where ω is the angular frequency, c is the speed of light in vacuum, and ϵ_d and ϵ_m are the dielectric constants of the dielectric medium and the metal, respectively. Once that the permittivity of the dielectric materials is positive, the real part of the metal material needs to be negative. For instance, metals such as gold, silver or aluminum show a negative real part in the visible and near-infrared regions of the spectrum. Moreover, those materials also show an imaginary part of the permittivity, thus causing the propagation constant of a SP to has a non-zero imaginary part. That imaginary part of the propagation constant is related with the attenuation of the SP in the propagation direction. Commonly, plasmonic sensors are typically performed on a prism coupler arrangement such as the *Kretschmann* configuration. The light wave passes through a high RI prism, being totally reflected at the prism base, thus evanescent wave that penetrates the thin metallic film excites the plasmon electromagnetic wave at the metal/dielectric interface. The evanescent wave propagates in the interface with a propagation constant which can be modified to match that of the SP by adjusting the angle of incidence [126]:

$$\frac{2\pi}{\lambda} n_p \sin(\theta) = Re\{\beta_{SP}\} \quad (4. 9),$$

where θ is the angle of incidence, n_p is the RI of the prism, and $Re\{\beta_{SP}\}$ is the real part of the propagation constant of the surface plasmon. However, this kind of sensors requires bulky setups, complicating *in situ* measurements, and are challenging to the development of small and low-cost point-of-care devices (e.g., useful for medical diagnosis) [125]. By other hand, optical fibers, due to their intrinsic characteristics, are attracting the researchers' attention to overcome the drawbacks presented by prism-based SPR sensors. As was well established in the previous subsections, the light propagates across the fiber core through the total internal reflection, but in standard fibers the evanescent field is almost zero in the cladding. Therefore, the SPR cannot be excited. The most common configuration is based on unclad fibers, where a segment of the fiber core is exposed by removing the fiber cladding and coating the fiber core with a thin metallic film (e.g., Au or Ag). When the light propagating in the core finds the unclad region and interacts with the metal interface in a certain angle of incidence θ , part of it is leaked out and forms an evanescent wave, creating the SPR phenomenon (Figure 4.7). The coupling condition of the guided mode and the SP is accomplished when both propagation constants are equal (equation 4.10):

$$\beta_{mode} = Re\{\beta_{SP}\} \quad (4.10),$$

where β_{mode} is the propagation constant of the waveguide mode. The SPR event induces a transmission dip or absorption band in the transmitted spectra depending on the characteristics of the measurand. As each measurand has a distinct RI or as each binding event (in case of biological entities measurements) changes the RI at the fiber surface, SPR-based configurations can acts as a (bio)sensor by monitoring the resonance wavelength position of the absorption spectra (shifting to higher wavelengths as the RI increases) [127]. The characteristics of the optical response, such as the resonance wavelength position, the FWHM of the resonance band as well as the sensitivity of the sensor, strongly depends on the coating material as well as its thickness (generally ranging from 30-50 nm).

Although unclad MMFs, with a core diameter ranging from 200 to 600 μm , are the most common fibers used to develop SPR-based optical fiber sensors, other fiber geometries such as tapered fibers, short- and long period fiber gratings, and D-shaped (etched or polished) fibers can be used to perform this type of sensors [128].

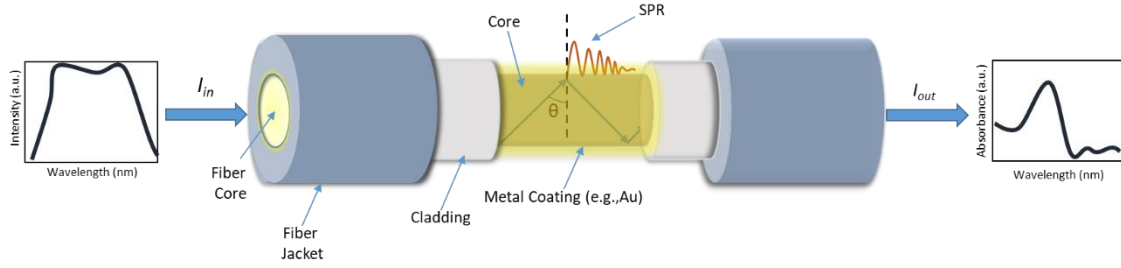


Figure 4. 7. Illustration of a SPR-based configuration performed in an unclad MMF, where are visible the propagating plasmonic wave and the absorption spectrum displaying the resonance wavelength band.

Furthermore, thin metallic films can be replaced by metallic nanoparticles to excite SPR. In those situations, the phenomenon is so-called localized surface plasmon resonance (LSPR). That localized resonances are characteristic of collective oscillations by electrons in small metallic nanoparticles. Depending on their size and shape the nanoparticles can behave as a dipole or even a multipole. When the incident light is absorbed by the particles, plasmonic signatures are created, in which the wavelength corresponding to the maximum extinction of the incident light on each nanoparticle strongly depends on the external RI [111]. The dependence of the LSPR peak with the surrounding medium's dielectric function can be expressed by the Drude model [129]:

$$\varepsilon_R = 1 - \frac{\omega_p^2}{\omega^2 + \gamma^2} \quad (4. 11),$$

where ε_R is the real part of the dielectric function of the metal, ω_p is the plasma frequency, ω is the angular frequency, and γ is damping factor. As $\gamma \ll \omega_p$ in the visible and infra-red regions, the Equation 4.11 can be exemplified to [129]:

$$\varepsilon_R = 1 - \frac{\omega_p^2}{\omega^2} \quad (4. 12).$$

Figure 4.8 exemplifies the light-nanoparticle interaction (in this example are illustrated gold nanospheres), and the resonance band resulted from light absorption.

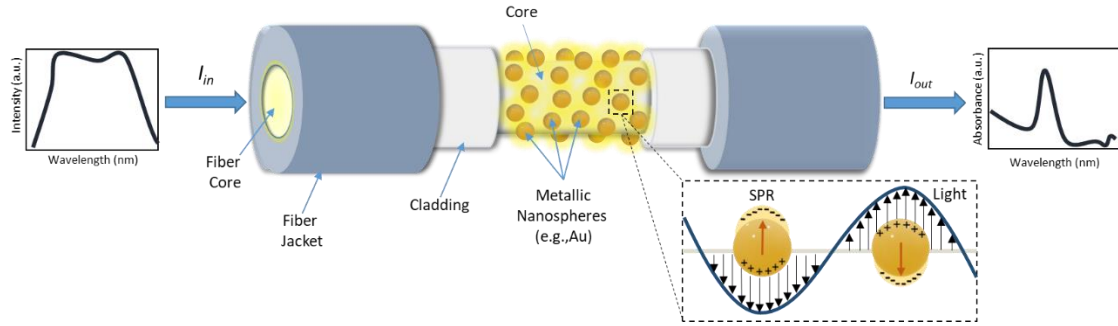


Figure 4. 8. Illustration of a LSPR-based configuration performed in an unclad MMF, where are visible the nanoparticles oscillation and the absorption spectrum displaying the resonance wavelength band.

The shape of the SPR and LSPR absorption (or transmitted) spectra are similar. However, for the same metal, LSPR spectra present a well-defined band with a FWHM smaller than the presented spectrum by SPR configurations. Thus, the resonance wavelength band position can be followed with higher accuracy. Moreover, the Figure of Merit (**FOM**) is an important parameter to evaluate a sensor performance that relates the sensitivity (**S**) of the sensing device with the **FWHM** of its spectral bands (Equation 4.13). Thus, if the sensitivity of two configurations, SPR- and LSPR-based, is similar, LSPR designs will present always a better FOM.

$$FOM = \frac{S}{FWHM} \quad (4.13).$$

As was reported for SPR-based configurations, uncladded MMFs are usually the choice to perform LSPR-based devices. Nevertheless, the other fiber geometries can also be used.

Another emerging phenomenon is the lossy mode resonance (LMR). While resonances based on SP are obtained when the real part of the metallic thin film permittivity is negative and higher in magnitude than both its own imaginary part and the material surrounding the thin film, LMRs are attained when the real part of the thin film permittivity is positive and higher in magnitude than both its own imaginary part and the material neighboring the thin film [130]. LMR phenomenon is caused by the coupling of optical waveguide mode and a particular lossy mode of the film at a finite thickness, inducing an increase in the imaginary part of the effective RI of the rest of modes propagating in the core. The lossy mode excitation results in an attenuation at a specific wavelength range, in which the guiding conditions is dependent on the RI neighboring the fiber optic sensing region [131].

4.2.2.2. Surface Enhanced Raman Spectroscopy

Different molecular structures can offer a unique signature by scattering light through their natural molecular vibrations. Then, the scattered light can be collected and transformed in optical information: the Raman spectrum [132]. Although Raman measurements are simple to obtain, the technique deals with low intensity of the provided Raman signals and has not gained significant attraction as a tool used for developing sensors. However, in the last decades, Raman spectroscopy was improved, by exploring plasmonic phenomena induced on the surface of a substrate, thus enhancing the Raman signal [132]. Signal enhancement arises when the incident light excites SP, creating an electromagnetic field extension (up to 20 nm) from the metal surface, thus improving the Raman signals of the exposed molecules by a factor of 10^6 [123]. SERS-based optical fiber sensors have caused great interest due to the possibility to extend SERS-sensors into practical applications. The different geometries that can be performed using optical fibers are of particularly attractive for minimally intrusive monitoring, such as biosensing applications [133].

4.2.3. Configurations based on Phase Modulation: Interferometric Sensors

The interest in interferometric measurements arises predominantly from the characteristic resolution offered by interference of light. Fiber optic interferometers can be classified as Fabry-Pérot, Mach-Zehnder, Michelson, and Sagnac interferometers [12]. As general definition, optical interferometers are devices in which the propagating light is divided in two or more distinct optical paths, resulting in a phase change.

Fabry-Pérot interferometers (FPI) consist in two parallel mirrors spaced by a certain length. The multiple reflections of a light beam between the mirrors cause an interference, which depends on the distance and the medium between them. The transmitted or reflected spectrum is modulated by the optical phase difference between the two transmitted or reflected beams [134]. In that perspective, a cavity can be fabricated by machining it perpendicularly to the fiber's axis. Another approach is splicing two independent fibers on both ends of a hollow core optical fiber, producing in both ways an optical fiber interferometer.

Other interferometric configurations are centered on light splitting between two arms, with further recombination producing an interference arrangement according to the optical path length difference between them: Mach-Zehnder and Michelson interferometers (MZI and MI, respectively). In a single fiber, MZI consists in the coupling

of the propagating light from the core to the cladding using a fiber coupler, being that light posteriorly re-coupled to the core by another coupler. Hence, LPFGs can be employed as a coupler to developed MZI configurations. In sensing applications, the coupled light from the core to the cladding can be used as a reference, and kept free of perturbations, while the re-coupled light from the cladding to the core can be exposed to the external medium and used as a sensor [135]. A similar process occurs in MI-based structures, where the light is split between two arms, being then reflected at the fiber ends, by reflecting on a mirror, forcing the light to travel back to the same split device. In a single fiber, a LPFG can acts as a coupler to split the light propagating in the core to the cladding and in the opposite way when the light is reflected [136]. Figure 4.9 shows some interferometric layouts based on: a) FPI; b) MZI; and c) MI.

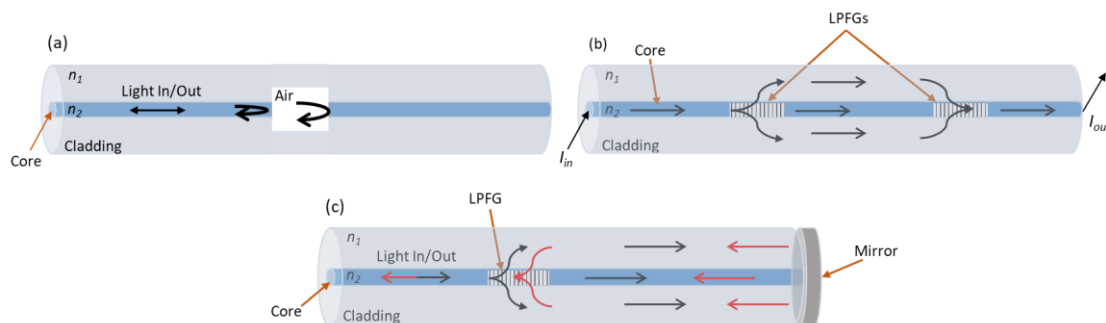


Figure 4. 9. Illustration of different interferometric layouts: a) Fabry-Pérot; b) Mach-Zehnder; and c) Michelson.

4.3 Coating Techniques and Materials

Several coating techniques have been implemented to modify the surface of optical fibers through the deposition of metallic, dielectric, or polymeric thin films. The coating processes can be performed by different techniques ranging from physical to chemical methods, such as physical vapor deposition (PVD), chemical vapor deposition (CVD), sol-gel, layer-by-layer (LbL), or self-assembled monolayers (SAM).

4.3.1. Coating Techniques

4.3.1.1. Physical Vapor Deposition

Physical vapor deposition (PVD) approach comprises evaporation, transportation, and precipitation methods. As main advantages, PVD procedures offers the possibility to obtain high levels of homogeneity; achieve thicknesses ranging from less than 1 nm up

to hundreds of microns; are very reproducible processes; allows the deposition of a wide selection of coating materials on various types of substrates; offers flexibility to the temperatures requirements of the substrate; permits multilayer deposition, allowing change the thickness and the coating material between layers; offers high levels of purity of the coating material; and low levels of environmental pollution [137]. The PVD techniques are based on physical processes to accomplish thin films deposition. The procedure is realized in a vacuum using the evaporation of a solid or molten source, or by the energetic gaseous ions in a gas plasma which knock down or sputter the atoms from the target. The sputtered atoms or molecules proceed them travel through the vacuum or a very low-pressure gas phase, colliding on the substrates and condense on the surface to form a film. In sputtering, it is not necessary to heat the source to high temperatures and its vapor pressure is not so problematic as it would be in vacuum-evaporation [138].

One of the most common method of PVD is *thermal evaporation* [139]. This method relies on the boiling or sublimation of a heated material onto a substrate in vacuum. Commonly, the electron-beam (e-beam) evaporation is one of the techniques used to evaporate the materials that will build the thin film. The beam is induced by an electron current supplied by a thermionic filament. Then, the electrons, with a certain charge, are accelerated by a strong electric field to reach the surface of the material to be deposited, increasing the temperature of the material, and causing its evaporation. E-beam systems allows high deposition rates, that depends on the source-to-substrate distance. Due to the high temperatures of the e-beam heaters, a large range of materials can be evaporated, therefore can be deposited, such as Au, Ag, Al, Cu, Si, Cr, Ti, Mo, W, Pd, Pt, etc, and several dielectric materials such as SiO₂, TiO₂, etc. Although its numerous advantages, the X-rays generated by the e-beam technique leads to the possibility of penetration in the surface layers, creating oxide-trapped charges [139].

Another example of physical vapor deposition is the *sputtering method*. Sputtering PVD offers better step coverage and adhesion to the substrate. In systems like these, an inert gas is released into the reactor at low pressure, and a plasma is created due to a voltage applied across two electrodes. In one of the electrodes (the cathode), that holds the material to be deposited, is applied a negative DC voltage. In the other side is placed the second electrode (the anode), that holds the substrate for the film deposition. The positive ions from the plasma (usually Argon, $\text{Ar} \rightarrow \text{Ar}^+$) are accelerated due to the potential gradient and strike the cathode electrode. The atoms close to the material surface become volatile and are moved as a vapor to the substrate, being then deposited

by condensation. As the target acts as an electrode, the rate of deposition is higher if the source material is a conductive material such as Al, Ti, Au, Ag, silicates or other metallic components but this technique allows the deposition of several dielectric materials [137].

4.3.1.2. Chemical Vapor Deposition

Chemical Vapor Deposition (CVD) is a technique to create thin films involving chemical reactions. The method involves the introduction of the constituents of a vapor phase, occasionally diluted with an inert carrier gas, into a reaction chamber, where, after chemical reactions, are adsorbed on a heated surface. Depending on the local where the chemical reactions take place, the deposition can result in heterogeneous or homogeneous films. Basically, if the chemical reactions occur on or close to the heated substrate, the resulted film will be a heterogeneous film; if the chemical reactions take place in the gas phase, the result will be a homogeneous film. Once the homogeneous films present poor adhesion properties, the heterogeneous reactions are highly desired. The most common CVD procedures includes atmospheric pressure and low-pressure CVD; plasma-enhanced CVD; high density CVD; and pyrolysis. CVD methods can create layers with a high degree of purity and control [137].

4.3.1.3. Sol-gel

The sol-gel method allows the elaboration of solid films, starting from a solution using either sol or gel as intermediary steps. It involves sol-gel chemistry, including the transformation of molecular precursors into oxide networks by hydrolysis and condensation processes. The process occurs at low temperatures and enables final products (e.g., glasses, ceramics, metal oxides, etc.) with high degree of purity and homogeneity [140]. Generally, the procedure starts by mixing the precursor in a water/co-solvent mixture (usually an alcohol) producing a homogeneous sol, followed by an acid or basic catalyzed hydrolysis and polycondensation. The properties of the formed gel depend on the type of precursor, pH, the nature and the concentration of the catalyst, type of solvents, temperature, and the drying process. Characteristics such as porosity, surface area, refractive index, thickness, and mechanical properties are strongly influenced by those factors. To be applied as sensors, chemical or biological elements can be added during the synthesis process. Thin films can be attained by using techniques such as spin coating, dip-coating, or spray-coating. The resulted matrices

can be transparent to wavelengths above 250 nm, being interesting materials to be applied to optic configurations [141].

4.3.1.4. Layer-by-Layer

One of the most used techniques to obtain multi-layered structures is the layer-by-layer (LbL) method. The LbL assembly has suffered considerable upgrades since its first reports, made by *Blodgett* and *Langmuir* (Langmuir-Blodgett method) when they introduced the transfer of insoluble monolayers from an air-liquid interface onto solid supports [142]. Later, emerged the electrostatic self-assembly (ESA) of multilayers. This method consists in the formation of multilayered structures by alternate deposition of oppositely charged materials. In this case it is imperative to think about the role of the electrostatic interactions between the charged materials, *e.g.*, polyelectrolytes, adsorbed in the adjacent layers as driving force of the assembly, considering some important aspects: the ionic strength, pH, or temperature of the solvent for electrolytes; and competence between electrostatic and entropic factor [143]. Briefly, the deposition of a layer with an opposite charge is not stopped when the neutralization of the adjacent charge covering the surface is reached. It continues till a certain degree of opposite charge occurs on that surface. This phenomenon leads to the charge inversion and to the formation of charged tailored layers that are projected to the solution. Thus, an overcompensation process begins, interfering with the adsorption of additional molecules due to repulsive electrostatic interactions. Nowadays, LbL-ESA it is considered an easy-to-do approach offering structures with well-defined thicknesses, enabling the production of materials with specific chemical, biological, optical, and mechanical or electrical properties [144].

Moreover, LbL deposition also includes covalent attached films. In LbL-covalent procedures, an assortment of chemical reactions is used to stabilize the multilayers, such as amide or anhydride formation through heating or catalyzed reactions or Schiff base reactions [145]. Usually, two different methods can be used to attain chemical bonds formation: post covalent reaction or consecutive covalent reaction. The first is based on a primary electrostatic immobilization followed by the application of covalent reaction conditions, by adding, *e.g.*, bifunctional reagents, increase the temperature, or using light irradiation (*e.g.*, UV) [145]. As consequence, the non-covalent immobilized films are converted into covalently immobilized ones. The second method relies on the development of multilayers using covalent reactions in each formation step [145].

4.3.1.5. Self-assembled Monolayers

Self-assembled monolayers (SAMs) are chemical structures with a thickness of a single or a few molecules. The self-formation is a thermodynamical process where the molecules react with each other, resulting in well-formed and organized structures. Under certain conditions, the molecular structures are vertically aligned on the substrate surface. Normally, the molecules used to accomplish SAMs have specific characteristics: the anchoring functional group, the spacer chain, and the terminal groups [146].

The anchoring group plays a fundamental role on SAMs formation. It is responsible for the interaction between the molecule and the substrate surface and needs to be carefully selected according to the type of surface. For instance, thiol (-SH) groups are widely used to build SAMs on metallic surfaces (e.g., Au), while organosilane compounds (Si-OR) are the choice to cover oxide substrates, such as silicon oxides [147]. The spacer chain also plays an important role in the quality of the SAM. It is the skeleton of the molecule and the bridge between the anchor group and the terminal functional group, being responsible for electronically isolating them. The orientation and organization of the formed SAM also depend on the lateral interaction between molecules. Finally, the terminal group is responsible for the interfacial chemistry with the air, overlayers, or target analytes. SAMs can easily work as a bridge to build multilayers or acts as a biosensing linkers to hold biological receptors useful in biosensing [148].

Among other applications, coatings are generally applied to components to extend their performances (e.g., optical fibers) and to avoid oxidation (e.g., metallic multilayers), exhibiting interesting characteristics to be used in the development of optical sensors.

4.3.2. Coating Materials

4.3.2.1. Metal and Oxides Materials

Metals and metal oxides can be categorized according to their intrinsic properties. Specific characteristics such as thermal expansion coefficients, dielectric constants, magnetic properties, and the capability to resist oxidation processes, need to be considered according to the required application. For instance, to generate surface plasmons in the visible spectra region, metals such as silver (Ag) or gold (Au) are the most attractive materials [149]. For physical parameters such as strain or temperature sensing, metals like aluminum (Al), tin (Sn), copper (Cu), lead (Pb), and zinc (Zn) can be employed due to their high thermal coefficient [96].

Metal oxides have been demonstrated its potential in several applications. These types of coatings have been successfully employed in biomedical fields, in food industry as antimicrobial surfaces for food safety, laser optics as micro mirrors, solar cells, etc [150-153]. For instance, for applications that requires electrical insulation the commonly used oxides are silicon dioxide (SiO_2) and titanium dioxide (TiO_2) due to their electrical insulating properties. For semiconducting materials, zinc oxide (ZnO), copper oxide (CuO), tin oxide (SnO), and indium tin oxide ($\text{In}_{18}\text{O}_{28}\text{Sn}$; commonly referred as ITO). The latter combines properties as transparency to visible light and electrical conducting [154].

4.3.2.2. Polymeric Materials

Currently, polymer engineering has been demonstrating a great flexibility to project innovative sensitive coatings that have been extensively applied in the chemical, biomedical, and biological field. Using a wide range of materials, shapes and sizes, polymeric matrices can hold a wide variety of transducers according to their final application [155].

For instance, the entrapment of luminescent materials for gas sensing is commonly made through LbL techniques. Polyelectrolytes such as polyethyleneimine (PEI), poly(diallyldimethylammoniumchloride) (PDMA) or poly(allylamine hydrochloride) (PAH) have been employed in optical fibers due to their ionic properties [155]. Other materials like tetraethoxysilane (TEOS) or methylethoxysilane (MTES) can also be used to synthesize xerogel layers entrapping metallic complexes for gas sensing [156].

Polymers can also act as sensitive elements by varying their RI that can be tracked using evanescent field configurations or by creating optical interferences by changing their morphology with the swelling phenomenon. Hydrogel-based polymers, poly(vinyl alcohol) (PVA), and poly(ethylene glycol) (PEG), among others, have been used to perform relative humidity sensors [157].

Another class of polymers are the molecularly imprinted polymers (MIPs). Briefly, MIPs are synthetic receptors produced by molecular imprinting [158]. Their combination with different optical fibers configurations (e.g., fiber gratings, thinned fibers, or etched fibers), highlighting phenomena such as evanescent field or SPR/LSPR, results in powerful devices that have been extensively used for chemical or biological sensing [159]. Further considerations about MIPs production techniques and their immobilization on optical fibers will be given in subsection 4.4.3.

4.3.3. Nanoparticles

Thin films are not the only way to coat a surface of a substrate. Nanoparticles (NPs) are also interesting materials to attain structures with thicknesses ranging from a few to small hundreds of nanometers. NPs can be synthesized by various techniques and can be made of different materials such as metals, ceramics, or polymers, assuming a considerable variety of shapes.

The fabrication of metallic NPs can be divided in two methods: (i) top-down methods and (ii) bottom-up methods [160].

- (i) top-down methods consist in a primary synthesis that results in a bulk material. Then, the bulk material is reduced to NPs by physical, chemical, or mechanical processes. Mechanical milling (*e.g.*, ball milling), laser ablation, and ion sputtering are techniques used to produce NPs via top-down methods.
- (ii) in bottom-up methods the NPs are formed from smaller molecules, creating nanostructured building blocks that are assembled to produce the final NP. The NPs can be produced by solid state methods such as the vapor deposition methods (PVD and CVD); liquid state methods (sol-gel); hydrothermal; solvothermal; or gas-phase methods (spray pyrolysis, laser pyrolysis, and flame pyrolysis).

Besides, environmentally friendly methods have emerged to reduce threat to health and to environment. Biological methods, also known as green synthesis methods, are very popular approaches to synthesize NPs in a “clean” way. They are considered methodologies with a clean chemistry and overcomes problems such as reaction complications, high cost and safety issues presented by the conventional methods [161]. The procedure involves the application of microorganisms such as bacteria, fungi, and plant products. The NPs formation is based on processes such as bioreduction and biosorption [162]. In the first case, the metal ions are chemically reduced into the biologically stable form by the microorganisms and their enzymes [163]. In the biosorption process, the metal cations (in aqueous media) are allowed to bind with the organism cell wall leading to the formation of stable NPs due to cell wall or peptide interaction [164].

Another very used type of nano-scaled materials are the silica-based NPs. *Stöber* method is a widely used technique to produce silica NPs and hollow silica NPs. The method consists in a homogeneous solution of a silica precursor (e.g., TEOS) dissolved in a water/alcohol mixture with a certain ratio, where is added a basic catalyzer (generally ammonia). Temperature, time, stirring, and nature of the catalyst, are important conditions to obtain the required spherical NPs with a certain size. Hollow core NPs can be obtained by this method by adding, for instance, a surfactant in the reaction composition [165].

An emerging class of nanomaterials are the polymer-based NPs. Polymer-based nanoparticles have revealed their potential to develop optical sensors, increasing their application in many fields. Two main strategies must be considered: dispersion of performed polymers and polymerization monomers. The dispersion strategy relies on solvent evaporation, salting-out, nanoprecipitation, dialysis, and supercritical fluidic methods. Direct polymerization of monomers can be attained by methods like micro-emulsion, mini-emulsion, surfactant free emulsion, and interfacial polymerization [166]. Some of these polymerization methods will be described in the next subsection, related to MIPs production.

4.4. Biorecognition Elements: Production Methods and Sensing Characteristics

4.4.1. Antibodies

Antibodies are, perhaps, the most used elements to develop biosensing configurations. They are produced by vertebrate animals as response of their immune system when exposed to pathogenic organisms or toxic substances. Briefly, when the immune system detects the presence of a foreign organism, B-cells react by producing Y-shaped immunoglobulins (Ig): the antibodies. The Y-shape is composed by two arms, each one contains a pair of heavy chains (55 KDa) and light chain (25 KDa) connected by interchain disulfide bonds. The antibody structure (Figure 4.10) is divided in two distinct regions: the variable region (located on the top) and the constant region (located at the bottom) [167].

The variable region is the place where the binding events, between the antibody and the antigen, can occur. This takes place at the antibody's idiotype region, also known as Fab region or fragment. The amino acids of that region vary from one antigen-antibody to another, while the amino acids of the constant region are quite similar (Fc fragment). The

variable region comprises a variable heavy chain (V_h) and a variable light chain (V_l), thus completing the idiotype region. Each variable chain is composed by six complementary determining regions (CDRs), which the amino acids can complement and relate with the amino acids present on the antigen. For instance, the positively charged amino acids of an antibody can interact with the opposite charged amino acids of an antigen. The V_h CDR region is responsible for the antigen-binding specificity, while the V_l CDR region is contributing to antibody specificity or antigen binding strength. The amino acids that are not involved in the recognition and binding processes, provide a framework (FW) to support the others. The binding event resulted from the interaction of an antigen and its specific antibody results in an immune complex [167]. The binding site of the antigen is known as epitope [167]. The production of antibodies results in two types of receptors: polyclonal and monoclonal antibodies. Polyclonal antibodies are generated by performing a mixture of Ig molecules with multiple specificities, directed to the epitope of interest. The procedure consists of injecting of a peptide or a full-length recombinant protein into an animal (usually a goat or rabbit). The animal's immune system responds to the foreign peptide or protein by creating antibodies with the ability to bound the immunogens. The range of Ig species contributes to the multiplexing flexibility of antibodies. The resulted polyclonal receptors are then isolated from the animal to produce a polyclonal antiserum [168]. However, this method of antibodies production, usually, results in heterogeneous antibodies with different specificity and affinity. In this context, monoclonal technology emerged to overcome those issues. For biosensing applications, monoclonal antibodies are preferentially selected instead the polyclonal due to their high affinity and specificity to a particular target. In this case, the B cells producing antibodies are isolated from the spleen of the injected animal after a few days. B cells are fused with myeloma cells to produce a certain number of hybridomas. These cells can proliferate in culture indefinitely and produce large amounts of antibodies with high reproducibility [169]. Over the years, several techniques have been investigated for more rapid, cost-effective, and simplify the production of monoclonal antibodies. For instance, recombinant techniques have made possible humanized antibodies, combining the DNA expressing the binding site of a mouse monoclonal antibody with the DNA of a human antibody. Another technique is based on bacterial expression of antibodies, by selecting the desirable antibody by phage display. The antibody fragments are generated from the plasma cells of human donors or from the spleen of an animal [170]. Although the effort and the progress in the research of new methods to develop antibodies, the procedures are still time consuming and requires biological fluids (from human donors or animals) to produce them.

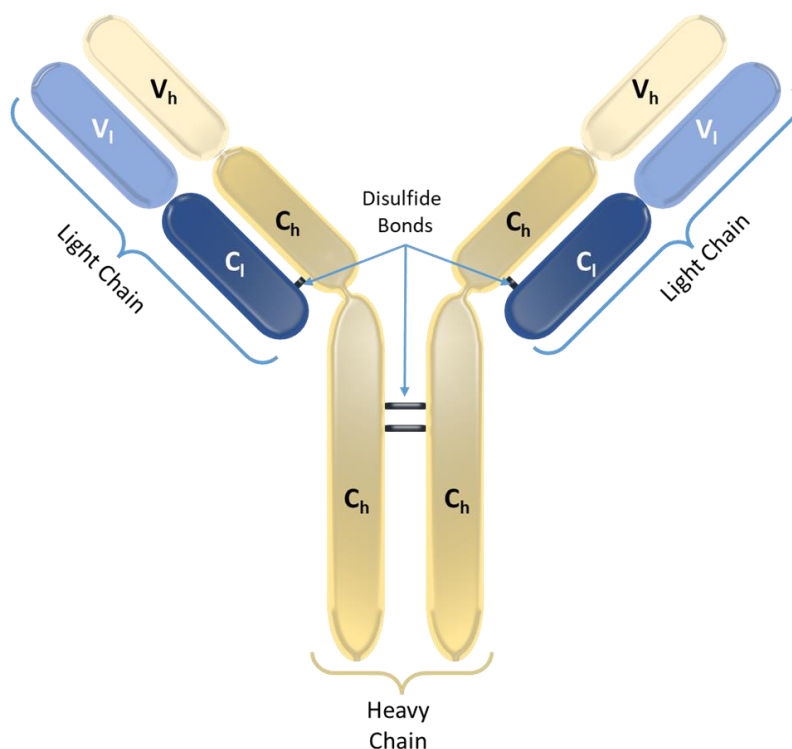


Figure 4. 10. Schematic representation of a Y-shaped antibody (based on Ig class), displaying the position of both heavy and light chains, and exposing the variable heavy and light (V_h and V_l , respectively), and the constant heavy and light (C_h and C_l , respectively) regions.

4.4.2. Aptamers

Briefly, aptamers are small sequences of single-stranded DNA or RNA (ssDNA or ssRNA) specially designed to bind a specific molecule, including proteins, peptides, amino acids, antibiotics, whole cells, viruses, bacteria, etc. The molecular engineering applied in the aptamers production is relatively simple due to the easy manipulation of the chemical synthesis, comprising high accuracy and reproducibility, and offering high purity degrees. One of the main advantages is the possibility to introduce desired functional groups in the terminals of the ssDNA or ssRNA sequences, thus enabling an easy process of subsequence derivatization with other molecules or substrate without compromising function. These characteristics allied to their high specificity and affinity to a wide range of targets, make aptamers a strong and reliable alternative for biological recognition in several analytical applications [171].

The aptamers production is mostly based on a combinatory biology technique known as SELEX (Systematic Evolution of Ligands by Exponential enrichment). The process relies on the capacity of the oligonucleotides to form 3D structures which can interact with a specific target through van der Waals surface contacts, hydrogen bonds and base

packing interactions. Typically, in the SELEX technique, a large random DNA or RNA library is exposed to an iterative process of *in vitro* selection and amplification in the presence of the target molecule (Figure 4.11). The separation strategy of the binding aptamers is a critical step and should be carefully selected according to the properties of the target molecule. For instance, small molecules, are frequently immobilized into a column and the unbound DNA is washed away. In case of protein targets, the process involves a filter membrane to retain the DNA protein complex, while the non-bound DNA pass throughout the filter. Larger molecules, the separation process relies on centrifugation steps. After separation, the isolated bound DNA is amplified by PCR to the next round of selection. The difficulty observed on the separation process, demands new strategies for aptamers separation without compromising their function. Therefore, several modifications have been made to offer more reproducibility and reliability in the separation process by using different methods such as electrophoresis, microfluidic devices, AFM tips, and magnetic beads [172]. However, there are still a large number of concerns directed at the SELEX methodology. In that context, new strategies such as cell-SELEX and tissue-SELEX have been developed, providing new promising analytical reagents [173-175].

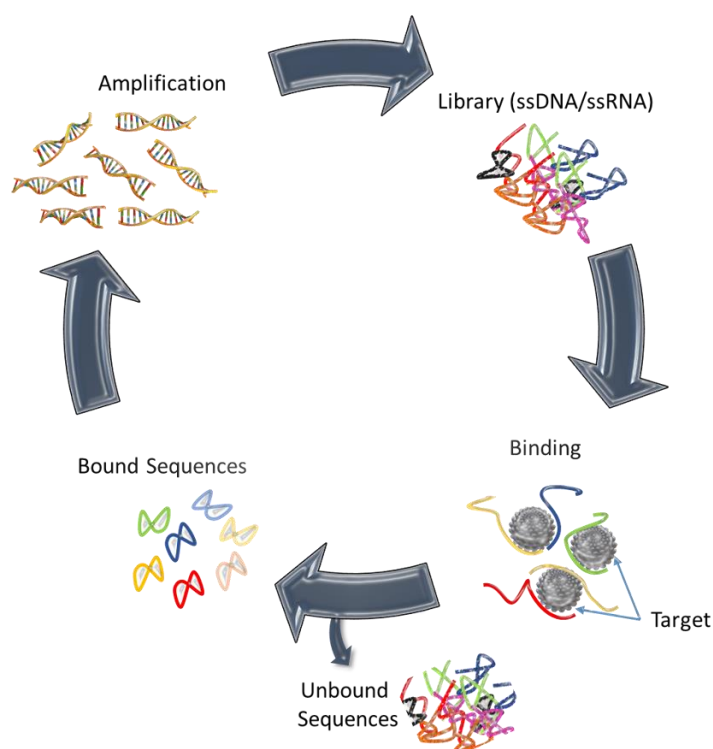


Figure 4. 11. Isolation of aptamers through SELEX method. In this example the target is immobilized on a bead surface. Adapted from reference [176]. ©Royal Society of Chemistry Copyrights.

4.4.3. Molecular Imprinting

The history of imprinting polymers started in 1972, when *G. Wulff* referred to an enzyme-analogue built polymer but, was in 1985 that *Wulff* published a paper [177] where he reports the production of chiral cavities in a polymer layer coated on wide-pore silica [178]. However, the first reference to a “molecularly imprinted polymer” appears only in 1992 reported by *S. Piletsky*, although some papers were published in the Russian literature describing the production of MIPs by *Piletsky* near 1989 [178]. Despite some doubts in the beginning of the molecular imprinting history, nowadays this technique is extensively used to mimic natural antibodies, starting from synthetic monomers [179]. MIPs are polymer made materials, allowing to obtain different formats of synthetic particles, ranging from bulk monoliths to surfaces, micro- and nanostructures. The MIPs produced on a nanoscale are particularly interesting, displaying a closer similarity to the natural antibodies: reduced number of binding sites per particle [180], fast kinetics of interaction and high affinities and selectivity [181, 182]. The process includes covalent and non-covalent interactions between a monomer and the target molecule, subsequently stabilized by a cross-linker agent [182, 183]. This technology can represent an ideal “plastic antibody” to be integrated in sensors, offering the possibility to detect changes in the recognition elements, with high sensitivity and selectivity to monitor (bio)targets in different applications [178, 184]. A brief description of some techniques used for molecular imprinting is presented in the following subsections.

4.4.3.1. Bulk Polymerization

Bulk polymerization is the easiest and most common technique to synthesize MIPs. In this method, the molecularly imprinted material is polymerized around the template molecule which is posteriorly removed (Figure 4.12). Additionally, the obtained bulk polymers are crushed mechanically to a suitable size, resulting in small particles within specific interaction sites [185, 186]. MIPs produced by bulk imprinting are adequate for molecular sensing with high selectivity and the method allows easy template removal and full reversibility which means the use of bulk-imprinted units for several rounds [187]. However, bulk polymerization has also some disadvantages such as the dispended time for crush and grind; irregular shape, heterogeneous particle size, and destruction of some binding sites resulting from grinding [186]. Besides, it is complicated remove the target molecule from the MIP due to troubled binding and in extreme situations, no binding occurs [185].

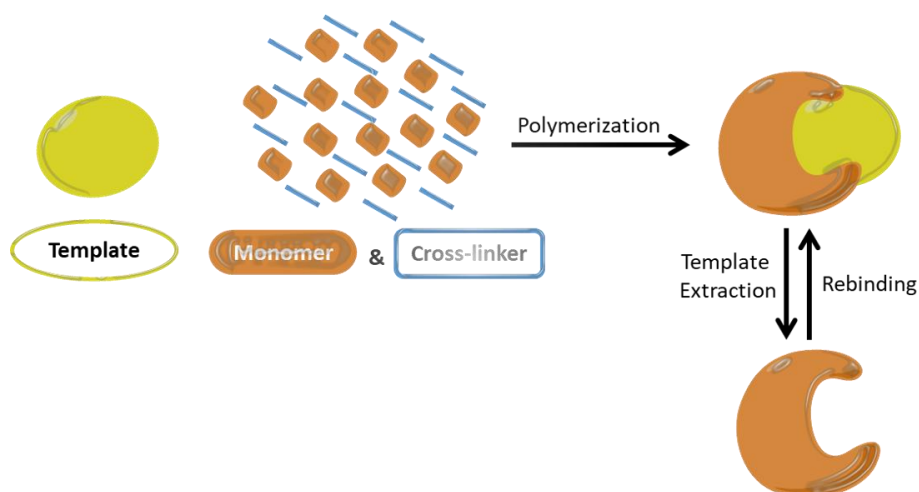


Figure 4. 12. General scheme of molecular imprinting process.

4.4.3.2. Precipitation Polymerization

Precipitation polymerization was introduced for the first time by Ye *et al.* (1998), synthesizing spherical nanoparticles targeting the pharmaceutical drug theophylline and the hormone 17β -estradiol [188, 189]. The general procedure (Figure 4.13) involves the stirring of the functional monomer, the cross-linker, the initiator, and a high amount of the porogen until a homogeneous solution is obtained. The polymerization process results in the production of particles ranging between 100 nm and 10 μ m. This technique generates uncontrolled MIP sizes and provides irregular shapes [190].

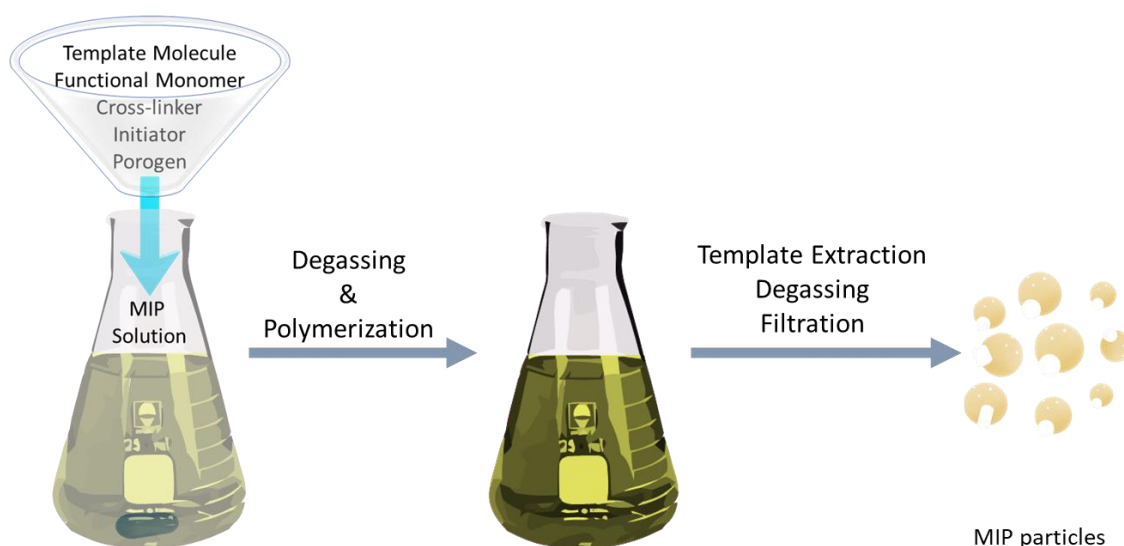


Figure 4. 13. General procedure to produce MIPs by precipitation polymerization process.

4.4.3.3. Dispersed-Phase & Mini-Emulsion Polymerization

The general processes of these type of surface imprinting comprises an emulsion step (two immiscible liquids) followed by a suspension step (liquid and solid phases) induced by polymerization (Figure 4.14). The polymerization solution containing the template molecule, the functional monomer, the cross-linker, and the initiator embodies one of the liquid phases and the dispersing solution containing the stabilizers represents the other liquid phase solution. In the dispersed-phase polymerization, the beads of one phase are formed due to rapid stirring in the second phase. This step is very important to control the size of the resulted particles. The polymeric reaction is started by UV radiation or by heating the solution. In the last step, the particles are filtered and washed. The main advantages of this technique is the high production of spherical particles and the simplicity of the fabrication process [191]. The first report on dispersed-phase polymerization was made by *Mayes et al.* in 1996 achieving microparticles sized between 5-50 μm [192]. The problem is that during the droplets formation, small beads can merging together creating bigger particles and, in that case, mini-emulsion polymerization allows a major control on the particles formation [193]. The fabrication process is very similar, but the droplets are formed via ultrasonication into a micron monomer droplet. Another important difference is the presence of a surfactant system that prevents the formation of bigger particles resulting from small particles agglomeration. The mini-emulsion polymerization allows the production of smallest particles, ranging from 100 to 500 nm [188].

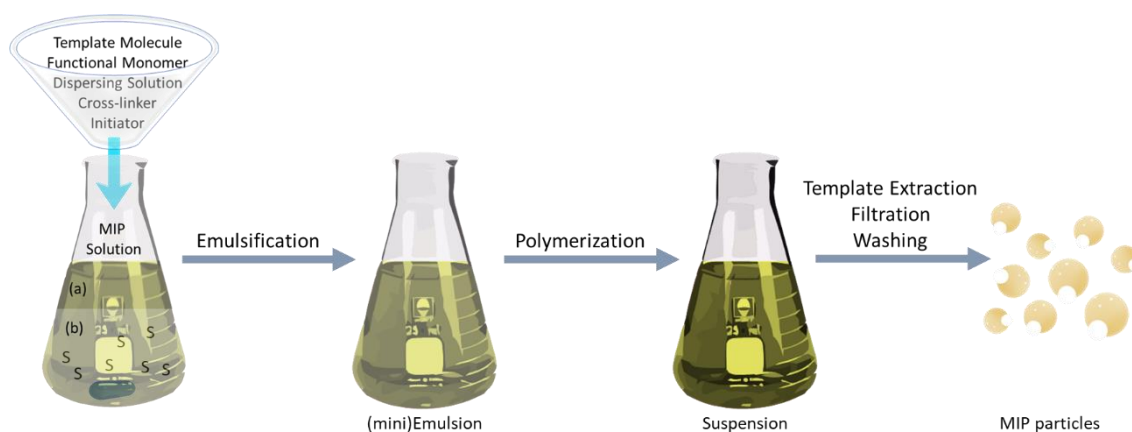


Figure 4. 14. Schematic of the general fabrication process of: (a) disperse-phase polymerization and (b) mini-emulsion polymerization with the presence of surfactant (S).

4.4.3.4. Surface Imprinting via Grafting Polymerization

The grafting of polymer layers on planar films or spherical micro- and nanoparticles is a common technique to produce MIPs [188]. *Norrlow et al.* reported for the first time the

use of this method to create a MIP film on silica beads in 1984, describing the development of a thin shell of acrylic polymer onto a 10 μm porous silica particle carrying acrylate groups [194]. In general, the procedure includes the covalent attachment or a non-covalent adsorption of the template molecule on the already grafted particle surface. In the first step, the particle surface is functionalized with specific functional groups (according with the template properties). The template molecule is immobilized onto the functionalized surface, starting then the polymerization process. After the synthesis, the template is removed and are obtained high selective imprinted core-shell beads [185, 188]. Figure 4.15 show the general process for the introduction of imprinting molecular recognition sites on spherical nanoparticles. The grafting process comprises several advantages such as improved affinity interactions due to the fast mass transfer induced by a high analyte mobility, better control of shape and morphology [185, 195]. In the literature, there are some reports combining this MIP imprinting process with optical transduction [196, 197].

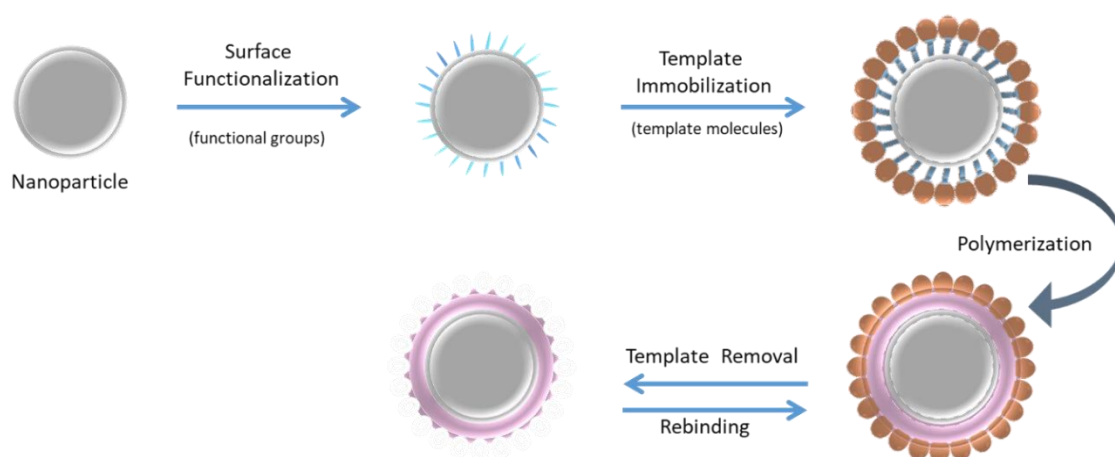


Figure 4. 15. General procedure of surface imprinting via grafting polymerization on spherical nanoparticles surface.

4.4.3.5. Molecular imprinting via solid-phase synthesis

Traditionally, MIPs are synthesized as a monolith, resulting in a polymer that requires to be crushed in small fragments, or as small particles, by using surfactants to avoid agglomeration, to obtain individual micro- or nanostructures. However, these techniques result in irregularly shaped particles and/or often produce MIPs with heterogeneous binding sites [198, 199]. In this context, MIPs produced via solid-phase synthesis (SPS) is a promising solution to overcome these issues. Nowadays, SPS is considered by several authors the most advanced technology to produce artificial receptors for various

diagnostic and life science applications [200-203]. The process comprises the covalent immobilization of a template onto a suitable solid support (generally glass beads) using specific functional groups (e.g., $-\text{COOH}$; $-\text{NH}_2$; $-\text{SH}$), previously fixed on the solid surface, considering the chemical interactions provided by the template. The modified particles are then placed in contact with the monomeric solution (in aqueous conditions or organic solvents) and the polymerization process is initiated under specific conditions that satisfies the formation of polymer nanoparticles around the template molecule [204]. The solid support is important during the polymerization process as well as in the purification step. It acts as an affinity medium, isolating the high-affinity nanoMIPs from the unreacted monomers and low-affinity nanoMIPs. In general, the nanoMIPs obtained from this technique are uniform, owning homogeneous and specific binding sites, analogous to monoclonal antibodies. Figure 4.16 shows, schematically, the process of production of nanoMIPs *via* solid-phase synthesis.

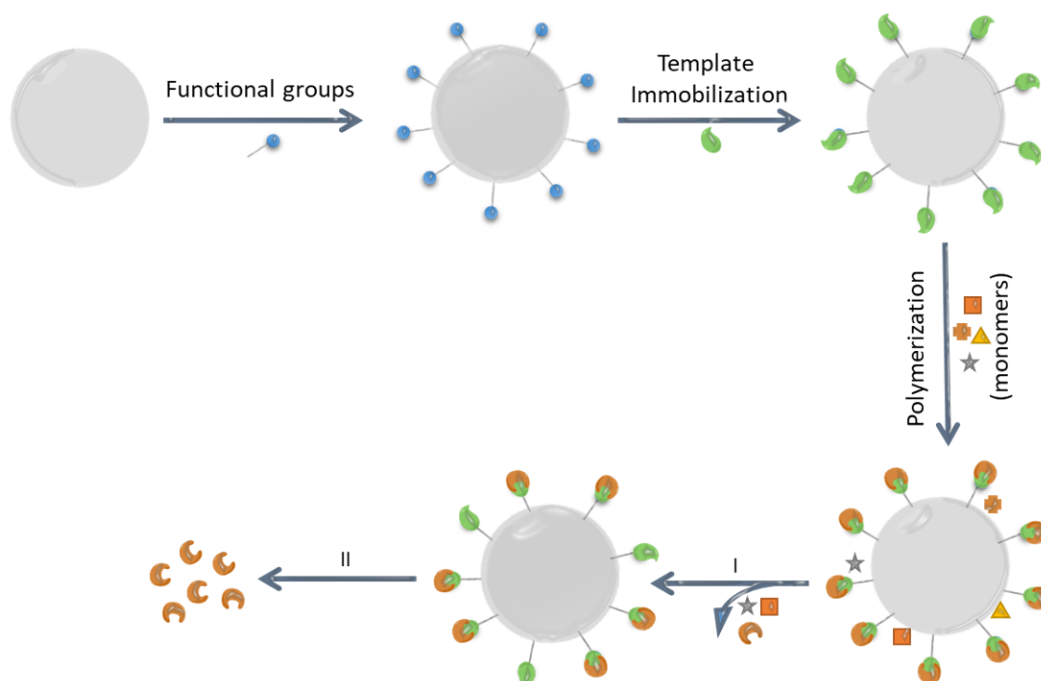


Figure 4. 16. Schematic representation of the production of nanoMIPs *via* solid-phase synthesis: I. first purification step (removal of unreacted monomers and low-affinity nanoMIPs); II. second purification step (collection of high-affinity nanoMIPs).

4.4.3.6. Epitope Imprinting

The production of a recognition shape for the whole material might not be ideal for several applications such as medical purposes, where a high level of discrimination is required [205]. Among other motivations, different approaches of imprinting techniques

are needed to overcome these issues. In this segment, the epitope imprinting method allows to produce specific recognition sites for particular components of the target analyte (Figure 4.17) such as proteins [206], lipids [207], saccharides [208], and their derivatives [205]. This technique minimizes the non-specific binding sites and improves the affinity of the resulted MIP. The interactions between the epitope and the functional monomer, involved in the MIP production, can be divided in two types: covalent interactions and non-covalent interactions [209]. The covalent interaction provides high selectivity in the recognition process, but present slow binding and slow dissociation resulting from the strong covalent bonds, which makes difficult to reach the thermodynamic equilibrium [210]. On the other hand, the non-covalent interactions present not so high selectivity in the recognition process due to the weaker interactions (when compared with the covalent interactions), nevertheless it is a simplest method, offer highest speed binding events and provides the, always convenient, template removal [209]. The epitopes can be produced via bulk polymerization or surface imprinting [211-214].

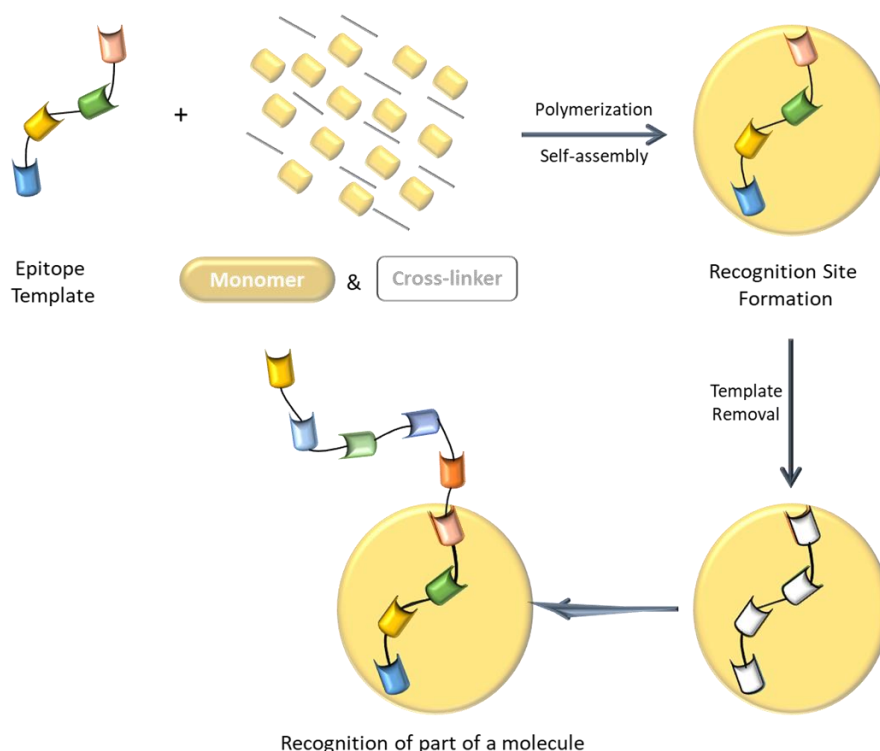


Figure 4. 17. General process of epitope imprinting by using a spherical nanoparticle.

4.4.4. Biosensors

The characteristics of biosensors should be carefully considered for the required application. Parameters such as selectivity, sensitivity, reproducibility, and reusability should be evaluated to attain a full sensor characterization.

Selectivity is the most important parameter that distinguishes a biosensor, mainly in clinical applications. The positive result should be detected just in the presence of the target analyte. A false positive result can be defined as a positive indication generated from a negative sample, *i.e.*, a sample that does not contain the analyte of interest. Consequently, low-selective biosensors tend to offer a high level of false positive rates, due to the complexity of the analytical samples. Antibody-based biosensors offer high selectivity in the biorecognition of elements due to their biological role in the immunological or other physiological functions [215]. Aptamers' selectivity is hindered by the inherent negative charge of the oligonucleotides subunits that constitutes the aptamer structure. When exposed to a complex sample, the negative charged subunits cause nonspecific interactions with competing analytes, inducing inaccurate positive results. However, the possibility of post-synthesis chemical modifications can be useful to reduce the nonspecific bindings [216]. Synthetic approaches, such as MIPs, have relevant benefits like low-cost and easy fabrication processes, and long-term stability, when compared with their "rivals". Nevertheless, molecularly imprinted bioreceptors have poor selectivity comparatively with antibodies or aptamers. MIPs' selectivity is hindered by nonspecific binding of the similar structures and/or functional groups due to the heterogeneity of the binding cavities [217].

The sensitivity of a biosensor is related to the changes induced by the target concentration in the transduced signal. The range of measurable concentrations is also linked to the biosensor sensitivity, that is defined by the analyte minimum or maximum concentrations that can be accurately measured. In a typical biosensor, the desired concentration range is established in the picomolar or nanomolar scale. The sensitivity and range parameters are defined by the number of binding sites per surface area, dissociation constant, and steric impediments. In this specific case, the size of the aptamers (1-2 nm) allows a higher surface loading density comparatively to antibodies (10-15 nm), thus providing a larger number of binding sites per surface area [218]. Moreover, steric hindrances from nearby biorecognition elements must be considered to avoid conformational changes, especially for antibodies [219]. In case of MIPs, their fabrication can occur directly on the surface of the transducer, avoiding the necessity of further immobilization by covalent or electrostatic interactions. Thicker surfaces offer an

acceptable surface loading density, *i.e.*, a great number of binding sites. However, the process needs to be carefully controlled in order to prevent an over thickness, especially in fiber optic biosensors. Higher thicknesses can result in a loss of sensitivity and lower concentration ranges [215].

The reproducibility of the method used to produce the biosensor is an important parameter, mainly for industrial manufacturing purposes. Reproducibility can be defined as the ability to manufacture multiple sensors that will produce the same response in the presence of the same analyte. Thus, understanding the characteristics of the biorecognition elements such as, long-term stability, structure, and fabrication processes, it is crucial to attain reproducible and reliable biosensors. Furthermore, the methods used to immobilize the bioreceptors at the transducer surface and the variability of the transducer constructs are other factors that can limit the reproducibility of a biosensor. Considering the bioreceptors, the activity of the antibodies is affected by several parameters that difficult the reproducibility of their production and the reproducibility of the antibody-based sensors as well. They present a problematic environmental instability, being sensitive to pH and temperature variations, that largely contributes to their degradation. Therefore, the storage and transport conditions become challenging processes for industrial production [220]. By other hand, aptamers are a useful tool to develop biosensors with high reproducibility. The chemical synthesis involved in their production are well-defined processes and the post-synthesis modifications are a great allied to induce long-term stability. The post-synthesis process is also a useful process to introduce desired functional groups in the aptamer chain to facilitate the immobilization of this biorecognition element onto the transducer surface. In a similar way, the well-defined chemical procedures to produce synthetic bioreceptors by molecular imprinting, offers biosensors with high reproducibility rates. The MIPs storage and transport conditions, their long-term stability, and extended self-lifetime are advantages comparatively to the aptamers and antibodies [221].

The ability to reuse a biosensor multiple times through its regeneration, *i.e.*, by analyte dissociation from the bioreceptor thus reopening the binding site, is also an important feature to be considered. For sensor regeneration, it is crucial to understand the forces that mediates the bio-interaction. Biosensors based on affinity interactions (antibodies or aptamers) are dominated by thermodynamic forces, where charge mediated events are controlled by enthalpy forces, while entropy drives the hydrophobic interactions [222]. These forces are affected by the solvent nature, thus changing the solvent conditions can conduct to sensor regeneration. For instance, by changing the pH of the buffer

solution, protein bindings can be disrupted due to the changing on the charge state of their constituent amino acids. This procedure is also conventionally used to regenerate MIP-based biosensors targeting proteins [222]. Their disruption will change the shape of the protein structure and the binding site can be reopened by washing processes. Of course, the regeneration of a biosensor is a challenging process by the complex nature of the various symbiotic forces of the bio-interactions between the receptor and analyte elements. Moreover, some procedures can damage the binding sites, disabling the site for further binding events or even unload the transducer surface [222].

4.5. Applications

The applicability of optical fiber-based sensors has been embracing a wide variety of scientific areas. In the last years numerous works were reported, giving to the scientific community the last developments on sensors employing optical fibers. In this section, some applications of sensors using optical fibers for physical, chemical, and biological entities evaluation are described.

4.5.1. Physical Parameters

Nowadays, the research on optical fiber sensors is mainly focused on the investigation of sensors capable to measure chemical entities like water pollutants or biological species such as virus or antibodies. However, besides its applicability in telecommunications, optical fibers have started to be used as sensors to monitor physical parameters such as temperature, strain, pressure, or vibration.

For instance, FBGs are a well-known allied to evaluate the long-term structural health of several types of constructions. Possible structural anomalies can be detected by strain monitoring. *Rodrigues et al.*, presented a strain monitor FBG-based system to be implemented into concrete during construction of a bridge, by placing two FBGs into a stainless-steel bar ($\varnothing$ 16 mm; L = 1 m). The calibration tests demonstrated a sensitivity of 1.2 pm/ $\mu\epsilon$ and a resolution of 0.8 $\mu\epsilon$ [223]. *Shen et al.* proposed a set of FBG strain sensors to monitor the hull health of a ship at long-term. The calibration tests showed a sensitivity coefficient of 0.89 $\mu\epsilon$ /pm by monitoring the wavelength position [224]. An interesting approach was adopted by *Leal-Junior et al.* by embedding a half of an FBG into a 3D-printed thermoplastic polyurethane (TPU) structure to obtain two FBG wavelength peaks. The sensing structure was applied as a multiparameter to monitor

strain, temperature, and force parameters, exhibiting sensitivities of 1.40 pm/ $\mu\epsilon$, 15.7 pm/ $^{\circ}\text{C}$, and 12.4 pm/N, respectively [225].

Zhao and his co-workers presented an FP interferometer sensor for strain monitoring with temperature compensation by combining the FP structure with an FBG [226]. The sensing configuration is based on a fiber Bragg grating placed in-line with a single mode fiber, both inserted into a capillary to form a sealed FP micro-cavity. The sensitivity demonstrated by strain sensor was about 1130 pm/ $\mu\epsilon$, while the FBG used for temperature compensation showed a sensitivity of around 55 pm/ $^{\circ}\text{C}$. Another interesting configuration was demonstrated by Xia *et al.* by creating an optical fiber composite FPI (CFPI) [227]. The authors fused a photonic crystal fiber with a transmission SMF creating a sensitive temperature intrinsic FPI, followed by the introduction of the resulted structure into a SMF ended quartz capillary to form an extrinsic FPI. The resulted CFPI demonstrated high-temperature stability (up to 1000 $^{\circ}\text{C}$), exhibiting a sensitivity to temperature of around 16 nm/ $^{\circ}\text{C}$, and a strain sensitivity of 0.0145 $\mu\text{m}/\mu\epsilon$ by measuring the cavity-length variation of the extrinsic FPI. Another interferometric structure was presented by Wang *et al.*, that developed an up-tapered MZI-LPFG sensor to measure strain [228]. The sensing theory is based on the MZI working principle. The light traveling in the core is coupled to the cladding by the up-taper region and it is re-coupled by the LPFG region. The strain variation is measured by monitoring the resonant wavelengths' intensities that decreases as increase the axial strain. The sensor sensitivity reported by the authors is 0.026 dB/ $\mu\epsilon$ in a working range from 0 to 590 $\mu\epsilon$.

Pressure is another valuable physical parameter to be measured, and FBGs appear again as a useful tool to do it. Hong *et al.* placed an FBG inside a polylactide (PLA)-made inner sensing unit, to evaluate the pressure effects on the sensing component [229]. The authors reported that the pressure sensor attained a maximum vertical pressure of 800 KPa with a sensitivity of 4.26 KPa/pm. Furthermore, pressure assessment can be used to obtain information about ocean depth as reported by Wang *et al.* [230]. The fiber Bragg grating was weld to a pressure diaphragm encapsulated into a metal structure. The working principle is based on the photosensitivity principle, *i.e.*, the core refractive index is periodically modulated in the axial direction. The calibration tests showed that the proposed sensor achieved a sensitivity of 1611 pm/MPa. The FBG-based system was tested in a real situation and compared with a commercial electrical pressure sensor, presenting good results.

Some investigations describing vibration-sensitive fiber optic sensors have been also present. Kaur *et al.* studied the applicability of an FBG-based sensor to monitor vibration

on a concrete beam [231]. The sensor was experimentally tested by placing the FBG sensor below the concrete beam and applying different loads by using a hydraulic jack. The vibration-sensitive FBG sensor exhibited a maximum vibration amplitude of 0.7 G at load values of 50 kN with a wavelength shift of 0.14 nm. *Song et al.* developed and investigated a 2D FBG vibration sensor based on an orthogonal flexure hinge structure [232]. The system is based on two FBGs arranged in vertical direction by two ends pasted setup. The platform intends to measure the vibration-induced structure displacement. The authors reported that the vibration-sensitive FBG sensor revealed a broad flat frequency ranging from 20 to 800 Hz and a sensitivity of 41.2 and 34.5 pm/g in the X/Y direction, respectively, with a cross-interference less than 6% in both directions.

4.5.2. Chemical and Biological Entities

Optical fibers have been emerged as excellent alternative to develop fast, low-cost and miniaturized fiber optic chemical- and bio- sensors. In this subsection, some examples of fiber optic chemical- and bio- sensors targeting chemical and biological species will be addressed, emphasizing the fiber optic configurations earlier described.

4.5.2.1. Chemical Entities

Gases

Volatile Organic Compounds (VOCs) can be evaluated by simply coating an unclad region of an optical fiber with a metal oxide thin film. *Renganathan* and *Ganesan* proposed multimode fiber optic gas sensor coated with a nanocrystalline zinc oxide [233]. The nanocrystalline ZnO was mixed with isopropyl alcohol to form a paste and the sensing configuration was obtained by dip-coating the unclad region. The sensing configuration is based on gas absorption by the ZnO which influences its refractive index, thus affecting the transmitted spectra by intensity modulation. The sensor was tested in the presence of acetone, isopropyl alcohol, and benzene, exhibiting sensitivities of -0.27 counts/100 ppm, -0.14 counts/100 ppm and -0.09 counts/100 ppm, respectively. A similar approach was adopted by *Prasanth et al.* by coating the unclad region with SnO₂ [234]. The sensor was tested in the presence of isopropanol showing a remarkable response time of 17s for the detection of 250 ppm, and 21s as recovery time. In both works, the authors used plastic-core optical fibers.

Nanoporous metal-organic frameworks (NM-OFs) are also attractive materials to perform fiber optic gas sensors. *Kim et al.* proposed a fiber optic configuration based on etched fibers coated with NM-OFs for carbon dioxide detection [235]. The authors performed two different sensing layers: one made of zeolitic imidazole framework, and another doped with cobalt (Co). The NM-OFs directly growth on the etched region by immersing the fiber in a methanolic solution containing the NM-OFs components. NM-OF presented better results when exposed to different concentrations of carbon dioxide (vs. nitrogen) than the Co/NM-OF by monitoring the intensity of transmitted spectra. However, the cobalt-doped NM-OF showed slightly wavelength red shifts as the carbon dioxide concentration increases.

Yang et al. proposed a hollow-core FPI based on photothermal effects for gas sensing [236]. To facilitate the gas intrusion, micro-channels were produced using a femtosecond laser. The transmission window of the sensor goes from 1500 to 1700 nm, comprising the absorption lines of several gases. *Wang et al.* demonstrated a Sagnac interferometer-based sensor using a methane sensitive film-coated D-shaped PCF, fused with a single mode fiber in both ends. The sensor demonstrated a sensitivity of around 13 nm/% [237]. An ammonia-sensitive MZI configuration was proposed by *Yao et al.* [238]. The sensor is composed by a graphene/microfiber hybrid waveguide and exhibits a sensitivity around 6 pm/ppm with a resolution of 0.3 ppm. The sensing principle is based on the graphene RI changes due to the ammonia absorption.

Bhatia and Gupta studied the performance of an ammonia sensitive SPR sensor by using bromocresol purple (BCP) as a sensitive element [239]. The interaction of the ammonia gas with the BCP layers forms a complex that induces a wavelength shift on resonant band. The sensitivity of the sensor was improved by the introduction of a very thin silicon layer between the metal (silver) and the BCP layers. The performance of the sensor was evaluated by measuring different ammonia concentrations ranging from 10 to 150 ppm. Others authors presented a nickel oxide/ITO-SPR sensor was tested for hydrogen sulfide (H₂S) monitoring [240]. The SPR configuration was attained by coating a small section (1cm) of a multimode optical fiber ($\varnothing$ 600 μ m) with 40 nm of silver. The nickel oxide/ITO doped material was coated over the silver layer. The sensor performance its linear between 100 and 1000 ppb.

An FBG modified with polyallylamine-amino-carbon nanotubes (PAACNTs) for sensing carbon dioxide was reported by *Shivananju et al.* [241]. The sensing configuration is constituted by a clad-etched FBG coated with PAACNTs and a second un-etched FBG to compensate temperature drifts. The sensor was tested by varying the carbon dioxide

concentration in range between 1000-4000 ppm, exhibiting a limit of detection of 75 ppm. Another grating-based sensor was reported by *Xu et al.* when they presented an ammonia-sensitive LPFG coated with graphene oxide/cellulose acetone (GO/CA) layers [242]. The methodology is based on the reaction of the nitrogen (N) element with the oxygens of the GO/CA matrix. This reaction affects the perimeter of the grating region, changing the coupling efficiency, which results in the shift of the resonance wavelength transmitted band. The sensitivity of this ammonia sensor was about -98 pm/ppm.

Heavy Metals

Heavy metals are polluting agents with great impact on the planet. They come from sources such as fertilizers used in agriculture and easily penetrate groundwater when washed away by the irrigation processes [243]. Heavy metals appear in their ionic form and most frequent are lead (Pb^{2+}), mercury (Hg^{2+}), copper (Cu^{2+}), cadmium (Cd^{2+}), and nickel (Ni^{2+}) [244]. Heavy metals are extremely dangerous for humans, and it is imperative to trace them.

Yang et al. developed a Ni (II) probe using a cascade FBG microfabricated in a SMF [245]. The authors used the LbL technique to coat the end of the SMF with bilayers of chitosan/poly acrylic acid (ChitPAA) by electrostatic interactions. The resulted sensing layer acts as absorbent for the nickel ions due to the presence of the amine group of the chitosan. This reaction forms a complex through the chelation effect and modulates the RI of the ChitPAA bilayers. As consequence the Fresnel reflectivity at the end of the fiber is modulated changing the reflectivity ratio of the FBG in the reflection spectra. The detection sensitivity exhibited by the sensor was around 40 dB/mM. *Tan et al.* used a similar approach by functionalizing the end-fiber FBG with EDTA. The EDTA was fixed on the end of the fiber following the EDC/NHS protocol and the sensor proved its applicability to detect Cd^{2+} and Pb^{2+} , exhibiting a sensitivity of 0.150 dB/10 ppm by varying the cadmium concentration from 1 to 200 ppm [246]. A GO-coated micro-tapered LPFG for Ni (II) detection was presented by *Wang et al.* [247]. The micro-tapered LPFG surface was chemically modified with (3-aminopropyl) triethoxysilane (APTES) to introduce amine functional groups ($-\text{NH}_2$) at the fiber surface. The NH_2 groups react with the epoxy groups of the GO structure, inducing the formation of GO nanosheets. The GO-Ni (II) interaction induces a wavelength shift in the LPFG transmitted spectra, displaying a sensitive of 5.12×10^{-4} nm/ppb and a limit of detection of 2.5 ppb. Another LPFG-based configuration for heavy metal monitoring was reported by *Tan* and his co-workers [248]. The grating was alternately covered with positive/negative/positive

polyelectrolyte (PE) layers that was used to electrostatically hold negatively charged gold nanoparticles (AuNp). A total of 1.34 nm of wavelength shift was observed in the presence of Hg^{2+} .

A Mach-Zehnder fiber interferometer for sensing nickel ions was demonstrated by *Tou* and his co-workers [249]. The optical configuration was obtained by splicing a photonic crystal fiber between two single mode fibers. The PCF segment of the SMF-PCF-SMF structure was dip coated with a PVA/PAA hydrogel solution, followed by chemical modification through the EDC/NHS protocol. The sensing methodology is based on the cross linkage of the Ni^{2+} with the hydrogel modified layer, inducing a phase shift in the interferogram. The Ni^{2+} -sensitive MZI sensor showed a sensitivity of 0.214 nm/($\mu\text{mol/L}$) and a limit of detection of 1 nmol/L.

Wang et al. proposed a DNAzyme modified TFBG-SPR sensor for lead ion detection [250]. The TFBG region was coated with a 50 nm of Au film by the sputtering technique, followed by the thiol modified DNAzyme immobilization. The TFBG-Au-DNAzyme fiber was placed in contact with MUA-AuNp, completing the sensor construction: TFBG-Au-DNAzyme-AuNp. The Pb^{2+} interacts with the DNAzyme inducing catalytic reactions (the double strand becomes a single strand), decreasing the distance between the Au film and the AuNp. This approximation changes the electric field distributions in the Au film, producing a change in the TFBG-SPR signal. The sensor showed its applicability to detect Pb^{2+} ion in a range from 0.01 to 10000 nmol/L with a limit of detection of 8.56 pmol/L. Other authors demonstrated a dual channel SPR sensor for copper (Cu^{2+}) detection [251]. The fiber sensor comprises two unclad sensing regions coated with different thicknesses of silver. The thicker Ag region was functionalized with a ChitPAA layer through LbL technique, while the thinner Ag region was used as reference. The reported sensitivity of the sensor was about 0.25 nm/($\mu\text{mol/L}$). *Dhara et al.* studied the response of AuNp in the presence of Pb^{2+} by monitoring the wavelength shift of the resonant band resulted from the AuNp localized plasmons. The determined sensitivity of the LSPR sensor was about 0.3 nm/(mmol/L) [252].

Drugs and Pharmaceuticals

Korposh et al. proposed a MIP-LPFG sensor to detect vancomycin (VA) [253]. In this work the LPFG was functionalized with MIP nanoparticles specially produced to be selective to VA. The sensor proved its selectivity over other antibiotics, presenting a binding constant of 43 ± 0.1 nmol/L.

Another LPFG sensor was demonstrated by *Mishra et al.* by immobilizing an enzyme-entrapped polyacrylamide gel on the surface of the grating [254]. The sensor is able to detect phenolic compounds through the enzymatic reaction of the immobilized enzyme (tyrosinase) in the presence of these chemicals. The enzymatic reaction induces a change on the gel layer according with the amount of the analyte. The sensor demonstrated a maximum sensitivity of 0.0088 pm/(μ mol/L) at a pH value of 7.

Cennamo et al. used an Au-coated plastic optical fiber (POF) to perform a SPR sensor to detect L-nicotine [255]. The Au-coated region was functionalized by a MIP layer that was directly formed on the gold surface in the presence of the template. After template extraction, the sensor proved its selectivity over other similar compounds. Moreover, the sensitivity exhibited by the sensor was 130 pm/(mol/L).

Other Parameters

Relative humidity (RH) controlling is an important demand for diverse industrial and scientific applications. *Dias et al.* proposed different approaches to measure this parameter [157]. The first approach relies on the formation of a Fabry-Pérot cavity by dip coating a tip of a single mode fiber in different RH responsive polymers such as PVA, PEG, and Hydromed D4 hydrogel (HD4). The authors observed a very good response of the FP/PEG sensor above to 80% of RH. The second approach relies on the coating of a LPFG using the same RH responsive materials. In this case the better performance was given by the LPFG/PVA combination, demonstrating better sensitivity in a larger working range (60-80% RH) when compared with the FP/PEG structure. *Wang et al.* studied the effect of different pore-foaming agents on polyamide coatings using an FBG configuration [256]. The authors reported a sensitivity improvement (2.76 times) by doping the polyamide with lithium chloride (LiCl) comparing to the FBG/polyamide combination. The sensitive of the FBG/LiCl configuration was about 1.7 pm/%RH (instead of 0.6 pm/%RH given by the FBG/polyamide).

For pH monitoring, different fiber optic configurations can be found in literature. *Zhao et al.* proposed an MMF-SMF-MMF configuration based on surface plasmon resonance [257]. The Ag-coated SMF was functionalized with a smart hydrogel synthesized by a set of monomers. The pH variations induce the dissociation of the carboxylic ions of the polymer matrix, resulting in the change of its volume and refractive index. These adjustments are monitored by following the SPR wavelength shifts. The sensor was tested in different pH solutions ranging from 1 to 12, attaining the maximum sensitivity of 13 nm/pH in the higher region (8-10 pH). *Yin et al.* proposed a tapered LPFG coated with

PAA for pH sensing [258]. The PAA hydrogel was patterned into periodic micropads on the fiber surface, applying a periodic strain modulation. The PAA hydrogel swells as the pH increases, decreasing its refractive index. The RI changes are monitored by the wavelength resonance band position of the transmitted spectra. The reported sensitivity of the sensor was 7.5 nm/pH. Another optical configuration was presented by *Hu et al.*, that developed a PCF interferometric pH sensor by splicing a PCF at the end of a SMF and coating the PCF region with a PVA/PAA hydrogel layer [259]. The sensor presented a sensitivity of 0.9 nm/pH in a pH range between 2.5 and 6.5.

Zhu et al. developed a glucose oxidase encapsulated zeolitic imidazole-based metal-organic framework on the surface of a LPFG for glucose detection [260]. The sensing methodology relies on the enzymatic catalysis caused by the glucose oxidase by converting the glucose into gluconolactone and hydrogen peroxide. As a result, the RI of the medium is changed and is monitored by the LPFG wavelength resonance band position. The sensor showed linearity to glucose concentrations in range of 1 to 8 mmol/L and a sensitivity of 0.5 nm/(nmol/L). *Sharma et al.* reported a LSPR biosensor for the detection of taurine, by immobilizing taurine dioxygenase enzymes on gold nanoparticles [261]. The sensing configuration was attained by modifying an unclad optical fiber with an aminosilane by inducing the formation of a SAM (in the end, the fiber surface was covered by amine groups). Thereafter, gold nanoparticles were immobilized onto the optical structure by electrostatic interactions between the positive charged amine groups and the negative charged AuNp, attaining the LSPR fiber optic configuration. For taurine dioxygenase immobilization, the AuNp surface was modified with cystamine dihydrochloride to allow the enzyme immobilization. The authors studied the behavior of the sensor by observing the effect of different concentrations of taurine (0 to 1 mmol/L) in the absorbance spectra given by the localized surface plasmons. They concluded that the sensor proved its selectivity over similar analytes (creatinine, lactate, and glutamate), reporting a sensitivity of 0.0190 AU/(mmol/L) (AU = absorbance units) and a limit of detection of 53 μ mol/L. *Kant and Gupta* reported very interesting work by fabricating a fiber optic SPR biosensor for the detection of the neurotransmitter acetylcholine (involved in human behavioral activities; its dysfunction is linked to several neurological disorders including Alzheimer's disease) [262]. The sensing probe was developed by depositing 40 nm thick silver layer over the unclad region of an optical fiber using the thermal evaporation technique. Thereafter, the silver coated fiber was immersed in acetylcholinesterase (AChE) enzyme entrapped solution of tantalum pentoxide (Ta_2O_5) nanoflakes to perform the sensing surface over the fiber probe by dip coating technique. The catalysis reaction between the enzyme and the analyte induces refractometric

changes on resonance band of the SPR configuration. The sensing probe exhibit a sensitivity of around 8.7 nm/($\mu\text{mol/L}$) and a limit of detection of 38 nmol/L. Soares *et al.* developed a SPR immunosensor for cortisol detection by using a D-shaped optical fiber [263]. The fiber structure was attained by polishing a standard single mode optical fiber by one side till the desired core diameter. Thereafter, a thin Au film of 50 nm thick was deposited on the polished side of the fiber. For cortisol detection, the Au-coated side was functionalized with cysteamine, by physicochemical interaction between sulfur (of thiol group) and the Au layer, allowing the immobilization of the anti-cortisol antibody. The sensor performance was experimentally assessed in a detection range of 0.01 to 100 ng/mL, exhibiting a sensitivity of 0.65 nm/log(ng/mL) and a limit of detection of 1.46 ng/mL.

4.5.2.2. Biological Entities

Antibodies

A Fabry-Pérot dfiber optic immunosensor was developed and characterized by Chan and his co-workers [264]. The FP cavity was attained by splicing a standard SMF with a hollow core optical fiber, forming a gap between both fiber ends. Thereafter, the end of the hollow core fiber was coated by layer-by-layer self-assembling of a suspended chitosan/polystyrene sulfonate membrane. Immunoglobulin G antibodies were immobilized on the membrane substrate, thus allowing the detection of anti-IgG molecules by monitoring the effective optical thickness of the substrate. The FPI configuration demonstrated a sensitivity of 0.033 $\mu\text{m}/(\text{pg/mm}^2)$ and a detection limit of 0.005 nmol/L.

A SPR configuration was presented and studied by Wang *et al.*, by developing an immunosensor based on GO and staphylococcal protein A (SPA) co-modified PCF for IgG detection [265]. The optical arrangement was attained by splicing a PCF between two multimode fibers, whose PCF section was sputtered with 50 nm thick Au film. The sensitivity of the SPR sensor was enhanced by the deposition of GO onto gold surface. For biological application, the GO layer was modified with SPA/anti-IgG conjugation allowing the detection and evaluation of IgG antibodies. The authors highlight the sensitivity improvement due to the introduction of the GO layer (≈ 1.7 times higher) and the limit of detection of 10 ng/mL (about 30 times lower than the Au-PCF configuration).

A similar approach for the same target was proposed by other authors, by coating the surface of an Au-PCF with GO/Au nanorods composite [266]. The sensing section of the

PCF-Au-GO/Au nanorods was chemically modified with 11-mercaptopundecanoic acid (MUA) that was used for the immobilization of the bioreceptor (anti-IgG). The IgG sensitive sensor exhibited a limit of detection lower than the previously reported SPR-sensor, attaining a value of 6 ng/mL.

Wu et al. developed some optical fiber platforms for the detection of three different antibodies: IgA, IgM, and IgG [267]. All designs are based on LPFG microstructures, operating around the phase matching turning point, functionalized with the respective antibody receptor: anti-IgA, anti-IgM, and anti-IgG. For the development of the sensing platform the LPFG was functionalized with multiple layers of PAH/SiO₂ nanoparticles *via* LbL formation, followed by the functionalization with APTES. Thereafter, gold nanoparticles were immobilized using the electrostatic forces between the positive charges of the APTES amine terminal groups and the negatively charged Au nanoparticles. For the bio-element immobilization, the Au nanoparticles surface was modified with 11-MUA, allowing the immobilization of the bioreceptor (bearing -NH₂ functional groups) by the EDC/NHS chemical protocol. The LPFG/anti-IgA, LPFG/anti-IgM, and LPFG/anti-IgG were tested in different working ranges (10-200 µg/mL, 10-320 µg/mL, and 1-90 µg/mL, respectively) reporting as minimum measurable concentration the same value, 10 µm/mL. All sensors proved to be specific to the target over the other two antibodies.

Proteins (Viruses and Biomarkers)

Luís et al. reported an aptamer-based fiber sensor for thrombin detection by presenting two different fiber optic configurations [268]. The first design comprises the sensitivity amplification of a LPFG by coating the sensing section with titanium dioxide. The second structure relies on an etched SMF coated by 16 nm thick gold layer followed by the deposition of 100 nm thick TiO₂ layer. Both configurations were electrostatically functionalized with poly-L-lysine (PLL) and the ssDNA probe (thrombin binding aptamer, TBA) was immobilized for specific recognition of the thrombin protein. The sensing configurations were used to evaluate the target analyte in range of concentrations from 10 to 100 nmol/L, exhibiting sensitivities of 0.025 nm/(nmol/L) and 0.35 nm/(nmol/L) for the LPFG and SPR configurations, respectively.

Another aptasensor based on the SPR phenomenon for thrombin detection was demonstrated by *Cennamo* and his co-workers [269]. The sensing configuration is based on a D-shaped plastic optical fiber where the D-shaped region was coated with 60 nm thick Au layer, followed by a biotinylated PEG SAM formation. Thereafter, a

biotin-modified ssDNA probe was immobilized *via* streptavidin binding. The sensing device showed a limit of detection of 1 nmol/L in a range of concentrations from 1.6 to 60 nmol/L.

The same author used a similar optical platform to immobilize nano-scaled molecularly imprinted polymers (nanoMIPs) for human transferrin (HTR) detection [270]. The Au surface was covalently derivatized with (R)-(+)- α -lipoic acid to further coupling the dipeptide Lys-Lys. The amine-functionalities of the used dipeptide allowed, by EDC/NHS protocol, the immobilization of the nanoMIPs. The sensing methodology is based on the monitoring of the wavelength shift of the SPR band induced by the nanoMIP deformation due to the MIP/HTR interactions, at the surface of the gold film. The sensing platform showed a linear dynamic range of concentrations from 1.2 fmol/L to 1.8 pmol/L and a detection limit of 1.2 fmol/L.

Pathak et al. demonstrated and characterized an ultrasensitive fiber optic SPR sensor based on molecular imprinting for real-time bovine serum albumin (BSA) detection [271]. The sensing configuration is based on an unclad-MMF coated with an Ag layer to excite surface plasmons. Thereafter, the Ag-layered region was coated with modified carbon nanotubes that acts as a platform for the MIP-layer formation. By monitoring the wavelength SPR band shift, the sensing platform showed a sensitivity of 0.862 nm/(ng/L) and a limit of detection of 0.386 ng/L, in a linear working range from 0 to 100 ng/L.

Kamil et al. presented a Dengue virus immunosensor by functionalizing a tapered optical fiber with Dengue virus E protein antibody [272]. The fiber structure was achieved by heat&pull a standard SMF, followed by the chemical modification of the tapered region with APTES molecules. Then, the fiber structure was treated with glutaraldehyde to allow a homogeneous immobilization of the dengue virus E protein antibodies. The sensor configuration displayed a sensitivity and a limit of detection of 5.02 nm/(nmol/L) and 1 pmol/L, respectively.

An immunosensor based on LPFGs, target the *norovirus* capsid protein, was presented and discussed by *Janczuk-Ritcher et al.* [273]. The LPFG was chemically etched (40% HF solution) to reach the dispersed turning point of the high-order cladding modes, followed by the covalently immobilization of 3-(Triethoxysilyl)propylsuccinic anhydride (TESPSA) for posterior immobilization of the anti-VP1 antibodies. The biorecognition occurs through the interaction between the antibody and the *norovirus* virus-like protein, inducing changes on the effective refractive index of LPFG cladding modes. The authors reported that this LPFG-based sensor was tested varying the concentrations of the virus-

like protein from 1 to 10 ng/mL, shifting a total of 2.8 nm for the highest concentration. They also showed that the sensor is selective to the target over other similar analytes.

Cennamo et al. presented a SPR design as an alternative for a rapid clinical diagnosis for the COVID-19 disease, by functionalizing the SPR region with MIPs targeting the SARS-CoV-2 virus spike protein [274]. The SPR structure was achieved by sputtering the exposed core of a D-shaped plastic optical fiber with 60 nm thick gold layer over a firstly layered 1.5 μm thick photoresist layer that was obtained by spin-coating. The spike protein specific receptor was directly formed over the gold layer by molecular imprinting. The optical platform exhibited a sensitivity of around 1.95 nm/($\mu\text{mol/L}$) and a detection limit of 0.37 $\mu\text{mol/L}$.

A immunosensor targeting a cancer biomarker, cytokeratins 17 (CK17), using a fiber optic configuration was reported by *Ribaut et al.* [275]. The sensing configuration is based on surface plasmons and a TFBG as optical transducer. The surface of the TFBG structure was coated with 50 nm thick Au film that was annealed for 2h at 200 °C. The Au-coated TFBG was chemically modified with Dithiolalkane aromatic PEG6-COOH ($\text{S}_2\text{-PEG}_6\text{-COOH}$) by SAM formation, followed by the immobilization of the anti-CK17 by the EDC/NHS protocol. The sensitivity of the SPR-TFBG-anti-CK17 configuration was studied in the presence of real samples: a healthy tissue and a tumoral tissue. In the presence of the healthy tissue was observed an unreliable evolution of the optical response (± 0.11 dB), while in the presence of the tumoral tissue the sensor reported an amplitude shift of 0.61 dB after 10 minutes of exposure time.

Loyez et al. studied the behavior of a SPR gold-coated fiber tip functionalized with an aptamer DNA probe for the detection of a breast cancer biomarker [276]. The sensor structure was performed by coating an uncladded multimode fiber tip with a gold layer (thicknesses ranging from 35 to 50 nm) and the chemical immobilization of a thiolated ssDNA probe targeting the HER2 breast cancer biomarker. The sensing configuration, working in reflection mode, was tested in the presence of different HER2 concentrations, exhibiting 9.3 ng/mL as the best limit of detection.

Bacteria

The performance of a fiber optic SPR immunosensor targeting the *Escherichia coli* (*E. Coli*) bacteria was studied by *Kaushik* and his co-workers [277]. The authors built the optical sensor by etching a small portion of a step-index multimode fiber, exposing it to a 48% w/w HF solution. Then, a gold layer was deposited on the etched section to induce

the SPR effect, followed by the formation of molybdenum disulfide (MoS_2) nanosheets (by dip coating), to improve the SPR sensitivity. Finally, the *E. coli* monoclonal antibodies were immobilized onto the MoS_2 nanosheets by physisorption via hydrophobic interactions. The performance of the sensor was evaluated by measuring the wavelength shift per unit of colonies formed (CFU) by the bacteria. The assessed sensitivity was about 3 nm.mL/1000 CFU and a limit of detection of 94 CFU/mL. Moreover, the sensor demonstrated its selectivity to the target over other similar bacteria.

A different approach was showed by *Janik et al.* by developing a Mach-Zehnder interferometer-based aptasensor for *E. coli* detection [278]. The MZI configuration was performed by fabricating cylindrical microcavities with 60 μm of diameter in a standard SMF through the cladding down to the core. Thereafter, the MZI surface was chemically modified by the introduction of aminosilanes, followed by the activated amine groups with glutaraldehyde. After the anti-*E. coli* aptamer immobilization, the sensor performance was evaluated with different concentrations ranging from 10 to 10^7 CFU/mL, exhibiting a detection limit of 10 CFU/mL.

Yang et al. demonstrated a LPFG-based configuration for *Staphylococcus aureus* bacteria detection [279]. Two types of LPFG designs were used. The first LPFG was modified with polyelectrolytes by coating the surface with PAH/PAA bilayers, followed by the final coating with PAH to induce positive charges at the fiber structure surface. The second LPFG was modified by the introduction of amine groups via APTES immobilization. On both configurations were immobilized *S.aureus* antibodies by glutaraldehyde linkage. The authors reported that the (PAH/PAA)-PAH-antibody displayed a better performance than the APTES-antibody structure in a range of concentrations from 10^4 to 10^8 CFU/mL. The better configuration showed a sensitivity of 0.478 ± 0.005 nm/log (CFU/mL) and a detection limit of 224 CFU/mL.

Zainuddin et al. developed an optical sensor based on a tapered fiber structure for the *Leptospira* bacteria DNA detection [280]. To fabricate the sensing configuration, the authors had reduced the diameter of a single mode fiber using the heat&pull technique. The tapered region was chemically modified by the covalent immobilization of APTES, followed by the introduction of glutaraldehyde that acts as an arm linker for the immobilization of the ssDNA probe. The resulted construction was tested with a complementary and a non-complementary DNA sequence. The optical fiber tapered sensor demonstrated its specificity to the complementary DNA (cDNA) over the non-complementary DNA. The cDNA hybridization was monitored by the wavelength shifts

of the transmitted spectra, exhibiting a linear sensitivity of about 1.29 nm/(nmol/L) and a limit of detection in the order of the femtomolar.

4.6. Conclusion

In this chapter, the basic principles and configurations for fiber optic detection of a wide variety of parameters were discussed, as well as the main techniques to improve the FOS performance for chemical and biological applications.

Due to optical fiber unique characteristics (long distance transmission with low loss and no electromagnetic interference and good biochemistry compatibility) and the possibility to perform miniaturized devices, FOS have been demonstrated their versatility to successfully measure a wide range of parameters such as chemical (gases, heavy metals, humidity, pH) and biological (antibodies, proteins, bacteria) species, sometimes in extremely harsh environments. Most FOCS and FOBS present in literature are based on the fabrication of microstructures, such as fiber gratings, micro-structured fibers, or fiber interferometers, and simple plasmonic configurations combined with chemical or biological elements to specific recognition of the target analyte. In several cases, the combination of both configurations results in sensitivity-enhanced platforms allowing detection of very lower concentrations, emphasizing effects such as evanescent field and surface propagating plasmons or localized surface plasmons. Optical fibers offer the advantage to build FOCS and FOBS over a microscopic platform, easily changing the surface material, sometimes to enhance its optical properties or to modify the surface biochemistry to assist or improve the strength of the immobilization of the recognition element. For instance, metal deposition techniques (PVD and CVD) are strongly used to protect against corrosion, but mainly to develop highly sensitive structures (e.g., TiO₂-coated LPFGs) or create effects as used by the fiber optic SPR-based sensors (e.g., Au or Ag coatings). A wide range of chemicals can be covalently employed at the fiber surface (coated or uncoated) using techniques such as SAM, acting as a “bridge” to immobilize elements for specific identification of different species. Other techniques such as LbL are used to perform multiple layered structures, using differently charged polyelectrolytes, to directly immobilization of the recognition element or to hold metal or sol-gel based nanoparticles. The introduction of nanoparticles offers different approaches. For instance, SiO₂ nanoparticles can be used to merge layers of polyelectrolytes to elevate the effective refractive index of a fiber optic structure and/or to carry the bio-elements used to develop FOBS. Another example are the gold (or silver) nanoparticles. Identically to the SiO₂ nanostructures, they can be used to immobilize the

recognition element as well as to direct detection of some chemical entities, but also to excite localized plasmons at a fiber structure surface.

Despite some reported research, most FOCS and FOBS only establish the use of one fiber structure for a specific analyte, which does not completely exploit the recognized advantage of the multiplexed detection capacity of fiber optic components. Optical fibers can be bundled for simultaneous applications by functionalizing the different fiber platforms for distinct sensing purposes. Furthermore, in a unique optical fiber can be produced different sensing sections, differently functionalized for different targets or to reduce the interference caused by non-specific species and/or physical interferences on the optical fiber itself.

Moreover, different from the predesigned conditions implemented in the research facilities, the analytes generally exist in a complex environment. Thus, is expected that several interferents may surround the analyte of interest. For instance, LPFGs are very sensitive to temperature, tension, and torsion variations. Hence, is anticipated that FOCS or FOBS based on LPFG structures deal with physical influences that interfere with their optical responses. Another situation is the fouling formation on the functionalized surfaces, mainly on FOBS, that can induce wrong conclusions when the sensor is applied to determine the existence or not of the targeted specie. This problem can be reduced by applying control sensors similar to the platforms used for sensing. For instance, a pair of fibers can be used to fabricate a reliable sensing platform: a main fiber for sensing and a second one to analyze the influence of interferents by being exposed to the same sample. The analytical data is obtained by crossing both optical responses, obtaining in that way the differential of both optical information. A method to accomplish such platform is the integration of optical fibers into microfluidic systems. Besides the advantage of to expose a sensing fiber and a control fiber to the same sample, microfluidic systems allow to reduce significantly the amount used for analysis. That is a particular advantage for biomedical applications.

In this work, were developed different intrinsic fiber optic configurations for reliable biological sensing, highlighting optical platforms based on long period fiber gratings, and plasmonic effects. All sensing configurations include the respective reference methods for the analytical signal correction, aiming to improve the optical response by calculating the differential responses of both sensing and reference signals, thus providing reliable and precise results. The next chapters are focused on the fabrication methods and materials for the development of the fiber optic configurations as well as the experimental procedures, the achieved results, and their discussion.

Chapter 5 – Fabrication Processes & Characterization Methods

This chapter describes the fabrication processes and the characterization methods of the sensing configurations used along the further chapters, namely the sensing layers production, the fiber structures, the thin metallic film deposition with physical vapor deposition system (PVD), and the fabrication methodologies of the used nanostructures.

5.1. Fabrication Processes

The fabrication of the fiber optic structures used in this thesis were carried out considering the background acquired over the years by the research groups involved in this work. The micro and nanofabrication processes were attained due to the extraordinary knowledge and specific instrumentation available in the facilities of both research units.

5.1.2. Long Period Fiber Gratings

As reported in chapter 4, LPFGs are structures that can be microfabricated by different processes such as UV laser-based techniques, CO₂ laser, IR femtosecond laser, and electric arc discharges. The LPFGs fabricated for this work were produced at INESC

TEC facilities, based on the induced electric arc technique [116]. Briefly, the fabrication setup comprises a light source to inject white light into a single mode fiber, an optical spectrum analyzer (OSA) to register in real-time the grating transmission spectrum, a motorized linear transitional carriage to move the fiber between electrical discharges, two tungsten electrodes to apply periodic arc discharges, and a power supplier. From this process results the formation of micro tapers between discharges, by increasing the temperature of the silica high enough to fuse the fiber to produce the required modulation, thus changing the local refractive index of the core. The LPFGs used in this work were produced applying this technique in common telecommunication single mode fibers (SMF28 Corning) by following the scheme in Figure 5.1. The optical fiber is prepared by removing 10 to 15 cm length of the fiber jacket followed by a cleaning step with acetone to remove any vestigial polymer. Thereafter, the fiber is placed between the electrodes (separated by 1 mm), being fixed to the carriage in one end and stretched in the other end by a mass of about 5 g to keep the same tension during the process. The power supplier and the transitional carriage are programmed to apply a current of 9 mA with a time duration of 1 second and move the fiber $415\text{ }\mu\text{m}$ ($\pm 0.1\text{ }\mu\text{m}$), respectively. These parameters were investigated and optimized by *Rego et al.* for this type of fiber [281]. The process is monitored in real-time by checking the transmitted spectrum displayed in the OSA and is stopped until the attenuated band of the LPFG reaches at least -20 dB.

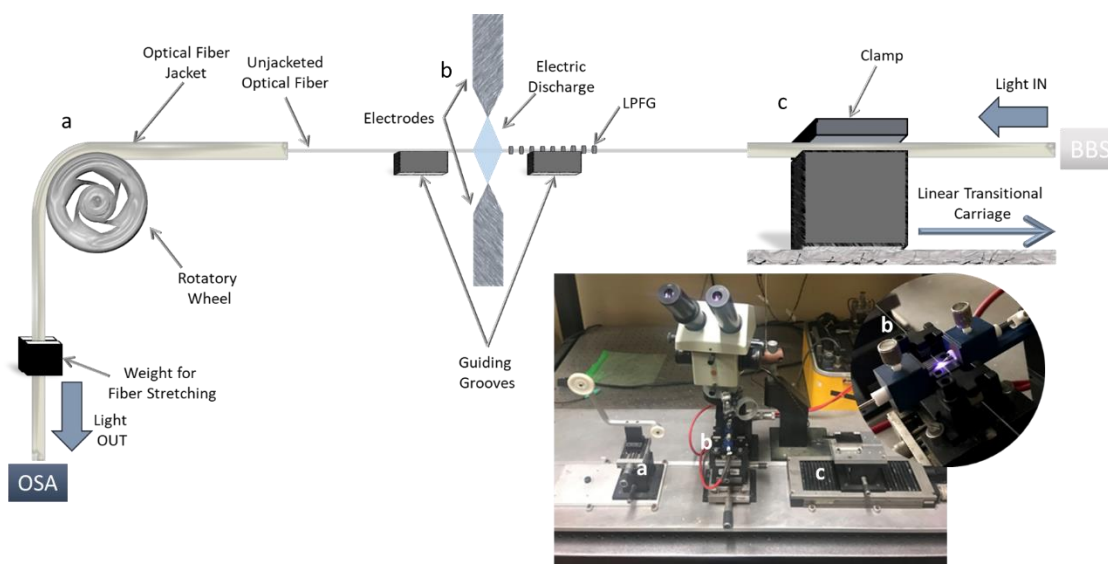


Figure 5. 1. Scheme of the long period fiber gratings production process using the electric arc technique and a picture of the real fabrication station, referencing the correspondent zones a, b, and c. BBS and OSA correspond to the broadband light source and optical spectrum analyzer position, respectively.

The produced LPFGs were used to develop different types of biosensors, namely an LPFG-immunosensor based on antibodies-covered silica nanoparticles, and a MIP-covered LPFG based on in situ polymerization. Both processes are theoretically described in section 5.2.

5.1.3. Etching Process

Etching is the process usually applied to reduce the diameter of an optical fiber. This technique allows to expose the core (or reduce the cladding to very close to the core), enhancing the sensitivity properties of an optical fiber configuration by assisting the access to the evanescent field to the external medium, enabling strong interactions with a measurand of interest [282]. To accomplish this, two main procedures are generally used to reduce the fiber diameter: mechanical polishing [283] or chemical etching [284]. However, mechanical methods usually result in hard and time-consuming processes. In the mechanical process the fiber is immobilized on a silica block, and the entire structure is polished with a certain grain size (or decreasing from a bigger grain to a smaller grain). Fiber slip, polished heterogeneous or small surface areas, are some issues that can result from the process. Chemical etching is a simple and faster process comparatively to the mechanical polishing.

Generally, silica etching can be achieved by applying one of two processes: reversible chemistry in hot water/alkaline or water/strong acid solutions, or irreversible etching with hydrofluoric acid (HF). In the first approach, an alkaline (e.g., NaOH, Na₂CO₃, ammonia) or strong acid (e.g., HCl, H₂SO₄) solution is performed in hot water to break the Si-O-Si bonds, forming less condensed oligomers of silica species. However, the formed silicate oligomers make this method a reversible process [285]. The chemical etching using HF, results in an irreversible and faster process and the main product resulted from the reaction is gaseous silicon tetrafluoride (SiF₄) [286]. *Saya et al.* have reported a strong correlation of HF etching rates with the acid concentration at room temperatures, reporting a quadratic dependence at lower concentration ranges (2 - 24% w/w in water). Thus, it is acceptable to assume that the etching rate increases as the HF concentration increases. The etching rate is also dependent of the temperature and the relative humidity [287].

During the process, the etchant reacts with the silica surface according with the reactions identified by Equations 5.1 and 5.2 where the latter reaction is dominating by the HF concentration [284].



Moreover, the reaction of the acid with the fiber is limited by the contact surface area. The dissolution rate of the fiber surface depends on the dissolution constant, surface area, and the HF concentration (Equation 5.3) [284]:

$$\frac{d}{dt} \left[\frac{\pi D_w^2 L_w \rho}{4} \right] = -k(\pi D_w L_w) C_{\text{HF}} \quad (5.3),$$

where D_w is the diameter of the fiber waist, L_w is the length of the exposed region, ρ is the density of silica, t is the reaction time, and C_{HF} is the concentration of HF. The first part of the Equation 5.3 (or the left side) describes the change of mass of the fiber, and the other part refers to the dissolution rate. Simplifying the equation 5.3:

$$\frac{d}{dt} \left(\frac{D_w}{D_i} \right) = -K \quad (5.4),$$

where K is a constant and that is equivalent to $K = 2kC_{\text{HF}}/D_i\rho$.

The investigation performed by *Shvartsev et al.*, determined that the dissolution of SiO_2 in HF strongly depends on three main parameters. The first is related with the four bridging oxygen atoms bonded to the silicon atoms. The replacement of the first oxygen by a fluorine ion is slow and regulates the rate of the reaction. Another important parameter is the pH. The surface equilibrium reactions of the silanol groups with its protonated and deprotonated forms is highly influenced by the pH evolution. The elimination of H_2O molecules, which occurs at low pH, is faster than that of OH^- . Finally, the fluoride-based species (HF_2^-) reacts with the silicon atom that lost the H_2O or OH^- group. This step promotes the elimination process [288].

In this work, the process was applied to reduce the diameter of a 600 μm core multimode optical fiber (core/cladding 600/630 μm from Thorlabs). The procedure was applied as described by *Coelho et al.*, when they reported a chemically etched single mode fiber sensing platform based on the SPR phenomenon [289]. In this case and due to the larger

fiber core, the HF was not diluted and was used as received (48%). The resulted etched optical fiber was coated with a thin gold layer following the process described in section 5.2.

5.2. Coating Processes and Materials

5.2.1. Spin-coating Technique

Spin-coating (SC) is a used technique to produce uniform films on planar substrates, offering high controllable and reproducible film thicknesses. Typically, a certain portion of a liquid sample is dropped on the center of a substrate and is applied a certain angular velocity to reach a homogeneous thin film (Figure 5.2). The centrifugal force initiates the liquid spreading, where the thickness of the film is dependent of the liquid viscosity, drying rate, percent of solids, surface tension, the nature of the used solvent, and the parameters selected for the spin course. The SC process can be divided in different stages [290]:

Dispense stage

The dispense stage defines how the liquid is dropped on the substrate and can be divided into a static dispense or a dynamic dispense. Static dispense consists in a simple deposition of the liquid to be spread on the immobilized substrate. Thereafter, is applied the desired velocity to spread the liquid. The sample amount and the applied velocity depends on the viscosity and the size of the substrate. Dynamic dispense involves the drop of the liquid when the substrate is already under a slow speed rotation (typically 300-500 rpm). This process allows to place the precise amount of the sample until the desired coverage of the substrate and reduce the waste of the material to be spread. If the fluid or substrate has poor wetting properties, dynamic dispense avoids the formation of uncovered small areas that can be formed by applying a higher starter velocity.

Substrate acceleration stage

This stage is related with the aggressive liquid expulsion from the substrate by the rotational motion. Due to the initial depth of liquid on the substrate surface, spiral vortices may occur caused by the inertia that the top of the liquid layer exerts while the rotational platform rotates faster and faster. The speeds used in this stage usually attains rotational velocities ranging from 1500 to 6000 rpm, depending on the properties of the material as

well as the substrate. Furthermore, the combination of the spin speed and the time of the spinning process strongly affects the thickness of the formed film in way that longer spin times and higher spin speeds produces thinner films.

Dependence of fluid viscosity and solvent evaporation at constant spinning rates

These stages are characterized by gradual thinning processes of the dropped liquid, being controlled by the viscous properties of the fluid and/or the solvent evaporation process. Depending on the surface tension, viscosity, and rotation rate, small fluid amounts can be formed on the edges of the substrate, presenting a different thickness comparatively with the thickness observed on the center. Mathematical investigations showed that if the liquid exhibits Newtonian viscosity and its thickness is uniform across the substrate, the spinning time does not affect the thickness of the final film [291]. As the latter stage advances, the thinning process reaches a point where the viscosity effects produce only a rather fluid flow. At this point the evaporation of the used solvent is the characteristic that dominates the thinning process. As the solvent is evaporated, the viscosity of the coating tends to increase, “gelling” the formed film. After the spinning processes, some coatings require further treatment such as heat treatment or dry in low humidity environments.

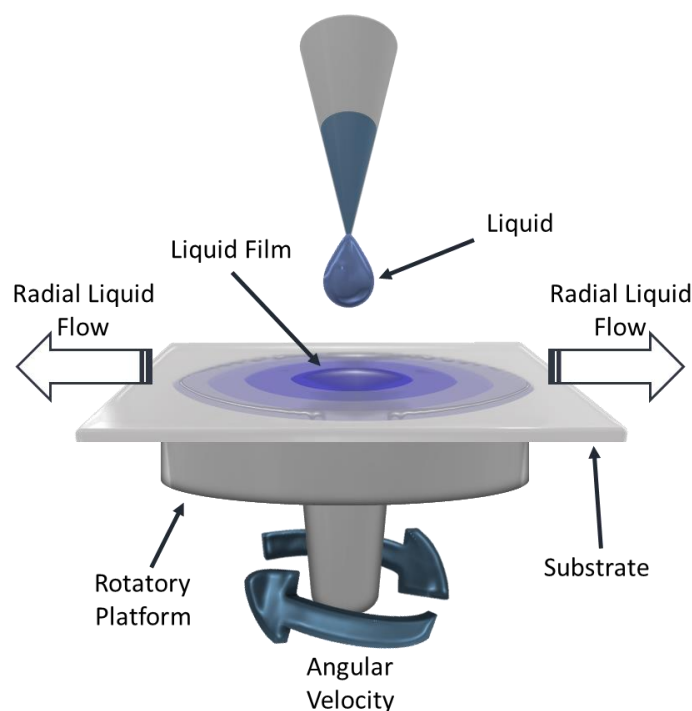


Figure 5. 2. Schematic figure of the spin-coating process.

This coating method is used in various applications such as coating of photoresist wafers, production of protective films, optical coatings, and fabrication of membranes used for sensing. Considering the latter application, one of the main purposes is the spin-coating of polymer films. [292].

In this work, the SC method was applied to spread a colorimetric cocktail based on a hydrogel polymer and another based on an organic polymer onto a transparent[®] Mylar foil to develop a gas-sensitive membrane. The process was accomplished using a homemade spin-coating equipment connected to a vacuum pump used to hold the substrate. The spinning process was performed using the dynamic dispense approach and the layer thickness was controlled by increasing gradually the spin speed and according to polymer concentration (*i.e.*, viscosity).

5.2.2. Nanoparticles Synthesis

Nano-scaled particles come up to with great success to revolutionize the sensors field. There are several research works focused on the development of nanoparticles to be applied in several scientific areas, such as chemical and biochemical, offering a wide variety of applications ranging from the enhancement of optical properties to basic structures holding different types of sensing elements. The materials to synthesize nanoparticles are often selected according to their intrinsic characteristics (such as optical), and/or biochemistry surface properties for further biochemical modifications [293, 294].

In the present work, were used silica and gold nanoparticles synthesized by the *Stöber* and seeding-growth methods, respectively. Both processes are following described.

5.2.2.1. Silica Nanoparticles Synthesis by *Stöber* Method

The *Stöber* method is used to produce monodispersed silica colloids by means of hydrolysis of alkyl silicates (typically TEOS), followed by the condensation of silicic acid in alcoholic solutions using a strong base (usually ammonia) as catalyst [295]. During procedure can or not employs a template (*e.g.*, sensing dye), resulting in solid or doped silica nanoparticles, respectively. Briefly, by action of the catalyst the hydrolysis reaction is initiated, where the ethoxy groups of TEOS reacts with water molecules to form the silica precursor, with the hydroxyl groups replacing ethoxy groups (Equation 5.5). Thereafter, the condensation reaction (Equation 5.6) occurs immediately, where the hydroxyl group of the intermediate reacts with ethoxy groups of other TEOS molecules

(condensation) or other hydroxyl groups of water molecules (hydrolysis), forming Si-O-Si bridges [296].



Along the years, have been made several investigations about the kinetics and characterization of the *Stöber* process, mainly on the control of the particle size, shape, and uniformity. Parameters such as the concentrations of TEOS, ammonia, water and the alcohol used as a solvent, the reaction time and temperature, and the rate of addition of TEOS, strongly affects the characteristics of the nanoparticles [296, 297].

In this work, the silica nanoparticles synthesized by the *Stöber* method were used for different purposes. The first approach consisted in the synthesis of silica nanoparticles in which was immobilized a colorimetric indicator for gas sensing, and the second approach included the amine-functionalization of the nanoparticles' surface for further immobilization of antibodies, both resulting in nanosized sensors. The sensing performance of the nano-sensors was evaluated by distinct optical platforms: i. based on intensity modulation; and ii. based on wavelength modulation; respectively.

5.2.2.2. Gold Nanoparticles Synthesis by Seeding-Growth method

Investigations on the synthesis of gold nanoparticles (AuNp) have been increased over the years due to their valuable contribution for applications photonics, catalysis, electronics, and biomedicine [298]. Typically, their production is related with the reduction of tetrachloroauric acid (HAuCl₄) by sodium citrate. This process was firstly reported by *Turkevich et al.* (in 1951) and posteriorly refined by *Frens et al.*, and remains the most common method for AuNp synthesis in aqueous media [299, 300]. This strategy allows to control the AuNp size in a range from 5 to 150 nm by varying the reaction conditions such as the sodium citrate/gold ratio, pH, and the nature of the solvent. However, the uniformity of the size and size distribution of the particles is quite imprecise, presenting irregular shapes, going far away from the required spherical form.

In this work, the used gold nanospheres were synthesized following the method presented by *Bastús* and his co-workers, based on the seeding growth method adapted from the classical *Turkevich/Frens* process. The method success relies on the kinetic control of growth process by adjusting the already reported reaction conditions, as well

as the seed concentration [301]. Briefly, the Au seeds were synthesized by boiling sodium citrate and HAuCl_4 . The second step includes the seed growth process that was controlled by adding small amounts, spaced in time, of sodium citrate and HAuCl_4 , after cooling down the reaction resulted from the first step to, approximately, 90 °C. The process was repeated until reaching the desired size.

The synthesized AuNp were used to develop coupled plasmonic fiber optic structures highlighting SPR and LSPR combined effects.

5.2.3. Molecularly Imprinted Layer Fabrication

For the development of the MIP-coated LPFGs based sensor studied in this work, was used an imprinting process based on the surface imprinting via grafting polymerization approach described in subsection 4.3.4.

As was already exposed here, the method comprises the covalent or non-covalent immobilization of the template molecule on the substrate surface prior the polymerization process. However, the procedure to remove the template can becomes a difficult process (in case of covalent attachments) or can destroy the binding sites of the polymeric layer, because during polymerization the polymer is not covalently attached to the substrate surface. Hence, the approach used in the present work includes the covalent attachment of the polymeric layer on the fiber surface to obtain a robust MIP structure (adapted from [274]). So, the LPFG region was chemically modified with alkoxysilane compounds holding allyl groups ($\text{R}=\text{CH}_2$). During polymerization, the allyl groups will work as a bridge structure between the fiber surface and formed MIP layer. The template molecule was placed in solution since the beginning of the polymerization process, allowing the polymer formation around the target molecule. After the template removal, specific and sensitive binding sites to that molecule were left behind. Moreover, the non-imprinted polymer (NIP) was performed using the same process, without adding the target molecule. All the polymerization processes were started by adding a polymeric initiator (avoiding heating or UV-light radiation processes) and were controlled in real-time by studying the wavelength position of the resonant band resulted from the grating fabrication. The MIP/NIP optical platform studied in this work was developed to be selective and sensitive to Immunoglobulin G antibodies.

5.2.4. Metal-film Deposition System

As was described in the previous chapter of this thesis, there are several coating techniques allowing the deposition of metallic films on a desired substrate. Due to the good chemical stability of the PVD technique and its good compatibility with optical fibers, this method was selected to fabricate the fiber coatings used in this work. The depositions were accomplished with the thermal evaporation system model Auto 306 electron beam source (Edwards, UK), from INESC TEC and CEMUP. Briefly, the vacuum chamber is connected to a mechanical rotatory pump (model E2M5, Edwards, UK) for strong pumping down to 10^{-3} mbar, achieving a high vacuum state with one diffusion pump (model EO4/160K) with a pumping rate of 600 L/s. A *Pirani* gauge (model PRL10K, Edwards, UK) capable to measure down to 10^{-4} mbar and a penning gauge (model CP25EK, Edwards, UK) capable of down to 10^{-7} mbar, were used to measure the vacuum pressure. The system also comprises an automatic pumping mode, allowing to switch to a manual mode for specific operations as depositions of different metals in different gaseous atmospheres.

The concept includes a low-tension power supply that is connected to a circular shape filament. The Joule effect leads to the filament heating to the point where thermionic emission takes place creating an electron beam [302]. The process follows the Richardson's law (Equation 5.7) that gives the current density $J_x(\text{A/m}^2)$ of the projected electrons in a perpendicular direction from the heated metal.

$$J = A_R \lambda_R T^2 \exp\left(-\frac{W_F}{kT}\right) \quad (5.7),$$

where A_R is the Richardson's constant ($1.2 \times 10^6 \text{ A/m}^2\text{K}^2$), λ_R is the correction factor from the used material and W_F is the minimum quantity of energy necessary for each electron leave the metal, *i.e.*, when the free electrons acquire enough thermal energy to overcome the energy barrier. Thereafter, a high-tension is applied to the filament (typically $>4 \text{ kV}$), causing a potential difference between the filament and the crucible high enough to accelerate the electrons to the crucible, thus transferring its energy directly to the target material. The generated electron beam has a conic shape, and the target should be moved to attain the point where the concentration of electrons is maximum. As the target material is negatively charged, it needs to be discharged by the crucible holder through the crucible must be conductive. The material composing the crucible can be used according to the material to be deposited. In case of gold, an intermetallic crucible was used.

The thin film thickness was controlled in real-time with a quartz crystal microbalance (Edwards, UK), formed by water cooled crystal holders, quartz crystals and oscillator units, enabling the direct read-out of the deposition rate with resolution of 0.1 nm. During the film growth, the mass of the deposited material increases which induces changes in the oscillating frequency of the quartz crystal. The deposition of different materials can be controlled by changing the unique properties of each material (density and impedance).

To produce homogeneous thin film coatings around the optical fibers, a rotation device was designed and fitted to the electron beam vacuum chamber. Briefly, the fibers were fixed at both ends with square magnets and metallic cylinders. The drive shafts were connected to a DC motor with a gearbox located outside the chamber through a combination of angle gears and a special rotatory feedthrough for high vacuum chambers.

In this work, the coatings were performed using the procedure and calibration parameters described used by *Coelho et al.* in their work [303]. The PVD system was used to coat etched optical fibers with thin Au films to create surface plasmon resonance-based platforms.

5.3. Characterization Methods

In this section will be briefly described the techniques used to characterize the materials used to fabricate the fiber optic sensing structures, namely UV-visible spectroscopy, Fourier-transform Infrared spectroscopy, and Dynamic Light Scattering.

5.3.1. UV-visible Spectroscopy

The absorption of light between 200 and 800 nm by molecules or solvated ions is related with energy changes at electronic levels atomic groups of such molecules. The energy change of a quantized system is usually described by Equation 5.8 [304]:

$$\Delta E = h\nu = \frac{hc}{\lambda} \quad (5.8),$$

where E is the quantized energy, h is the Plank's constant (6.6256×10^{-34} J.s), ν is the frequency of the electromagnetic radiation, c is the speed of light in the vacuum (3×10^{10} cm/s), and λ is the wavelength in the vacuum and can be obtained by:

$$\lambda = n\lambda_{air} \quad (5.9),$$

where n is the index of refraction. In this range of wavelength, there are several information about the properties of the molecules or solvated ions that can be obtained. For instance, in case of zeolite hosts, it is possible to acquire information about the band gap resulted from transitions between the top valence band and the bottom of the conduction band; by the electronic transitions associated with the d orbitals of transition metal ions; and by the charge transfer associated to the oxidation states of metal ions. By other hand, in specific types of molecules such as chromophores it is possible to obtain information about the $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ transitions. Generally, rotational and vibrational motions are also simultaneously excited. The position of a rotation/vibration/electronic transition is established by the change in the electronic energy, while the changes of the other states only give rise to transitions nearby and affect the rotational and vibrational structures of the electronic bands. As was already established, the Beer's law defines the intensity of these absorption bands. The electronic transitions are characterized by Gauss-shaped absorption peaks (plotted as absorbed radiation vs. wavelength), providing qualitative evidence of the presence of the target species, where the absorption band is dependent on the concentration of the analyte. The absorption spectrum can show more than one absorption peak caused by several electronic transitions of the absorbing species or by different entities with absorption properties.

In this work, the UV-VIS spectroscopy was used, first to characterize the absorption properties of a pH-sensitive colorimetric dye, and second to evaluate the performance of a gas-sensitive colorimetric membrane developed from the pH-sensitive dye. Both optical configurations include multimode optical fibers as a light transporter. Figure 5.3 displays the fiber optic UV-VIS spectroscopy setup used to evaluate the absorption properties of the dye (the setup used to evaluate the gas-sensitive membrane performance will be described in detail in the chapter 6). The setup also comprises a halogen lamp light source (model LS-W, Sarspec, Portugal) and a UV-VIS spectrometer (Spec Speed+, Sarspec, Portugal) operating in a wavelength ranging from 250 to 900 nm.

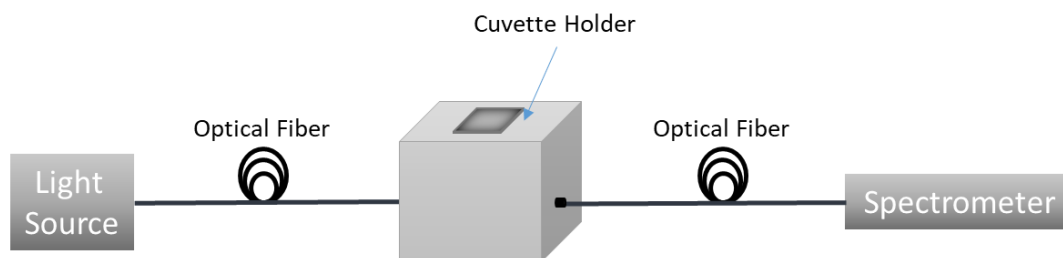


Figure 5. 3. Scheme of the fiber optic UV-visible spectroscopy configuration including (from left to right) the light source, the cuvette box, and the spectrometer.

5.3.2. Fourier-transform Infrared Spectroscopy

Infrared (IR) spectroscopy is a widely used technique that includes interaction of IR radiation with a sample, in which one part is absorbed and the other part is transmitted out of the sample. The resulting spectrum reveals the molecular absorption and transmission. That spectrum can be defined as the molecular fingerprint of the measured sample, giving absorption peaks that correspond to the vibrational and rotational frequencies of bonds between the atoms and the material. Due to the multiplex composition of the molecules and the different arrangement of the atoms, each material will provide a unique spectrum. Initially, the IR instruments were of the dispersive, individually dividing the frequencies of the emitted energy from the IR source. That separation was possible using a prism or a grating. Although IR spectroscopy is a very useful technique, the method presents some drawbacks such as slow scanning caused by the individual measurement of the IR frequencies. In that context, Fourier-transform infrared (FTIR) spectroscopy emerged to overcome those limitations [305]. Briefly, the concept includes the inclusion of an optical interferometer, resulting in the production of a unique type of signal comprising all the IR frequencies. The design includes a light source and a beam splitter that divides the main beam in two distinct beams, launching one of the beams in direction to a fixed flat mirror and other one in direction to a flat mirror that comprises a mechanism that allows the mirror to move a very short distance away from the splitter (typically a few millimeters). Due to this latter characteristic, one path length traveled by the beam is fixed (established by the fixed mirror) and the other path length is periodically changing (controlled by the moving mirror). As the beams interfere with each other, an interferometer is achieved, emitting an optical signal called interferogram. The IR photons reach the molecule with enough energy to cause groups of atoms to vibrate with respect to their bonds. Similar to electronic transitions, the vibrational transitions are related to distinct energies, and the IR radiation is absorbed

by the molecules only a certain wavelength and frequencies. However, the interferogram signals cannot be interpreted directly. A decoding process is applied to the individual frequencies according to Equation 5.11 [306]:

$$S(\nu) = 2 \int_0^{\infty} I(x) \cos(2\pi \tilde{\nu} x) dx \quad (5.10),$$

where $S(\nu)$ is the spectrum, x is the retardation, $I(x)$ is the interferogram, and $\tilde{\nu}$ the wavenumber. This method is the well-known mathematical technique Fourier transformation (FT), that presents a final spectrum with the spectral information related to measurand. The interpretation of the FTIR spectra allows the identification of functional groups and compounds, and also permits to evaluate the purity of the measured material [305]. The information given by the FTIR technique can be divided in two types: qualitative and quantitative.

The qualitative analysis is possible due to the unique combination of the fundamental vibrations or rotations of the different functional groups comprising the molecule under study. The successive interactions of these functional groups with the other atoms of the measurand result in unique and complex IR spectrum of each compound. Thus, IR spectroscopy can be used for structural elucidation.

The FTIR analysis was used in this work to evaluate the surface modifications induced on spherical silica nanoparticles, and the structural modifications caused by the synthesis polymerization of the pH-sensitive dye, using an FTIR spectrometer with a spectral range from 7500 to 370 cm^{-1} and a standard KBr beam splitter (Bruker Tensor 27 IR). The purity of both compounds was also assessed.

5.3.3. Dynamic Light Scattering

The detection of light scattering from matter in solution is a useful tool to study specific properties of solutions/dispersions, by exposing the sample to be measured to a monochromatic wave of light that is further collected by a detector. The light scattering process has been studied and improved over the years, as result of a series of seminal discoveries. *John Tyndall*, studied the scattered light from colloidal suspensions where the particles size was larger than the wavelength of the incident light (known as Tyndall effect) [307]. Later, *Rayleigh* studied the behavior of the light scattered from particles

where their size was smaller than the wavelength of the incident light (Rayleigh scattering), concluding that the refractive index of the scattering medium has a considerable influence in the scattering phenomenon. However, *Gustav Mie* reported research where he describes the scattering of light from absorbing and non-absorbing particles (larger than the wavelength of the incident light), considering the shape of the particles and the difference of the refractive index between the particles and the medium where they were dispersed [308]. *Einstein* and *Smoluchowski* had considered that the liquid is a continuous medium where thermal fluctuations produce heterogeneities, resulting in density and concentration fluctuations. *Einstein* also reported that particles are affected by random forces due to constant collisions with the solvent molecules, flipping the particles in random directions. This random movement was called as Brownian motion. Later, he established the relationship between the particle's diffusion coefficient with their translational friction. This fact was also previously studied by *George Stokes* that suggested that the friction applied by a particle in movement is proportional to its radius and the viscosity of the solvent [308]. Although the reporting of valuable research on light scattering characteristics along the 20th century, was in the middle of the century that *Zimm* concluded the investigations that led to the establishment of the modern light-scattering approaches that have been used to determine the size, shape, and molecular weight of molecules in solution [309, 310]. After some improvements on *Zimm's* work, since its inception, dynamic light scattering (DLS) has proven particularly importance in defining hydrodynamic behavior in several types of solutions/dispersions.

The concept is based on the measurement of the intensity of light scattered from a distribution of particles into a given scattering angle as result of interference on the surface of a square-law detector between light that is scattered from various particles in a certain medium. The phases at the detector of the light scattered depend on the position of the particles in relationship to the direction of the incident and scattered light beams. As the particles are constantly in movement (Brownian motion), their position is frequently changing, thus, consequently, the scattered intensity also fluctuates. These intensity fluctuations occur on the time scale that it takes each particle to move a significant fraction of the wavelength of light. So, to extract valuable information from the scattered signal, its time correlation is computed, usually, using an autocorrelator (a computer specially equipped to allow rapid and real-time calculation of the scattered intensity). Moreover, modern DLS apparatus often uses optical fibers as waveguide to transport the light from the source to the sample and to collect the scattered light and guide it to the detector [311]. In the present work, DLS technique was used to access the size of synthesized silica nanoparticles.

Chapter 6 –Ratiometric Detection of Dissolved Carbon Dioxide in Aquaculture Systems

This chapter aims to study the dissolved CO₂ dynamics in environments similar to aquaculture ecosystems by using a fiber optic colorimetric sensor based on a pH indicator. Firstly, will be described the experiments performed to attain the best combination of materials for CO₂ detection in gas-phase, being than applied to the aqueous phase. Finally, will be displayed and discussed the achievements resulted from the sensor calibration process as well as the results obtained from dynamic studies performed in environments simulating real situations.

6.1. Introduction

Dissolved carbon dioxide (herein noted as dCO₂) is a very important parameter in many different fields, e.g., monitoring oceans, rivers, streams, and lakes, food industry (including aquaculture), and clinical analysis [312-319]. Besides the actual concern with ocean acidification due to the increasing atmospheric carbon dioxide (CO₂) levels [320, 321], dCO₂ evaluation and control is particularly vital in aquaculture, as cultivated species are highly sensitive to the excess of this gas [322]. A detailed knowledge on short-time dCO₂ variations in intensive fish production systems (as are recirculating aquaculture systems, RAS) has been hindered, among other factors, by the long response time of

the commercial sensors that are in use, as they do not allow highly frequent measurements. Moreover, this parameter can change rapidly with normal fish farm operations (e.g., feeding events, which increased fish and bacterial metabolism and thus dCO₂ production), or with performance changes of the recirculated water treatment components, such as the biofilter, gassing (O₂), or degassing (CO₂) units, which are more pronounced in seawater [323]. So, sensors are needed with fast response time to obtain more dCO₂ data per unit of time, which is crucial in studies of dynamic systems, as are the recirculating aquaculture systems [324]. Besides fast response time, high sensitivity is another aspect of interest in intensive aquaculture, not only to assess irregularities in system functioning, but also to monitor dCO₂ concentrations in the very low range (e.g., 1 mg/L), which are expected, for example, in hatchery and nursery production facilities [324]. Nevertheless, dissolved CO₂ detection is difficult because when CO₂ dissolves in water, physical and chemical equilibria occur, and different products are generated [319]. Moreover, commercial sensors for dCO₂ detection and evaluation in aquaculture environments are scarce, and adequate sensors for more demanding operations, such as real-time monitoring or low operation ranges, are missing [325]. So far, known methods for dCO₂ measurements in an aqueous medium, include gas chromatography [319, 326], colorimetric sensors (fluorescence) [327, 328], amperometry [329], potentiometry [315, 330, 331], UV/Vis spectrophotometry [332, 333], and IR spectrometry [319, 334]. However, it is reported that evaluating dCO₂ using some of the techniques referred above is difficult or not adequate for field work, and other analytical techniques are preferred [319]. In aquaculture, the utilization of commercial dCO₂ analyzers (such as OxyGuard, model Portable CO₂ Analyzer, Denmark) has been thoroughly studied and described based on dCO₂ partial pressure detection via IR absorption, needing a certain flow under the probe membrane for best performance [323, 325, 335]. More recently, optical sensors gained strength in the sensing area and for a wide variety of analytes, with no exception for dissolved carbon dioxide. These sensors can be more robust, compact, fast responding, and cheaper [319, 336]. Usually, these optical devices are fluorescence-based (light emission) or colorimetric-based (light absorbance) sensors [319]. An example of the first method utilization is referred by *Atamanchuk et al.*, who used an optrode for measuring the partial pressure of dCO₂ in natural waters [337]. The sensor is based on pH changes originated by CO₂ diffusion and fluorescence signal response. The authors reported that the sensor was able to detect the partial pressure of CO₂ (pCO₂) in a range of 0 – 50000 µatm, exhibiting a precision of about ± 2 µatm in a range of 200 – 1000 µatm. *Contreras-Gutierrez et al.* developed a dCO₂ sensor using a polymer matrix directly combining a fluorescent dye with a

polymer molecule [338]. The resulted membrane exhibited sensitivity to dissolved CO_2 in a range of 4 – 60% (v/v) and a limit of detection of 1.3%. The authors also tested the fluorescent membrane in a fiber optic architecture that was only tested in gas-phase CO_2 . *Thomas et al.* reported the development of a fluorescence based optrode for pCO_2 monitoring, exhibiting sensitivity in a concentration range of 300 – 20000 μatm [339]. More recently, *Li et al.* had presented a hybrid CO_2 ratiometric fluorescence sensor by decorating tetraphenylethene (a phenolic compound) and Au nanoclusters into disulfide functionalized poly(amido amine) skeleton. The fluorescence sensor showed its efficiency by detecting dCO_2 in a range of 0.38 – 19 mmol/L , with a LOD of 7.8 $\mu\text{mol/L}$ [340]. Colorimetry is also a widely used method to detect carbon dioxide. For instance, *Mills and Yusufu* reported a promising colorimetric-based sensor using a pH-sensitive dye, the thymol blue [312]. Nevertheless, the thymol blue membrane decreases its sensibility in water, which can be a problem for applications requiring high sensitivity. *Demirezen and Yilmaz* reported the development of a colorimetric sensor for real-time monitoring of dCO_2 . The architecture is based on a pH-sensitive indicator composed by anthocyanin and PVA coated green iron oxide nanoparticles [341]. The sensor was tested in dCO_2 concentrations ranging from 600 to 3200 ppm, revealing fast detection and complete reversibility. *Lee et al.* applied a colorimetric sensor to monitor dCO_2 in natural waters [342]. The optical platform is based on an acrylic reactor and a cresol red pH indicator solution protected by a gas porous membrane. The sensor was tested in concentration range of 2 – 800 mg/L , exhibiting a response time of around 60 seconds between 10 and 411 mg/L . However, the fact that the sensor uses a liquid solution as a transducer could be seen as a disadvantage for some applications. The examples described above are based on “wet sensors” or “dry sensors”, *i.e.*, are based on CO_2 -sensitive liquid solutions or based on solid-state membranes, respectively. The later type, uses a solid-state system that itself uses a quaternary ammonium, QA^+OH , as a transfer agent (TA) to detect dissolved carbon dioxide, and these form a very popular system these days [319]. However, it is crucial to choose a suitable material according to the required applications. Therefore, a material is required that keeps its sensing properties in different application environments. In the present study, a new sensing device was developed, combining a solid-state membrane based on a colorimetric indicator derivative material, poly *p*-nitrophenol (poly-pNPh), with multimode fiber optic patch cords and a dual wavelength LED. This sensing platform works with a ratiometric detection scheme to measure the absorbance changes caused by the dCO_2 interactions with the chemical membrane. The sensing chemistry comprises a thin layer formed by a colorimetric indicator/solvent/TA/plasticizer generated by spin-coating on

the top of a transparent [®]Mylar foil (as substrate). The resultant layer contains a sensitive dye in its deprotonated anionic form, which is subsequently covered by a hydrophobic silicone layer fixed in a proper support specially designed for the purpose. The sensor was tested in gaseous CO₂ (gCO₂) and for low dissolved carbon dioxide concentrations (needed for adequate water quality monitoring and control) with the goal of studying its viability for aquaculture applications.

6.2. Materials and Methods

6.2.1. Chemical Reagents

For the CO₂-sensitive cocktails synthesis were used the following chemical reagents: *p*-nitrophenol (pNPh, Sigma-Aldrich ReagentPlus[®], ≥99%); tetraoctylammonium hydroxide solution (TOA-OH, Sigma-Aldrich, 20% in methanol); polyurethane Hydrogel D4 (AdvanceSource Biochemicals) and poly(2-hydroxyethyl methacrylate) (pHEMA, Sigma Aldrich). For the pNPh-derivative indicator were used formaldehyde (Sigma Aldrich, 38% in H₂O) and sodium hydroxide (NaOH, reagent grade, ≥97%). For the silica nanoparticle synthesis were used tetraethyl orthosilicate (TEOS; Sigma Aldrich) and ammonia hydroxide (NH₄OH; Supelco, EMSURE[®] ISO) solutions. For the immobilization of the indicator, was used the 3-triethoxysilyl propyl isocyanate (3-IPTES; Sigma Aldrich, 95%).

As primary solvents were used deionized water (Wasserlab, Micromatic – Type II Analytical Grade Water), ethanol (EtOH; AGA, 96% and Absolut), methanol (MeOH; Sigma Aldrich, ≥99.9%), and dimethyl sulfoxide solution (DMSO; Sigma Aldrich, ReagentPlus[®], ≥99.5%).

Sylgard 184 (Dow Corning, 9:1 w/w) was used to fabricate a polydimethylsiloxane (PDMS) gas-permeable membrane to protect the CO₂-sensitive films.

Dry Nitrogen (N₂), supplied from a 50 L bottle (Linde, ≥99.99%) and CO₂, supplied from a 30 L bottle (Linde, ≥99.99%), were used to produce well-known CO₂ concentrations in the gas-phase tests. For the calibration of the sensor in aqueous media, citric acid (C₆H₈O₇, Merck[®]) and sodium carbonate (Na₂CO₃, Sigma-Aldrich, >99.5%) were used. Other chemicals such as hydrochloric acid (HCl, ACS reagent, Sigma Aldrich, 37%) were also used.

6.2.2. pH-Sensitive Dyes

6.2.2.1. *p*-Nitrophenol derivative dye: poly *p*-Nitrophenol

The CO₂-sensitive membranes were fabricated by using the pH indicator *p*-nitrophenol and its derivative obtained by applying a polymerization process. The method intends to extend the pH sensitivity of the base material. The polymerization procedure was accomplished in a close collaboration with members of the Faculty of Sciences of the University of Pécs, Hungary, and following one of the procedures described in the European Patent EP3201619B1 (International publication number: WO 2016/051217) [343].

Describing the procedure, the pNPh-derivative polymer was obtained by a one-step synthesis (Figure 6.1). The pNPh was pulverized using an agate mortar, placed into a double neck round bottom flask and melted using a paraffin oil bath at 116 °C. Then, was added, drop-by-drop, a certain amount of an aqueous solution of formaldehyde and NaOH elevating the temperature to 150 °C that was kept for 24h, resulting in a thick, fluidic, and brown solution. Thereafter, the solution was allowed to cool down at room temperature, followed by the addition of acetic acid until total dissolution of the previous resulted resin. The precipitation of the polymerized indicator was induced by adding distilled water, being then stirred for 24h. The purification of the new material was reached by a dialysis process. After that, it was centrifuged at 5000 rpm for 30 minutes and allowed to dry at 60-70 °C in a desiccator. Both *p*-nitrophenol and pNPh/formaldehyde condensed polymer (poly-pNPh) were evaluated by FTIR spectroscopy.

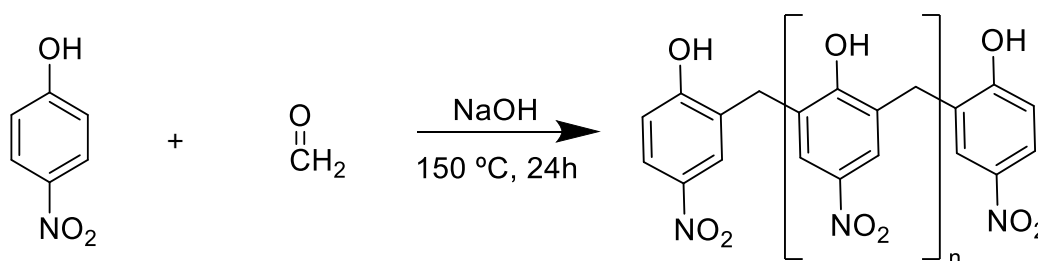


Figure 6. 1. Chemical reaction resulting in the formation of the pNPh/formaldehyde condensed polymer ($0 < n < 20$).

6.2.2.2. Dye absorption properties

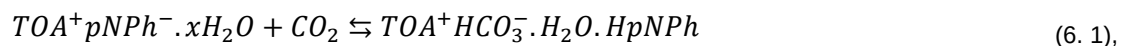
The pH working range and the maximum absorbance wavelength of the pH-sensitive materials was evaluated by applying a titration procedure. A pNPh 20 mM aqueous

solution was prepared in suitable flask equipped with a magnetic stirrer. Then, the solution was acidified (until reach 2-3 pH units) with a 0.01 M HCl solution, and its pH was measured by using a commercial pH meter (Hach, HQd Portable Meter). Under stirring, were added small amounts of a 0.01 M NaOH solution, until titration was completed. The same procedure was applied to the poly pNPh material. The polymer maximum absorbance wavelength was assessed by UV-vis spectroscopy. The absorbance evaluation was executed by transferring 1 mL of the poly pNPh solution to an optical poly(methyl methacrylate) (PMMA) UV cuvette ([®]Brand) and measuring the light absorption emitted by a 600 μ m silica core multimode optical fiber (Thorlabs). On the other side, was placed a similar optical fiber to collect the light and drive it until a mini spectrometer (Hamamatsu C12880MA), that processed the light in a wavelength range between 300-900 nm. A tungsten continuous light source (LS-W20; Sarspec, Portugal) was used as light source.

6.2.3. Sensing Methodology and CO₂-sensitive Membranes Fabrication

6.2.3.1. Sensing Chemistry

The chemistry behind the sensitivity of this material to CO₂ involves an ion-pair formation between a quaternary ammonium (TOA-OH) and the pH-sensitive colorimetric indicator (TOA⁺pNPh⁻). The interaction of the pNPh with the TOA-OH converts and keep the sensitive dye in its deprotonated anionic form. The interaction with the gas seems to be linked to the association of this ion-pair with a few molecules of water (TOA⁺pNPh⁻.xH₂O) absorbed from the air humidity following the Equation 6.1 (adapted from [312]):



where the first part of the equation characterizes the pH-sensitive dye in its anionic form, stabilized by the presence of the cationic form of the TOA-OH. The CO₂ interacts with the membrane, reacting with the molecules of water present in the polymeric matrix, resulting in the formation of carbonic acid. In this form, the system can protonate the pNPh (HpNPh), thus decreasing the internal pH which results in a color change. The excess of the quaternary ammonium stabilizes the system by forming a stable ion pair with the carbonate resulted from the reaction between the gas and water. The reaction stops when the equilibrium between the film matrix and the external medium is established. When that equilibrium changes, favoring a decrease of the gas concentration in the external medium, the gas is forced to leave the film internal system

to reach a new equilibrium, thus decreasing the internal pH, leading a color recovering by the membrane.

6.2.3.2. Sensitive Membranes Fabrication

For the CO₂-sensitive films preparation the pNPh and the poly-pNPh pulverized materials were dissolved in the respective solvent (or mixture of solvents) and combined with the quaternary ammonium to create the CO₂-sensitive cocktails. All the cocktails were mixed with a hydrogel solution to obtain cross-linked gel mixtures. Table 6.1 shows all the cocktail combinations tested in this work.

Table 6. 1. Description of the composition of the CO₂-sensitive cocktails tested in this work.

CO ₂ -sensitive cocktail	Material	Solvent	Phase Transfer Agent	Hydrogel Material
C1	pNPh	MeOH:H ₂ O	TOA-OH	Hydrogel D4
	(Mw = 139.11 g/mol)	(3:2, v/v)	(20%, in methanol)	(10%, in ethanol)
C2	poly-pNPh	MeOH:H ₂ O	TOA-OH	Hydrogel D4
	(Mw = 903.66 g/mol)*	(3:2, v/v)	(20%, in methanol)	(10%, in ethanol)
C3	poly-pNPh onto silica	DMSO	TOA-OH	pHEMA
	nanoparticles	(99.5 %)	(20%, in methanol)	(20%, in DMSO)

*chain length was estimated with n = 4.

The CO₂-sensitive cocktails described in table 6.1 were accomplished as following:

- **C1** and **C2**: respective amounts of each pH-sensitive materials were dissolved, in suitable containers, in a MeOH:H₂O mixture using a magnet stirrer. After total dissolution, was added, to each container, the respective volume of a 0.5M TOA-OH solution (20% in methanol) and the mixture was stirred for 1h. The volume of the quaternary ammonium was calculated to be, at least, 20x higher (molar ration) than the molar quantity of each pH-sensitive material. Thereafter, a certain volume (comprising 10% of the final cocktail volume) of a hydrogel D4 solution (10% in ethanol) was added and the solution was stirred for another 1h to form the networking chain. The

CO₂-sensitive membranes resulted from the cocktails **C1** and **C2** were named as **M1** and **M2**, respectively.

- **C3**: the silica nanoparticles (SiNP) were synthesized according with the *Stöber* method. Absolute ethanol and TEOS were mixed (7:1 v/v) in a proper container equipped with a magnetic stirrer. Under stirring, was added, drop-by-drop, a certain volume (10% of the ethanol/TEOS volume) of a 25% NH₄OH solution. After 30 minutes of continuous stirring, the mixture was placed into an ultrasonic bath for 30 minutes, followed by 23h of continuous stirring at room temperature and 1h at 80 °C. After cooling down, the resulted solution was centrifuged at 5000 rpm for 20 minutes, followed by two washing processes with ethanol. After final centrifugation, a small amount of absolute ethanol was added to the nanobeads, and the suspension was sonicated for 30 minutes. The final silica nanobeads solution was decanted on a suitable support and allowed to dry at 40 °C.

For the indicator immobilization, the poly-pNPh was dissolved in a DMSO solution, followed by the addition, drop-by-drop, of 3-IPTES (3:1 v/v; 3-IPTES:DMSO) and was stirred for 24h. Thereafter, the dried nanoparticles were resuspended in absolute ethanol where was added, under stirring and drop-by-drop, a certain volume of the IPTES-modified poly-pNPh solution (1:1.5), followed by the addition of the same volume of 25% NH₄OH solution. The resulted solution was stirred for 24h, followed by washing processes with ethanol (including centrifugation) and was allowed to dry at room temperature. As result, the poly-pNPh molecules are attached to the silica nanobeads surface (Figure 6.2).

For the cocktail preparation, the dried poly-pNPh/silica beads were mixed with the TOA-OH solution and stirred for 1h. Then, was added, under stirring, a certain volume of a pHEMA solution (20% in DMSO) comprising 10% (v/v) of the final cocktail solution. The CO₂-sensitive membrane resulted from the cocktail **C3** was named as **M3**.

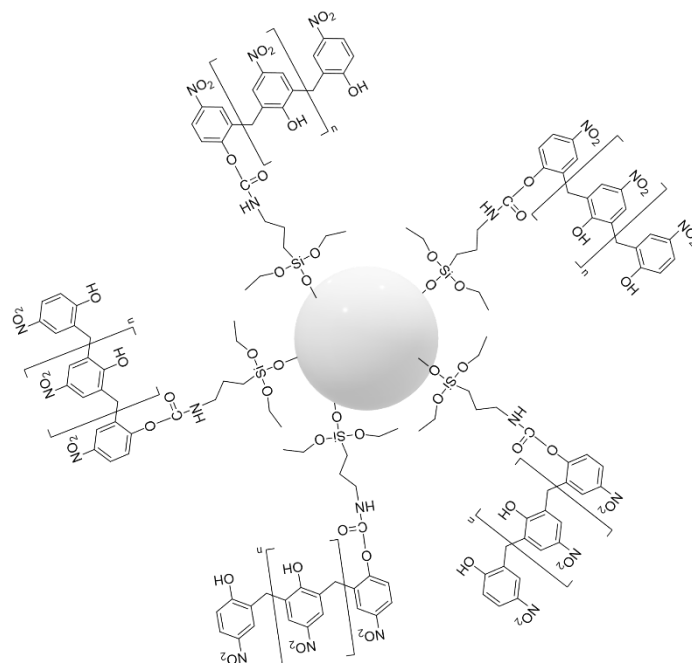


Figure 6. 2. Poly-pNPh immobilized on a SiNP surface.

All the membranes were attained by spreading the respective cocktail on the [®]Mylar foil by spin-coating (homemade equipment) at 600 rpm for 60 s and allowed to polymerize at room temperature and high relative humidity for 2h. The resulted membranes were kept at 4 °C until use. Before use, the main membrane was divided in small membranes to better fit in the sensing device. The fabrication process is schematized in Figure 6.3.

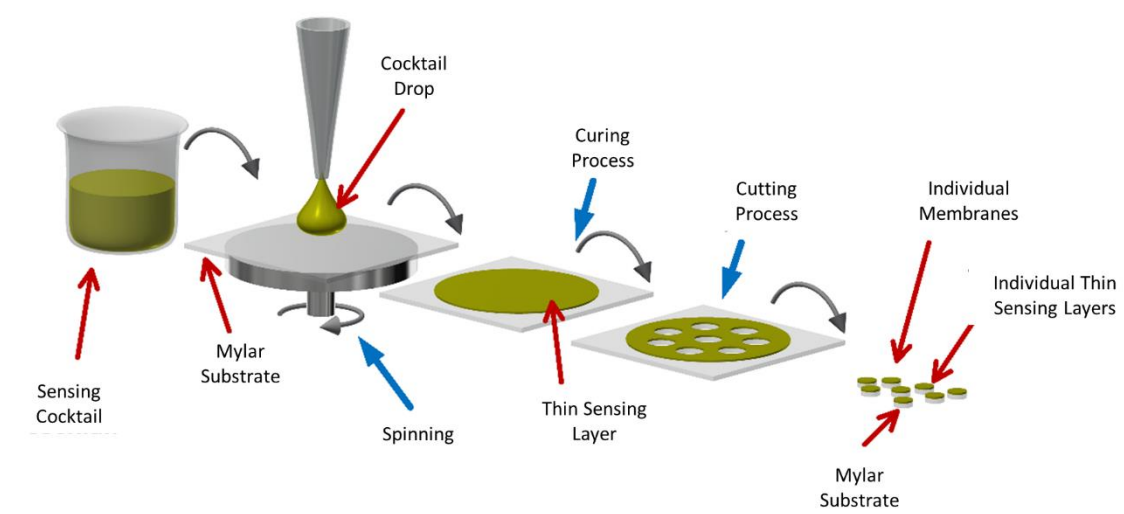


Figure 6. 3. Schematic representation of the CO₂-sensitive membranes fabrication process.

6.2.4. Optrode Configuration

The sensing platform is based on a transmission colorimetric setup combining a sensing layer placed between a dual wavelength LED and a large core optical fiber (200 μm , $\varnothing$). To protect the CO_2 -sensitive membranes from air dust or from water (in gCO_2 and dCO_2 measurements, respectively), the sensitive films were encapsulated between two gas-porous PDMS membranes. For that process, two plastic supports covered by thin films (approximately 90 μm) of Sylgard 184 (base:curing agent, 10:1), previously cured, were used (Figure 6.4b). The sensing membrane was placed between the plastic parts that were sealed with fresh silicone and allowed to dry overnight at 25 $^{\circ}\text{C}$. The plastic supports and the sensor head structure were 3D printed (Zortax M2000; material: Z-Ultra T). As shown in Figure 6.4a, the sensor composed by a light-emitting diode (LED) box containing an additive color model in which red, green, and blue light are added together in various ways to reproduce a broad array of colors (a.k.a. RGB LED; Kingbrighth Europe), powered by an electric wire, working in two specific wavelengths with maximum absorbance at: 460 nm for blue light (detection band) and 645 nm for red light (reference signal). Thus, the optical configuration allows the ratiometric detection of CO_2 by measuring the absorption changes caused by the gas in the established detection range and the absorption changes caused by other species or spectral fluctuations in the reference range. A fiber optic connector was placed in the other extreme to attach a fiber optic cable to guide the light to a mini spectrometer (Hamamatsu C12880MA) in the UV-Vis band incorporated into an electronic platform specially designed for this purpose. Both sides of the sensing head are separated from the external medium by an optical window (glass) that also contains two plastic lenses (Roithner LaserTechnikGmbH) inside, one to collimate the beam of the emitted light to the center of the membrane, and other to improve the collected signal by collimating the transmitted beam to the optical fiber. This configuration allows a great proximity (approximately 90 μm distance) between the aqueous medium and the sensing surface, which measures without stirring (similarly to potentiometric electrochemical sensors and some optrodes). The use of fiber optic cables allows for potentially real time, multipoint, and continuous measurements as well as the construction of a miniaturized sensing platform. As the mini spectrometer needs to be connected to a PC, the data acquisition is possible at a larger scale. For data acquisition and real-time data observation, homemade LabView interactive software was designed to process the optical signals.

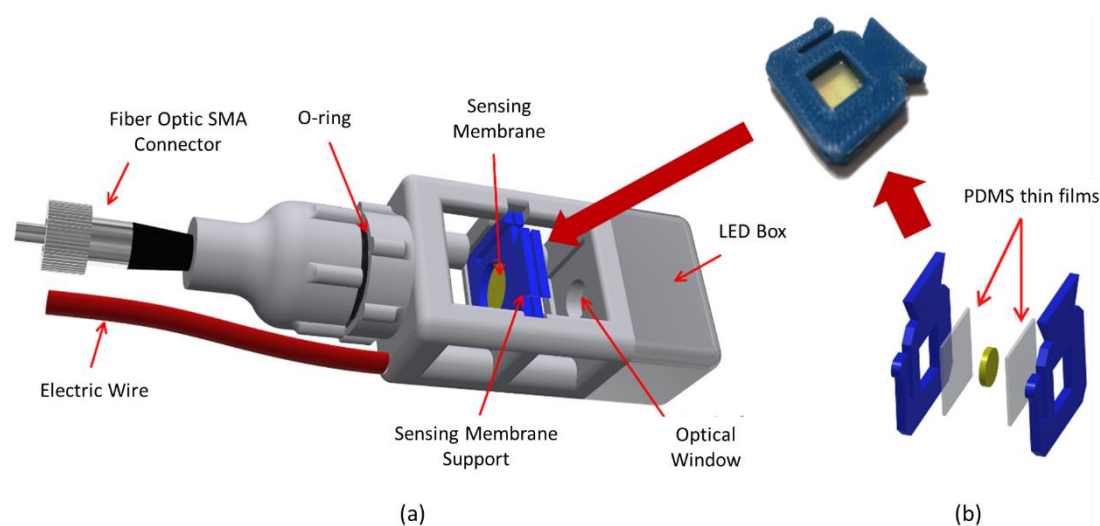


Figure 6. 4. Schematic illustration of: (a) sensor head arrangement, including the encapsulated CO₂-sensitive membrane placed between the LED box and the light receiver components; and (b) the encapsulation method and a picture of the final encapsulated membrane.

6.2.5. Experimental Procedure and Setup for gCO₂ Assessment

A set of experiments was completed to evaluate the sensitivity of the fabricated membranes, M1, M2, and M3, in gas-phase CO₂. Therefore, using the sensor head described in the previous section, the membranes were tested with a humidified gas mixture composed by CO₂ and N₂ supplied by gas bottles. The percentage of the gases in the mixture was varied using high-resolution mass-flow controllers (Brooks, SLA5800 Series). The gases were previously mixed in a proper vessel, and then the mixture was transferred to the humidification vessel. The humidified gas-mixture was then transferred to the experimental container where was placed the sensor head. Figure 6.5 exemplify the assemblage of the experimental setup. The performance of the CO₂-sensitive membranes was assessed by mixing different amounts of CO₂ and N₂, keeping the flow constant, and varying the CO₂ concentration from 0 (*i.e.*, 100% of N₂) to 0.7% of CO₂. All the three experiments had the duration of 35 minutes, changing the gas concentration every 5 minutes.

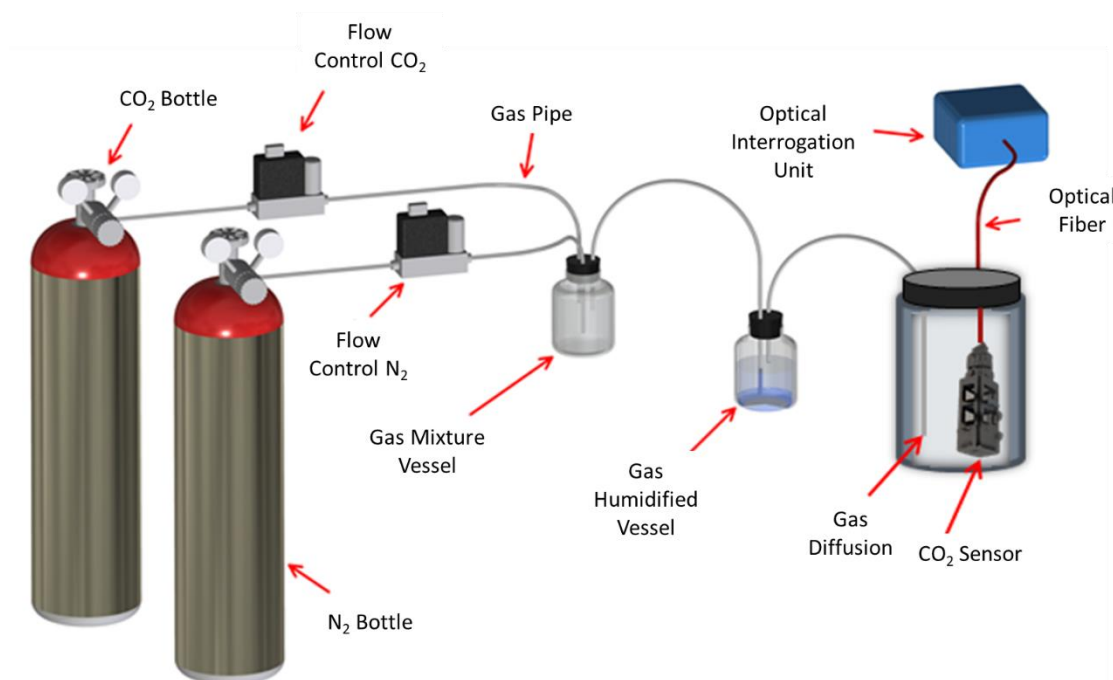


Figure 6. 5. Experimental setup for humidified gCO₂ assessment.

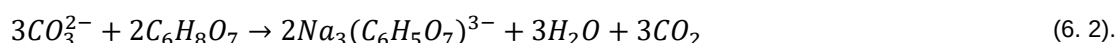
6.2.6. Experimental Procedure and Setup for dCO₂ Optrode Characterization

In order to develop an optical sensor for dissolved carbon dioxide detection in fish culture applications, the membrane with the best performance in the gaseous carbon dioxide tests was selected. The experimental procedure to attest the applicability of the developed sensor for dCO₂ detection was conducted using the same protocol used for the gCO₂ assessment. However, the experimental container was filled with 0.2 L of deionized water to dissolve the gas mixture. To calibrate the sensor in order to be used in aqueous applications, a chemical calibration was completed. The calibration procedure as well as the assessment of the dCO₂ optrode parameters in different experimental conditions are described in the further subsections.

6.2.6.1. Optrode calibration for dCO₂ detection

The sensor was calibrated in two types of water: fresh and saline water (salt concentration = 2.5%). In both situations the calibration carried out using CO₂ standard solutions ranging from 1 to 20 mg/L (maximum concentration recommended for fish farming [344]). The CO₂ standard solutions were attained by using a standard Na₂CO₃ solution (0.300 ± 0.001 g dissolved in 50.00 ± 0.05 mL of ultra-pure water) with well-known dCO₂ concentrations in deionized water and in the saline solution, which were

previously acidified with citric acid ($\text{pH} \approx 3$; $V = 200 \text{ mL}$). The sensor was placed into a proper and semi-closed container equipped with a magnetic stirrer apparatus. Here, the agitation is just required to promote the acidic reaction of the Na_2CO_3 and generate the CO_2 . Hence, proper amounts of Na_2CO_3 were added to the acidified water, stirring the mixture for 10 seconds. The stirrer was stopped for data acquisition. The formation of carbon dioxide results from the stoichiometric reaction of sodium carbonate in an aqueous citric acid solution, as shown in Equation 6.2.



The procedure was adopted to compare the results with the calibration procedure implemented by a commercial dCO_2 analyzer recommended for fish culture (OxyGuard CO_2 Analyzer[®] with data logging). Through the calibration curve it was possible to determine the sensor limit of detection (LOD) and the limit of quantification (LOQ) using the “calibration curve parameters method” [345]. The mathematical Equations 6.3 and 6.4 were used for LOD calculation, where Y_{LD} is the instrumental signal of the LOD, a_0 is the zero intercept value, t is the unilateral critical value for a 90% confidence interval for $(n-p)$ degrees of freedom (n is the number of points of the calibration curve; p is the number of parameters), SD_{a_0} is the standard deviation of the zero intercept value, X_{LD} is the concentration corresponding to the LOD instrumental signal, and b is the slope of the calibration curve. Equations 6.5 and 6.6 were used for LOQ, where Y_{LQ} is the instrumental signal of the LOQ, and X_{LQ} is the concentration corresponding to the LOQ instrumental signal (adapted from reference [344]). All the other symbols for Equations 6.5 and 6.6 are described in the description of Equations 6.3 and 6.4.

$$y_{LD} = a_0 + 2 \times t_{0.10(n-p)}^u \times SD_{a_0} \quad (6.3).$$

$$x_{LD} = \frac{y_{LD} - a_0}{b} \quad (6.4).$$

$$y_{LQ} = a_0 + 3 \times 2 \times t_{0.10(n-p)}^u \times SD_{a_0} \quad (6.5).$$

$$x_{LQ} = \frac{y_{LQ} - a_0}{b} \quad (6.6).$$

6.2.6.2. Assessing to optrode parameters: precision, accuracy, response time, and resolution

The calibration procedure was followed by sequential tests to evaluate precision (in the form of coefficient of variation; CV, % = standard deviation (SD)/mean $\times$ 100), accuracy ((real value – experimental value)/real value), and response time (signal time stabilization to 95% of equilibrium response, $t_{95\%}$) in the following water conditions: deionized water and saline water (2.5%). Different dCO₂ concentrations were tested in the range from 1 to 20 mg/L. Three independent runs were done for each concentration, and the data acquisition was made every 100 milliseconds. As the response time, it was considered the time taken by the sensor to reach a new concentration starting from the immediately preceding concentration (cumulative time). With the goal of comparing the new sensor response time with the response time of the used commercial sensor, tests using the two different devices were made in deionized water. The optrode resolution (defined as the smallest variation that the sensor can distinguish) in very low dCO₂ concentration increments was also tested in deionized water. So, known dCO₂ concentrations were varied by the incremental addition of 0.25 mg/L between 1 and 2 mg/L until total signal stabilization. For this study, standard solutions were used as described in subsection 6.2.6.1.

6.2.7. dCO₂ Dynamics in a Recirculating Shallow Raceway System (SRS+RAS) Prototype

6.2.7.1. Prototype Characteristics

One of the aims of this work was to contribute to a solution for the dCO₂ monitoring needs of the aquaculture industry. Nevertheless, when working in real aquaculture facilities, several uncontrolled events occur, which need to be avoided to guarantee consistent testing and the monitoring of dCO₂ in water. This can only be achieved in controlled laboratory conditions. Therefore, to be able to simulate diverse intensive aquaculture situations, a laboratory prototype was needed. Therefore, a shallow raceway tank (SRS) was used with 0.4 m² of area and 7 cm of water height, as reported by *Borges et al.*, which was linked to a water treatment loop (with sump, mechanical filter, and aerated biological filter), representing a total working volume of 100 L [325]. The whole system mimicked a recirculating aquaculture system (RAS) working at >95% water recycling.

6.2.7.2. *dCO₂ dynamics studies in the SRS+RAS prototype*

Gas injection was done using a special cylindrical diffuser (Dupla CO₂ reactor 400), which was connected to an Eheim Hj-731, 550 L/h pump. Carbon dioxide was delivered by an aquarium-type compressed CO₂ system (Dupla CO₂ Set Delta 400). The injection device was placed near the bottom of the tank, after the inlet pipe. The continuous monitoring of dCO₂ was made using the dCO₂ optrode as well as the OxyGuard CO₂ Analyzer® with logging capabilities. Both devices were placed near of the prototype tank outlet. Two independent runs were done, injecting gaseous CO₂ for 1 h and 15 min with a flow rate of approximately 10 mL/min. The next run was carried out after complete CO₂ degassing, as indicated by zero readings in the sensors. In both tests, trout food was introduced into the water, and after some time, the excess was removed to simulate the fish feeding process and study the behavior of the optrode under these conditions. The experiments were performed in a controlled temperature room at 15 °C, using dechlorinated tap water.

6.2.8. Continuous dCO₂ Monitoring in an Experimental Fish Culture System with Water Recirculation

Short time monitoring of a fish culture system was performed in a laboratory fish culture system using water recirculation in order to evaluate the behavior of the sensor under in situ conditions and continuous dissolved CO₂ monitoring. The aquaculture recirculation system consisted of two fish tanks of 300 L each and contained a mechanical filter, a sump tank with a trickling filter, and a third tank equipped with a moving bed biofilter (MBBR). The system, with a total volume of 1000 L of freshwater, was operated at 80% RAS. Cultivated fish were rainbow trout (*Oncorhynchus mykiss*), with 110 g average weight and a density of 10.6 kg/m³. Sampling points comprehended one fish tank and the tank containing the MBBR biofilter. The dCO₂ optrode and the commercial sensor Oxyguard CO₂ Analyzer® were used simultaneously for dCO₂ evaluation and results were compared.

6.3. Results and Discussion

6.3.1. pH-sensitive Dyes

After polymerization and in order to understand the main differences between their chemical structures, both pNPh and poly-pNPh were evaluated by FTIR spectroscopy in

a wavenumber frequency ranging from 600 to 4000 cm^{-1} (Figure 6.6). Considering Figures 6.4a and 6.5b, the peaks observed at 3300 cm^{-1} and 1330 cm^{-1} are caused by the O-H stretching vibration and O-H bending (characteristic in phenol compounds), respectively. Moreover, the peaks observed at 3085 cm^{-1} , caused by C-H stretch, confirm the presence of the aromatic ring as well as the existence of peaks at 1590 and 1500 cm^{-1} that are attributed to the aromatic ring C-C stretching vibrations. The IR peaks attributed to the presence of nitro compounds usually appear around 1500-1550 cm^{-1} . However, when such groups are attached to aromatic rings, the N-O stretching bands shift to lower wavenumbers [346]. Thus, the peaks observed at 1490 cm^{-1} can be attributed to the N=O asymmetric vibrations. Furthermore, the C-N bonding IR mode (from the C-NO₂ bond) is proved by the peaks observed at 1100 cm^{-1} . Finally, the main different between both IR spectra can be found at 910 cm^{-1} (highlighted in blue color). The peak observed in Figure 6.5b (that is not observed in Figure 6.5a) is attributed to vibrations of the polymer chain, proving the presence of several molecules of pNPh constituting a polymeric sequence [343].

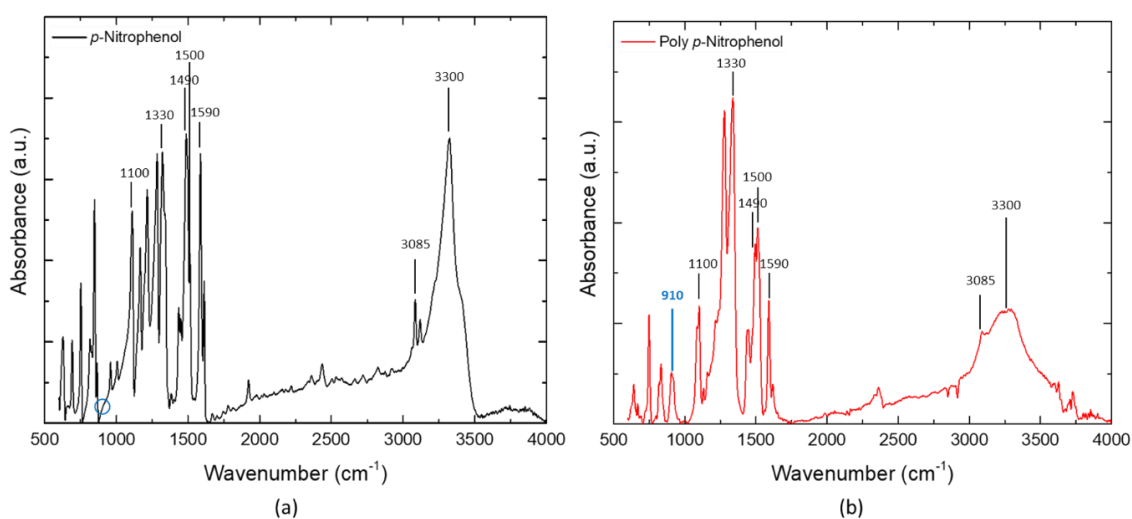


Figure 6. 6. Fourier-transform Infrared spectra: a) p-nitrophenol; and b) poly p-nitrophenol. Black lines attributing peak positions represent the peaks shared by both spectra. The blue circle and blue line attributing peak position, characterizes the main structural differences between spectra.

The pH-sensitivity of both materials was assessed by realizing a titration procedure. Hence, were prepared two distinct aqueous solutions that were posteriorly acidified with a certain amount of a 0.01 mol/L HCl solution until the pH meter point a pH value between 2 and 3. The titration proceeded by pipetting small amounts of a 0.01 mol/L NaOH aqueous solution and recording the pH variations. Figure 6.7 show both titration curves.

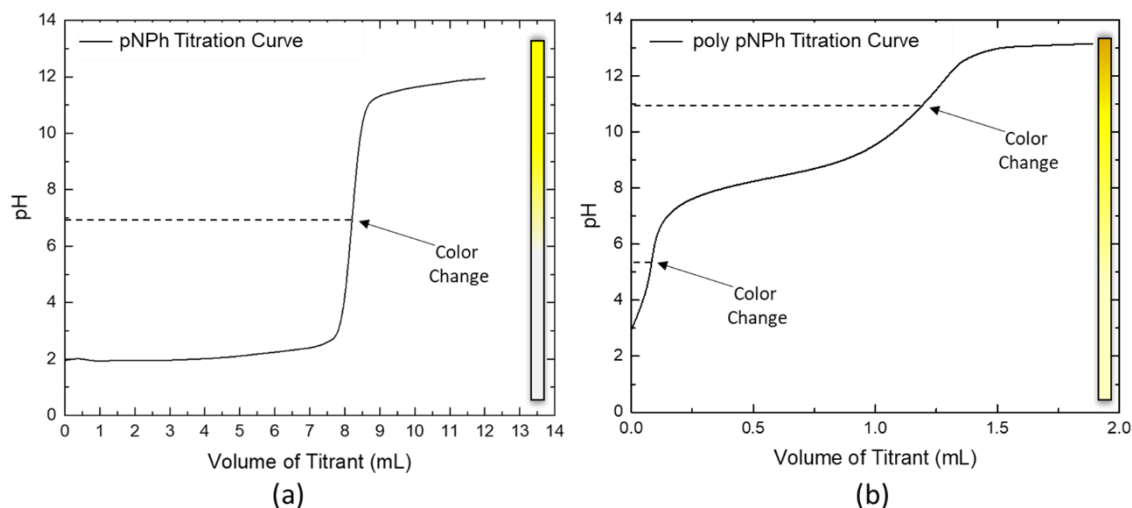


Figure 6. 7. Titration curves obtained for pNPh (a) and for poly-pNPh (b), pointing at which pH value each solution changed its color.

From the titration curves, it is evident the difference in the range of operation of each indicator. The poly-pNPh (Figure 6.7b) allows to distinguish pH values in a wider range with higher resolution, in which the color of the solution varies from light yellow to dark yellow between pH 3 and pH 10, changing to yellowish brown at pH > 10. Moreover, the shape of the titration curve reveals a better performance to detect small pH variations in the typical working pH range, and healthy for fish growth, used in fish farms [347]. In order to realize in which wavelength range the poly-pNPh cocktail registered maximum absorbance, were prepared aliquots at each pH value and the absorbance was measured in a spectral range from 300 to 900 nm (Figure 6.8).

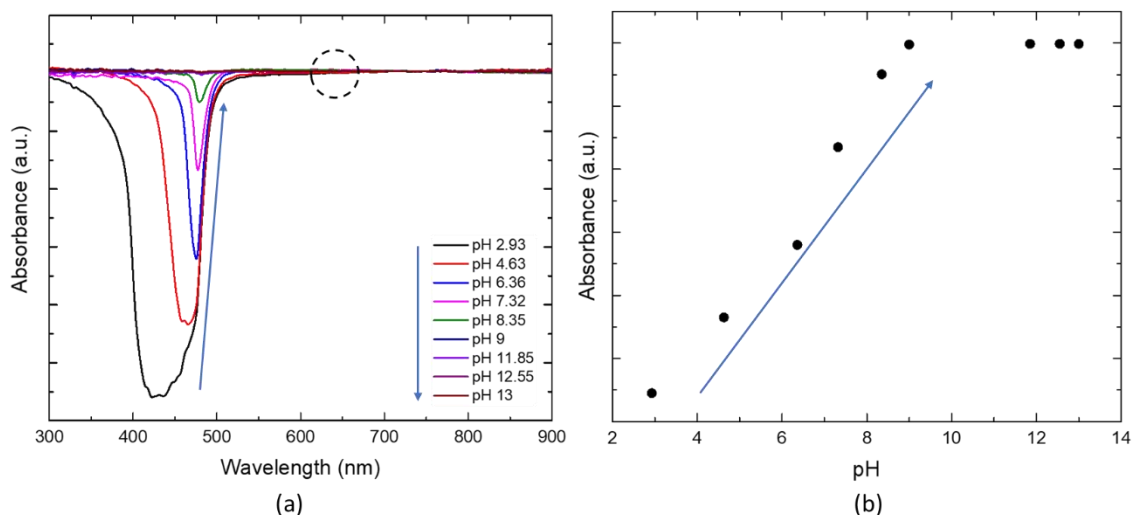


Figure 6. 8. Absorbance spectra (a) obtained by measuring the absorbance of the solutions resulted from the titration of the poly-pNPh at different pH values, and the absorption variation in function of the pH variation (b). The arrows indicate the ascendent direction. The dashed circle represents the wavelength zone chosen to perform the ratiometric measurements.

Considering Figure 6.8 is visible that the colorimetric cocktail registered maximum absorbance between 420 and 475 nm. It is also perceptible that at higher wavelengths was not registered any absorption of light at the different pH values. So, to develop a CO₂-sensitive device offering reliable measurements, the results in the next subsections were obtained using the optical platform described in subsection 6.2.4. The light is provided by a RGB system covering the characteristic absorption wavelength range exhibited by the indicator dye, also comprising a light beam with maximum intensity at 635 nm (given by the red part of the RGB LED). By measuring the changes in both wavelengths, was possible to implement a ratiometric method in data treatment. From here, the colorimetric tests were obtained by using the fabricated solid-state membranes. Figure 6.9 show the aspect of the graph using the RGB system (in which the green light was disabled) and the spectral response variation of a generic membrane exposed to a different concentrations (in %) of gCO₂. From figure, is visible the variation caused by the gas in the indicator characteristic absorption spectral zone, whereas the reference signal (at 635 nm) remains stable.

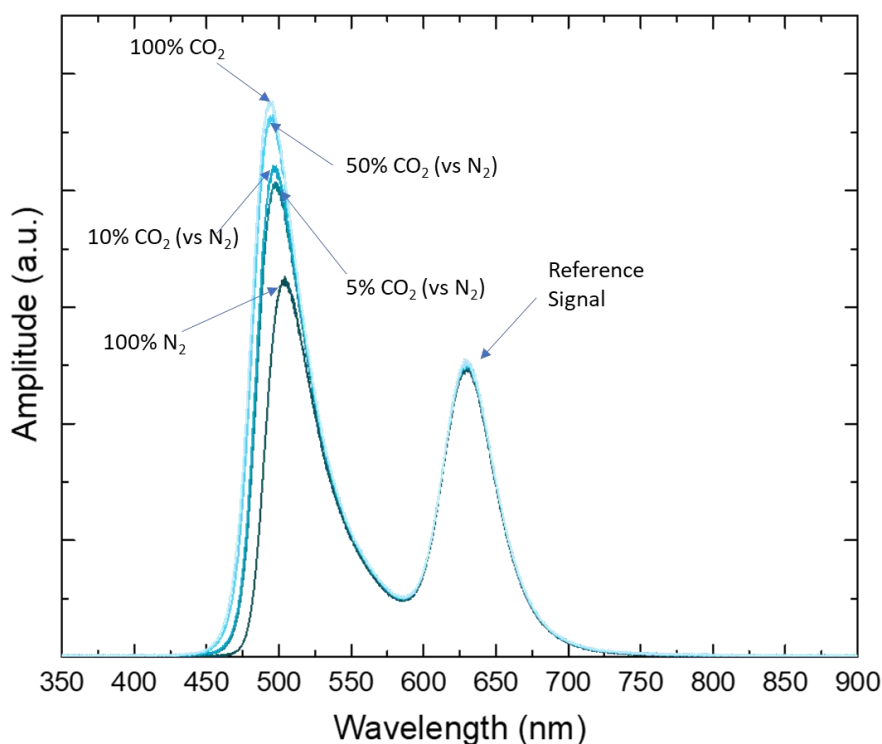


Figure 6. 9. Illustration of the spectral response variation of a generic membrane using the RGB system.

6.3.2. CO₂-sensitive Membranes Performance in Gas-phase CO₂

The performance of the membranes M1, M2, and M3, were evaluated in gas-phase media. The experiment intended to demonstrate which configuration provides better response to carbon dioxide. So, using the experimental setup described in subsection 6.2.5, the tested membranes were exposed to a different gCO₂ concentrations ranging from 0 to 0.7% (vs. N₂). Figure 6.10 show the obtained results. The response of each membrane is quite different. While the M1 membrane (based on the pNPh) responds linearly, the M2 and M3 provides a logarithmic response (both based on the poly-pNPh). That difference can be explained by the highest sensitivity exhibited by the poly-pNPh materials, especially at lower gas concentrations (showed by the steeper slope from 0 to 0.3%). The abrupt change in sensitivity results from the polymerization process which induces modifications on the chemical properties of the indicator [348]. As this system is based on internal pH changes, the acid-base equilibrium between the membrane and the external media is very important [349]. Recently, *Hefferman et al.* reported a new type of polymeric synthesis and realized that the pK_a seems to be linked to the sensory capabilities of the material [350]. This conclusion matches with the results obtained from the titration experiments where were observed more than one states of equilibrium. Polymerization stimulated new chemical properties on the sensing material related to the acid-base equilibrium, increasing the sensory features and the stability between pH changes caused by the membrane-gas interaction.

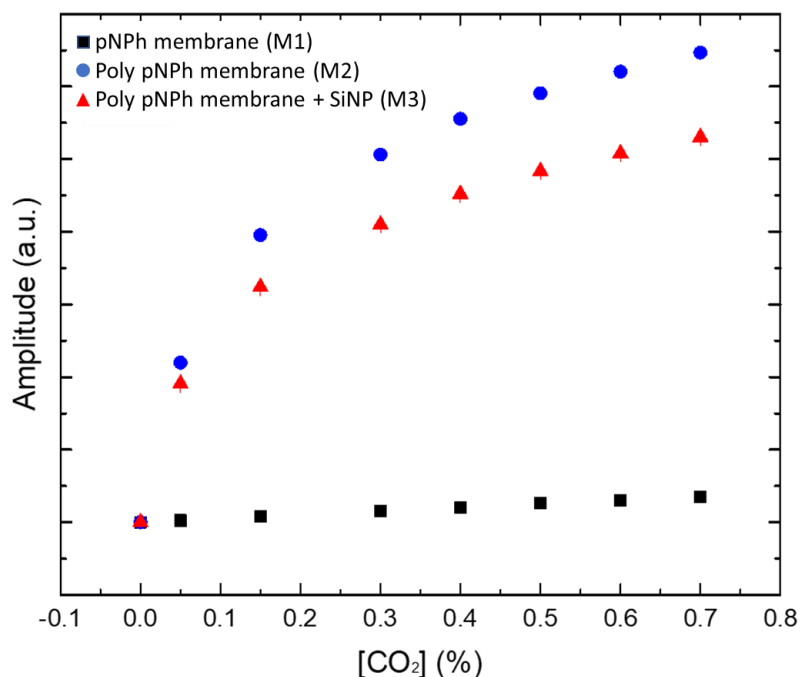


Figure 6. 10. Performance of the M1, M2, and M3 membranes at different gaseous carbon dioxide concentrations.

Comparing the configurations based on the poly-pNPh (M2 and M3), is undeniable that the M2 membrane exhibits a better performance in sensitivity. Although the similar slope at lower concentrations, the M1 membrane stands out in the full range of concentrations. This difference could be explained by the introduction of the IPTES structure used for the covalent immobilization on the silica particles. The introduction of the IPTES molecules inhabit, at least, one -OH group of the poly-pNPh structure to perform amide bonds. Once the sensing methodology depends on the protonation of the pNPh monomeric structures, the introduction of IPTES seems to limit the number of anionic pNPh molecules ($\text{NO}_2\text{-Ph-O}^-$) able to be protonated.

Considering the results obtained from this study, the protocol used to produce the membrane M2 was selected to proceed for the dissolved carbon dioxide evaluation.

6.3.3. CO_2 -sensitive Membranes Performance in dCO_2

As mentioned, for experiments in aqueous media it was required for the membrane to be waterproof. This was a difficult and lengthy process, and several approaches were tried to encapsulate the sensing membrane. The first method attempted consisted of spin coating the used silicone directly on the top of the sensing membrane [351]. However, some reactions between the sensing layer and the silicone were observed, which affected the silicone-curing process. A second attempt used the “knife technique”. At this phase, the silicone was spread on the top of the sensing layer using a spatula, controlling the silicone layer thickness using mechanical guides with known height. As the problems reported before were also verified, a new approach was required. Hence, the solution was to spin coat a clean substrate ([®]Mylar foil) with the silicone, forming a thin cured silicone porous membrane that was subsequently detached from the substrate and then attached onto the sensing membrane using specially designed 3D-printed supports. To study the sensor stability in water, a glass container with 200 mL of deionized water was used and different dCO_2 concentrations (% vs. N_2) were bubbled. Several cycles were done between 0.33% and 1.67% of dCO_2 (always keeping the same flow rate), during more than 3 h. A timeline graph was plotted, which is shown in Figure 6.11a. The stability of the sensor was confirmed. However, some fluctuations are visible when the sensor achieves the target concentration. These variations can be explained by the turbulence produced by the bubbles of gas in water. To compare the behavior of the sensor in the two environments (dCO_2 vs. gCO_2), a test in gas atmosphere, using humidified air, was performed (Figure 6.11b). A total of six complete cycles were done in each experiment, and it is possible to see that in air, the six cycles were completed in 1 h and 40 min against the 3 h and 20 min of the test in water. This

increase in the signal response time is expected due to the time that the gases take to dissolve in water and diffuse through the porous silicone membrane. The resistance offered by the water to the degassing from the membrane also contributes to the increase in the signal response time. As the CO_2 in the system was gas injected in this test, the time observed cannot be measured as the sensor response time and should be considered the setup response time. Moreover, the sensor response time was estimated using a the dCO_2 standard solutions and the resultant data is shown in subsection 6.3.3.2.

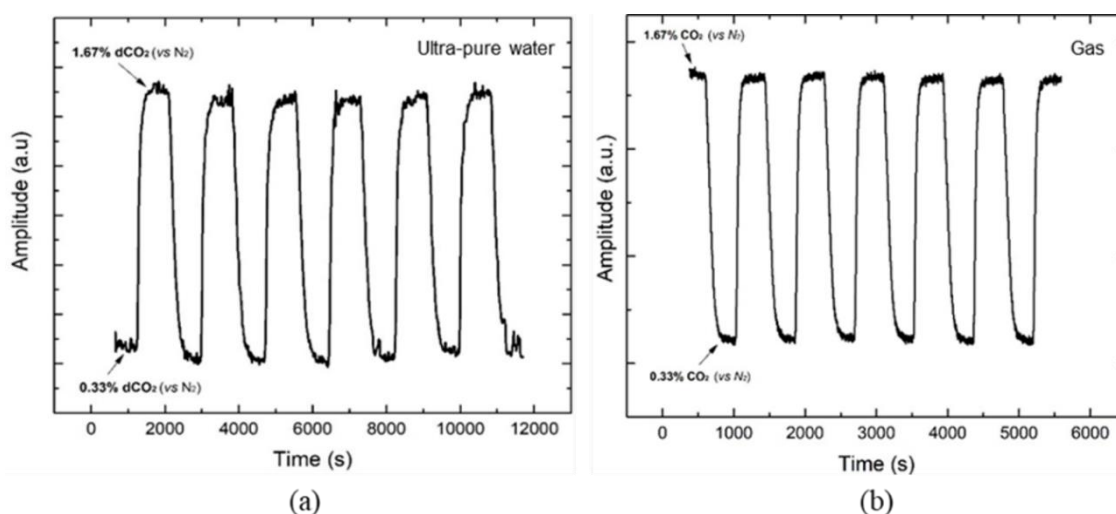


Figure 6. 11. Gassing and degassing cycles of dCO_2 (a) and gCO_2 (b) assessing the stability of the sensor in different media.

Once the dissolution of carbon dioxide acidifies the water, it is acceptable to assume that the sensor responds to the media pH variations. Therefore, to prove that the sensor response in aqueous media is caused by carbon dioxide and not by the acidification of the water, two experiments were executed. In a first place, were made several cycles by bubbling CO_2 into 200 mL of water, registering the variations caused by the gas in the signal of the sensor and the water pH variations, by using a commercial pH meter. The second experiment consisted in immerse both sensors (CO_2 Optrode and pH meter) into the same volume of water, in which the water pH was forced to change by adding citric acid. Figure 6.12 show the data obtained from these trials. In Figure 6.12a is visible both responses according with the gas concentration in water. As expected, when the CO_2 quantity increases the water pH decreases. Observing Figure 6.12b, it is noticeable that the signal given by the optrode is caused by the CO_2 that diffuses through the silicone membrane and reacts with the colorimetric membrane, does not responding to water pH variations.

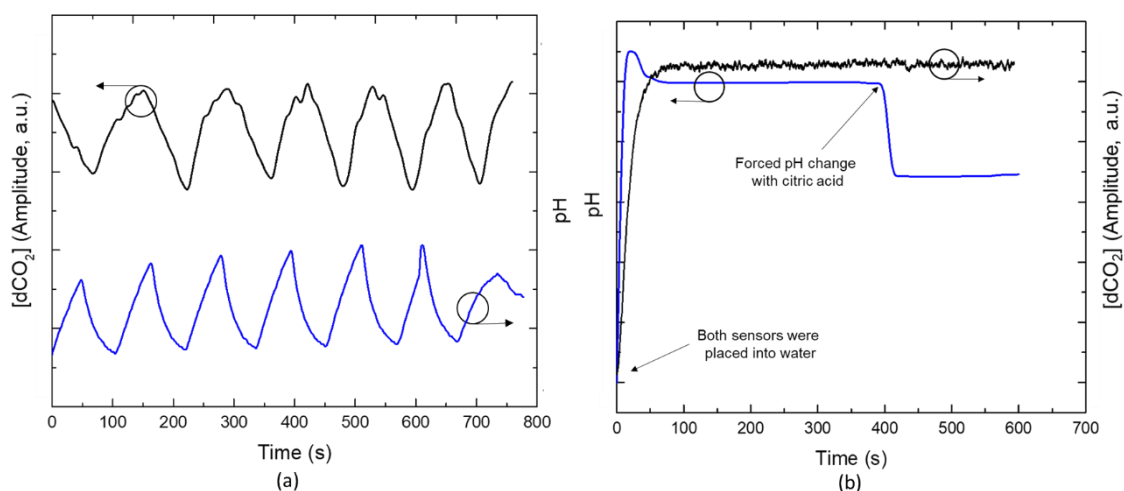


Figure 6. 12. (a) Optical response given by the optrode by applying several CO_2 cycles in water, and respective pH variation; and (b) forced pH change by adding citric acid, recording the changes in both sensing devices.

6.3.3.1. Optrode calibration for dCO_2 detection

The sensor was calibrated for an operation range between 1.00 and 20.00 mg/L dCO_2 in different aqueous solutions (deionized water and saline water at 2.5%). As referred before, this is the relevant concentration range for aquaculture operations. Calibration curves and calibration data can be found in Figure 6.13a and Table 6.2, respectively. The sensor response to the successive standard dCO_2 solutions is logarithmic, so the data were plotted as a function of dCO_2 logarithmic concentration to attain linearity. The results show a slight difference in the response given for fresh and saline water. This interfering process resulted in unexpected results obtained for the calibration curve in saline water, which should not meet the deionized water calibration curve in the complete concentration range. As referred in literature, the solubility of CO_2 in fresh water is higher than in saline water (at the same pressure and temperature), decreasing as the gas concentration increases for both waters [352]. Eventually, the presence of salt is affecting the silicone membrane permeability and interfering with the calibration process. The understanding of this process needs more extensive study. To understand the impact of the ratiometric system on the analytical data treatment, Figure 6.13b displays the difference between the calibration curves, obtained for deionized water, applying the ratiometric method (corrected data) and the raw data (normal data). It is undeniable the impact of the ratiometric method on the linear regression (R^2) of the curves.

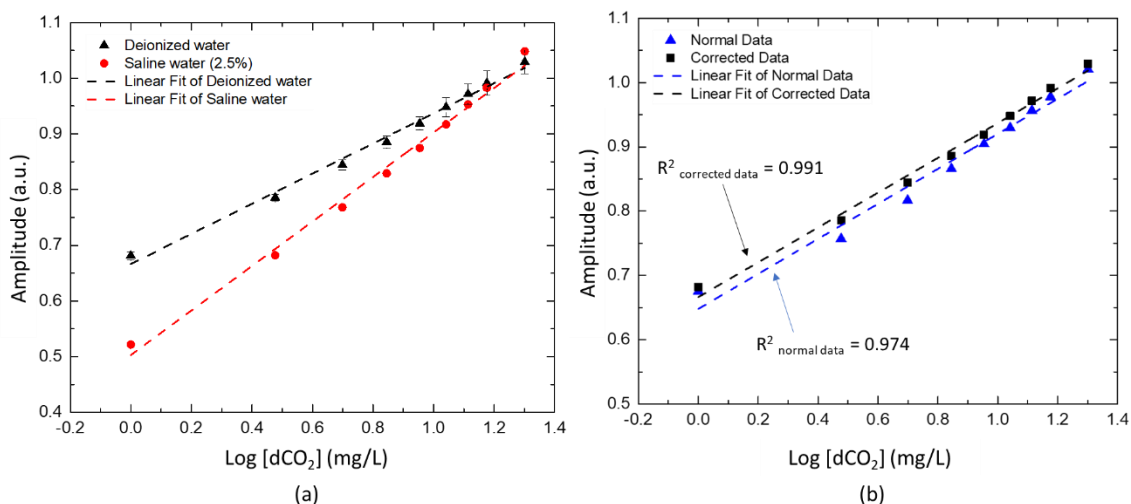


Figure 6. 13. (a) Optrode calibration curves for deionized (black triangles) and saline (2.5%; red circles) water; and (b) comparison between the calibration curves plotted with the raw data (blue triangles) and by applying the ratiometric method (black squares). The dashed lines represent the linear fit of each curve.

The LODs and LOQs (Table 6.2) resulted from the calibration curves obtained for deionized and saline water (Figure 6.11a) are also different. Nevertheless, these results suggest that the sensor is suitable to work in the concentration range found in aquaculture for both types of water [353].

Table 6. 2. Calibration data obtained for the chemical calibration of the new optrode and the respective limit of detection (LOD) and limit of quantification (LOQ).

Calibration	Intercept	Slope	P	R ²	Adj. R ²	LOD	LOQ
$y = a + bx$	(a)	(b)				(mg/L)	(mg/L)
Deionized water	0.666 ± 0.008	0.271 ± 0.009	0.996	0.992	0.991	1.225	1.843
Saline water (2.5%)	0.501 ± 0.013	0.405 ± 0.014	0.996	0.992	0.991	1.048	1.154

P: Pearson's; R²: R-Square;

6.3.3.2. Optrode precision, accuracy, and response time in deionized and saline water (2.5%)

The dCO₂ optrode performance data for a range of concentrations between 1.00 and 19.8 mg/L in different aqueous solutions can be found in Table 6.3. There are different ranges for precision in the different aqueous solutions. For deionized water, the range of precision is from 5.87% to 19.1%, while for saline water (2.5%), the range of precision is from 1.19% to 1.43%. These results show that the optrode is more precise for measurements in saline water. It is possible to see that the precision in both cases is

better in the range from 1.00 to 8.97 mg/L, which is the less dangerous range to fish life and that which is normally is found in aquaculture waters. The accuracy changed from the extreme concentrations (1.00 and 19.8 mg/L) to the intermediate concentrations: 2.99 to 10.9 mg/L for deionized water and 2.99 to 14.9 mg/L for saline water (2.5%).

Table 6. 3. Optrode performance (mean $\pm$ standard deviation; n = 3) in different aqueous solutions at different dCO₂ concentrations. CV: coefficient of variation.

[dCO ₂] (mg/L)	Deionized Water (0%)			Saline Water (2.5%)		
	M.C. ¹ (mg/L)	P ² (CV%)	Acc ³ (mg/L)	M.C. (mg/L)	P (CV%)	Acc (mg/L)
1.00	1.14 $\pm$ 0.10	5.87	0.14 $\pm$ 0.07	1.13 $\pm$ 0.01	1.19	0.13 $\pm$ 0.01
2.99	2.77 $\pm$ 0.14	5.15	0.08 $\pm$ 0.05	2.81 $\pm$ 0.03	1.00	0.06 $\pm$ 0.01
4.99	4.56 $\pm$ 0.34	7.52	0.09 $\pm$ 0.07	4.58 $\pm$ 0.07	1.50	0.08 $\pm$ 0.01
6.98	6.47 $\pm$ 0.62	9.56	0.09 $\pm$ 0.06	6.49 $\pm$ 0.09	1.51	0.07 $\pm$ 0.01
8.97	8.61 $\pm$ 0.88	10.2	0.09 $\pm$ 0.03	8.41 $\pm$ 0.11	1.31	0.06 $\pm$ 0.01
10.9	11.1 $\pm$ 1.7	15.1	0.11 $\pm$ 0.10	10.7 $\pm$ 0.2	2.70	0.02 $\pm$ 0.02
12.9	13.5 $\pm$ 2.2	15.9	0.11 $\pm$ 0.11	13.1 $\pm$ 0.2	1.51	0.02 $\pm$ 0.01
14.9	16.1 $\pm$ 3.2	19.8	0.11 $\pm$ 0.11	15.6 $\pm$ 0.4	2.62	0.05 $\pm$ 0.03
19.8	22.2 $\pm$ 4.2	19.1	0.14 $\pm$ 0.19	22.6 $\pm$ 0.3	1.43	0.14 $\pm$ 0.02

¹ measured concentration; ² precision; ³ accuracy (absolute values).

The response time ($t_{95\%}$) of the sensor is almost the same in both types of water, with a slight increase for saline water (2.5%) (Figure 6.14a). The degassing time, t_g , recorded was about twice as long as the response time observed in the reaction to CO₂ increase ($t_g = 2t_{95\%}$). Comparing the dCO₂ optrode and the commercial sensor behavior, in Figure 6.14b, it is possible to see that the time that the optrode takes to respond to concentration increments of 1.00 mg/L and 2.00 mg/L decreases as the concentration increases, but it is on average around $t_{95\%} \approx 3$ min. On the other hand, the response time of the commercial sensor increases along the experiment. With this test, it is concluded that the dCO₂ optrode is faster than the tested commercial sensor in the range of 2.99 to 19.8 mg/L.

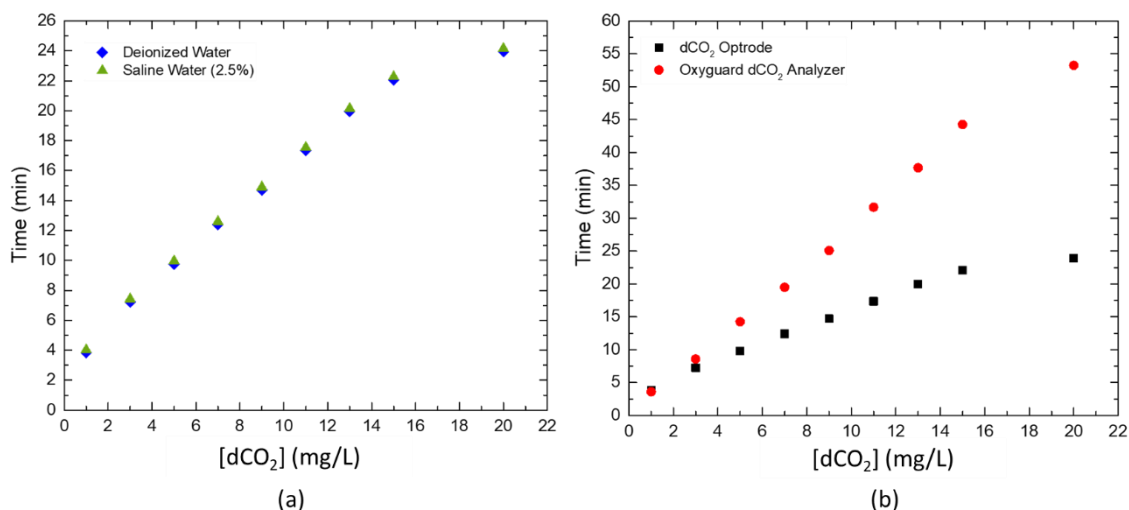


Figure 6. 14. (a) Optrode response time ($t_{95\%}$) evaluation in deionized (blue diamonds) and in saline (2.5%; green triangles) water; and (b) comparison of the response time ($t_{95\%}$) between the colorimetric optrode and the used commercial sensor in the same range of concentrations of dCO₂.

The resolution (defined as the smallest variation that the sensor can detect) of the dCO₂ optrode in deionized water was assessed, by increasing the CO₂ concentration in intervals of 0.25 mg/L starting from 1.00 to 2.00 mg/L. As result, the optrode showed a suitable sensitivity response to small increments of dCO₂ concentrations in deionized water, as displayed in Table 6.4. The performance of the optrode considering the accuracy parameter, improves as the dCO₂ concentration increases. That result was expected, once as the dCO₂ concentration increases, the measured concentration value is moving away from the limit of the calibration range. The response time in this range is similar to the results observed in higher concentrations.

Table 6. 4. Sensitivity of the new dCO₂ sensor to 0.25 mg/L increments (starting from 1.00 mg/L) until signal time stabilization to 95% of equilibrium response ($t_{95\%}$) in deionized water.

[dCO ₂] _{final} (mg/L)	M.C. ¹ (mg/L)	Acc ² (mg/L)	Response Time (s)
1.25	1.39	0.56	103
1.50	1.55	0.11	152
1.75	1.76	0.01	137
2.00	2.02	0.02	146

¹ measured concentration; ² accuracy (absolute values).

6.3.4. dCO₂ dynamics in an SRS+RAS prototype

The aim of this experiment was to simulate the conditions present in fish farms after the feeding events, where it was supposed to have the hardest conditions for monitoring.

The results of a monitoring experiment with a duration of 4 h and 15 min (with two complete cycles of gassing and degassing) can be seen in Figure 6.15. It is observable that both sensors showed the pre-existence of some dissolved CO₂ in the system water before the gaseous CO₂ injection. The optrode showed a starting concentration around 0.75 mg/L, and the used commercial sensor (OxyGuard CO₂ Analyzer®) showed a dCO₂ concentration around 1.00 mg/L (however, the displayed value varied successively between 1.00 and 2.00 mg/L before the gas bottle opening). In run 1, after 10 min of CO₂ injection, the commercial sensor showed a concentration around 3.75 mg/L, while the dCO₂ optrode only detected 2.00 mg/L. This can be explained by the higher initial value displayed by the OxyGuard sensor. However, after 20 min, the values given by both sensors were the same (around 5.00 mg/L), and from this point on, the optrode was able to reach the next concentration in a shorter period. Furthermore, the discrepancy between both sensors at the same instant should be related to the different response time. The same was observed in the monitoring of the degassing process after the CO₂ supply was cut (around 60 min).

In run 2 (executed 1h50 min after the first run), the dCO₂ optrode showed a starting concentration of 1.58 mg/L, and the OxyGuard sensor showed again variations between 1.00 and 2.00 mg/L. When the CO₂ supply bottle was opened, the new sensor response was always faster than the used commercial sensor, achieving 4.00 mg/L in 10 min, while the Oxyguard sensor only displayed 2.00 mg/L. Even in the degassing period (started at 170 min), the dCO₂ optrode showed around 6.00 mg/L after 30 min, while the commercial sensor showed 9.00 mg/L. The two sensors showed again different maximum concentrations. Nevertheless, the dCO₂ optrode showed the same maximum concentration 1h after injection in both assays, registering 12.00 mg/L, while the commercial sensor registered 10.00 and 11.00 mg/L, respectively.

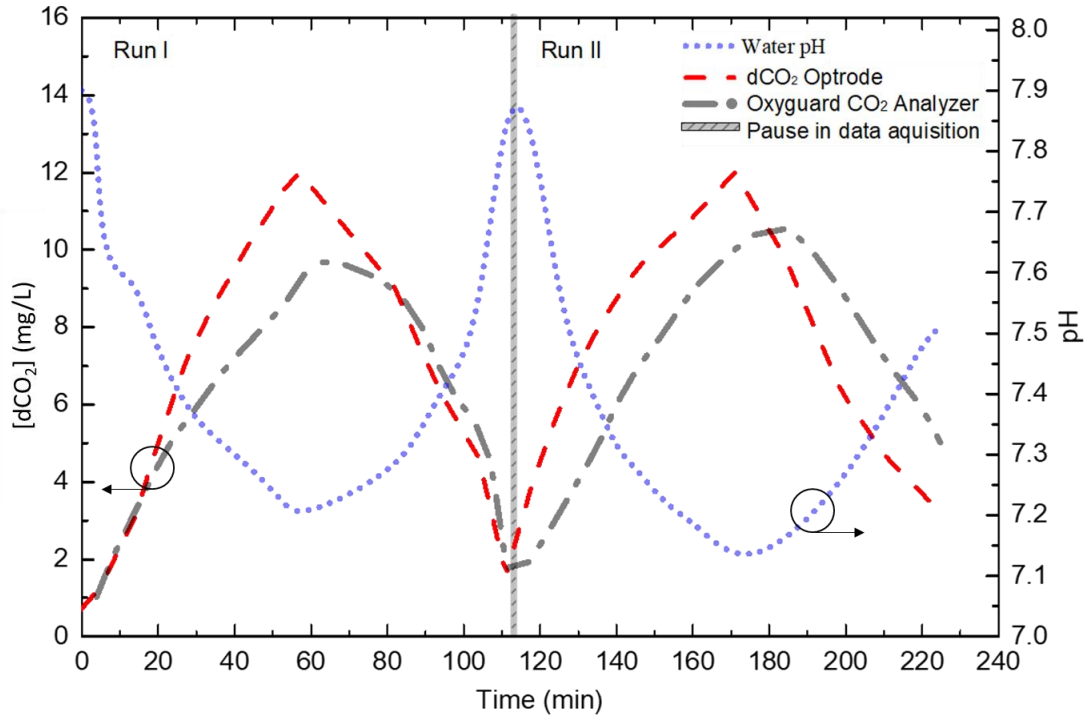


Figure 6. 15. Continuous dissolved CO₂ profile in the recirculating shallow raceway system (SRS+RAS) prototype, using the dCO₂ optrode (dashed line) and the OxyGuard CO₂ Analyzer (dotted line), and concomitant water pH variations (short dot line).

Considering these results, it is noticeable that the dCO₂ optrode has shown a better performance than the used commercial sensor, mainly considering the response time. The pH was also monitored, and 10 min after the first CO₂ injection, it decreased from 7.90 to 7.60, reaching 7.19 in the maximum CO₂ registered concentration. In run 2, the water pH also started around 7.90 and attained 7.12 in the same experimental time, which is a result that can be considered a match between both runs. The experimental error for the executed assays using the dCO₂ optrode was around $[dCO_2] \pm 0.50$ mg/L. The error was calculated using Equation (6.7), where $S_{y/x}$ is the standard error of the estimate, $(y_i - y_i')$ is the deviation of each measurement, and n is the number of points of the calibration curve; and Equation (6.8), where S_{x_0} is the x error, y_0 is the experimental signal of the instrument for which x_0 is to be determined, and m is the number of repetitions [345]. The OxyGuard CO₂ Analyzer[®] experimental error was given by the manufacturers in the data sheet of the equipment (± 1.00 mg/L).

$$S_{y/x} = \sqrt{\frac{\sum_i (y_i - y_i')^2}{n - 2}} \quad (6.7).$$

$$S_{x0} = \pm \frac{S_{y/x}}{b} \times \left(\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 \sum_i (x_i - \bar{x}_i)^2}} \right) \quad (6.8).$$

6.3.5. Continuous dCO₂ Monitoring in an Experimental Fish Culture System with Water Recirculation

In this experiment, it was planned to study the performance of the dCO₂ optrode in a real aquaculture situation with the CO₂ being produced by fish respiration. The resultant data are shown in Figure 6.16. The dCO₂ optrode and Oxyguard CO₂ Analyzer[®] were placed close to each other in the sampling locations (A – clean water; B – fish tank; and C – biofilter tank), and the samples were taken at the same time. The data logging results showed that the optrode was sensitive to small amounts of dissolved CO₂ and the readings took around 2 min and 30s to stabilize, reaching 3.00 mg/L (Figure 6.14 section B). Simultaneous commercial sensor readings did not change from 1.00 mg/L during the experiment (dashed line in Figure 6.16). After about 10 min of reading, the sampling point was changed, as the sensors were placed in the biofilter tank (logging was stopped during this switch). As expected, the dCO₂ optrode showed a decrease in the dissolved gas concentration, reaching 2.35 mg/L (Figure 6.16 section C). Theoretically, these are acceptable values for dCO₂ concentrations in those situations, as some degassing occurs in the sump, prior to the biofilter. The commercial sensor maintained the 1.00 mg/L reading. Although there was some curiosity of the fish by the new sensor presence (when it is on, a light is seen), no noticeable behavioral changes were observed.

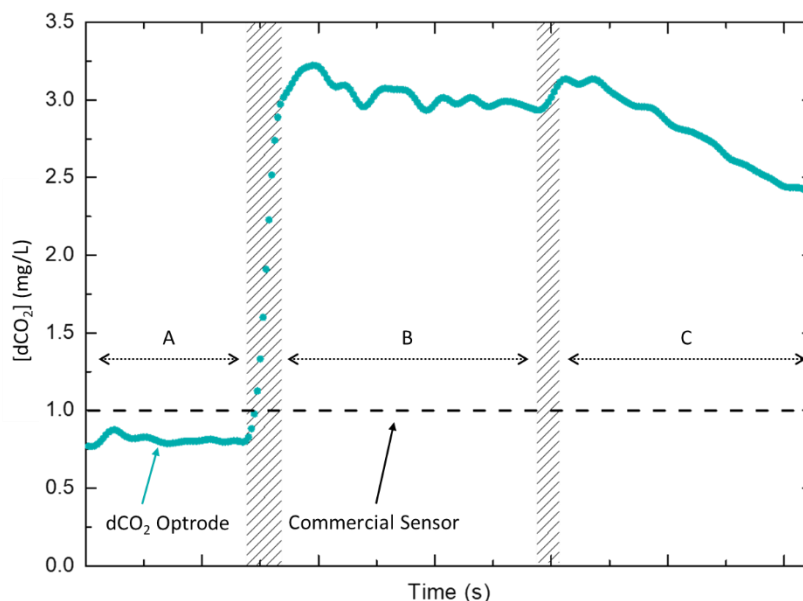


Figure 6. 16. Experimental results of the dCO₂ optrode in a fish culture system obtained by measuring the dCO₂ produced by rainbow trout respiration in fresh water (section A), and after water biofiltration (section B), and after water biofiltration (section C).

6.3.4. Discussion

The response of the new dCO₂ optrode was studied with promising results for real-time monitoring applications. Using the reaction of the sodium carbonate in acid media, it was possible to produce known and precise amounts of dissolved carbon dioxide to calibrate the new optrode in the required range of operation [351]. Moreover, the sensor could work in a larger range of dCO₂ levels with the respective calibration. This method shows that this sensor is capable of operating in environments where the pH is quite low ($\text{pH} \leq 3$) in contrast with most other naked optical CO₂ sensors [312].

This type of calibration was also used by *Fritzsche et al.* to calibrate a sensor for marine applications [354]. However, at low CO₂ concentrations, the sensor had a response time of over 30 min. which is not suitable for applications in aquaculture, in contrast with the new optrode (response in the range of ≈ 120 s for a small variation of concentration ≈ 0.25 mg/L). Sensitivity studies showed that the new optrode can detect and evaluate small dCO₂ variations, not just in a low range but also in higher dCO₂ concentrations. Furthermore, the sensitivity of the membrane is not affected by changing the work environment. In other works, the change from air to water causes a decrease in the CO₂ sensitivity, which is not observable in the results presented for the new optrode [312]. The precision of the new optrode was also attested, showing a better performance in saline water (2.5%), especially for higher concentrations (>10 mg/L).

Although the precision reported by *Borges et al.* for the commercial sensor in saline water is similar, the new dCO₂ optrode shows superior accuracy in the experimental tests [325]. In addition, in what concerns shelf life, the sensing film can be stored in a sodium carbonate solution at low temperatures ($2 < T < 10$ °C) for at least 6 months, and can be used after a new calibration [355]. As stirring is not needed, the sensor is suitable to be used for continuous dissolved CO₂ monitoring, contrary to the systems used by other authors [325, 356].

6.4. Conclusion

A new dCO₂-sensitive ratiometric optical platform was demonstrated and validated, showing its suitability and precision for continuous direct measurements of dissolved CO₂ concentrations in different aqueous solutions.

The previous assays in gas-phase CO₂, had demonstrated that the membrane fabricated with the pNPh/formaldehyde condensed polymer has an outstanding performance when compared with the pNPh-based membrane. Those experiments were a crucial step to understand which material fits better for the detection of low concentrations of carbon dioxide with high resolution.

In the dCO₂ experiments, the best performance was found in saline solutions followed by the deionized water solutions. Sensor validation in an SRS+RAS prototype and in an experimental fish culture system with water recirculation showed its versatility and appropriateness to be used in aquaculture systems as well as in a larger field of applications. Although the sensor body is 3D printed, it was proved that this material resulted in a robust enough apparatus that can be used in real aquaculture environment, as SRS+RAS systems. In addition, its size is appropriate to be used in such working conditions. The electronic part needs to be connected to a PC, which acts as an energy supplier and also allows interaction with the specially designed control software, which processes and stores the data in the memory with a minimum acquisition time of 100 ms. Overall, it was demonstrated a compact portable solution that could be used to realize multipoint readings in different parts of the recirculating system. In future embodiments, this configuration can become even more compact and robust using more durable materials and a micro-controlled electronics module with wireless capabilities.

Chapter 7 – Differential Refractometric Platforms Based on Long Period Fiber Gratings for Biosensing

Chapter 7 describes the fabrication of two different types of biosensors using LPFGs. In a first place, are addressed results obtained from the characterization of an immunosensor by combining a LPFG structure with antibody-modified silica nanoparticles. Antibody/antigen binding events are evaluated by a self-compensated LPFG system. In a second place, the binding kinetics between a MIP layer and its specific target are investigated by using a MIP/NIP self-compensated system. Both configurations were tested in the presence of their specific targets and other similar species to assesses the selectivity of the sensors.

7.1. Introduction

Biosensors are powerful allies for food safety, drug discovery, environmental monitoring, and clinical diagnosis [357-359]. The sensing methodology comprises a bioreceptor, a recognition element, and a transducer whose properties changes upon analyte binding [360]. Typically, the bioreceptor is immobilized on the surface of the transducer and the binding event can be, e.g., mechanically, electrically, or optically transduced producing an increase in mass, a change in electrical resistivity or changes in the refractive index

at the surface of the used material allowing to be measured [361, 362]. Considering optical transduction, optical biosensors are an active field of research worldwide, presenting rapid progress [363-365]. Taking different sizes and forms, optical biosensors can assume a preeminent role to transform chemical or biological interactions between a sensing platform and an analyte into qualitative and/or quantitative information. Advanced methods for fast and real-time detection [366, 367], targeting chemical and biological contaminants in water [9] or pathogens in health-care [368], are important to control the water quality or trace rapid and reliable medical diagnosis. In this perspective, optical biosensors based on refractometric architectures have been developed with great successes in the last decades [363]. Moreover, single mode optical fibers are an excellent platform to develop high-stability and high-sensitive refractometric biosensors [4]. Additionally, SMF are robust, low-cost, corrosion resistant, have low attenuation at long distances, present immunity to electromagnetic interference, are easy to install and easy to move, allowing *in situ* measurements [2]. The quantitative and/or qualitative measurements result from the effect caused by the analyte/receptor interaction on the evanescent field of light at the fiber surface. The well-known silica chemical and biochemical compatibility makes them appropriate for biochemical functionalization, creating very sensitive structures targeting viruses, drugs, antibodies, proteins, among others [127]. Additionally, some works using biofunctionalized optical fibers with specific receptors such as aptamers [369, 370], antibodies or anti-antibodies [371, 372], and DNA probes [373] have been recently reported. *Liyanage et al.* had developed a label-free sensitive tapered optical fiber plasmonic biosensor targeting microRNAs. The sensing platform comprises different types of gold nanoparticles immobilized on the surface of the fiber to enhance the evanescent mode, followed by self-assembled ssDNA probes [374]. Other authors showed a plasmon-assisted TFBG based biosensor for non-enzymatic D-glucose using polydopamine-immobilized concanavalin A [375]. *Lee et al.* have developed and investigated the applicability of LPFGs for the SARS-CoV-2 spike protein detection [376]. The sensor architecture comprises a phase-shifted LPFG chemically modified by the covalent immobilization of the spike protein antibody. The optical platform demonstrated to be suitable to detect the proposed analyte at a concentration of 100 pg/mL. However, the production of these bioreceptors presents drawbacks related with lack of simplicity, cost-effectiveness, time consuming, expensive and have limited shelf lifetime [214, 377]. In this perspective, the efforts and progress achieved in the research of molecular imprinting techniques helped in the development of synthetic materials for specific recognition.

Currently, MIPs are widely used to mimic natural receptors, assuming different formats, sizes and thicknesses, ranging from nano- to micro-structures [210, 378, 379]. The most common technique to synthesize MIPs is the “Bulk Polymerization”. Briefly, the imprinted material is polymerized around the template molecule (target analyte) which is posteriorly removed. The resulted bulk polymers are crushed mechanically to a suitable size, resulting in small particles with specific recognition sites [185]. However, this technique has some disadvantages due to the crushing process, leading to irregular shapes, heterogeneous particle size, and partial or total destruction of the sites [186]. Despite those drawbacks, some researchers have been reporting fluorescent sensors based on MIPs produced by this method, by doping the resulted beads with amine-reactive groups according with the target analyte [380, 381]. Besides, have been reported some progresses to overcome the problem of the quality and reproducibility in the production of these synthetic materials, by improving the polymerization techniques. *Pluhar* et al. used a two-phase technique, applying a dispersed-phase to polymerize spherical particles and a mini-emulsion phase to control the particle size avoiding agglomerations, producing pepsin selective MIPs. The performance of the resulted sensing structures was evaluated by UV-VIS spectrometry [382]. Moreover, MIPs mimicking monoclonal antibodies have been attracting the interest of the scientific community [181, 182]. The solid-phase synthesis has been applied to produce artificial receptors for various diagnostic and life science applications [202]. The process starts with the covalent immobilization of the template onto a suitable solid support (e.g., spherical glass beads). The modified solid support is placed into the monomeric solution and the polymerization initiates under specific conditions, resulting in the formation of uniform spherical nanoparticles around the templates. As main advantages, this technique allows the formation of just one binding site per particle (mimicking monoclonal antibodies) and the process to extract the template molecule allows the isolation of the high-affinity from the low-affinity nanoMIPs [204]. *Canffarota* et al. developed an ELISA-like format device (enzyme-linked immunosorbent assays) for vancomycin detection, based on molecularly imprinted nanoparticles using solid-phase polymerization [200]. Other works involving pseudo-ELISAs using MIPs in substitution of natural antibodies can be found in literature [383-385]. Other interesting technique relies on surface imprinting via grafting polymerization. This method allows to create polymeric layers on planar or spherical (and cylindrical) substrates by direct polymerization (covalently or non-covalently) on the surface of the substrate (e.g., spherical particles, planar surfaces, optical fibers) in the presence of the template molecule (with or without immobilization) [386]. The grafting process comprises some advantages such as improved affinity interactions due to the

fast mass transfer induced by high analyte mobility, better control of the shape and morphology [185]. *Riskin et al.* presented an enhanced sensitivity SPR technology by grafting gold nanoparticles to detect different chemical compounds, *He* and his co-workers presented a MIP-coated optical fiber to evaluate dabrafenib, and *Cennamo et al.* applied a similar approach to develop a system for SARS-CoV-2 detection [196, 274, 387]. Overall, MIPs can be considered to be more versatile, cost-effective, and have extended shelf lifetime compared with the common antibodies. However, the lack of toxicity studies and their scarce use for practical applications leads to a demand for further investigations. Nevertheless, these synthetic molecules have been showing many potential advantages to developing a new family of biosensors, especially in fiber optical sensing [158].

Considering this, researchers in the field of optical sensing have directed their works to combine MIPs together with optical transduction systems to develop new sensing platforms such as optical fibers-based biosensors [388-390]. Additionally, fiber optic evanescent wave (FOEW) sensors, where the LPFGs can be included, are an interesting and promising technology to develop MIP-based optical sensors for bio-applications [391, 392]. However, despite the advantages linked to LPFGs for label-free detection, these optical structures can work as true multi-parameter devices, being sensitive to strain, torsion, and temperature changes [393, 394]. Considering this, the optical response of the LPFGs-based sensors needs to be carefully evaluated, namely in biosensing.

In this work were developed and investigated two distinct optical platforms, based on the differential refractometric detection of anti-immunoglobulin G and immunoglobulin G antibodies (anti-IgG and IgG antibodies, respectively) using a different recognition approach from each other. Moreover, the conventional methods for anti-IgG/IgG identification are based on antigen-antibody specificity using methods such as ELISA or cell/tissue immunofluorescence [395]. Despite their high specificity and selectivity, those methods involve laborious procedures, have limited multiplexing options and, require centralized laboratory equipment and specialized personnel, besides the respective drawbacks associated with natural antibody production [396]. Considering these drawbacks, several researchers have been reporting their works to the scientific community in order to improve IgG quantitative and/or qualitative evaluation. *Yang et al.* demonstrated a SPR gold-coated prism coupler sensor doped with a single layer of graphene to detect IgG molecules by antigen-antibody specificity in a lower range (0-250 ng/mL; unknown LOD); *Shen et al.* reported an electrochemical sensor based on

functionalized carbon nanotubes with ionic liquid, detecting IgG molecules in a range of 0.1-15 ng/L (LOD, 0.02 ng/L); *Choi et al.* showed a nanoporous hydrogel photonic crystal modified with protein A, displaying a range of operation from 0.5 to 10 mg/L (LOD, unknown) [397-399]; *Chiavaioli et al.* reported a D-shaped single mode optical fiber nanocoated with different metals for IgG/anti-IgG assays, reaching limit of detections (LOD) around to the femtomolar values [400]; a similar approach (considering the fiber architecture) was presented by *Chen et al.*, where the authors had sputtered a D-shaped section of a multi-mode OF with a thin gold film, and performed a electrostatic immobilization of the bioreceptor (anti-IgG) for IgG detection (sensitivity to IgG was about 91 nm.mL/mg) [401]; and *Wang et al.* developed and investigated a dual channel self-compensation optical biosensor by coupling functionalized gold nanoparticles with IgG antibodies with an anti-IgG-functionalized gold film, using a silver film acting as reference [402].

Here, the first investigated platform presents a method based on silica nanoparticles (prepared based on *Stöber* [295] method), immobilized on the surface of a LPFG, serving as a base structure for the recognition elements immobilization. The nanoparticles surface was functionalized by the introduction of an aminosilane (APTMS) followed by the covalent immobilization of human-IgG molecules. The antibody was activated by the EDC/NHS protocol to allow the interaction of the amine exposed groups, located on the surface of the silica nanoparticles, with the activated carboxyl acid groups of the IgG molecules. The resulting template was immobilized onto the surface of an OF by electrostatic interactions between the negative charges of the fiber surface and the positively charged amine groups located in the IgG molecules. The optical platform includes a real-time correction system based on a second LPFG (bare) to correct temperature and refractive index drifts. This work aims to detect anti-IgG antibodies.

The second sensing scheme proposed in this segment combines LPFGs and MIPs, produced by surface imprinting, carrying specific binding sites targeting the detection of IgG antibodies. The platform includes a real-time correction system, based on the NIP, allowing reliable detection of the target analyte.

The behavior of both sensors was successfully evaluated, assessing their sensitivities, selectivity, and limit of detections.

7.2. Label-Free Anti-Human IgG Immunosensor

7.2.1. Materials and Methods

7.2.1.1. Chemical reagents

Silica nanoparticles were prepared following the *Stöber* method [403], using tetraethyl orthosilicate (TEOS; Sigma-Aldrich, $\geq 98\%$) and ammonium hydroxide solution (NH_4OH ; Sigma-Aldrich, 28% m/m) as reagents. The functionalization of the nanoparticles surface was attained using the following reagents: (3-Aminopropyl)trimethoxysilane solution (APTMS; Sigma Aldrich, 97%), anhydrous toluene (Sigma-Aldrich, 99.8%), phosphate buffered saline (PBS; pH 7.4, tablets, Sigma-Aldrich), 2-(N-Morpholino)ethanesulfonic acid (MES; Sigma-Aldrich), N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC; Sigma Aldrich, $\geq 99\%$), N-Hydroxysulfosuccinimide (sulfo-NHS; Sigma Aldrich, $\geq 98\%$), and immunoglobulin G from human serum (human-IgG; Sigma-Aldrich, $\geq 95\%$). For the LPFG surface cleaning were used sodium hydroxide anhydrous (NaOH ; Sigma-Aldrich, $\geq 98\%$) and hydrochloric acid (HCl ; Sigma-Aldrich, 37%). For surface activation were used sulfuric acid (H_2SO_4 ; Sigma-Aldrich, 95% - 98%) and hydrogen peroxide solution (H_2O_2 ; Sigma-Aldrich, 30%) to perform piranha solution. For the affinity and selectivity assays were used the human IgG, the human serum albumin (HAS; Sigma-Aldrich, $\geq 98\%$), and the anti-human IgG (Fab specific; antibody produced in goat, Sigma-Aldrich). Ultra-pure water (type II-analytical grade, $< 1 \mu\text{S}\cdot\text{cm}^{-1}$) and ethanol (Labchem, 96%) were also used.

7.2.1.2. Silica nanoparticles preparation and characterization

The SiO_2 nanoparticles were synthesized according with the *Stöber* method. Briefly, in a proper container the ethanol, ultra-pure water and the TEOS, were mixed in that order. The mixture was sonicated for 20 minutes and was added, under stir, the ammonia hydroxide and the final mixture was stirred for 24h at room temperature. The resulted solution was centrifuged at 6000 rpm for 10 minutes and the beads were redispersed/centrifuged (five times) in deionized water and acetone. The resulted beads were dried at 40°C for 12 h in the oven. The average size of the nanoparticles was determined by W130i Dynamic Light Scattering (AvidNano, UK), showing an average diameter ranging from 300-400 nm, and were evaluated by Attenuated total Reflectance.

7.2.1.3. Immobilization of the biorecognition element onto the SiO₂ nanoparticles surface

Silica nanoparticles were redispersed in a freshly prepared piranha solution and gently swirled for 20 minutes. After successive washing processes with deionized water and ethanol, the beads were allowed to dry at 40 °C for 30 minutes. Thereafter, the dried material was incubated in a freshly prepared 2% (v/v) APTMS solution in anhydrous Toluene for 24h at room temperature in a closed container, using 10 mg/mL of beads concentration. After incubation, the nanoparticles were centrifuged at 6000 rpm for 10 minutes and redispersed/centrifuged for five times in acetone and ethanol. From this step resulted amino-functionalized silica nanoparticles that were verified by ATR-IR. The beads were redispersed in a PBS solution (pH 7.4) in a concentration of 5 mg/mL. For the receptor immobilization, a solution of IgG antibodies in MES buffer (pH 5.5) was prepared. Then, the activation of the carboxylic acid functional groups was attained by adding a 1:1 EDC/sulfo-NHS (10x molar excess) solution and incubating for 30 minutes at room temperature. The process comprises a first reaction of the EDC with the carboxyl groups of the IgG molecules, resulting in the formation of an unstable amine-reactive intermediate. Then, the sulfo-NHS is used to stabilize that intermediate, converting the previous amine-reactive ester into a semi-stable active ester. Here, the sulfo-NHS plays an important role. In a first place prevents intra and intermolecular cross-linking of the antibody (once it has both amine and carboxylic groups). In a second place, the use of the sulfonated NHS ester (instead normal NHS ester), prevents the neutralization of the positive charged amine groups of the IgG antibody. The sulfo-NHS ester is reactive towards the amine groups of the APTMS molecules, generating stable amide bonds [404].

The previous prepared beads solution was added to the activated IgG solution and the pH was adjusted to 7.4 - 8.0. The incubation carried out for 4h at room temperature without stirring (just swirled the mixture every half-hour). The resulted modified nanoparticles were centrifuged at 6000 rpm for 10 minutes and redispersed/centrifuged in deionized water. The IgG-modified silica nanoparticles were kept in PBS buffer solution (pH 7.4) until use and the chemical modifications introduced on the nanoparticles were assessed by FTIR-ATR. Figure 7.1 illustrates the all the process.

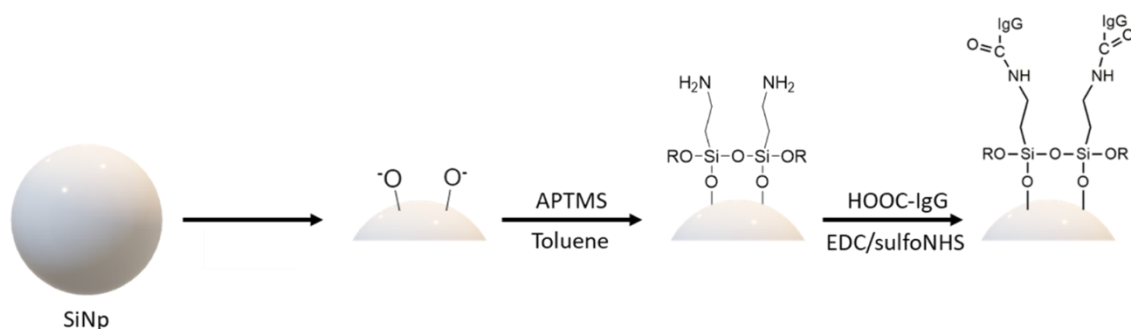


Figure 7. 1. Scheme of the chemical modification process of the silica nanoparticles (SiNP) surface.

7.2.1.4. Working principle and LPFG surface modification

The LPFGs used in this study were microfabricated by the induced electric-arc technique following the protocol described in section 5.1. Moreover, the following parameters were applied: electric-arc current discharge - 9 mA; duration time - 1 second; period – 415 μm . The sensitivity of the fabricated LPFGs was evaluated by examining the respective resonance wavelength shifts using refractive index standard solutions ranging from 1.332 to 1.375.

Afterwards, the LPFG used as sensor was chemically modified by the immobilization of the biorecognition molecule on the fiber surface. The sensitive section of the optical fiber was cleaned with a 2 mol/L NaOH solution for 10 minutes followed by immersion in a 0.5 mol/L HCl solution for 2 h. After washing with deionized water, the sensitive surface was activated with piranha solution (3:1 v/v) for 1h at 60 °C. Finally, the LPFG was washed with deionized water and kept in the oven for 10 minutes to completely dry and was cooled with pure nitrogen. From this process had resulted a negatively charged surface, allowing the electrostatic immobilization of the IgG-modified silica nanoparticles (Figure 7.2). The bioreceptor/analyte interaction is transduced by the LPFG by analyzing the resonance wavelength shifts caused by the binding event. So, considering that the effective refractive index increases as more binding events follow, is expected that the resonant band shifts to lower wavelengths (also known as “blue shift”).

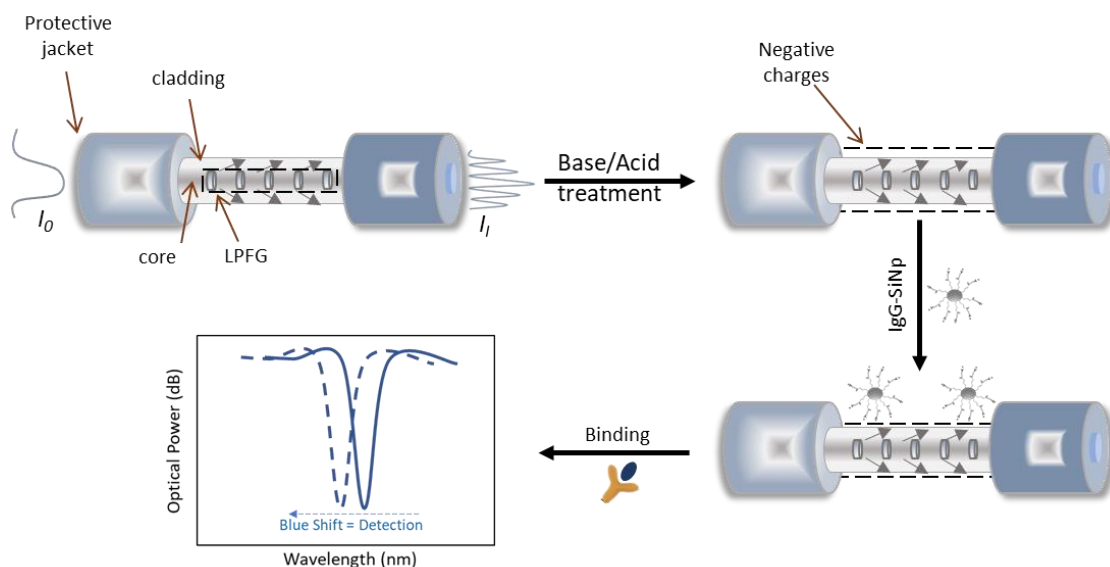


Figure 7. 2. Schematic figure of the surface modification of the LPFG comprising (following the arrows) the bare LPFG; the activated and negatively charged LPFG surface; the receptors immobilization; and the binding event shifting the resonant wavelength band.

7.2.1.5. Sensor performance evaluation

The modified and reference LPFGs were tested in the presence of the anti-human IgG in different concentrations ranging from 0.015 to 15 $\mu\text{g/mL}$ to attest the affinity of the sensing platform. To verify the selectivity of the modified optical fiber, the sensing scheme was exposed to a 9 $\mu\text{g/mL}$ solution of human IgG, a 9 $\mu\text{g/mL}$ solution of HSA and a 9 $\mu\text{g/mL}$ solution of anti-human IgG, in the same experimental conditions. In order to obtain the most trustable results, all data will be presented as the differential between IgG-modified LPFG (LPFG_{sensor}) and the LPFG used as reference (LPFG_{Ref}), in which the difference between both LPFGs sensitivities was fully compensated.

7.2.2 Results and Discussion

7.2.2.1. Evolution of the silica nanoparticles chemical modification: FTIR-ATR spectroscopy

To confirm the introduction of the new functional groups after each step on the SiO_2 nanoparticles surface, FTIR-ATR spectroscopy was used. Figure 7.3 shows the infrared absorbance spectra of the bare SiNP, the aminated SiNP (SiNP-NH_2), and the obtained nanospheres after the incubation of the IgG ($\text{SiNP-NH}_2\text{-IgG}$). The absorption peaks at $3000\text{-}3500\text{ cm}^{-1}$ are related to the stretching $-\text{OH}$ bands, whereas the absorption peaks at $1000\text{-}1150\text{ cm}^{-1}$ are assigned to the Si-O-Si asymmetric stretching bands, and the

peaks at 800-950 cm^{-1} are appointed to the asymmetric bending of Si-OH. In the figure 7.3a, is displayed the asymmetric deformation vibration at around 1550 cm^{-1} , usually linked to the presence of amine groups ($-\text{NH}_2$). Thus, its suggest that the APTMS molecules were successfully fixed in the silica nanoparticles surface. Moreover, figure 7.3b show a peak at around 2900 cm^{-1} that is attributed to C-H stretching of the APTMS structure. Finally, figure 7.3c show the carboxylate peak at 1650 cm^{-1} , assigned to the amide bond between the IgG molecule carboxylic acid and the amine groups of the APTMS. This evaluation is similar to the evaluation made by *Feifel* and his co-worker when they proved the possibility to create electro-active cytochrome C multilayers by using carboxyl-modified SiO_2 nanoparticles [405]. Moreover, *Hernandez-Leon et al.* also showed parallel spectra resulted by the modification of a core-shell SiO_2 nanobeads for capture low molecular weight proteins and peptides [406].

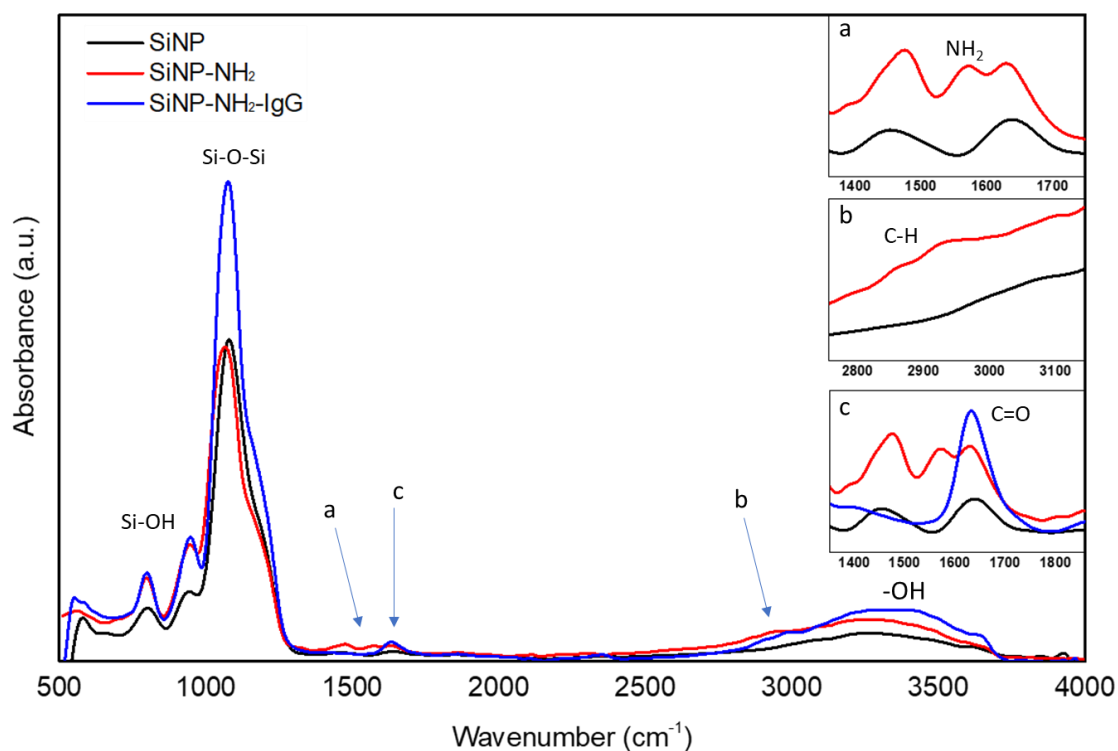


Figure 7. 3. Obtained absorbance spectra from FTIR-ATR analysis of bare SiNP (black line); amino-functionalized SiNP (red line); and after IgG immobilization (blue line). Inset graphs magnifies the main absorbance peaks after the introduction of APTMS (a and b) and IgG antibodies (c).

7.2.2.2. Long-period fiber gratings sensitivity to refractive index standard solutions

As mentioned, the optical system is based on differential measurements between the LPFG used for the specific detection of the target analyte and a second LPFG acting as reference for non-specific fluctuations. Thus, to an accurate relation between both

LPFGs it is necessary to assess their sensitivities. Therefore, the LPFGs were placed side-by-side and their sensitivities were tested by immersing the sensing regions into a set of aqueous ethylene glycol (EG) solutions with well-defined refractive indices ranging from 1.332 to 1.375. Furthermore, the refractive index of the EG solutions was evaluated by a digital refractometer (DR-A1, Atago CO., LTD). Figure 7.4 shows the experimental results, in which figures (a) and (b) exhibit the resultant data for the LPFG_{ref}, and (c) and (d) reveal the data for the LPFG_{sensor}. Table 7.1 displays the data related with the respective calibration curves.

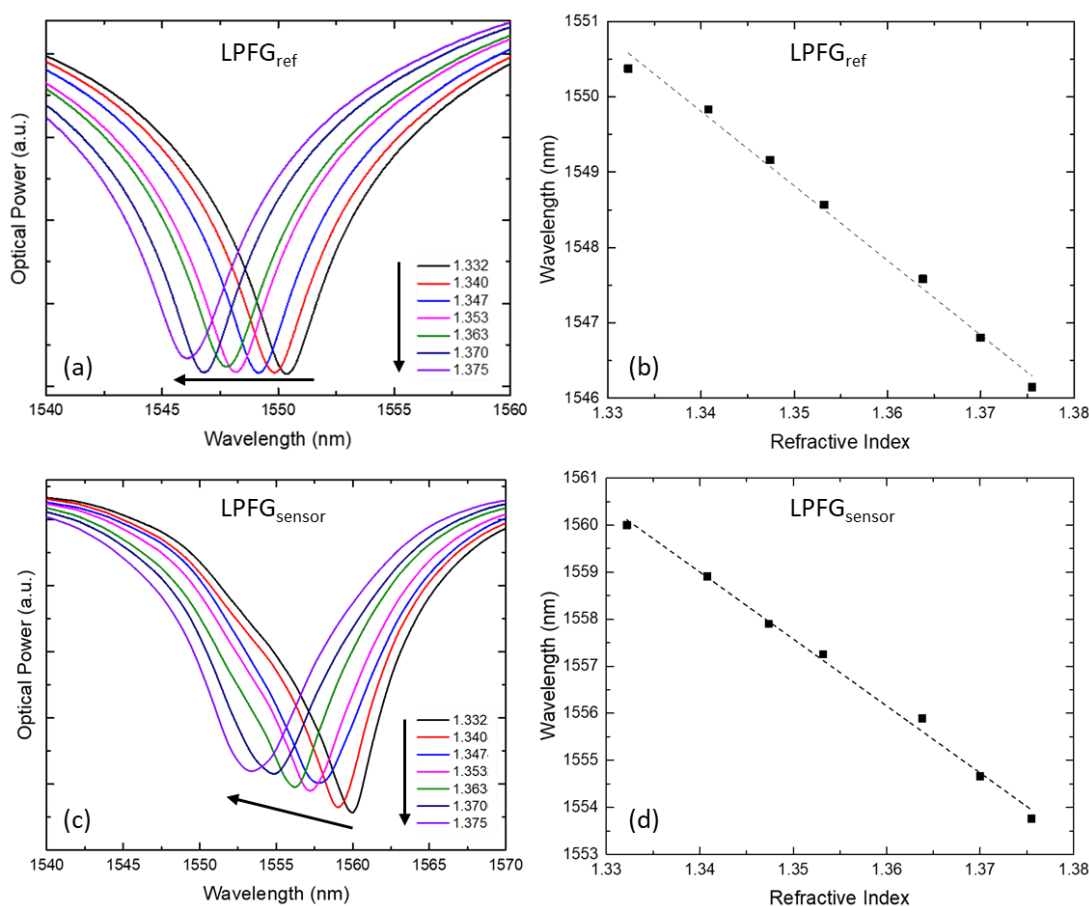


Figure 7. 4. (a and c) Spectra of the resonant wavelength bands and respective variation caused by the different refractive index solutions for the LPFG_{ref} and LPFG_{sensor}, respectively; and (b and d) refractive index calibration curves for the LPFG_{ref} and LPFG_{sensor}, respectively.

Table 7. 1. Experimental data from the refractive index calibration curves for the used LPFGs

	Slope	Intercept	R ²
LPFG _{ref}	-99.27	1682.79	0.99
LPFG _{sensor}	-142.33	1749.72	0.99

Considering the linear regression, the slope of each curve reveals the sensitivity of each LPFG. Thus, the sensitivities of $LPFG_{Ref}$ and $LPFG_{sensor}$ are: $|S_{LPFG,Ref}| = 99 \text{ nm/RIU}$ and $|S_{LPFG,sensor}| = 142 \text{ nm/RIU}$, in which RIU is the refractive index unit. Therefore, the data obtained from the subsequent experiments is processed as following (Equation 7.1):

$$Experimental\ Data = LPFG_{sensor} - 1.43 \times LPFG_{Ref} \quad (7.1),$$

in which the value 1.43 is the ratio between both sensitivities ($LPFG_{sensor}/LPFG_{ref}$).

7.2.2.3. IgG-modified SiNP immobilization onto LPFG surface and sensor performance evaluation

The IgG-modified SiNP were immobilized onto the LPFG surface by electrostatic interactions between the positively charged amino-groups of the IgG antibody and the negatively charged silica surface. Figure 7.5 show the both resonant wavelength bands of the bare fiber and the SiNP/IgG-modified LPFG after several washes with PBS solution and deionized water (both spectra were acquired in deionized water). From Figure 7.5, it is observable a change in the wavelength band position (of about 805 pm), shifting to lower wavelengths. This fact suggests that the effective refractive index of the LPFG has changed due to the nanoparticles' immobilization.

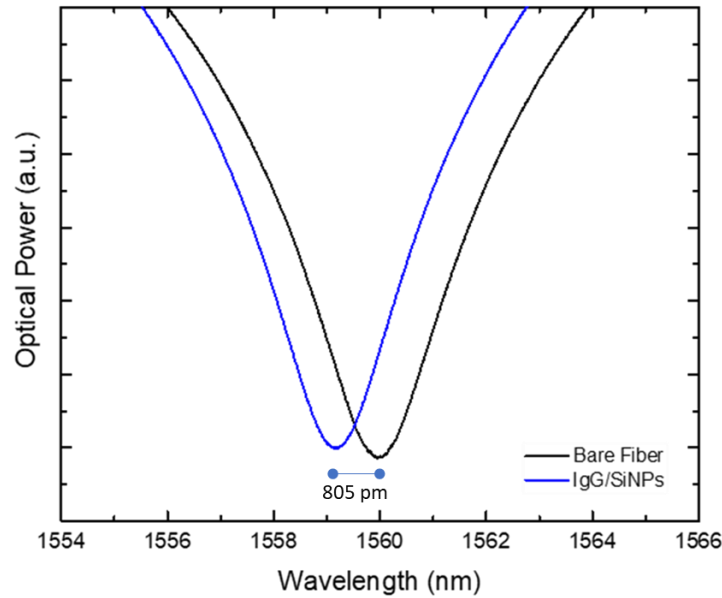


Figure 7. 5. Resonant wavelength band position shifting to lower wavelengths due to the IgG-modified SiNP immobilization at the LPFG surface.

Then, the SiNP/IgG-modified LPFG probe was tested in the presence of different concentrations of anti-human IgG to attest the affinity of the sensing platform, considering the target analyte. Both gratings were exposed to a freshly prepared standard anti-IgG solutions ranging from 0.015 to 15 $\mu\text{g/mL}$ in PBS. After 10 minutes of exposure time, the LPFGs were washed three times with fresh PBS and three times with deionized water. All the data was obtained by recording the resonant wavelength band position of each LPFG in deionized water at 22 $^{\circ}\text{C}$. Figure 7.6a show the resonant wavelength band shifting (to lower wavelengths) after exposing the LPFG_{sensor} to successive anti-IgG solutions. It is noticeable the wavelength shifts caused by the successive binding events between the IgG/SiNP and the target analyte. The resonant wavelength band shifted between the first standard solution and stopped at 9 $\mu\text{g/mL}$, revealing a total shift of around 270 pm as is visible in Figure 7.6b. The experimental data observed in Figure 7.6b show the resonant wavelength band variation (in pm) *versus* the concentration of each anti-IgG standard solution and was obtained according with Equation 7.1.

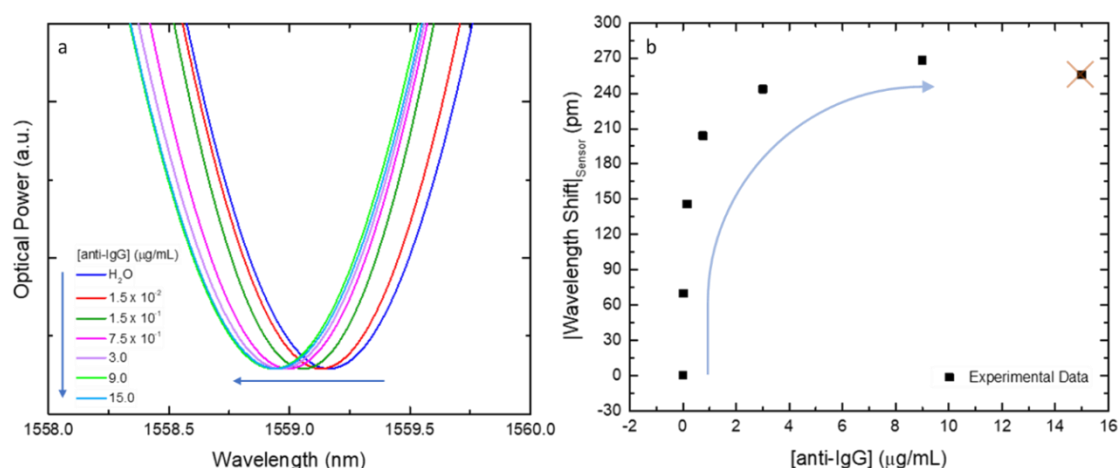


Figure 7. 6. (a) Resonant wavelength band (normalized to the minimum) shifting after successive expose to anti-IgG standard solutions; and (b) resonant wavelength band variation as the concentration of the target increases. The arrows reveal the “blue shift” and the red cross represents the concentration in which the sensor stopped to respond.

As is perceptible from Figure 7.6b, the sensor responds logarithmically to the presence of the anti-IgG standard solutions. So, to attain linearity, the experimental data was plotted in the form of a semi-log scale as is shown in Figure 7.7a. To understand the importance of the reference platform to correct some fluctuations, Figure 7.7b shows the resultant calibration curve (also in a semi-log scale) without applying the correction system. Both graphs also show the respective equations resulted from the calibration curves. Data is reported as a mean value with standard deviation ($n = 3$). It is evident

the impact of the correction method in the performance of the optical platform. As is visible, the optical system (differential method) provides a better sensitivity (that is given by the slopes of the calibration curves) than the single LPFG_{sensor}. Moreover, the statistical data of the calibration curve offers more reliability for the detection of the proposed target in this working range. Furthermore, the limit of detections (calculated as 3 times of the standard deviation of the blank measurements divided by the slope) are quite different. The limit of detection calculated for the corrected system was 0.12 µg/mL while the limit of detection for the uncorrected system was 0.24 µg/mL.

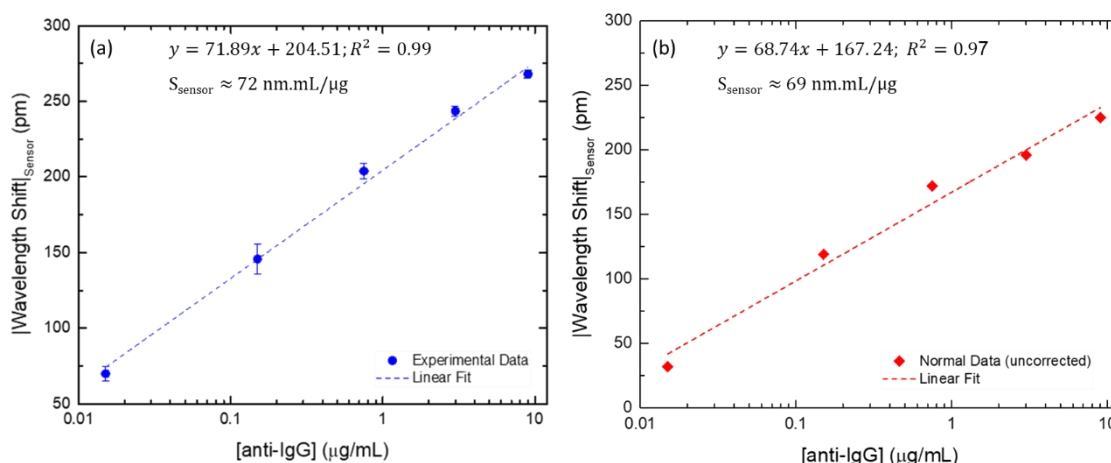


Figure 7. 7. Experimental data plotted in a semi-log scale, exhibiting the wavelength variation as the concentration of the target increases for the differential system (a) and for the uncorrected system (b).

Other works using optical fibers were described recently such as the interferometric optical fiber biosensor for IgG/anti-IgG immunosensing presented by *Wang et al.*, reporting a limit of detection around of 50 ng/mL [407]. Other approach was demonstrated by *Han et al.* that combined a Bragg acoustic reflector with an Au electrode and an aluminum nitride piezoelectric thin film, to develop a biosensor for anti-human IgG detection by immobilization of the human IgG antibody onto the modified Au electrode. The sensing platform was able to detect anti-IgG concentrations smaller than 0.4 mg/mL [408]. A similar approach to this work was reported by *Wu* and his co-workers when the authors immobilized silica nanoparticles, by electrostatic interactions between positively charged polyelectrolytes and the negative surface of the nanoparticles, onto a LPFG surface. After the immobilization of anti-IgG receptors, the optical platform demonstrated its applicability for the detection of IgG antibodies in a range of concentrations between 1 to 90 µg/mL with a LOD of 10 µg/mL [267].

To validate the specificity of the sensing platform, the same protocol was followed in the presence of human IgG antibody, the HSA protein, and finally the anti-human IgG. Figure 7.8 reveals the resulted data after 10 minutes of incubation time for each target

in 9 $\mu\text{g/mL}$ (in PBS). The results are reported as a mean value with standard deviation ($n = 3$). These results proved the specificity of the built sensing platform to the proposed target, revealing a very relevant wavelength shift when exposed to it. By other side, the shifts showed by the LPFG in the presence of the other targets are not relevant. It is also noticeable that the applied differential method decreases the response to the interferents at, at least, 50%, which is a remarkable result, especially for immunosensing.

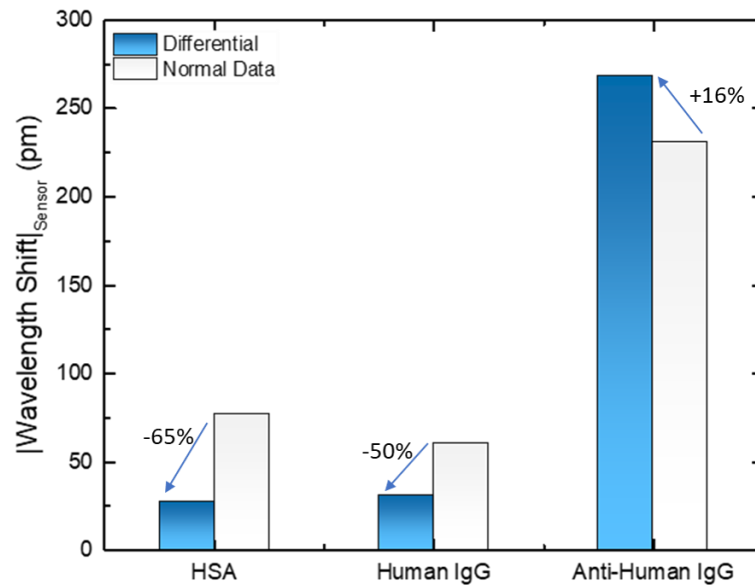


Figure 7. 8. Cross-reactivity studies assessing the selectivity of the optical platform by exposing the device to different analytes with the same concentration, discriminates the experimental data obtained by the differential system (blue columns) and the uncorrected system (white columns).

7.3. Differential Refractometric Biosensor for Human IgG detection based on Molecular Imprinting and Long Period Fiber Gratings

7.3.1. Materials and Methods

7.3.1.1. Chemical Reagents

Sulfuric acid (p.a. 95–97%; Merck) and hydrogen peroxide solution (30 wt. % in H₂O; Sigma-Aldrich, Burlington, MA, USA) were used to prepare piranha solution in 3:1 ratio, respectively. Allyltrimethoxysilane (ATMS, 97%, RI@20 °C = 1.4036; Gelest) was used for self-assembling monolayer (SAM) formation. Acrylamide (Aam, >99%; Sigma-Aldrich), N-terc-Butylacrylamide (TBAam, 97%; Sigma-Aldrich), 2-Hydroxyethyl methacrylate (HEMA, 97%; Sigma-Aldrich), N,N'-Methylenebis(acrylamide) (BISAam, 99%; Sigma-Aldrich), N-(3-Aminopropyl) methacrylamide hydrochloride (APMA, 96%; Sigma-Aldrich), ammonium persulfate (APS, ≥98%; Sigma Aldrich) and N,N,N',N'-tetramethylethylenediamine (TEMED, 99%; Sigma-Aldrich) were used for the MIP fabrication process. IgG from human serum (≥ 95%; Sigma-Aldrich) was used as a template molecule and as target to build and assess the sensor. Albumin from human serum (HSA; ≥97%, Sigma-Aldrich) and transferrin from human blood plasma (HTR; ≥95%, Sigma-Aldrich) were used for cross-reactivity tests. Phosphate buffered saline solution (PBS, 0.01 mol/L, pH 7.4; Sigma-Aldrich), deionized water (DIW; Wasserlab, type II Analytical Grade), ethanol (96%; LabChem), sodium dodecyl sulfate (SDS; Sigma-Aldrich), sodium chloride (NaCl; Sigma-Aldrich), and ethylene glycol (EG; >99%, Sigma-Aldrich) were also used. A standard single mode optical fiber (SMF28, Corning®) and a BraggMeter unit (HBK, Fibersensing) were used to develop the sensor and to process the light signal, respectively.

7.3.1.2. Long-period fiber gratings and experimental setup

The LPFGs used in this study were microfabricated by the induced electric-arc technique following the protocol described in section 5.1. Moreover, the following parameters were applied: electric-arc current discharge - 9 mA; duration time - 1 second; period – 415 μm. The sensitivity of the fabricated LPFGs was evaluated by examining the respective resonance wavelength shifts using refractive index standard solutions ranging from 1.3322 to 1.3716.

The LPFGs were placed under tension into a fluidic chamber (Figure 7.9) specially designed for this purpose. The fibers were connected to a BraggMeter unit working in

transmission mode and the light signal was transformed in analytical data through a dedicated LabView routine in a spectral range from 1500 nm to 1600 nm. Furthermore, a second chamber includes a bare LPFG to explore its behavior under the same experimental conditions (not shown in figure).

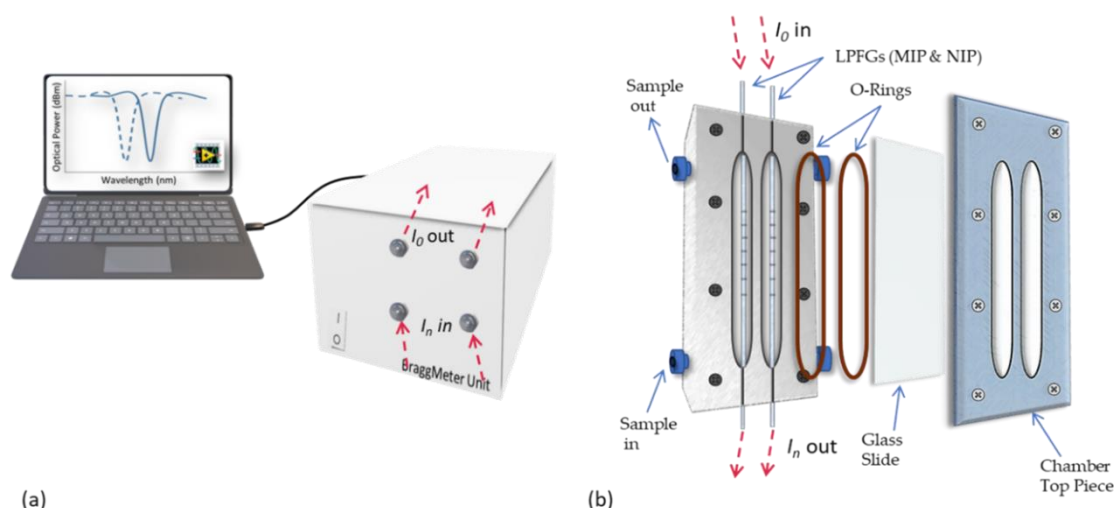


Figure 7. 9. Experimental setup used for LPFG surface preparation, template extraction, and sensor performance evaluation, including the interrogation unit (a) and the fluidic chamber components (b).

7.3.1.3. Long-period fiber grating surface modification

Silanization processes are commonly used to chemically modify silica optical fibers [273, 279]. In this work, both LPFGs were cleaned by immersing the silica sensing region in a piranha solution for 30 min at room temperature. After washing several times with deionized water, the optical fibers were placed in the oven for 2 h at 60 °C to remove all the water from its surface and generate hydroxyl groups (-OH). This step is extremely important to the success of the following reaction between the silicon group of the ATMS with the -OH previously created, in order to cover the fiber surface as much as possible. The LPFGs were placed into the fluidic chamber where the sensing region was isolated from the external medium. The chamber was filled with a 2% ATMS solution in an ethanol/water mixture (96% ethanol) and reacted for 16 h at 4 °C, allowing a SAM formation in all the fiber sensing region. The sensing surface was washed with fresh ethanol and DIW for several times and dried for 2 h at 60 °C enabling covalent bonds formation (Figure 7.10). This chemical modification will help to hold the polymeric layer to the LPFG surface by C-C bonds. In order to observe the progress of the fiber surface modification, the spectra after each introduced change on the LPFG were acquired (in deionized water) and labeled as following: H₂O (reference); H₂O@Allylsilane (after

silanization process); H₂O@Polymerization (after MIP layer formation); and H₂O@TemplateExtracted (after template extraction).

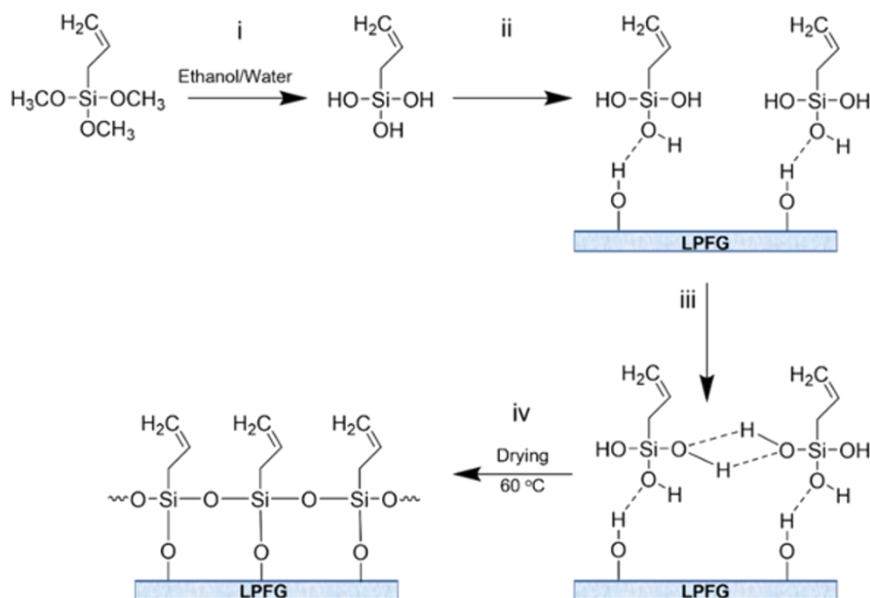


Figure 7. 10. Schematic figure of the fiber surface allylation process: i. hydrolysis (formation of -OH groups); ii. chemisorption (reaction of the allylsilane with the LPFG surface); iii. cross-polymerization (reaction between different allylsilane molecules at the LPFG surface); iv. covalent bond formation (monolayer formation).

7.3.1.4. MIP/NIP layer formation and template extraction

The synthesis of the molecularly imprinted polymer layer was adapted from a recently reported strategy for molecular imprinting [274]. A pre-polymeric mixture including Aam (0.68% w/v), TBAm (0.24% w/v), HEMA (0.1% v/v), BIS (0.58 w/v), and APMA (0.12% w/v) in 10 mmol/L PBS solution, was prepared. After mixing all components, the pre-polymeric mixture was ultrasonicated for 15 min and bubbled with nitrogen (N₂) for 1h. After that, the template was added to the solution in a final concentration of 0.5 $\mu\text{mol/L}$ followed by the addition of APS (0.2% w/v). Then, 1 mL of the mixed solution was dropped onto the LPFG followed, immediately, by a suitable amount of TEMED over the sensing section, performing a final concentration of a 0.12% (v/v), to catalyze the reaction. The reaction started immediately, and the polymerization took place for, approximately, 10 min being stopped by washing the LPFG surface with copious amounts of deionized water. For the template extraction, the sensing region was immersed in deionized water at $60\text{ }^\circ\text{C}$ for 1 h (replacing the warm water at each 5 min) and then washed with a 5% SDS solution (in PBS) for 10 times. Finally, the LPFG was

immersed in a 0.5 mol/L NaCl solution (in 5% SDS) overnight, washed three times with SDS and five times with DIW. After template extraction, the MIP layer built on the LPFG surface was characterized and sensitivity and selectivity towards human IgG antibodies was evaluated. Figure 7.11 exemplifies the polymerization process. The interaction receptor/target analyte was evaluated on an LPFG covered with a non-imprinted polymer layer (NIP). The NIP layer was built in the same conditions of the MIP without adding the template.

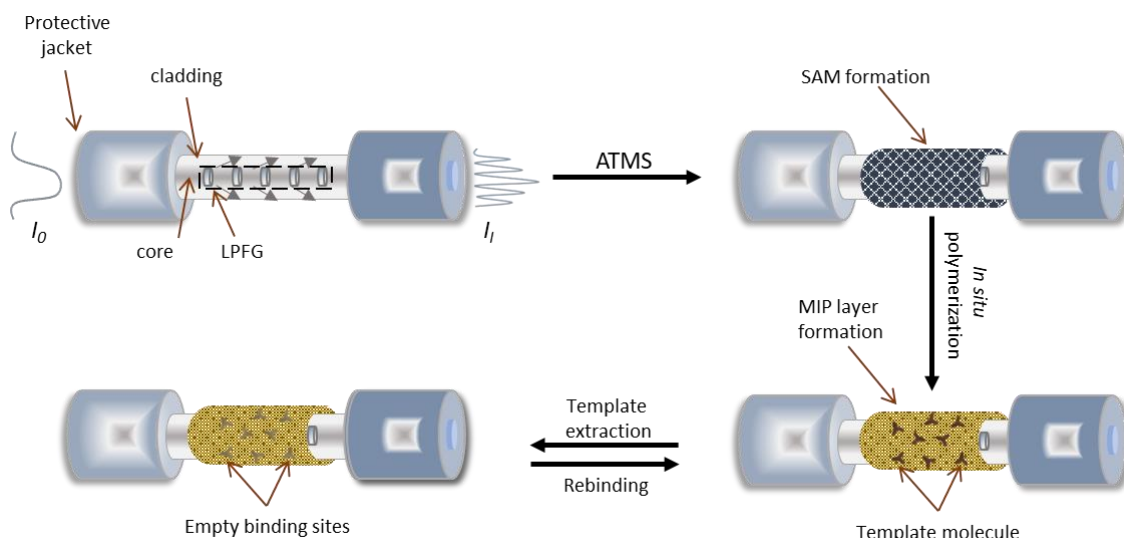


Figure 7. 11. Schematic figure of the in-situ polymerization, sequentially showing the process of the fiber surface chemical modification from the allylation to the template extraction. NIP-coated LPFG has the same procedure without the presence of the template and, consequently, template extraction step.

7.3.1.5. Binding experiments and selectivity evaluation

For the binding experiments, standard solutions of IgG antibody (Mw = 150 KDa, [409]) in PBS (pH 7.4) were prepared at different concentrations, ranging from 1 to 100 nmol/L. The LPFGs, MIP-coated and NIP-coated, were immersed in buffer solution and the respective spectra were acquired (reference spectra). Each IgG concentration was placed over the sensing region for 5 min followed by the washing process with buffer solution (5x). Then, the respective spectra were acquired also in buffer solution to evaluate the wavelength shift ($\Delta\lambda$) considering the initial position measured in the buffer solution. The spectra of the NIP-coated LPFG were used to correct RI changes caused by “bulk effect” and/or temperature and/or tension changes, subtracting its $\Delta\lambda$ to the $\Delta\lambda$ of the MIP-coated LPFG. The non-imprinted layer $\Delta\lambda$ was also tested in the same conditions versus the $\Delta\lambda$ of a bare LPFG fiber to understand the impact of the nonspecific RI variations. The selectivity (MIP vs. NIP vs. (MIP-NIP)) of the optical platform was also

accessed using a single concentration for the competitors in presence of half that concentration for the IgG antibody.

7.3.2. Results and Discussion

7.3.2.1. LPFGs sensitivity at different refractive index standard solutions

The LPFGs sensitivity was assessed by measuring different refractive index solutions attained by mixing deionized water and EG in different portions. The refractive index at 589.3 nm (sodium D line) of each solution was evaluated by a digital refractometer (DR-A1, Atago CO., LTD) displaying, increasing the EG concentration, the refractive indices from 1.3322 to 1.3716. Each solution was placed over the sensing region for 5 min before spectra acquisition. The mean of 10 spectra acquired for each solution (after exposure time) as well as the calibration curve of the experimental values obtained by fitting the respective spectra with a Gaussian curve versus the wavelength position are shown in Figure 7.12. The sensitivity (S) is provided by the slope of the respective calibration curve that was about $|S| = 130$ nm/RIU and $|S| = 107$ nm/RIU for the LPFG used for MIP coating (Figure **a** and **b**) and for the LPFG for NIP coating (Figure **c** and **d**), respectively. Thus, the data obtained from the subsequent experiments is processed as following (Equation 7.2):

$$|\Delta\lambda|_{\text{sensor}} = |\Delta\lambda|_{\text{MIP}} - 1.21|\Delta\lambda|_{\text{NIP}} \quad (7.2),$$

where $\Delta\lambda_{\text{sensor}}$ is the differential mode between the modules of the MIP-coated and NIP-coated LPFGs wavelength shifts, $|\Delta\lambda|_{\text{MIP}}$ is the module of the wavelength shift of the MIP-coated LPFG, and $1.21|\Delta\lambda|_{\text{NIP}}$ is the module of the wavelength shift of the NIP-coated LPFG, in which the value 1.21 is the ratio between both sensitivities ($\text{LPFG}_{\text{MIP}}/\text{LPFG}_{\text{NIP}}$).

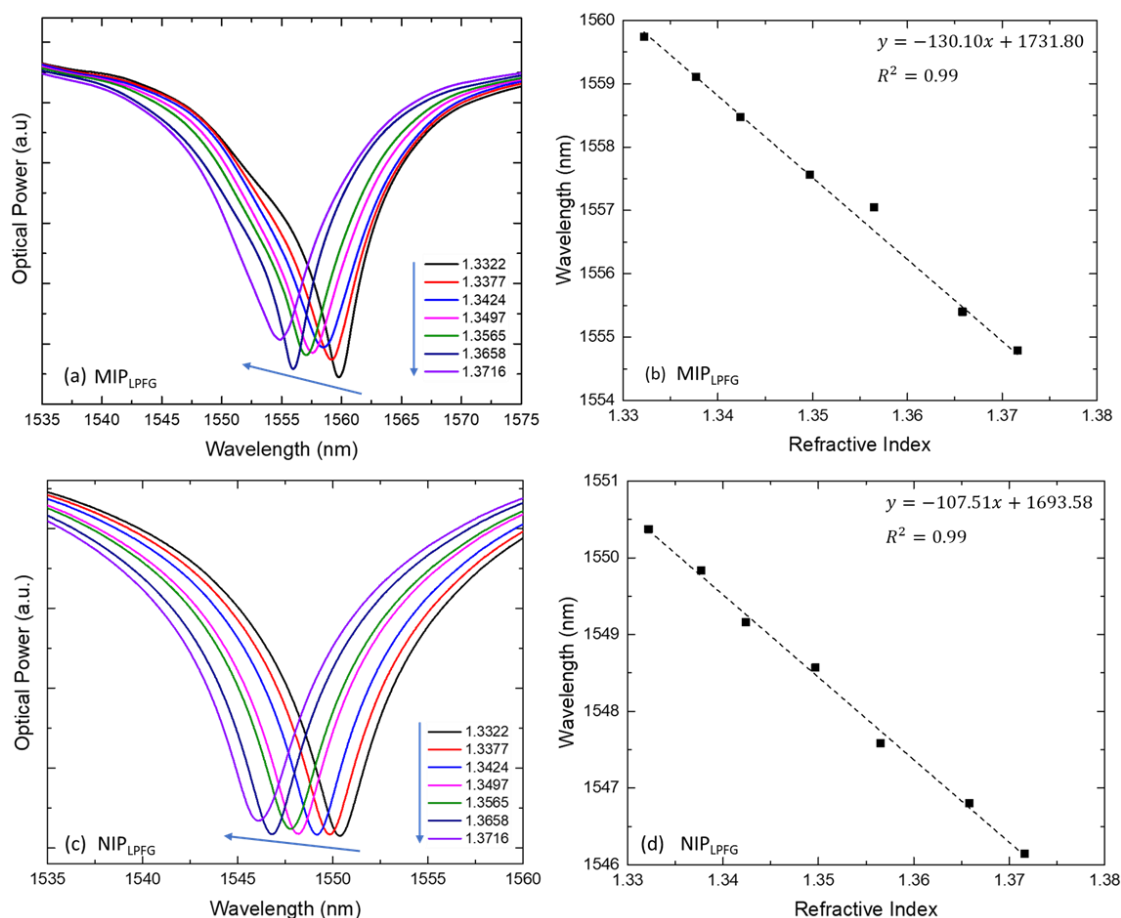


Figure 7. 12. Evaluation of the sensitivity of the LPFGs used for MIP coating (a and b) and for NIP coating (c and d).

The FOM of the LPFG used for MIP coating was also assessed, displaying a value around 16 RIU^{-1} in the refractive index range from 1.3322 to 1.3658.

7.3.2.2. Step-by-step of MIP-sensor preparation

In the allylation process, a self-assembled monolayer was covalently formed at the LPFG surface causing a variation of the RI (by increasing it) in the surrounding medium of the optical fiber. This RI increment is proved by the resonance wavelength blue shift (125 pm to lowest wavelengths) observed in Figure 7.13 (from **a** to **b**) which confirms the layer formation. The same behavior was observed by *Gupta* after immobilizing APTES on an LPFG surface for posterior soil fungi detection [410].

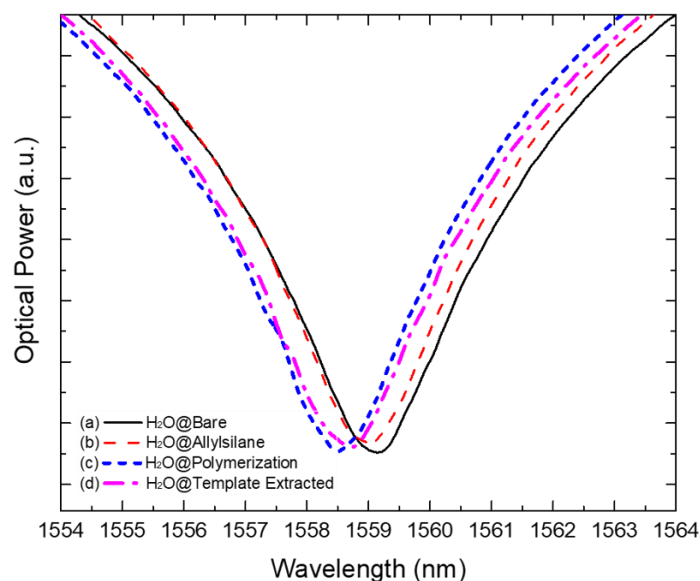


Figure 7. 13. Acquired spectra showing the wavelength resonance band position resulted from measurements in pure water of (a) bare LPFG (solid black line); (b) allyl-silanized LPFG (dashed red line); (c) MIP-coated LPFG (short dashed blue line); and (d) MIP-coated LPFG after template extraction.

Moreover, a shift was also observed resulting from the polymerization process (from **b** to **c**). The spectral evolution of the polymeric layer formation was controlled in real-time by monitoring the LPFG wavelength shift during all the process (Figure 7.14). As a result of the polymerization and further washing process, the resonance wavelength band stabilized, fixing its position at 575 pm and 450 pm away from the H_2O and the $H_2O@Allylsilane$ spectra, respectively (Figure 7.13c). This result match the observation reported by *Arcadio et al.* for a surface plasmon resonance (SPR) sensor for bovine serum albumin (BSA) detection [411]. Similarly, *Verma and Gupta* used a similar approach to develop a MIP layer onto a silver-coated silica optical fiber for antibiotic recognition using the SPR phenomenon [412].

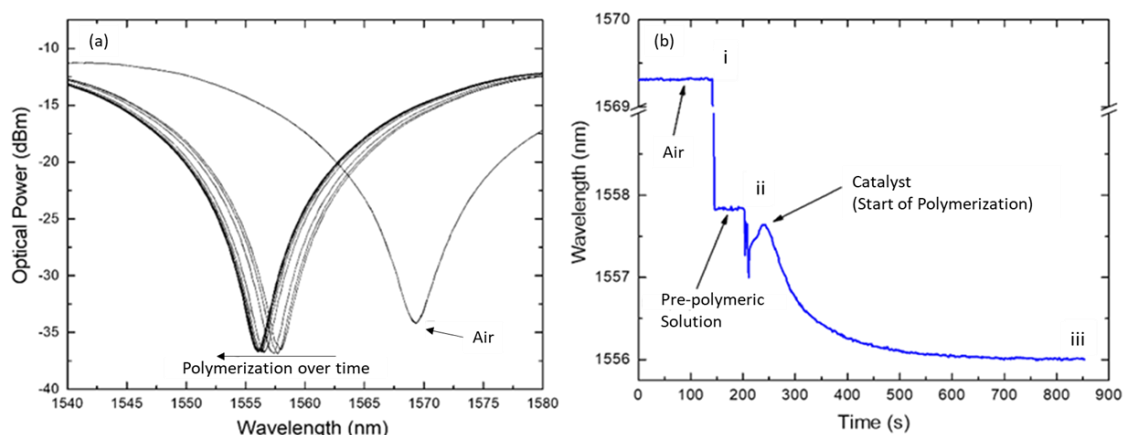


Figure 7. 14. (a) Position of the spectrum changing over time and (b) tracking of the band position versus the deposition time where: (i) is the time when the polymeric solution was dropped onto the LPFG followed by the consequent shift due to RI change in the surrounding medium; (ii) is the time when the catalyst was placed on the previous solution followed by the beginning of the polymerization process; (iii) is the time when the polymerization process was interrupted (after about 10 min from step ii).

After template extraction, a decreasing on the effective refractive index around the LPFG (shifting 300 pm to higher wavelengths) was observed which can be related to the IgG molecules removal from the MIP layer (Figure 7.12d). In literature, similar procedures to remove electrostatically immobilized templates using NaCl were reported by other authors, *e.g.*, by Schwark *et al.* to remove IgG molecules from imprinted macroporous membranes; Matsumoto *et al.* to remove prostate-specific antigen (PSA) from a synthesized MIP layer on an SPR chip; and by Yang *et al.* when developing a selective and sensitive impedance sensor targeting the BSA protein, using a NaCl/SDS solution in the template extraction stage [413-415]. The resulted MIP receptors were tested in the presence of IgG antibodies and competitors. The NIP formation process was carried out in the same experimental conditions and the resulted data is exhibited in Figure 7.15.

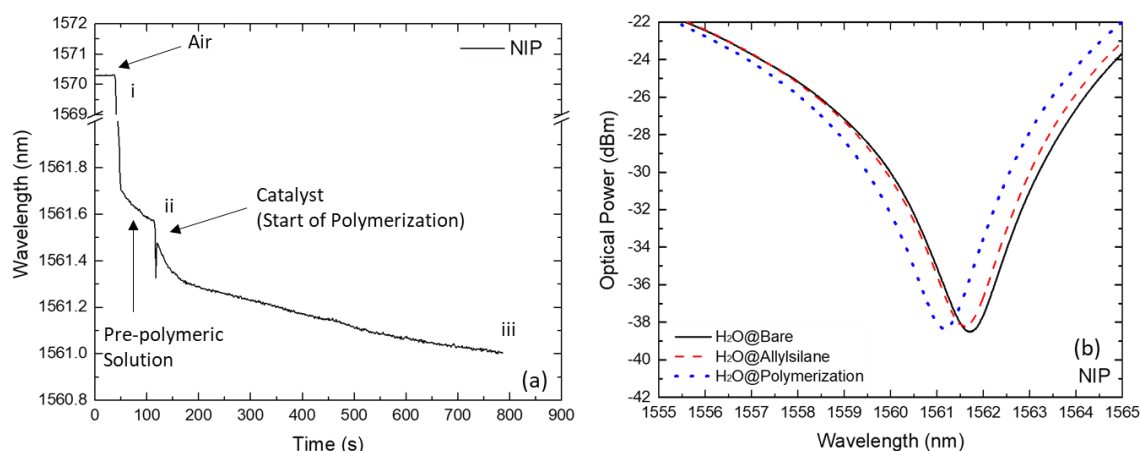


Figure 7. 15. (a) Real timeline of the resonance wavelength position of the NIP polymerization process: i. the pre-polymeric solution was placed; ii. the catalyst was placed; iii. the polymerization process was stopped. (b) Acquired spectra showing the wavelength resonance shift after allyl-silanization process (dashed line) and MIP layer formation (dotted line). Solid line is the bare fiber spectrum in water.

7.3.2.3. Binding experiments

The binding experiments were carried out placing the MIP-coated and the NIP-coated LPFGs in different grooves but using the same standard IgG solution to assess its spectral behavior. The exact position of the wavelength band is given by a gaussian fit. The bare LPFG was also exposed to the same standard solutions and its $\Delta\lambda$ was also evaluated. In that way, Figure 7.16 displays the MIP-coated LPFG transmitted spectra for different concentrations of IgG antibodies and reveals a systematic blue shift of the resonance wavelength when the analyte concentration increases.

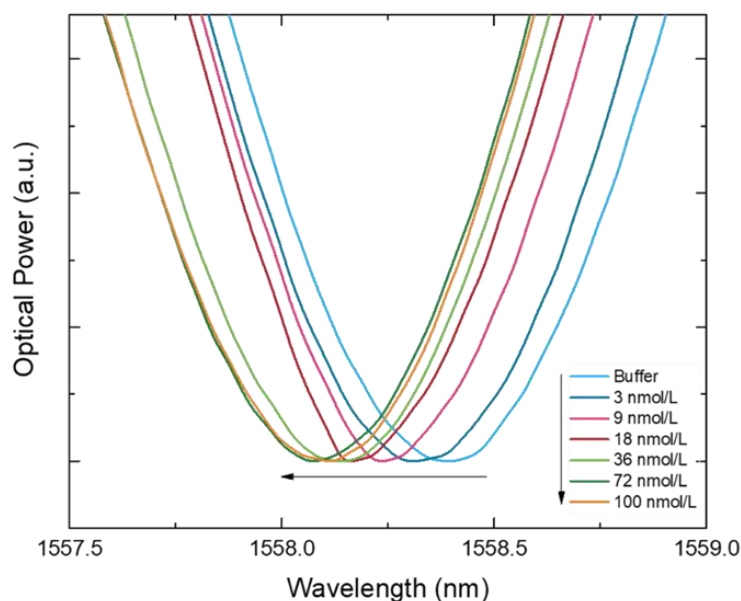


Figure 7. 16. Transmitted spectra (normalized to the minimum) shifting according with the increase of IgG concentration ($n = 10$). Some curves were removed (1; 6; and 13 nmol/L) to provide the clearest image for a better evaluation of the spectral behavior.

To understand the impact, on the LPFGs, of the refractive index deviations of the external medium, caused by “bulk effect” and/or temperature fluctuations, the responses (in the format of $\Delta\lambda$) of the NIP-coated LPFG (NIP@IgG) and the bare LPFG (Bare LPFG@IgG) were measured and compared (Figure 7.17).

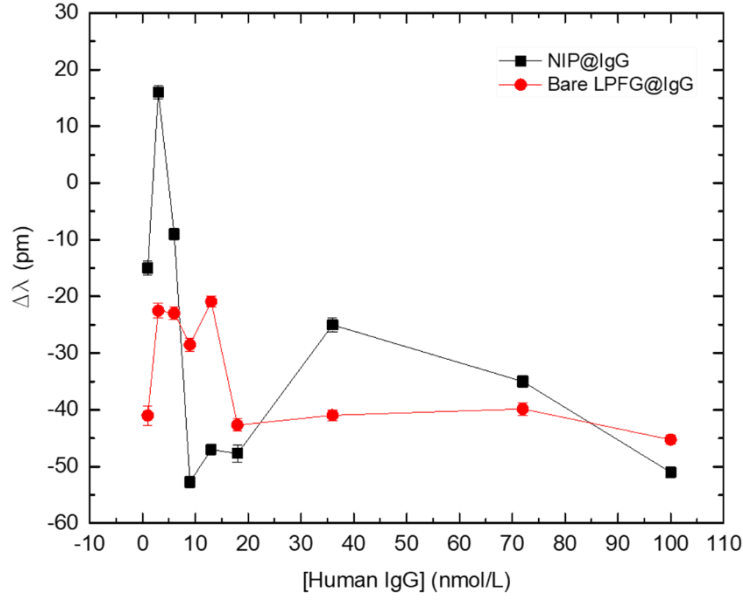


Figure 7. 17. Wavelength variation ($\Delta\lambda$) of the NIP-coated LPFG (black squares) and the bare LPFG (red circles) in the presence of different IgG concentrations. Errors bars were obtained from standard deviations.

Evaluating the results, obtained in the presence of the same analyte solutions, it is possible to conclude that they display similar behaviors. However, it is also noticeable that, along with the similar general trend, there are fluctuations on $\Delta\lambda$ that could be attributed to external effects (e.g., temperature variations, physical tension) as well as to “bulk effect” for the NIP-coated LPFG.

Considering the results from Figure 7.17, this configuration allows a correction to external parameters in real time making the interaction of the MIP-coated LPFG with different concentrations of IgG antibodies (MIP@IgG) with increased reliability of the sensing scheme. The proposed correction consists in subtracting the response of the NIP-coated LPFG to the signal of the MIP-coated LPFG layer, according with Equation 7.2. Raw experimental data and corrected data are plotted on Figure 7.18. The calibration curves were fitted using the Hill model (Equation 7.3):

$$\Delta\lambda_c = \frac{\Delta\lambda_{max} \times C^n}{(K^n + C^n)} \quad (7.3),$$

where $\Delta\lambda_c$ is the wavelength variation at the concentration C , $\Delta\lambda_{max}$ is the wavelength variation at the saturation point (or response to the infinite); K is the ligand concentration at the $1/2 \Delta\lambda_{max}$; and n is the Hill coefficient.

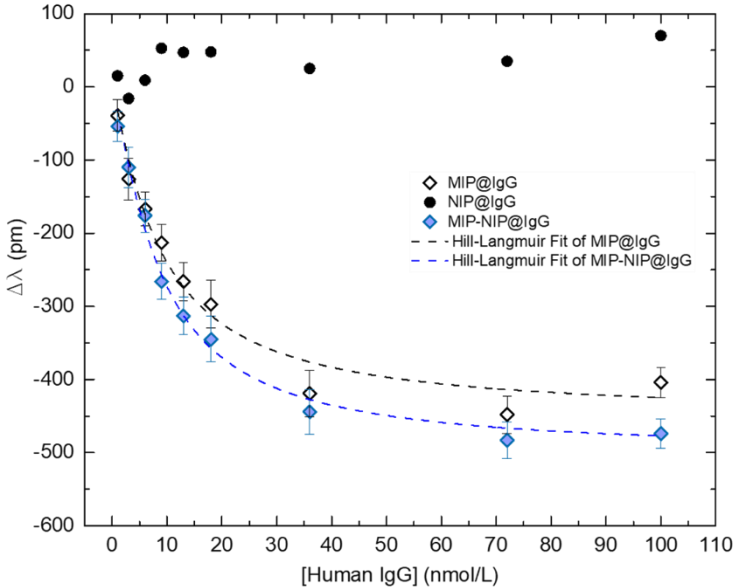


Figure 7. 18. Wavelength variation ($\Delta\lambda$) of the MIP-coated LPFG (green diamonds), NIP-coated LPFG (black squares), and the differential “MIP-NIP” (blue triangles) in the presence of different IgG concentrations. The dashed blue line and the pointed green line are the respective fittings, using the Hill model equation, of the MIP-coated LPFG and NIP-coated LPFG.

It is noticeable that the $\Delta\lambda_{\text{Sensor}}$ mode reveals a better fitting to the Hill-Langmuir model, improving the performance of the sensor targeting the analyte and providing more reliable results and this is a clear demonstration of the importance of using the NIP-coated LPFG for real time correction of disturbances caused by “bulk effect” and external influences in the measured solution. Moreover, Table 7.2 shows the statistical data from both fittings, revealing a small Residual Sum of Squares (RSS) proving that the differential “MIP-NIP” is the better method to use in this kind of measurements.

Table 7. 2. Statistical data resulted from the respective Hill fittings of the curves presented in Figure 7.17.			
Model	Reduced Chip-Sqr	Residual Sum of Squares	R-Square (R^2)
$ \Delta\lambda _{\text{MIP@IgG}}$	0.9189	6.4322	0.973
$\Delta\lambda_{\text{Sensor@IgG}}$	0.4067	2.8470	0.990

However, to assess the full sensitivity of the sensing platform we need to resort to the Hill-Langmuir equation by plotting the experimental data in the lin-log form, *i.e.*, the wavelength variation (in nm) versus the logarithm of the IgG concentration (in nmol/L),

to achieve symmetrical confidence intervals for the experimental parameters [255, 416, 417]. Therefore, the respective Hill fitting (Figure 7.19) was plotted through Equation 7.4, where $\Delta\lambda_c$ is the wavelength variation at the concentration C ; $\Delta\lambda_{max}$ is the wavelength variation at the saturation point (or response to the infinite); K is the ligand concentration at the $1/2 \Delta\lambda_{max}$; and n is the Hill coefficient, with: $K = 8.61$ nmol/L, $n = 1.24$, and a $\Delta\lambda_{max} = 490$ pm.

$$\Delta\lambda_c = \frac{\Delta\lambda_{max}}{1 + 10^{n(\log K - \log C)}} \quad (7.4).$$

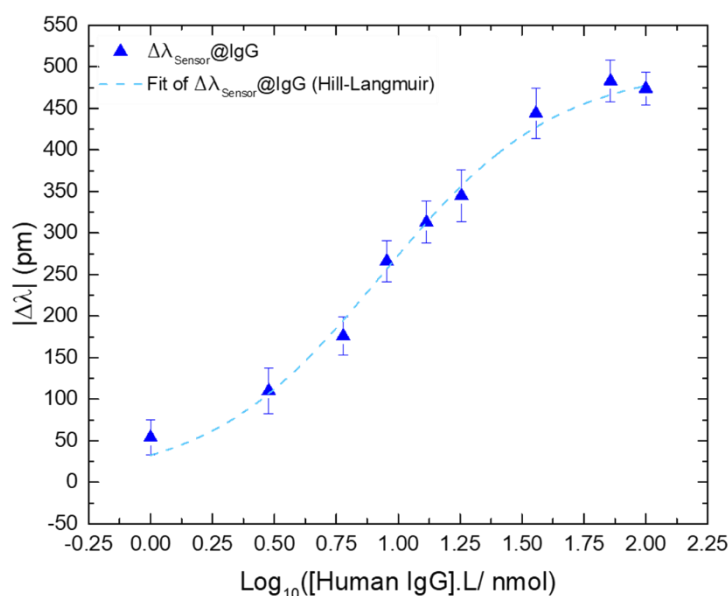


Figure 7. 19. Wavelength variation of the differential $\Delta\lambda_{\text{Sensor @IgG}}$, as a function of the logarithmic concentration of IgG (blue triangles); and the respective Hill fitting ($R^2 = 0.9912$; dashed blue line). Errors bars were obtained from standard deviations.

The calculated sensitivity at low concentrations (for $C < K$) was $|S| = 57$ pm.L/nmol for the differential system and $|S| = 46$ pm.L/nmol for the MIP system ($|S| = |\Delta\lambda|_{max}/K^n$), in the range from 1 to 36 nmol/L. The affinity constant (K_{aff}) was also assessed revealing a value of $K_{aff} = 0.12$ L/nmol ($K_{aff} = 1/K^n$). The limit of detection (LOD) was calculated based on a proposed method for non-linear sensors, using the signal-to-noise (S/N) approach [418]. This method was suggested for an ion-selective electrodes (ISEs) model through Equation 7.5.

$$LOD_{S/N} = \beta_2 \left(10^{\frac{3\sigma}{\beta_1}} - 1 \right) \quad (7.5),$$

where β_2 relates with the selectivity and activity of the ions, β_1 is the slope parameter, and σ is the standard deviation of the blank. Adapting the LOD definition for non-linear sensors to Equation 7.4, considering that the n parameter is, geometrically, the factor that characterizes the slope of the curve at the midpoint and σ is the standard deviation of the blank, the LOD for this kind of curves can be defined as [417]:

$$LOD = \log K (10^{\frac{3\sigma}{n}} - 1) \quad (7.6).$$

Therefore, the calculated LOD for the proposed sensing platform was 0.25 nmol/L for the $\Delta\lambda_{\text{Sensor@IgG}}$ system and 0.35nmol/L for the $\Delta\lambda_{\text{MIP@IgG}}$ system. Additionally, the reversibility of the sensor was also evaluated, by applying the same protocol used for template extraction. However, as is shown in 7.20, the sensor wasn't totally regenerated (just about 82%) and further research needs to be done to improve the regeneration step.

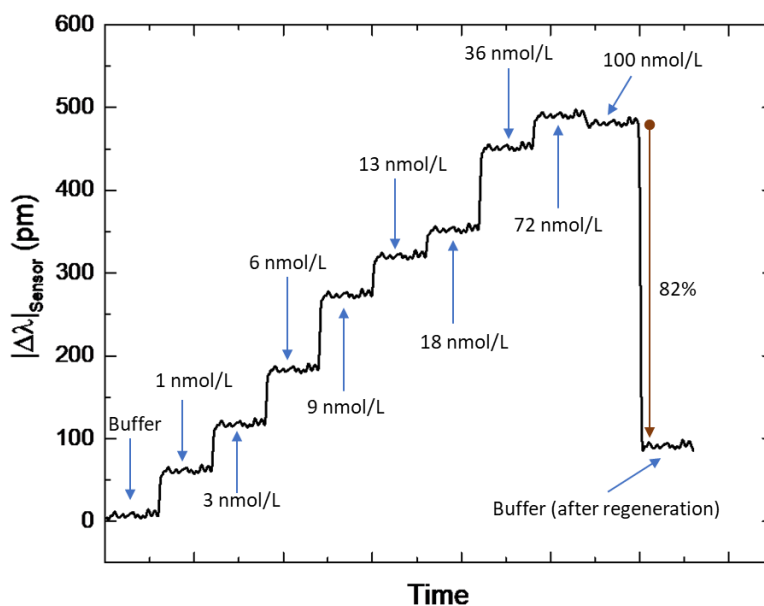


Figure 7. 20. Wavelength peak position of the differential (MIP-NIP) shifting over time throughout reversibility trials, including the calibration timeline.

Table 7.3 shows a comparison between the presented sensor with others in the literature for IgG (or anti-IgG; human or others) using different configurations, working ranges and LODs based on different configurations using optical fibers.

Table 7. 3. Comparative analysis of different platforms for human IgG detection based on optical fibers sensors.

Sensor Configuration	Transduction Method	Analyte (Receptor)	Detection Range	LOD	Ref.
TFBG modified with GO and staphylococcal protein A.	SPR	Human IgG (protein A)	30–100 µg/mL	0.5 µg/mL	[419]
Au film coated photonic crystal fiber dopped with AuNPs modified with protein A and anti-human IgG.	SPR + LSPR	Human IgG (anti-IgG)	1–30 µg/mL	0.037µg/mL	[420]
H-shaped optical fiber coated with an Au thin film and modified with anti-IgG receptors	SPR	Human IgG (anti-IgG)	10-100 µg/mL	3.4 µg/mL	[421]
S-tapered fiber modified with dopamine and Protein A.	Mach-Zehnder interferometer	Human IgG (protein A)	0.25–2 µg/mL	0.028 µg/mL	[422]
Thin core single mode fiber sandwiched two single mode optical fibers modified by anti-IgG immobilization.	Mach-Zehnder interferometer	Human IgG (anti-IgG)	100–1000 µg/mL	not reported	[423]
Sol-gel-based titania-silica over coupled LPFGs modified by mouse IgG immobilization.	LPFG	Anti-mouse IgG (mouse IgG)	0.1–100 µg/mL	0.025 µg/mL	[424]
GO-coated-U-bent LPFG inscribed in a two-mode fiber modified by anti-human IgG immobilization.	U-bent LPFG	Human IgG (anti-IgG)	3–20 µg/mL	0.023 µg/mL	[425]
MIP-coated LPFG for Human IgG detection	LPFG	Human IgG (MIP layer)	0.15–15 µg/mL (1–100 nmol/L)	0.037 µg/mL (0.25 nmol/L)	this work

TFBG-tilted fiber Bragg grating; SPR-surface plasmon resonance; LPFG-long period fiber grating; GO-graphene oxide; LOD-limit of detection; Ref.-reference.

Additionally, the sensor proposed in this work is an easy device to build, requiring fewer protocols for its development. Furthermore, despite using synthetic materials for specific recognition, the proposed sensing platform offers a similar LOD, and a significant resolution (considering the lowest evaluable concentration) compared to the majority of the sensors listed in Table 7.3. Moreover, the synthetic receptors are an advantage compared with the natural receptors considering the production requirements. Finally, the included correction system improves the sensor performance by offering a more accurate response.

Despite the difficulties in finding LPFG-based sensors coupled with MIPs for IgG detection, other sensing platforms such as electrochemical-based sensors or SPR-based sensors using planar gold chips, applying molecular imprinting for its selective detection have been reported. Table 7.4 compares some of those investigations based on MIPs for biorecognition of the IgG molecule.

Table 7. 4. Comparative analysis of different platforms for human IgG detection based on molecular imprinting.

Sensor Configuration	Transduction Method	Analyte (Receptor)	Detection Range	LOD	Ref.
SPR Au chip modified with a MIP nanofilm	SPR (planar Au chip)	F _{ab} fragment (MIP nanofilm)	2 – 15 µg/mL	0.056 µg/mL	[426]
nanoMIPs-coated SPR gold chip	SPR (planar Au chip)	IgG, F _c domain, peptide epitope (nanoMIPs)	0.003 – 1 µg/mL	not reported	[427]
Electrochemical biosensor based on graphene quantum dots covered with a MIP layer	Cyclic Voltammetry	IgG molecule (MIP layer)	10 ⁻⁴ – 0.05 µg/mL	2 × 10 ⁻⁵ µg/mL	[428]
MIP layer interfaced with a SAW chip	Surface Acoustic Wave	IgG molecule (MIP layer)	0.06–8 µg/mL	Not reported	[429]
MIP-coated LPFG for Human IgG detection	LPFG	Human IgG (MIP layer)	0.15–15 µg/mL (1–100 nmol/L)	0.037 µg/mL (0.25 nmol/L)	this work

The parameters displayed in Table 7.4 show that molecular imprinting materials applied to biosensing can compete directly with the sensors based on antigen-antibody specificity. Moreover, the sensing device proposed in this work has similar performances, although more investigations need to be conducted in order to improve specificity and sensitivity to achieve similar performances that are presented by electrochemical sensors.

7.3.2.4. Selectivity evaluation

The selectivity of the sensing platform was tested in the presence of IgG competitors acting as interferents and selected due to their abundant presence in the human blood plasma as well as the IgG antibody. Therefore, the negative controls were performed with HSA protein [430] and the HTR glycoprotein [431]. Other authors such as

Aylaz *et al.* or Ruiz *et al.* used the same blood plasma components to attest the selectivity of their platforms [432, 433]. Figure 7.21 show the information resulted from the sensing region incubation in 60 nmol/L (in PBS) of each interferent and in 36 nmol/L of the IgG standard solution, for five minutes. Between each step, the LPFG was washed several times with the buffer solution. The results are presented as mean of 10 measurements acquired after the exposure time.

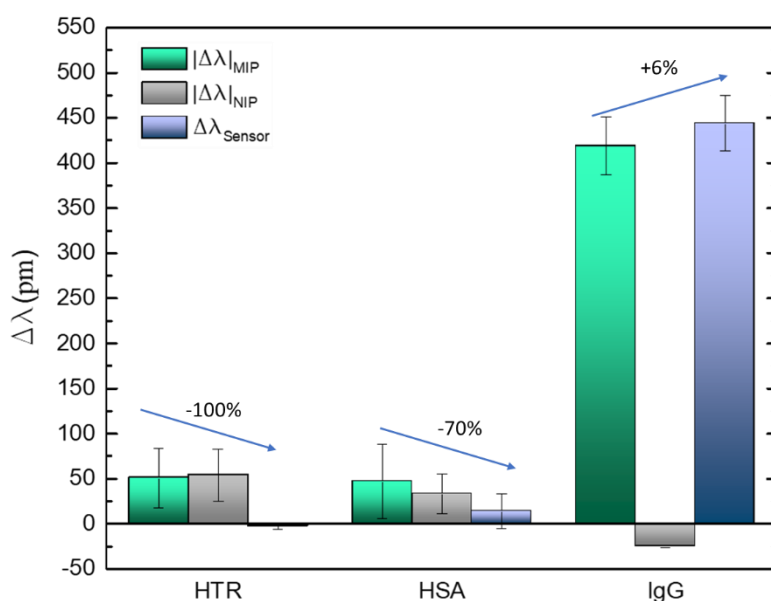


Figure 7. 21. Selectivity evaluation in the presence of the competitors (HTR and HSA) and the target analyte (human IgG). Errors bars were obtained from standard deviations.

In case of the control tests, slight shifts are visible for each competitor comparing with the specific binding towards IgG. It is undeniable that the sensitivity and selectivity of the imprinted cavities in the presence of the IgG antibody, revealing a wide variation for about half of the concentration used for the interferents assays. The impact of the proposed correction system is also noticeable, reducing the response to the interferents in, at least, 75%, allowing reliable measurements by improving the rejection of “false positives”.

7.4. Conclusion

This investigation aimed to demonstrate different approaches using LPFGs to develop biosensing devices. Additionally, the inclusion of a correction system, suitable for each platform, is also included in the main achievements resulted from this research.

In first place, a sensing platform for the detection of the anti-human IgG antigen was developed by chemical modification of a LPFG surface. The sensing methodology is based on refractometric changes caused by antibody/antigen interactions. The surface of the optical fiber was changed by the electrostatic immobilization of IgG-modified silica nanoparticles when were previously immobilized IgG antibodies that was used as receptor. The FTIR-ATR spectra proved that the biorecognition molecule was successfully attached onto the silica nanoparticles surface. The optical platform exhibited good performance targeting the antigen and had demonstrated its specificity by differentiating the analyte of interest from other similar species in solution. Despite the good results obtained from these preliminary results, this approach is dependent on the production of antibodies, which is a disadvantage. Thus, the second optical platform try to overcome these drawbacks associated to the use of this kind of bioreceptors.

Therefore, an optical (bio)chemical sensor system coupling LPFGs and molecular imprinted binding sites for specific detection of human IgG antibodies was developed and experimentally investigated. The sensor comprises the advantages associated to the optical fibers, combining them with the advantages linked to the molecular imprinting. The experimental results suggests that this platform reveals good performances in terms of selectivity. However, the sensitivity can be improved by reducing the polymerization time, increasing the possibilities to obtain a larger number of binding sites at the LPFG surface and avoid fast saturation, and by coating the LPFG surface by metal-oxide films to increase its sensitivity. It was noticed that the sensor recovery after template extraction is somewhat different from the shift observed after the calibration process. This situation could be explained by RI deviations or by the existence of low- and high-affinity binding sites. In the washing process, to remove non-reacted monomers, some IgG antibodies attached to low-affinity binding sites could be removed from the polymer surface. However, during calibration, those sites are still available for interaction with the analyte. Nevertheless, it is evident that the proposed label-free sensor, is based on a robust and emerging technique for MIP fabrication requiring a short incubation time for target detection. Moreover, the correction system seems to improve the sensor performance and its reliability, being a very attractive approach to be implemented in clinical diagnosis. Additionally, the sensing platform used in this work is simple to realize and employs

green strategies for biological recognition. Furthermore, the implementation of the proposed system is not limited to being applied in the medical field. Its combination with low-cost interrogation units, with several reading channels, results in very attractive devices for multiplexing different analytes of interest in water quality control, namely water contaminants. Moreover, SPR-based optical fibers can be used to perform MIP/NIP differential measurements using a single optical fiber employing two distinct sensing regions (MIP-coated and NIP-coated). In this way, is possible to obtain miniaturized devices with high sensitivity and selectivity for reliable chemical and biological sensing.

Chapter 8 – Hybrid Plasmonic Platform: Studies for Biosensing Applications

The coupling between SPR and LSPR phenomena on an etched-core MMF has been explored through, where was built a secondary plasmonic waveguide by immobilizing spherical nanoparticles on a gold layer surface. The plasmonic configuration was characterized by varying the external refractive index as well as the size of nanoparticles in order to evaluate the impact of that parameter on the sensor performance. Finally, biosensing experiments were conducted to evaluate the suitability of this novel plasmonic device for biological detection, using the thrombin protein as a biological model.

8.1. Introduction

Nowadays, biosensing devices have demonstrated great potential in a wide range of areas such as medicine, food industry (mostly in food safety), and environmental monitoring. Mainly for medical diagnosis or contaminants detection, biosensors need to include characteristics such as high accuracy, high sensitivity, and high specificity for the analyte of interest. In general, the simplest architecture of a biosensor involves a bioreceptor for specific analyte recognition (e.g., antibody/antigen, aptamers, or receptors based on molecular imprinting) and a transducer to convert the

receptor/analyte binding events into a measurable signal. Based on the transducing system, biosensors can be categorized as electrochemical, magnetic, piezoelectric, or optical [364, 434-436]. Optical biosensors have been attracting great interest due to their enormous potential to develop highly sensitive and specific, multiplexing, and low noise background devices. Plasmonic structures have offered a wide variety of sensing schemes, demonstrating their applicability in diverse areas [359, 437, 438].

As reported in previous sections of this document, the plasmonic phenomenon investigates the interaction of light with metals ranging from thin metallic films to metallic nanostructures, by combining techniques from photonics and electronics, thus generating surface plasmons. Coupled to biosensing, those interactions are dependent of the surrounded medium enabling the detection of a biomolecule of interest through the measurement of the effective RI variations caused by modifications on chemical structures or binding events. The concept and the ability of SPs and SPPs to guide and confine light into small subwavelength scale has been significantly investigated in the past decades in the context of nanophotonic research [439]. Such investigations have resulted in several plasmonic designs, playing a significant role in the development of highly sensitive and selective plasmonic biosensors, mainly based on two plasmonic techniques: SPR and LSPR.

Currently, the most used techniques to develop SPR or LSPR sensing platforms are based on prism coupling, gratings, and optical fibers. Despite some notable performances reported for prism-based [440] and gratings-based [441] SPR or LSPR sensors, optical fibers came to offer several benefits and overcome some drawbacks, mainly related with device miniaturization, *in situ* implementation and real-time detection. Optical fibers offer advantages such as strong anti-electromagnetic interference and microscopic size, offering designs such as single mode fibers SMF, MMF and PCF, based on high-order cladding mode, different air hole arrangement, fiber core mismatch fusion-type, and asymmetric fiber structures, allowing a strong investigation on the performance enhancement of plasmonic biosensors [442]. Basically, the detection concept relies on changes in the molecular mass of the receptor bound on the sensor surface, inducing a change on the refractive index at the surface of the sensor which causes a shift in the resonance wavelength, enabling the quantitative and/or qualitative real-time detection of the analyte. In literature, several researchers have reported the performances given by different approaches to develop fiber optic SPR-based sensors in numerous applications [443-446]. Other authors, drove their investigations to find new fiber architectures to enhance the performance of fiber optic plasmonic sensors. An

example of that was demonstrated by *Hu* and his co-workers by fusing a gold-coated no-core fiber between two multimode fibers to enhance the sensitivity of the fiber structure to the external refractive index variations [447]. The authors reported that the studied fiber configuration had reached a maximum sensitivity of about 11800 nm/RIU by measuring successive RI standard solutions ranging from 1.333 to 1.410. A similar approach was adopted by *Wang et al.* by splicing a microstructured optical fiber between two multimode fibers and coating the MOF region with a thin silver film [448]. The sensitivity of the MMF-MOF-MMF configuration was studied by varying the external refractive index with sucrose standard solutions ranging from 1.3328 to 1.3990, exhibiting a sensitivity of about 3223 nm/RIU. An interesting investigation was reported by *Liu et al.* by fabricating an optical fiber in which the refractive index of the core varies along the fiber, by employing a UV curable adhesive with distinct RIs and hollow capillary fibers [449]. The authors had studied the sensitivity performance of SPR sensors developed from the reported fiber architecture, concluding that the region with the smallest core RI provides better sensitivities (approximately 3500 nm/RIU) in a RI range between 1.335 and 1.385. In addition, other examples based on numerical results can also be found in literature [450-453]. Despite the advantages of those fiber optic structures, mainly associated with the enhancement of their sensitivity to external RI variations, there are easiest ways to increase the fiber optic plasmonic (bio)sensors performance. As reported, the detection of the analytes relies on the relationship between the number of receptors immobilized at the sensor surface and the resonance wavelength. Thus, the treatment of the sensor surface is crucial to the sensor performance [454]. Therefore, exploring combination of different materials with specific optical properties to coat the substrates is an attractive way to enhance the performance of sensors. For instance, dielectric materials with high refractive indices such as GO, metal oxides or transition-metals dichalcogenides (TMDCs) have been used to enhance the SPR optical properties of fiber optic plasmonic sensors and easily can be found in literature [455-460]. A different approach includes the use of plasmonic nanostructures. Accordingly, metal nanoparticles can be applied to SPR biosensors, resulting in the increase of the local electric field strength due to the coupling effect between localized and propagating plasmon resonance. The phenomenon leads to a sensitivity enhancement in relation to individual SPR or LSPR phenomena [461]. In systems comprising metallic nanoparticles localized very close to the metal surface, the modes of the surface plasmons and the modes of the localized surface plasmons interact between each other, leading to the formation of normal modes in the space between the metal layer and the nanoparticles in which the electric field, associated with those modes,

is strongly localized in the nanoparticles-metallic film surface system [454]. This interaction causes a wavelength shift on the SPR resonance band and, consequently, enhances the sensitivity of the sensor to the variations of the external RI. In literature, several investigations have been reported using fiber optic plasmonic sensors based on SPR-LSPR coupled effects to study the kinetic profiles of numerous biological interactions using different types of nanoparticles [129, 462]. Moreover, the enhancement of the optical properties of the SPR configurations by nanoparticles strongly depends on their shapes, sizes, and type of metal [463].

Accordingly, and focusing on metallic nanospheres, one of the most used methods includes the detection of the target analyte using the so-called “sandwich” assays. While the surface of the thin metal film is decorated with the specific receptor, the target is immobilized onto the nanoparticle surface that works as a tag molecule. When the binding event occurs, the coupling between the nanoparticle and the receptor at the metal surface results in a larger refractive index change, thus increasing the shift of the SPR curve. Several works comprising “sandwich”-type fiber optic SPR biosensors, have been described in literature [464-468]. Another approach includes the direct immobilization of the metallic nanospheres directly on the thin metallic film and further immobilization of the specific receptors on the surface of the nanostructures. For instance, *Wang et al.* studied and compared the performance of three different SPR sensors fabricated on a photonic crystal fiber: i) PCF/Au film/receptor; ii) PCF/Au film/AuNPs/receptor; and iii) PCF/Au film/AuNPs/Protein A/receptors; in which the used receptors were anti-IgG antibodies [420]. The authors concluded that the PCF/Au film/AuNPs sensor reached highest sensitivity (about 1.6 times higher) than the PCF/Au sensor. They also reported that the use of Protein A leads to a better orientation of the anti-IgG receptors compared with the direct immobilization of the PCF/Au film and the PCF/Au film/AuNPs fiber sensors, exhibiting lower limits of detection. A different approach was adopted by *Niu et al.* by using a side polished MMF (105/125 μm ; core/cladding) with a sputtered 50 nm thick gold film on the polished zone [469]. The NPs were immobilized on the Au film surface assisted by the sulfhydryl groups (-SH) of the 1,6-hexadithiol compound. The authors reported a sensitivity enhancement of about 1.4 times higher than the sensitivity exhibited by the Au coated side-polished fiber. A similar method was used by *Cheng et al.* by side polishing a single mode fiber (8.2/125 μm ; core cladding) in which the polished region was coated with a 100 nm thick of a dielectric-matching-layer, followed by the deposition of a 40 nm thick Au film [470]. The Au film surface was then decorated with Au-nanoshells using Au-S bonds, followed by the immobilization of goat anti-human IgG antibodies. The authors had compared the

performance of the described platform with a similar sensor where the Au-nanoshells were not included, reporting a better performance exhibited by the Au-nanoshells modified SPR sensor by targeting human IgG antibodies. And many other research works using metallic nanoparticles for SPR sensitivity enhancement can be found in literature, including experimental and/or numerical studies [471-475].

In this investigation, was developed and studied a new methodology to fabricate a fiber optic plasmonic sensor, based on the coupled effects between plasmonic thin films and plasmonic nanostructures. The resulted configuration includes an optical fiber etching process to reduce the core diameter and the coating of a thin gold film by physical vapor deposition induced by electron beams. Moreover, gold nanospheres, capped with sodium citrate, were immobilized on the metallic layer surface by electrostatic interactions using a three-layered polyelectrolyte structure. In addition, the optical response of the resulted platform was studied by varying the size (diameter) of the used nanospheres, in which their sensitivity enhancement, final resonance band position, and the figure of merit were assessed and compared. Moreover, the sensor was validated for biosensing applications using as a model the thrombin protease, an important active involved in the coagulation cascade, thrombosis, and hemostasis [476].

8.2. Working Principle

The working principle of the proposed plasmonic configuration is based on the excitation of a SPR through light scattering on the nanoparticles, that gets coupled to the thin metallic film. However, such excitation can only occur if some of the core guided-light modes reach the external medium to interact directly with the nanoparticles, which can be achieved by a reduction of the MMF core via chemical etching. The etching process (reached by applying concentrated hydrofluoric acid to the exposed core) causes very smooth transition regions between the core and the polymeric cladding (that it is not affected by the acid) (Fig. 8.1).

The geometrical changes to the fiber core structure leads to some light propagation towards the external medium, affecting the guided modes supported by the fiber structure. Part of that light will then interact with the nanoparticles attached to the thin film surface and via scattering effects, the light will excite an SPR on the thin film/external medium interface. Similar effects can be seen in light harvesting devices using NPs where light scattering by them gets trapped more efficiently [477, 478]. Also, due to the

small thickness of the metallic film, light will reach the film/core interface, where it will be recoupled to the fiber (Fig.8.1b, inset figure).

Although this coupling mechanism is based on the scattering of each individual NP-film interaction, the surrounding NPs also affect the excited SPR, by two mechanisms: plasmonic coupling between NPs and medium refractive index change, produced by the total density of NP over the film.

To account for the later, the optical properties of such random distribution of NP can be described by effective medium approximations, such as, the Maxwell-Garnet (MG) effective medium hypothesis [479]. Thus, the inhomogeneous medium, created by the random distribution, can be considered a homogeneous medium with an effective RI, given by the relation between the external medium and the NPs refractive index (Fig. 8.1a, inset figure) [480]. Hence, the excited SPR features are affected due to the presence of the complex and high effective RI layer, inducing an enhancement of the sensitivity of the plasmonic sensor to the external RI changes [481]. This phenomenon can be of great practical relevance since by changing the NP density, one can tune the SPR band characteristics, as RI sensitivity and band position [480, 482]. There is also another relevant, and perhaps more important, tuning mechanism, related to NP size. Since the light-scattering angular distribution is deeply linked with the NPs geometry and size, it is possible to change the wave-vector that will excite the SPR in the NP/film interface, thus changing the wavelength position of the excited SPR band. This wavelength tuning mechanism can be seen in Fig. 8.1c, where it can either locate the resonance wavelengths band close to the SPR initial band or shifting it to higher wavelengths by increasing the size of the metallic NP.

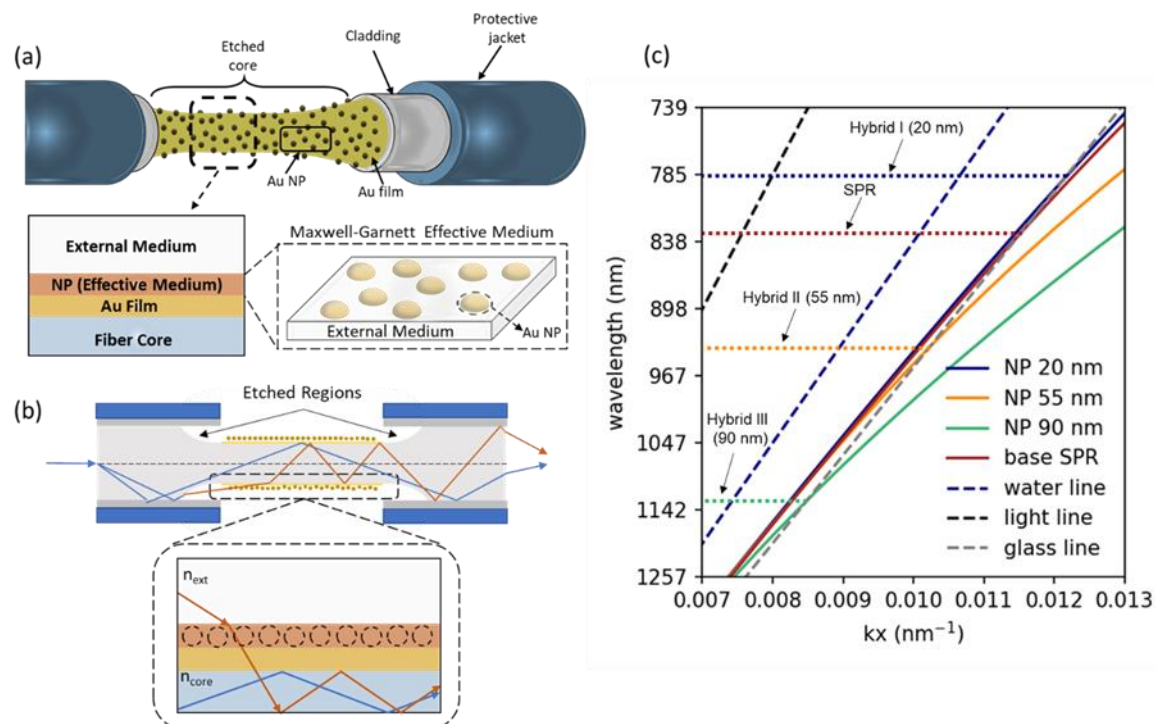


Figure 8. 1. (a) Illustration of the optical fiber plasmonic sensor arrangement, including the types of materials composing the layers from the silica core to outwards as well as the Maxwell-Garnett Effective Medium representation (inset figures); (b) illustration of how the waveguided modes propagate along the etched core, including the light escaping to the external medium and consequently light recoupling through the NP structure (inset figure); and (c) dispersion relation of the plasmonic modes for the three different NP diameters with equal densities and for a 50 nm thick Au film (solid lines) and of the dispersion curves for the water, light, and silica (dashed lines). Dotted lines display the wavelength position, at $n_D = 1.333$, in which each hybrid band should arise, by localizing the interception points between the silica line and each plasmonic modes.

8.3. Materials and Methods

8.3.1. Chemical reagents

The followed chemical reagents were used for the gold nanospheres (AuNS) synthesis and for further immobilization on the optical fiber: gold salt ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), tannic acid ($\text{C}_{76}\text{H}_{52}\text{O}_{46}$), trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), polyethylenimine (PEI, 750 KDa; $\text{H}(\text{NHCH}_2\text{CH}_2)_n\text{NH}_2$), and poly-sodium 4-styrenesulfonate (PSS, 70 KDa; $(\text{C}_8\text{H}_7\text{NaO}_3\text{S})_n$). Dextrose (D-(+)-Glucose; $\text{C}_6\text{H}_{12}\text{O}_6$) was used to produce the standard refractive index (RI) solutions. Hydrofluoric acid (HF; ACS Reagent, 48%) was used for the chemical etching process. Poly-L-lysine (PLL, 0.1% (w/v) in H_2O), SSPE buffer solution (20x concentrate), Tris base ($\text{NH}_2\text{C}(\text{CH}_2\text{OH})_3$; for molecular biology, 99.9%;), potassium chloride (KCl; Bioextra, 99%), sodium chloride (NaCl; Reagent Plus, 99%), magnesium chloride (MgCl_2 ; anhydrous, 98%), phosphate buffered saline tablets (PBS), bovine serum albumin (BSA; lyophilized powder, 96%), hydrochloric acid (HCl, ACS Reagent, 37%), α -thrombin from human plasma (THR; lyophilized powder, >2800 NIH units/mg protein), and amine-modified thrombin binding aptamer ($\text{H}_2\text{N-TBA}$;

reverse phase HPLC purification) were used for the biosensing assays. Albumin from human serum (HSA; $\geq 97\%$) and transferrin from human blood plasma (HTR; $\geq 95\%$) were used for cross-reactivity tests.

All the used chemicals were purchased from Sigma-Aldrich (Merck Group), except for the Tris base and the $\text{H}_2\text{N-TBA}$ which were purchased from Fisher Scientific (Thermo Fisher Scientific Group) and Eurogentec, respectively. Moreover, all the chemicals were used without additional purification. Ultra-pure water (analytical grade), ethanol (EtOH; 96%), and acetone were also used.

8.3.2. Gold Nanospheres Synthesis

The AuNS were synthesized by a kinetically controlled seeded growth process based on the work developed by *Bastus et al.*, also described in this document in subsection 5.2.2.2 [301]. From the synthesis had resulted AuNS with different diameters: i) 20 nm; ii) 55 nm; iii) 90 nm. Moreover, the synthesized AuNS were capped with sodium citrate allowing negative charges formation on their surface.

8.3.3. Fiber Optic Platform Fabrication and Characterization

The fiber optic configuration proposed in this work was fabricated in a MMF (FT600EMT, Thorlabs), with a core/cladding diameter of 600/630 μm , respectively, and a 10 mm long sensing section, in three steps: i) chemical etching process with HF to reduce the silica fiber core diameter; ii) coating with a thin Au film using the PVD method achieved by EBID; and iii) AuNS immobilization on the surface of the Au film. A brief explanation of the experimental procedure applied in each step is described as follows:

- i. After exposing the fiber core, by mechanical removal of the plastic fiber jacket and the Hard Fluoropolymer cladding, the uncladded section was subjected to a chemical etching process, by immersing the exposed core in a 48% hydrofluoric acid during 40 minutes at 22 °C and 60% of humidity until the etched region became $\sim 500 \mu\text{m}$ diameter. This process resulted in a soft transition from the thicker region to the thinner core region due to the meniscus interface between the etching liquid and the silica fiber.
- ii. The etched region was coated with $\sim 3\text{nm}$ chromium (Cr) layer to enhance the adhesion properties of Au, followed by the deposition of a $\sim 50 \text{ nm}$ thick Au film. Both coatings were attained by using an electron beam evaporator equipment as described in subsection 5.2.4. Briefly, the etched optical fibers were placed on a homemade rotatory system to rotate the fibers inside of the

vacuum chamber with a velocity of ~ 5 rpm, producing homogeneous films around the cylindrical optical fiber. The Au films deposition carried out at high vacuum, at a base pressure of 2×10^{-6} mbar, and with a deposition rate of 1 nm/s. The thickness was controlled in real-time by a quartz crystal microbalance (Edwards, UK) along with a previously made calibration procedure.

- iii. The AuNS immobilization onto the Au film surface was assisted by electrostatic interactions between the negative charges at the AuNS surface (provided by the sodium citrate) and positively charged polyelectrolytes, using the layer-by-layer method. Initially, the Au-coated region surface was washed with acetone and EtOH, and dried with pure nitrogen. Then, the sensing region was immersed into a PEI solution (1 mg/mL in ultra-pure water) for 30 minutes, ensuring positive charges at the Au film surface. Thereafter, the same portion of the fiber was immersed into a PSS solution (1 mg/mL) for 20 minutes, followed by new immersion into a PEI solution for 20 minutes. From the procedure, have resulted a positively charged surface, with a homogeneous charge distribution. Moreover, between immersions, the sensing region was washed with ultra-pure water for three times.

Additionally, Figure 8.2 illustrates the procedures described above, exemplifying the procedure from the simple uncladded fiber to the final structure (Figure 8.2a), also displaying a microscope picture (Figure 8.2b, obtained from a Microscope – DM500, Leica), highlighting the transition zone from the full fiber core to the etched region as well as the diameters of the presented distinct zones of the fiber.

Furthermore, this procedure was applied at three distinct optical fibers, keeping the etching process and the thickness of the Au coating, but changing the size of the AuNS, resulting in the fabrication of three different platforms labeled as follows: i) Hybrid I (AuNS 20 nm diameter); ii) Hybrid II (AuNS 55 nm diameter); and iii) Hybrid III (AuNS 90 nm diameter). Moreover, was developed an additional fiber optic structure fabricated under the same procedure in which the etching process was removed, to obtain an AuNS-modified SPR configuration for further performance comparisons.

The sensitivity of all platforms was determined by immersing the sensing regions on mixtures of dextrose and ultra-pure water at different concentrations, offering different RI from 1.333 to 1.380, evaluate by a digital refractometer (DR-A1, Atago CO., LTD).

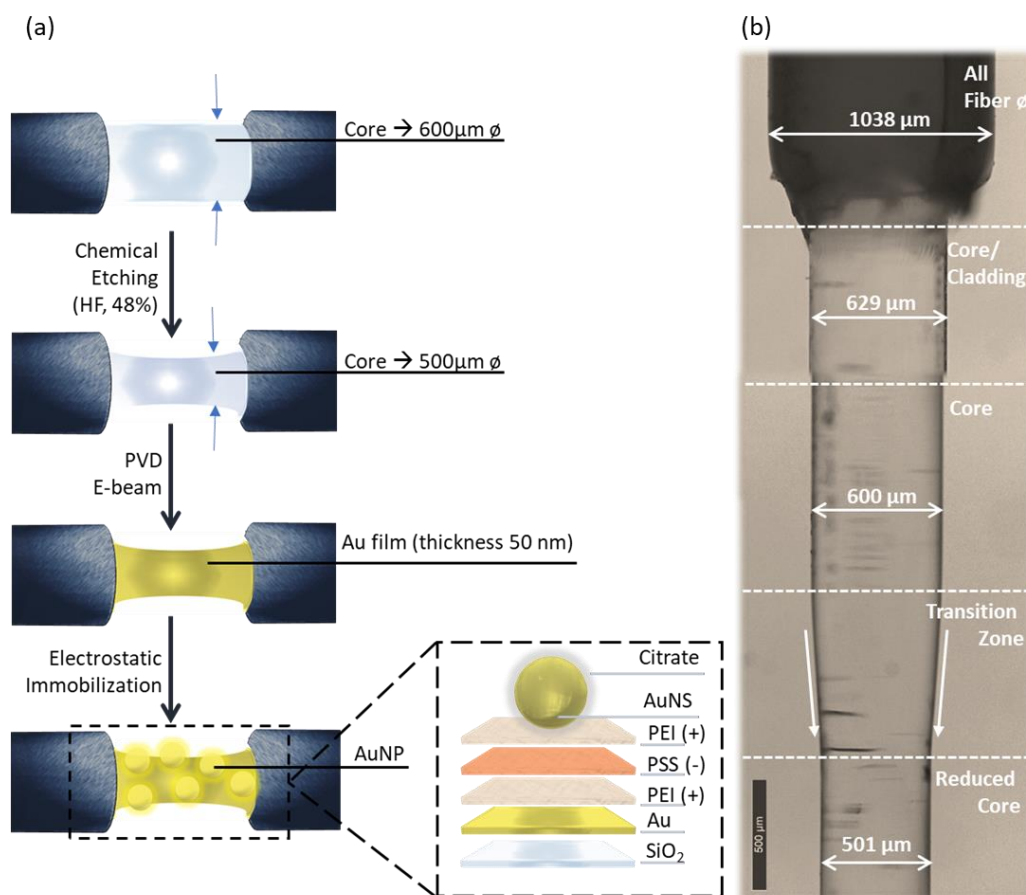


Figure 8. 2. Schematic illustration of the procedure applied to the optical fiber from the simple uncladded fiber to the final hybrid structure (a), and a microscope picture of one of the used fibers, highlighting the transition zone from the full fiber core to the etched region as well as the diameters of the presented distinct zones of the optical fiber.

8.3.4. Instrumentation and Experimental Setup

The optical fiber comprising the sensing region was connected to the main arm of a MMF bundle (Y-shaped with fiber core of 600 μm diameter; BFY600S02, Thorlabs) through SMA905 fiber connectors, whereas one of the secondary arms was connected to a tungsten continuous light source (LS-W20; Sarspec, Portugal) and the other secondary arm was connected to a high-resolution spectrometer (Speed+, Sarspec, Portugal). On the other end of the sensing fiber was fabricated an aluminum (Al) mirror to reflect the light, thus providing a second passage of the light in the sensing region and drive it into the arm connected to the spectrometer (Figure 8.3). The absorption spectra were acquired on a wavelength range from 200 nm and 1050 nm. In order to enhance the spectral range, in some cases, the output fiber was connected to another MMF bundle in which the secondary arms were connected to the reported spectrometer and to a

near-infrared spectrometer (NIRQuest512, Ocean Insight, Germany) with a spectral working range from 900 to 1700 nm.

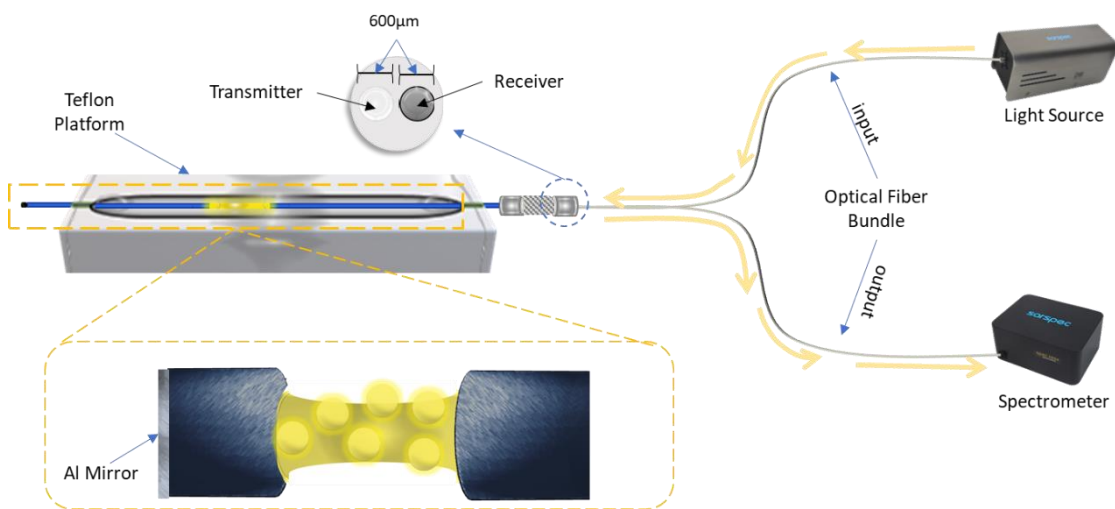


Figure 8. 3. Illustration of the experimental setup used for all the experiments realized with the SPR and Hybrid configurations.

8.3.5. Biosensing Validation Assays

To understand the applicability of the proposed plasmonic configuration in biosensing applications, biosensing assays were conducted. Therefore, the hybrid structure surface was functionalized with amine modified thrombin binding aptamer (TBA) receptors to study the kinetics of the binding events between the TBA and the thrombin (THR) protein. The NH_2 -TBA were immobilized onto the AuNS surface assisted by the well-known electrostatic properties of poly-L-lysine (PLL). The polycationic nature of PLL leads to the electrostatic immobilization onto the negatively charged AuNS surface (citrate capped), thus acting as a bridge for the covalent immobilization of the amine-modified aptamer. The TBA incubation was conducted at room temperature, by immersing the sensing region in a 0.75 $\mu\text{mol/L}$ TBA solution in a 10x SSPE buffer solution for 30 minutes. Thereafter, the functionalized surface was washed with a cleaning buffer ($\text{Tris}_{\text{clean}}$) solution and the spectral band was acquired in a measurement buffer ($\text{Tris}_{\text{measure}}$) solution. The specific binding experiments carried out by applying the THR standard solutions, by crescent order, in a range of concentrations from 0.01 nmol/L to 100 nmol/L. Additionally, the THR standard solutions were produced by successive dilutions from a stock solution ($C = 100 \mu\text{mol/L}$, in PBS; stabilized by a 1% BSA solution) by using an affinity buffer solution (Tris_{aff}). Moreover, the THR incubation on the TBA receptors was allowed for 40 minutes, leading to the formation of a TBA/THR complex,

consequently causing an increase in the effective RI (Figure 8.4). The sensor surface was washed three times with the Tris_{clean} solution, and the hybrid band position was acquired (five samples spaced by 20 seconds) in the Tris_{measure} solution.

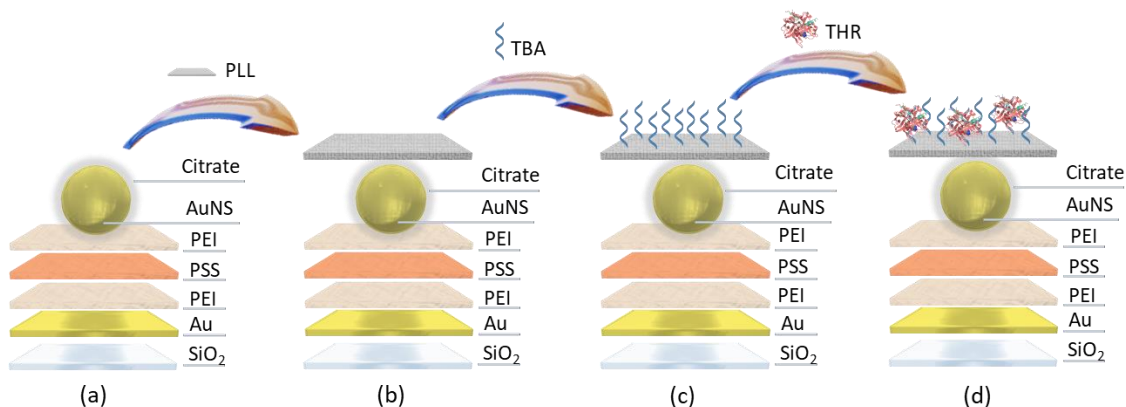


Figure 8. 4. Schematic illustration describing the process of the sensor surface functionalization (a to c), and the TBA/THR complex formation (d).

8.4. Results and Discussion

As was well established before, the transition zone shaped by the etching process plays an important role in the working principle of this new configuration. To prove the significance of that feature in this novel plasmonic structure, Figure 8.5 displays the initial and final spectra of two distinct optical fibers (a regular MMF and an etched core MMF), coated with a 50 nm thick Au film where AuNS were immobilized with the same size and with the same deposition time. For the full core MMF (Figure 8.5a) the SPR band shifts towards higher wavelengths, which is the expected behavior for this type of plasmonic platforms. It is induced by the interaction between the modes of the SPPs and the modes of the localized SPs, thus creating a large EM-field in the space between the film and nanoparticles surfaces [454]. Furthermore, this phenomenon was also observed in the works reported by *Niu et al.* [469] and *Wang et al.* [420]. However, for the etched core MMF structure (Figure 5b), it is observable that with the increase of the AuNS density at the Au film surface, the SPR band totally disappears and a new plasmonic band arises (herein noted as hybrid band). The phenomenon is caused by a gain of energy induced by the light previously leaked from the etching transition zone, which becomes guided through the gap (between the Au film and the AuNS) formed by the PE structure. These results prove the importance of the etching process in the development of this configuration and confirm the interaction of the leaked light with the external medium, and subsequent recoupling back to the fiber.

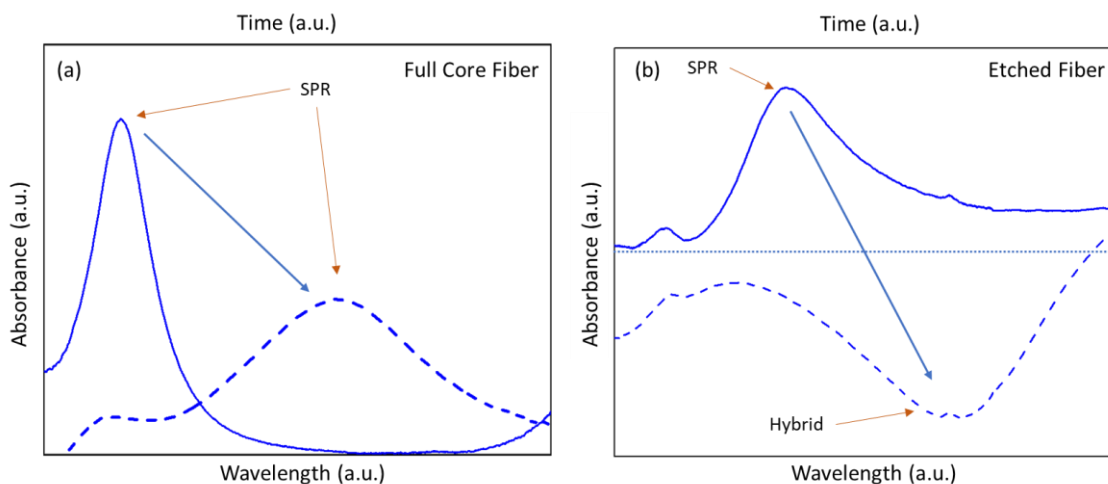


Figure 8. 5. Comparison of the spectral behavior between a regular MMF (a) and an etched MMF (b), both coated with a 50 nm thick Au film and decorated with AuNS. Solid and dashed lines represent the initial and the final spectra, respectively. The dotted line marks the zero in the absorbance axis.

As in modified SPR configurations (similar to the presented in Figure 8.5a) the shift of the SPR band strongly depends, among other features, on the size of the nanostructures, thus it would be expected that the spectral position of the band where the hybrid band is formed be affected by this parameter. Therefore, the spectral dependence of the hybrid configurations was studied by varying the size of the AuNS from 20 to 90 nm. Figures 8.6a-c show the spectral behavior of the different configurations during the AuNS deposition (spectra acquisition at each 10 minutes). It is noticeable that the position in which the hybrid band is formed strongly depends on the size of the used AuNS. It is also observable that the hybrid band formation only starts when the SPR band is almost extinct. The phenomenon can be explained by the increase in the density of the AuNS at the Au film surface, reaching a moment in which the structure is able to guide the leaked light through the PE channel. It was also observed, at a certain moment, that the hybrid band formation stops and only shifts to higher wavelengths. At that instant, it is assumed that the new plasmonic waveguide formation is completed and the AuNS deposition is interrupted. The sensitivity to the external RI variations of the developed hybrid configurations ($\text{Hybrid}_{\text{sens}}$) as well as the SPR platforms (SPR_{sens}) was assessed to establish a full comparison between them. Figures 8.6d-f show the spectral variation of the hybrid bands, by increasing the external RI. It is visible that the hybrid configurations using the 55 nm and 90 nm AuNS (Figure 8.6 e and f) display a working range in the NIR region (~ 875 to 1100 nm and ~ 1200 to 1400 nm, respectively). These are remarkable results using AuNS, once plasmonic sensors based on NPs working in that spectral range are just possible by changing the characteristics of the nanostructures such as shape (e.g., nanorods or bipyramids) [483, 484] or combining different materials

[485]. Finally, Figures 8.6g-i show the respective calibration curves, and respective linear fittings, for both SPR and hybrid configurations. Additionally, Figure 8.7 shows an overview of the results displayed in Figure 8.6.

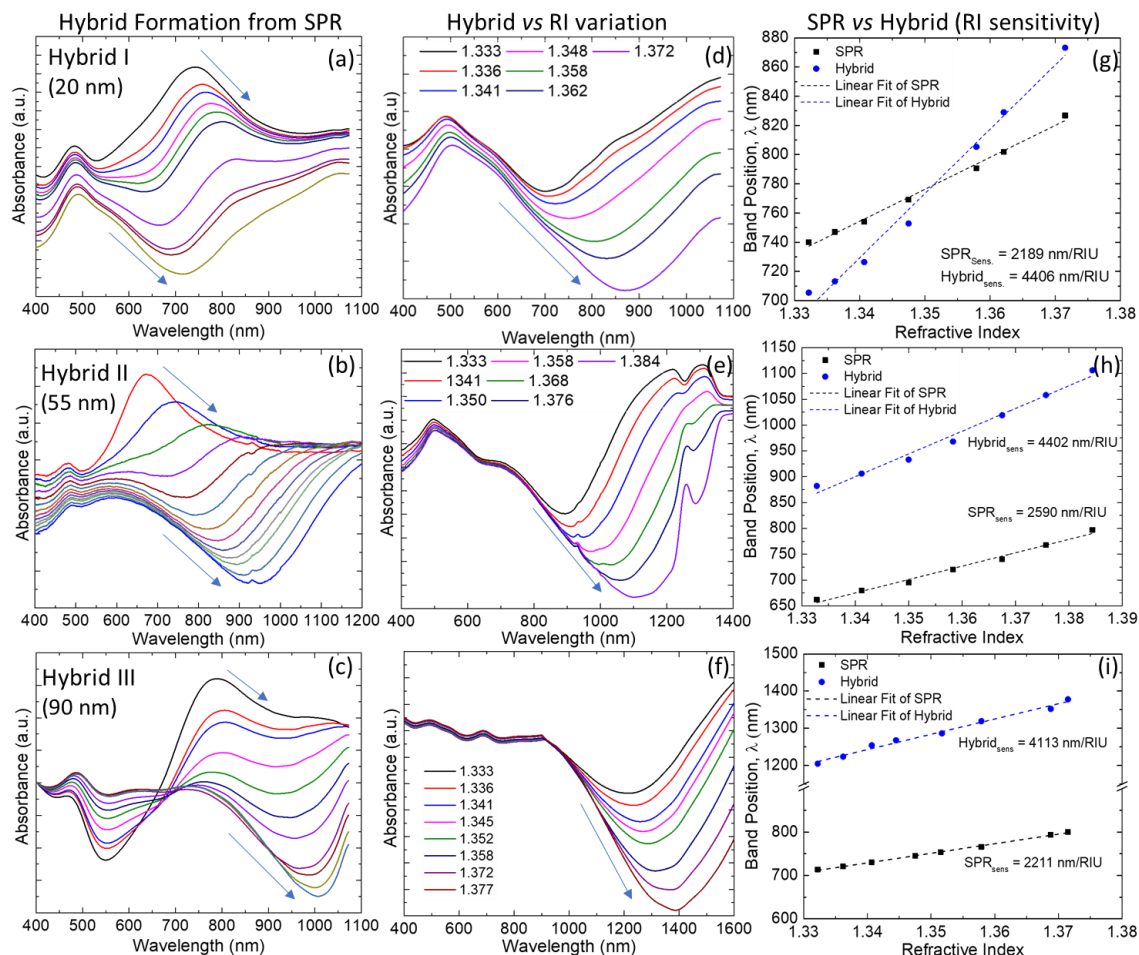


Figure 8. 6. Summary of the experimental results revealed by the Hybrid I (a,d, and g), Hybrid II (b, e, and h), and Hybrid III (c, f, and i) configurations, displaying the Hybrid bands formation (a, b, and c); the Hybrid platforms characterization by varying the external RI (d, e, and f); and the calibration curves obtained from the SPR configurations (black squares) and from the hybrid configurations (blue circles). Dashed lines are the respective linear fittings (g, h, and i).

Figure 8.7a exhibits the curves following the wavelength hybrid band position as the external RI increases from 1.336 to 1.372 for the Hybrid I, from 1.341 to 1.384 for the Hybrid II, and from 1.336 to 1.377 for the Hybrid III configuration (in relation with the band position in water), fitted to third-order polynomial trend, which is the most convenient and suitable procedure for this type of curves [289]. Figure 8.7b describes the initial SPR band position (marked by the empty circles) and the position on which the hybrid band was formed (marked by the empty triangles). Both positions were acquired in the same RI (1.333). It is visible that for the Hybrid I configuration, the hybrid band appears prior to the SPR band. So, it is supposed that the hybrid system is totally controlled by the AuNS and their characteristics (including, the packaging system), leading to the

conclusion that the sensitivity provided by the hybrid configurations (Table 8.1) is related to the interactions between the EM wave and the structure formed by the AuNS.

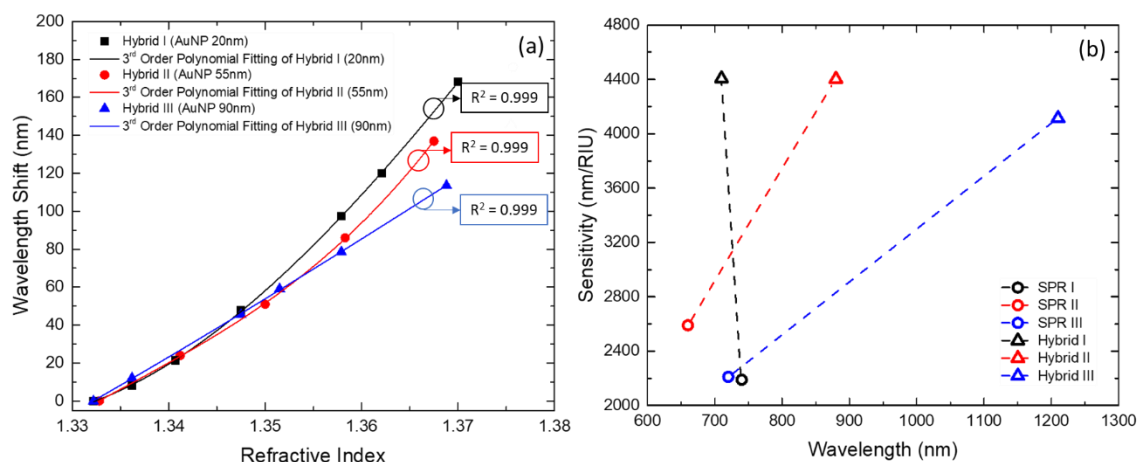


Figure 8. 7. (a) Wavelength shift (in relation to the blank solution (water)) that resulted from the hybrid structures calibration by changing the external RI with the RI standard solutions, and the respective 3rd order polynomial fitting; and (b) the comparison between the SPR band position (circles) and the position where the hybrid band had formed (triangles), and between the sensitivities offered by all configurations (dashed lines).

Additionally, Table 8.1 also exhibits the FWHM of the hybrid bands and the respective FOM. In order to compare the performance of these new plasmonic platforms with the performance of a traditional modified SPR structure, Table 8.1 also includes experimental data obtained from a modified SPR configuration produced with the 55 nm AuNS immobilized onto a regular MMF covered with a 50 nm thick Au film. Comparing the performances of the Hybrid II and modified SPR configurations (both fabricated in the same conditions and AuNS size, except for the etching process that was only applied to the hybrid) it is obvious the difference in the final sensitivity and in the FOM parameter (despite the similar values for the FWHM), in favor of the hybrid arrangement. Between the hybrids, the provided sensitivities are close to each other, but the observed enhancement in relation with the initial SPR (revealed by the % values in Table 8.1) assume distinct values, which suggests, one more time, that the features of this novel configuration are controlled by the AuNS. Considering the FWHM parameter, it is quite different, mainly for the Hybrid III configuration, which was fabricated with the biggest AuNS. That fact could be related to the concentration of the AuNS solution used for the immobilization process. Once the 90 nm AuNS have a higher mass, the possibility to occur aggregation between them is higher (compared with the smallest AuNS), thus the FWHM of the final hybrid band will be affected by it. A solution that can be applied to overcome this problem is to reduce the initial concentration of the AuNS (in solution) and replace it, if necessary, by a new solution during the process.

Table 8. 1. Analytical data obtained from the characterization of the hybrid structures, including experimental data obtained from the analysis of a modified SPR architecture, to external RI variations. The % values reveal the enhancement on sensitivity in relation to the initial SPRs.

Configuration	NS Size (nm)	S to ERI (nm/RIU)	FWHM at $n_D = 1.333$ (nm)	FOM (RIU ⁻¹)
Hybrid I	20	4406 (+101%)	217	20
Hybrid II	55	4402 (+70%)	172	26
Hybrid III	90	4113 (+86%)	398	10
Modified SPR	55	2248 (+21%)	165	14

NS – nanosphere; S – Sensitivity; ERI – External Refractive Index; FWHM – Full Width at Half Maximum; FOM – Figure of Merit.

To conclude the investigation and prove the suitability of this novel plasmonic architecture for biosensing applications, the Hybrid II configuration fabricated with the 55 nm AuNS (the one that revealed the better FOM) was selected for this purpose. Therefore, as described in the previous sections, the AuNS surface was decorated with amine modified TBA, using PLL as a bridge between the AuNS and the aptamers, followed by the successive measurements of different concentrations of THR standard solutions by crescent order. The process was monitored in real-time by following the hybrid band position (Figure 8.8a). Figure 8.8b displays, chronologically, the wavelength position of the hybrid band after each modification introduced at the hybrid sensor surface. The experimental data are presented as a mean (solid lines) of five distinct measurements (dots). It is visible the hybrid band shifting toward higher wavelengths (also known as “red shifts”) as these changes were introduced. The first two shifts are related to the immobilizations of the PLL and the TBA, which induced an increase of the RI close to the AuNS surface. The remaining shifts are caused by the specific binding events between the TBA and the THR. The connection receptor-analyte causes a change in the molecular mass of the receptor which induces an increase in its RI. This behavior was observed by several authors elsewhere when they analyzed the performance of their fiber optic plasmonic sensors studying a wide variety of receptor-analyte interactions [263, 274, 374, 486].

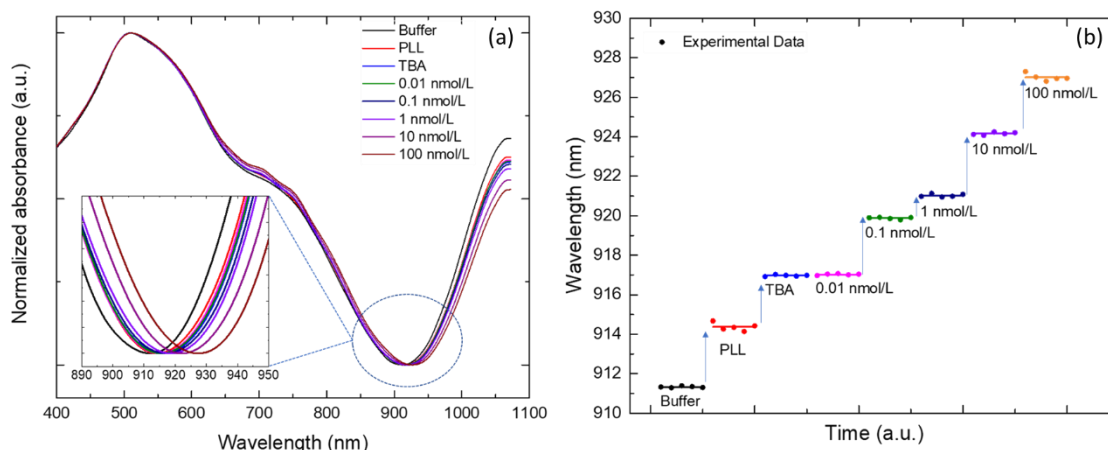


Figure 8.8. (a) Resonance wavelength curves displaying the shifts (towards higher wavelengths) caused by the Hybrid structure surface modification, followed by the shifts caused by the TBA-THR binding events; and (b) timeline plot describing the wavelength band position (solid lines), resulting from the mean of five measurements (dots), after each experimental step.

The experimental data resulting from the shifts caused by the binding events were plotted as wavelength shifts (in relation to the blank; for the blank was considered the band position after TBA immobilization) in the function of the concentration of the analyte (Figure 8.9a). The experimental values were fitted using the Hill-Langmuir model, a useful method to assess the parameters of this type of sensors, following the Equation 8.1 [416]:

$$y = \frac{\lambda_{max} \times C^n}{K^n + C^n} \quad (8.1),$$

where λ_{max} is the total shift observed for the entire calibration, C is the analyte concentration, K reveals the concentration of the analyte at the $1/2 \lambda_{max}$, and n is the Hill coefficient. Figure 8.9b shows the used parameters obtained from the Hill-Langmuir fitting as well as the parameters that describes the analytical performance of the hybrid aptasensor, in particular the sensitivity and the LOD. The former was calculated as the *sensitivity at low concentrations*, i.e., for values of $C \leq K$ when the curve assumes a linear trend [269], whereas the LOD was calculated by the ratio of three times the standard deviation of the blank and the sensitivity at low concentrations.

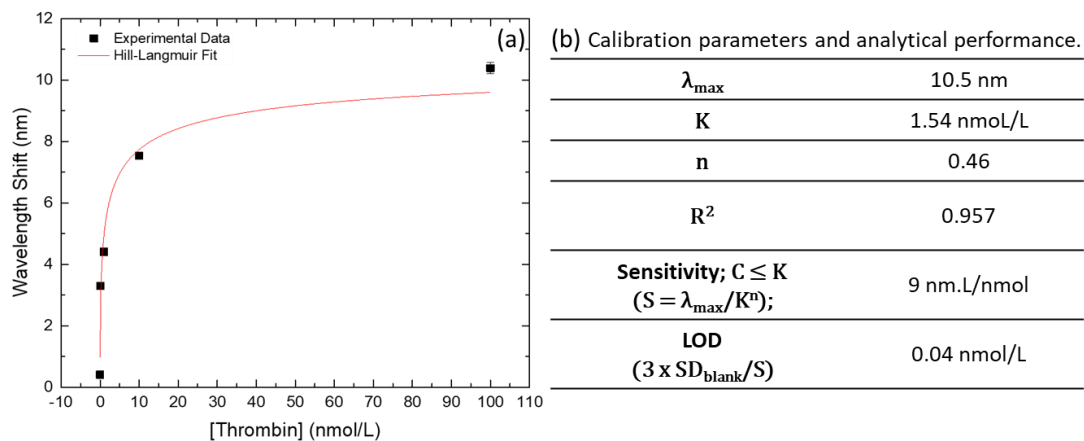


Figure 8. 9. (a) Calibration curve exhibited by the sensor by plotting the wavelength shifts, caused by the specific binding events (in relation with the blank), and respective fitting (red line) using the Hill-Langmuir model; and (b) Calibration parameters of the Hill-Langmuir fitting and analytical performance resulted from the statistics given by the Hill-Langmuir model.

For a better understanding of the achievements revealed by this new plasmonic platform, Table 8.2 shows the information related to the performance of different types of fiber optic sensors targeting thrombin, including the description of the sensor configuration, the transduction method, the detection range that was investigated, the sensitivities to the external RI and to the THR detection, and the respective LODs.

Table 8. 2. Performance of different types of fiber optic sensors targeting thrombin, including the description of the sensor configuration, the transduction method, the detection range, the sensitivities to the external RI and to the THR detection, and the respective LODs.

Sensor Configuration	Transduction Method	Detection Range (nmol/L)	S to ERI (nm/RIU)	S to THR	LOD (nmol/L)	Ref.
Hybrid plasmonic sensor on an etched MMF functionalized with TBA	Hybrid SPR/LSPR	0.01 - 100	4400	9 nm.L/nmol	0.04	This Work
Gold-coated D-shaped optical fiber functionalized with TBA	SPR	1.6 - 60	-	0.14 nm.L/nmol	1.6	[269]
Gold-coated MMF derivatized with TBA assisted by aptamer complementary element (ACE) functionalized AuNS	SPR	0 - 2000	-	-	36	[487]
Gold-coated TFBG for sandwich-type biosensing by coupling SPR and LSPR effects	TFBG/SPR/LSPR	1 - 35	-	0.03 dB.L/nmol	1	[488]
Fiber optic tip-ball resonator coated with gold and functionalized with TBA	Backscatter Reflectometry	0.0004 - 0.1	1274	-	0.0016	[489]
TBA-functionalized etched TFBG	TFBG	2.5 - 40	24	0.0033 nm.L/nmol	0.075	[490]
Tapered fiber optic interferometer functionalized with TBA	Fiber Interferometer	100 - 5000	1660	-	100	[476]

S – sensitivity; ERI – External Refractive Index; THR – Thrombin; LOD – Limit of Detection

Despite some authors do not reveal the sensitivity of the developed configurations to variations of the external RI or for the analyte detection, a noticeable difference, in favor of the plasmonic sensor fabricated in this investigation, is undeniable, mainly for the detection of the analyte. The obtained LOD is also highlighted, considering the presented

working range. The work presented by *Ayupova et al.* [489], using the backscatter reflectometry method, reveals a LOD of around 1.6 pmol/L (against to the 40 pmol/L presented by this investigation), however, the range of concentrations studied is quite lower, which can explain that difference. To conclude the investigation, the sensor was placed in solutions containing other blood plasma constituents, namely HTR and HSA. The results revealed in Figure 8.10 demonstrates that the developed plasmonic aptasensor is selective for the required analyte, presenting a considerable shift in the presence of THR, whereas just presented very slight shifts in the presence of HTR and HSA.

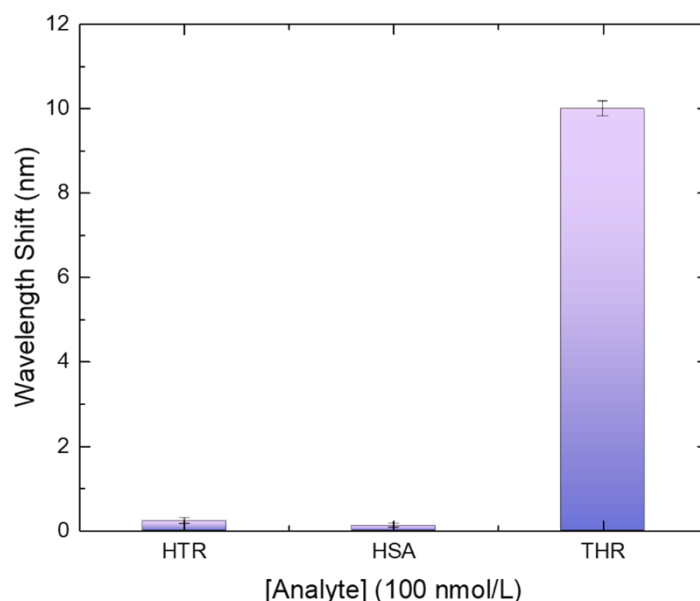


Figure 8. 10. Wavelength shifts presented by the hybrid plasmonic aptasensor in the presence of THR and of other blood plasma constituents (HTR and HSA).

8.5. Conclusion

A new methodology to fabricate sensitivity-enhanced fiber optic plasmonic sensors was developed and studied. The detection of thrombin was used as biological model for biosensing validation, and the results indicate the viability of the fabricated fiber optic plasmonic sensor for point of care biosensors for diagnostic purposes.

Preliminary results suggest that the light propagating in the fiber is leaked out of the core due to a physical modification induced by the etching process on the core structure, in which the conditions to occur total internal reflection are changed. The working principle of the proposed plasmonic configuration seems to be related to the excitation of a SPR through light scattering on the nanoparticles, that gets coupled to the thin metallic film.

The light leaked by the etched region interacts with the NPs arrangement, then exciting an SPR on the Au film/external medium interface via scattering effects. The structure that is in contact with the external medium is formed by an AuNS well-defined system that acquires the properties of thin layer based on the description given by the EMA-MG theoretical model.

The investigation on the sensor fabrication, reveals that the size of the AuNS strongly affects the spectral position (in wavelength) in which the hybrid band will be formed. □

Moreover, it was observed that the NP density can tune the SPR band features, as RI sensitivity and band position. Another relevant tuning mechanism is related to the NP size. Since light scattering angular distribution depends on the shape and size of the NPs, a change in the wave vector that excites the SPR at the NP/film interface will result in a change in the wavelength position of the excited SPR band.

The biosensing experiments carried out by decorating the sensing region with aptamers targeting thrombin. The functionalization of the sensing region with the cationic polymer PLL, followed by the immobilization of the TBA receptors, causes a change on the optical properties of the sensing device. The refractometric platform showed its suitability for the detection of thrombin by identifying the changes in the external RI variations caused by the TBA-THR bindings, therefore, the concept could be applied for other specific bindings. Additionally, the plasmonic device exhibited high specificity for the proposed target, suggesting high reliability to rapid and real-time clinical diagnosis.

Chapter 9 – Conclusions and Future Work

The work presented in this thesis has as core elements the knowledge and competencies acquired in fiber optic technology associated with the conception, development, and characterization of combining fiber optic structures to develop sensors for differential optical sensing with the goal to generate sensing devices operating with high reliability. The combination of highly sensitive chemical or biological materials with fiber optic structures, including optical fiber bundles or sensing structures relying on long period fiber gratings and surface plasmon resonance, offers promising possibilities in fiber optic technology.

The performance of a novel material for dissolved carbon dioxide detection using a ratiometric extrinsic fiber optic sensor based on multimode fibers was investigated. The CO₂-sensitive material is based on a chemical pH colorimetric indicator, the *p*-nitrophenol, exhibiting a yellow color at alkaline pH values (> pH 7.5), becoming colorless at acidic pH units (< pH 6). Additionally, a pNPh-derivative material was synthesized (*via* formaldehyde condensed) and was observed an improvement on its performance, mainly in the pH range from 7 to 10. The new polymeric material shows a color variation ranging from light yellow (pH 3 to 5), to yellow (pH 5 to 11), and finally to yellowish brown (> pH 11).

Additionally, it was found that from the chemical mixture between the indicators with a quaternary ammonium, the TOA-OH, have resulted an ion pair (TOA⁺pNPh⁻) that remains stable into its anionic form, thus able to be protonated by protons (HpNPh) generated from the interaction between the and the molecules of water associated to the

relative humidity in the TOA-OH/pNPh/hydrogel matrix. Moreover, it is crucial to keep the CO₂-sensitive film with a certain relative humidity level, otherwise this reaction will not occur. The performance of both materials targeting gCO₂ was assessed, demonstrating the enormous potential of the poly-pNPh to detect CO₂ at low levels, as determined by the shapes of the calibration curves, mainly from 0 to 0.15% of CO₂ (vs. N₂).

The performance of the polymer made CO₂-sensitive film in aqueous solutions was also evaluated. Firstly, it was found that the optical response of the sensitive film does not depend on the pH of the medium, just responding to the variations caused by the addition of CO₂, also presenting a full reversibility in the degassing assays. Moreover, the ratiometric fiber optic sensor was successfully calibrated for applications in fresh and saline water. It was found that the ratiometric system improves the linear relation in the studied concentration range, offering low LODs. The investigated sensor has revealed to be suitable for applications in both types of water, however, has showed higher accuracy for the saline water (2.5%). Additionally, the sensor has demonstrated to be able to detect small dCO₂ variations as 0.25 mg/L. The experimental tests in the laboratorial environment, in the SRS+RAS prototype, and in the experimental fish culture situation, revealed a better performance of the ratiometric fiber optic platform when compared with the used commercial sensor, displaying levels of dCO₂ in accordance with the expected values for each situation.

The differential detection of anti-IgG and IgG antibodies was investigated by assembling two distinct refractometric platforms based on long period fiber gratings. The label free immunosensor for anti-IgG detection was fabricated by preparing individual nanosensors using nano scaled silica nanoparticles as substrate for the immobilization of the bioreceptors *via* APTMS covalent attachment. The FTIR-ATR spectra proved the addition of the bioreceptors on the SiNP surface by displaying the absorbance bands characteristic of each functional group associated with the respective elements. The electrostatic immobilization of the produced nanosensors on the LPFG surface was monitored by following the LPFG resonance band. The observed shift, towards lower wavelengths, proved the immobilization of the nanosensors at the fiber surface, and was understandable that the bath in a strong basic solution followed by the acidic treatment induces enough negative charges at the fiber surface to hold the nanosensors. The sequential experiments using the target analyte showed systematic “blue” shifts, proving the changes in the RI caused by the specific bindings. Moreover, the differential system using the bare LPFG, has improved the sensitivity of the sensor, however, the main achievement was the decrease of the LOD in 50%. However, this approach could not be

the optimal methodology to develop immunosensors on fiber optic surfaces once the procedure strongly depends on the pH and temperature of the used medium as well as the purity of the fiber surface in terms of cleanliness. Moreover, the random (and uncontrolled) distribution of the receptors around the SiNP could bring some disadvantages, *i.e.*, some of them could be not accessible to the target analyte. This problem could be solved, in principle, by directly decorating the fiber surface with the receptors, however, the sensitivity of the sensor will probably decrease since the presence of SiNP tends to offer higher sensitivities.

The differential detection of IgG antibodies was accomplished by measuring the changes in RI of a MIP layer induced by the specific interaction with the antibodies. The *in situ* polymerization allowed to monitor the synthesis of the MIP layer in real-time, by following the position of the LPFG resonance band (in wavelength) as the RI at the LPFG increases. This procedure is very useful to avoid the formation of very thick layers and prevent the entrapment of the used template at inferior levels, being inaccessible for further extraction. Moreover, with a proper calibration, the changings in the RI caused by the layer formation could be used, in principle, to estimate the thickness of the MIP film. Additionally, it was found that this method has high reproducibility due to the very similar results observed for the NIP fabrication ($|\Delta\lambda_{\text{MIP}}| = 575 \text{ pm}$ and $|\Delta\lambda_{\text{NIP}}| = 560 \text{ pm}$).

The microfluidic platform proved to be very useful for template extraction. The slow but continuous flow of the solutions used for the template extraction seems to drag out the template from the MIP layer, leaving empty the binding sites. The shift of the LPFG resonance band towards higher wavelengths, caused by the decrease in the RI of the MIP layer, demonstrates the successful extraction of the template molecule. The rebinding trials have revealed good performance of the LPFG/ $\Delta\lambda_{\text{MIP-NIP}}$ system, especially in IgG concentrations ranging from 1 to 13 nmol/L (0.15 to 2 $\mu\text{g/mL}$). It was found that the $\Delta\lambda_{\text{MIP-NIP}}$ system offers better sensitivity to the target and improved the LOD, when compared with the LPFG/ $\Delta\lambda_{\text{MIP}}$ arrangement. Additionally, the LPFG/ $\Delta\lambda_{\text{MIP-NIP}}$ refractometric platform revealed a remarkable behavior in differentiating the target from other species present in human blood, almost eliminating the response to “false positives”.

A distinct configuration of a surface plasmon resonance sensor in a multimode fiber section chemically etched was developed and studied. In the proposed method, a MMF core is reduced through chemical etching in order to allow some guided-light modes from the core to reach the external medium. Then, the leaked light is allowed to interact directly with metallic nanoparticles immobilized on the thin Au film, generating several

scattering effects, thus inducing the excitation of a SPR in the NP/Au film interface. It was found that the chemically etched region plays a crucial role in the operation of this novel configuration. Sensitivities to the RI variations higher than 4100 and 4400 nm/RIU were reached considering the different nanospheres diameter. It was found that the size of the AuNP strongly interferes in the wavelength position in which the hybrid plasmonic band is formed, however, no considerable impact in the sensitivity was observed. The larger AuNS (in diameter) transport the hybrid band to the NIR region.

The applicability of the hybrid plasmonic structure was investigated in the biosensing field with the detection of thrombin. The electrostatic immobilization of the PLL layer as well as the covalent attachment of the thrombin binding aptamers on the PLL structure, caused a shift of the hybrid band towards wavelengths indicating the immobilization of each respective element. The binding of the THR molecules to the aptamer leads to changes on the molecular weight of the TBA and, consequently, changes in its RI. The hybrid platform has revealed a very good performance targeting the thrombin protein, presenting high sensitivity for the detection of low concentrations and a LOD in the picomolar range.

The sensing platforms addressed in this thesis were developed according to the best transduction scheme considering the final application. Although it is expected that the reaction of the gas-sensitive film changes its RI with the interaction with the CO₂ molecules, those changes are expected to be insignificant, due to the very low molecular weight of the target molecules, and, therefore, such variations only be detected, in theory, by extremely sensitive devices. Therefore, absorbance (or even fluorescence) fits better for this type of applications. Moreover, the equipment required for the signal processing tends to be less expensive.

Considering the refractometric structures in optical fibers, the plasmonic structure have shown several advantages over the LPFG arrangements. The highest sensitivities presented by the plasmonic devices allows to obtain lower LODs and cover wider ranges of concentrations, however, the multimodal excitation provide wider bands which difficult the interrogation process. In that context, the narrower attenuation band provided by the LPFG system turns that job easier. Additionally, the LPFG system allows the use of light sources and detectors with reduced wavelength window range.

The research developed in this PhD thesis has pointed out topics that require further investigation, whereas opened spaces for new studies.

Colorimetric sensors are very promising tools to assess the gas levels in diversified fields. Combining highly sensitive colorimetric materials with optical fibers allows to fabricate miniaturized devices for real-time monitoring of gases of interest such as carbon dioxide. It is an important parameter to control, for instance, ocean acidification that was proved to be related with the high emission of greenhouse gases to the atmosphere. Another emerging application is to control the water quality in aquaculture facilities with a very high fish density in low levels of water such as the SRS+RAS systems. As high levels of carbon dioxide cause fish poisoning, the selective, accurate *in situ*, and real-time evaluation of its concentration in water have attracted great interest. However, other elements of interest are necessary to be monitored to accomplish the better quality in fish production. Perhaps, the combination of different sensing materials with optical fibers emitting light in a wide spectral range and an array of photodetectors, processing at specific wavelengths, could result in the development of multiplexing colorimetric sensors targeting two or more gases of interest.

Refractometric optical fiber sensors, namely those based on LPFG or (L)SPR, exhibit a wide range of possibilities to develop highly sensitive and selective devices for the biological field. Concerning LPFGs, the sensitivities provided by these gratings are not outstanding, however, offer the highest resolutions due to their sharp attenuation bands. They cannot be the better choice for quantitative measurements but proved their efficiency to detect the presence/absence of the measurand of interest, exhibiting their versatility to be combined with different types of receptors. With a proper design, LPFG arrangements could be an interesting tool to monitor biochemical changes in foodstuff, or even to attest to the purity of some pharmaceuticals.

Fiber optic plasmonic structures have been the subject of more investigation due to the wide range of possibilities in their fabrication. Plasmonic designs on optical fibers are in continuous expansion, combining different types of metallic films and/or nanostructures with different optical fiber shapes and types, ranging from single mode to multimode. Here, was presented a configuration on which were used gold nanoparticles to excite surface plasmon resonances on a thin gold film *via* scattering effects. The high sensitivity detection of refractive index variations in liquids with refractive indices ranging from 1.333 to 1.380, an ideal range for the detection of biological targets after proper surface functionalization with the most adequate bioreceptors, offers a distinct methodology for fiber optic plasmonic detection. Further investigations need to be conducted in order to understand how to improve the sensing performance and or to obtain narrowed plasmonic bands to improve the sensitivity/interrogation relationship. Will be also

significant to investigate the possibility to develop a fiber optic differential system, in the same way as the one developed for the LPFG, however, in the same optical fiber by fabricating two plasmonic sections.

In overall, is crucial to understand the characteristics and in which situation the (bio)sensor will be applied considering all the advantages and disadvantages in terms of the bioreceptor or the transducer performance. If for some applications the selectivity is the vital parameter to distinguish an analyte from other similar species, for others the sensitivity and low limit of detection are the main requirement to monitor a specific type of analyte. The production methods, cost-effectiveness, storage, transport conditions, and long-term stability are important characteristics that need to be considered for the development of a fiber optic (bio)sensor. In fact, fiber optic technology applied to sensors is a multidisciplinary investigation field involving different areas of sciences such as physics, chemistry, biology, and nanotechnology. Thus, only a strong collaboration of researchers from distinct disciplines can accelerate the development of fiber optic (bio)sensors. The investigation reported in this thesis is a simple contribution for this expanding field which offers new approaches for the development of fiber optic sensors.

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