

Development of optical fibre sensors for real time measurements of nutrients in water

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Abstract

The research described in this thesis explores the possibility of the development of an optical fibre sensor (OFS) for the detection of nutrients in the water. The nutrients targeted were nitrites, ammonia and phosphates. The sensing platform to measure these targets was based on absorbance and long period grating optical fibre sensors. The long period grating sensors operating in the visible range for chemical measurements using sensitive dyes were also explored.

For nitrite sensing, optical fibre was modified with the sensitive layer based on Brilliant cresyl blue (BCB) sensitive dye embedded into a base catalysed sol gel. The response of the dye to nitrite was studied in both free solutions and entrapped in sol gel matrix. The response and repeatability of the developed sensors in solution and sol-gel matrix were compared. The response was found to be not fully reversible in free solution, but reversible in base catalysed sol gel. The influence of pH as an interfering parameter was also compared with nitrite response and was proven to be opposite to the sensor's response, but a factor that needs to be taken into account in practical deployment. A ratio of two absorption peaks with opposite behaviour was used to analyse the dynamic response and repeatability. The BCB embedded sol-gel was deposited onto a U-shape and straight OFS via dip-coating. The sensor was found to give a repeatable response to nitrites in (deionised) DI water with 21.81 μM limit of detection (LOD) in the case of 0-50 μM detection range (DR), and 36 μM in case of 0-500 μM DR. However, the lifetime of the sensor appeared to be limited to few days, with complete insensitivity after 7 days of shelf life.

Ammonia sensing was achieved using tetrakis-(4-sulfophenyl) porphyrin (TSPP) dye via OFS based on two different sensing platforms: U-shape and LPG OFS operating in the visible range. The response of TSPP dye to ammonia was examined in DI water, salt aqueous solution and seawater, and it was found to be not responsive in seawater, which makes the development of seawater sensors challenging. The TSPP was immobilised on the surface of the glass substrate using electrostatic layer by layer deposition of positively charged diazonium resin (DAR) – TSPP followed by UV treatment to create covalent bonding between DAR and TSPP. The ratio of intensity values at different wavelengths with opposite response

behaviour was used to analyse the dynamic response and repeatability. 3 layer U-shape DAR-TSPP OFS sensitivity parameters to ammonia in DI water were $3.01 \mu\text{M}$ LOD at 0-10 μM detection range and $\text{LOD}=25.94 \mu\text{M}$ 10-200 μM . The sensor showed a repeatable response. The research described in this chapter played a significant role in a sensor prototype, which was further industrially developed and is currently at its final stage of completion.

The link between the complex refractive index and absorption governed by Kramers-Kronig relation opens the possibility of RI sensitivity in the ranges close to TSPP's attenuation bands (eg. ~ 500 and 700 nm). In order to fabricate such LPG, with phase matching turning point (PMTP) close to TSPP's attenuation bands, fibres with cut off wavelength in the visible wavelength range ($488\text{-}633$ for SM450 and $633\text{-}780$ for SM600, both by Thorlabs) were chosen. In order to increase the photosensitivity of the optical fibre, they were loaded with hydrogen and UV-laser amplitude mask fabrication method was used to inscribe LPGs. The deposition and ammonia response of DAR-TSPP film was compared between „visible” and NIR LPGs, and only the „visible” LPG was found to be responsive to ammonia in DI water. The assumed explanation for this phenomenon is in the DAR/TSPP's complex refractive index change behaviour in the visible range. The difference in the complex RI caused by DAR-TSPP ammonia response was calculated and analysed. Based on the response of the visible LPG modified with DAR/TSPP two different approaches to track ammonia response was proposed. One approach is intensity-based, - by taking the intensity ratio between 678.5 and 662 nm wavelengths ($\text{LOD}\sim 3.24 \mu\text{M}$), the other by taking the sum of the PMTP central wavelength shift ($\text{LOD}\sim 4.81 \mu\text{M}$). However, the proposed LODs are not superior to the existing detection techniques such as electrochemical, chromatographical, spectrophotometric and other optical detection techniques. The usage of the visible LPGs in combination with sensitive dyes is a novel approach that can be further investigated and significantly improved to increase sensor performance.

Phosphate aqueous detector development was conducted via molecular imprinting. A molecularly imprinted polymer was synthesised based on N-allylthiourea monomers and PPA template. The template removal from MIP was evaluated via UV-VIS spectroscopy. The MIP film was deposited onto an LPG

operating in reflection mode via dip-coating in a nitrogen atmosphere using UV enhanced dip coater setup. A novel approach to quantifying the response of an LPG OFS using the areas ratio of the PMTP was applied, where the ratio factor compensates for any intensity caused changes. Temperature compensation via thermocouple was performed. The test results show the response of LPG to phosphate concentration in water from 100 μ M to 10 mM, however, the response is not linear and wasn't able to be replicated with other similar attempts.

Phosphate detection was also targeted with phosphate imprinted nanoparticles (called MIP materials) based on the unknown to author polymer provided by MIP Diagnostics Ltd using a U-shape sensor. The synthesised nanoparticles were claimed to be $\sim$ 350 nm in size and colour change was used to detect phosphates. 3 types of nanoparticles were designed with different binding functional groups (via silanization) and one type without any for matrix entrapped usage. MIP materials were examined for response to organic and inorganic phosphates in water and organic solvents as well as in their 50/50 mixture. It was established that water prohibits any response of the MIP materials. The scanning electron microscope (SEM) evaluations of MIP materials showed very few actual nanoparticles in the MIP materials. Filtration weighting showed only $\sim$ 8-10.5% (depending on the type) of actual nanoparticles in the MIP materials provided. Due to a large amount of polymer residue, the deposition of the actual nanoparticles was problematic. SEM images of glass fibre coated with MIP materials confirm massive coverage of residues among rare nanoparticles. U-shape OFS coated with MIP grown directly on its surface in the facilities at MIP diagnostics was examined, unfortunately, none of the U-shapes found to be responsive to phosphates in water.

In summary, the novelty of the work is in deploying VIS LPGs in combination with sensitive dye, first-time nitrites sensors with BCB sensitive dye on optical fibre sensor, an attempt to develop MIP based sensor for phosphates on OFS.

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List of acronyms

ACN	Acetonitrile	MINP	MIP nanoparticles
APTES	3-Aminopropyltriethoxysilane	MIP	Molecularly imprinted polymer
BCB	Brilliant cresyl blue	MOPA	4-methoxyphthalaldehyde
BSA	Bovine serum albumin	MZI	Mach-Zehnder interferometers
CCD	Charge-coupled devices	NA	Numerical aperture
CE	Capillary electrophoresis	NED	N-(1-naphthyl) ethylenediamine
CL	Chemiluminescence	NHS	N-hydroxysulfosuccinimide
CNT	Carbon nanotubes	NIP	Non-imprinted polymer
CS	Chitosan	NIR	Near infra red
DAR	Diazonium resin	NP	Nanoparticles
DI	Deionised	OFS	Optical fibre sensor
DLS	Dynamic light scattering	OPP	o-phthalaldehyde
DMF	Dimethylformamide	PMTF	Phase phase matching turning point
DMSO	Dimethyl sulfoxide	PPA	phenylphosphonic acid
DR	Detection range	PPG	photoplethysmogram
ECL	Electrochemiluminescence	PTT	Pulse Transit Time
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide	PVC	Polyvinyl chloride
EDTA	Ethylenediaminetetraacetate	RI	Refractive index
EGDMA	Ethylene glycol dimethacrylate	SD	standard deviation
EPM	Electrophoretic mobility	SEM	Scanning electron microscope
EU	European Union	SF	Spectrofluorimetric
FBG	Fibre Bragg grating	SG	Sol gel
FPI	Fabry–Perot interferometer	SPE	Screen printed electrode
FT	Fourier transform	SPM	Spectrophotometric
GC	Glassy carbon	SPR	Surface plasmon resonance
GDME	Gas diffusion micro-extraction	SR	Synthetic receptor
HPLC	High Performance Liquid Chromatography	TBAPh	Tetrabutylammonium phosphate monobasic
HPTS	Hydroxy pyrene trisulfonate	TEMS	Triethoxymethylsilane
IPA	Isopropyl alcohol	TEOS	Tetraethyl orthosilicate
IR	Infra red	TFBG	Tilted Fibre Bragg Grating
ISE	Ion selective electrodes	TMOS	Tetramethyl orthosilicate
LBL	Layer by layer	TSPP	tetrakis-(4-sulfophenyl) porphyrin
LED	Light emitting diode	UK	United Kingdom
LOD	Limit of detection	USA	United States of America
LPG	Long period grating	UV	Ultra violet
MES	2-(N-morpholino) ethanesulfonic acid		

Chapter 1 Introduction

The condition and quality of natural waters are very important for health and safety reasons. Along with several bacterial factors of water health, there are some important nutrients, such as phosphates, nitrates, nitrites and ammonia. Nutrients play a fundamental role in metabolic reactions in the aqueous environment. A high level of those may cause plants to overgrowth, increase bacterial activity and decrease dissolved oxygen levels. High concentrations of nutrients could be hazardous for human and animal health as well. Hence the detection and monitoring of nutrients are crucial for environmental and safety reasons. The actual detection methods, such as spectrophotometric, colourimetric, spectrofluorometric, electrochemical suffer from various disadvantages. They are mainly bulky, not stable and often require expensive devices, reagents and highly trained personnel.

The development of a nutrient sensing method based on optical fibre has various advantages. Optical fibres are small, robust, relatively cheap, and do not suffer from electromagnetic and electrical interaction. Due to their size and physical properties, optical fibre based chemical sensors are perfect candidates for environmental and industrial measurements of nutrients in both fresh and sea water. It's well known that some materials are sensitive to the presence of nutrients [1]. With proper modification and treatment of those materials, it should be possible to use optical fibre as a sensor. Several types of optical fibre sensors were studied and applied in this research, such as evanescent based and long period grating optical fibre sensors. The general principle behind optical fibre sensing lays in the detection of the optical properties difference as a function of the target value.

The work contains novelty and contribution to knowledge in deploying VIS LPGs in combination with sensitive dye, first time nitrites sensors with BCB sensitive dye on optical fibre sensor, an attempt to develop MIP based sensor for phosphates on OFS. This PhD project became possible as a collaboration with "Gill research and development limited". The PhD research was partly funded by Gill R&D. As was agreed with Gill R&D, this research targeted the development of three nutrients, nitrites, ammonia and phosphates.

1.1 Aims and objectives

1.1.1 Aims

The main aim of this PhD is to explore the possibility of the development of an optical fibre sensor (OFS) for nutrients detection in the water environment. The research looked in detail into various deposition methods such as layer by layer deposition, sol-gel matrix, dip coating, silanization and molecular imprinting to endow optical fibre sensors with the required specificity and sensitivity towards target compounds. A combination of the existing deposition techniques with sensitive elements to particular ions provided a research foundation and enabled the development of such sensors.

Attention also will be given to study the possibility of long period grating (LPG) sensors operating in the visible range for chemical measurements using sensitive dyes.

1.1.2 Objectives

The main objectives of this PhD research were to develop optical fibre sensors for the detection of nitrites, ammonia and phosphates in water laboratory-based.

To meet the particular specification for application in different environments the following sensing parameters are required:

- for nitrites the LOD should be lower than 70 μM , this would allow monitoring in wastewater ;
- for ammonia the LOD should be lower than 5 μM , this would allow monitoring in natural coastal waters, drinking waters and waste waters quality;
- for phosphates, the LOD should be below 30 μM , this would enable wastewater quality monitoring;

Also, the following learning objectives had been chosen to achieve the aims of this PhD:

- Literature review to obtain appropriate knowledge with main focus on:
 - the types and operational principles of fibre optic sensors;

- Benchmarking against existing nitrite, ammonia and phosphate sensing techniques and methods.
- Functional coatings and deposition techniques applicable to optical fibre;
- Deposition of functional coatings onto the surface of the optical fibre sensors using different techniques such as:
 - Layer by layer technique and thickness optimisation;
 - Sol-gel approach with thickness control, and concentrations optimization of the sensitive compound;
 - Molecular imprinting technique
- Development of evanescent based optical fibre sensors:
 - Fabrication and optimisation of the performance in terms of sensitivity;
 - Surface modification using different sensitive elements;
 - Characterisation of the sensor performance such as sensitivity, response time and selectivity in the lab by exposing to different target analytes;
- Development of LPG optical fibre sensors:
 - Obtain the necessary theoretical background and practical skills in order to fabricate LPGs via UV laser beam technique using amplitude masks;
 - Study the possibility of LPG fabrication at a visible wavelength range;
 - Study and deploy hydrogen loading photosensitivity enhancement approach;
 - Use LPGs for chemical sensing by functionalising them via sensitive elements deposition on the surface;
- Data acquisition and processing, surface characterisation techniques (scanning electron microscope (SEM)):
 - data processing using appropriate software packages such as Origin, SpectraSuite, Matlab, Labview etc.
 - Learn the essentials of SEM imaging and apply this knowledge in practice by taking SEM pictures of the coatings for evaluation of the film properties in order to investigate the quality of the coating.

1.2 Structure of the PhD thesis

This thesis consists of 7 chapters:

Chapter 1, - Introduction covering main aims and objectives.

Chapter 2, - covers the literature review of existing nutrient sensing techniques their main features pros and cons. Also includes a brief description of the optical fibres principle of operation and an overview summarising some examples of chemical and biochemical OFS reported in the literature.

Chapter 3, - explains the main experimental methods used in this thesis. Explains the coatings and deposition methods used in this PhD research.

Chapter 4, - describes the research and development of nitrite OFS, using BCB dye as a sensitive element, and sol-gel as an entrapment matrix. This chapter covers the BCB dye examination, deposition on a glass substrate and straight and U-shape cladding replaced OFS.

Chapter 5, - describes the research and development of ammonia aqueous sensors, as well as the deployment of LPGs operating in the visible in combination with sensitive dyes, and their fabrication and comparison with “regular” near infrared counterparts.

Chapter 6, - describes the research attempt to develop a phosphate sensitive aqueous sensor using molecular imprinting technique. The first half of the chapter focuses on MIP coated LPG sensor operating in reflection mode. While the second part describes the evaluation and deployment of phosphate sensitive MIP nanoparticles received from MIP Diagnostics Ltd.

Chapter 7, concludes this thesis with a brief summary and conclusion to each chapter and proposes future research tasks to be completed.

Chapter 2 Literature review

2.1 Introduction

This chapter provides a literature review on the recent nutrient sensing techniques targeted in this PhD. Each review on nitrate, ammonia and phosphate is summarised with a comparative table with detection limit and detection range for each particular sensing method. Also in the last section of this chapter, an overview of the principle of operation of optical fibre sensors, with a brief literature review of recent sensing applications. The section concludes with a summary table of some optical fibres sensors reported in the literature.

2.2 Nutrient sensing techniques

The condition and quality of coastal waters is very important for ecological, health and safety reasons. Along with several bacterial factors of water health, there are some important nutrients, such as phosphates, nitrates and nitrites [1]. Nutrients play a fundamental role in metabolic reactions in aqueous environments. High levels of those may cause plants overgrowth, increase bacterial activity and decrease dissolved oxygen levels [2]. The by-products of water eutrophication, as well as high concentrations of nitrites, nitrates, phosphates and ammonia, could be hazardous for human health [3, 4]. Hence, monitoring the level of nutrients in sea and fresh water is extremely important from environmental and health points of view.

Table 2-1 Usual concentrations of nitrites, ammonia and phosphates in natural waters, μM [1]

Analyte	Surface ocean	Deep ocean	Estuarine	Coastal
Nitrite	0.0001–0.2	0.0001–0.005	0.5–1.5	0.1–2
Ammonia	0.05–1.5	0.02–0.05	0–600	5–30
Phosphate	0.02–0.20	1–3.5	0.5–3	0.02–1.5

The usual concentration of nitrates, ammonia and phosphates in natural water can be seen in **Table 2-1** [1].

2.2.1 Nitrite detection

This section is a brief overview of the existing nitrite sensing techniques published in the literature.

Nowadays there are various nitrite sensing techniques [5, 6] such as spectrophotometric, chemiluminescence (CL), electrochemical, chromatography, capillary electrophoresis, spectrofluorometric and electrochemiluminescence. A brief overview of existing methods and their advantages and disadvantages is discussed below.

2.2.1.1 Spectrophotometric (SPM) detection

The SPM way of detecting nitrite ions was proposed in 1879 by German chemist Johann Peter Griess [7]. It is based on a reaction between nitrites and sulfinic acid under an acidic environment, which results in a diazonium cation. Afterwards, this cation couples to the aromatic amine 1-naphthylamine and produces a red-violet coloured ($\lambda_{\text{max}} \approx 540 \text{ nm}$), water-soluble azo dye. Later, however, the 1-naphthylamine was found carcinogenic and was replaced with N-(1-naphthyl) ethylenediamine (NED) as a coupling component [8]. The NED was found to have even more advantages over 1-naphthylamine such as higher sensitivity, faster response, reproducibility and wider pH independence of the colour in pH range 1-2. Currently, this modified Griess reaction is the most frequently used Griess assay [9] and has been recognized as a European standard for nitrite detection in an aqueous environment [10]. The schematic of the reaction is shown in **Figure 2-1**.

Along with the simplicity, low cost and as a result a wide usage of the modified Griess method, it has a few disadvantages such as bulkiness, it requires a fresh supply of chemicals and suffers from cross interactions with some other ions: Fe^{3+} , Cu^{2+} , I^- and S^{2-} [11]. There are also other modified Griess reactions used to determine nitrites [6].

Along with Griess diazo-coupling assay, nitrosation based methods suitable for nitrite detection have been reported. Among a lot of colours indicating substances available for nitrosation reaction a few could be mentioned as an example: barbituric acid transforms into violuric acid in acidic solution [12] and interaction between nitrite ion and phloroglucinol (1,3,5-trihydroxybenzene) [13].

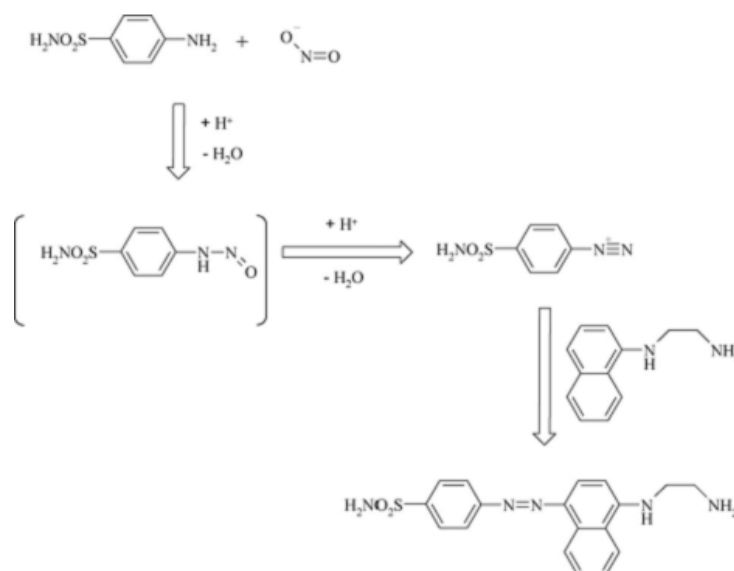


Figure 2-1 The modified, currently most commonly used Griess reaction [10].

In the past decade also, several catalytic SPM methods have been reported. These type of detection assays are mainly based on the catalytic effect of nitrite on the oxidation of some indicating substances with proper oxidizing agents. Among those agents the most recent is potassium bromate. Some of the indicators which were published are: triarylmethane alkaline dyes (crystal violet [14]); substituted phenothiazine derivatives (chlorpromazine [15], perphenazine [16]); azo dyes (Tropaeolin OO [17]; Methyl red [18]; Sulfonazo III [19]); triarylmethane acidic dyes (pyrogallol sulfonephthalein [20]; Pyrogallol red [21]); quinone imine dyes (gallocyanine [22]; Brilliant cresyl blue [23]). Unfortunately, many of these catalytic methods require strict control of dosage, acidity, temperature and suffer from interferences with other ions: Br^- , Fe^{2+} , Fe^{3+} , SO_3^{2-} , I^- and Ag^+ [24].

As a summary of spectrophotometric detection methods, it can be stated, that nitrite detection in this way is possible due to the colour change of the reagent. This colour change then is a subject of measurement by a spectrometer and compared with sample spectra with known concentration values.

2.2.1.2 Chemiluminescence

Recent technological advances have made possible the rise of the popularity of fluorescence based optical sensing. The operational principle of this method lies in the light emission as a result of a chemical reaction. Two chemicals form an excited high energy level intermediate substance. This substance then emits photons while dropping the energy level to normal. These emission wavelengths can be observed and quantified by modern sensors and spectrometers.

Two main CL based methods can be distinguished. The Gas-phase CL and the Luminol CL.

The Gas-phase Chemiluminescence uses the nitrite reduction to nitrogen oxide which couples with ozone and was firstly applied by Garside [25] in 1982 (**Equation (2-1)**) [26].



Where γ is a quantum of light with a certain wavelength. Within a certain range, the amount of emitted light is proportional then to nitrite concentration. This method can be applied with a flow injection system for a lower detection limit. The gas-phase CL requires a high combustion temperature, around 600 °C, and even a small amount of oxygen can't be neglected because it will affect the results by reacting with NO to form NO₂.

There are other CL methods based on reagents such as luminol (5-amino-2,3-dihydrophthalazine-1,4-dione), lucigenin, tris (2,2-bipyridyl)ruthenium(II) and peroxyoxalate derivatives. The most frequent CL reaction used to detect nitrites is luminol oxidation in a high pH environment. As a result, excited 3-aminophthalate anion appears and emits 425 nm wavelength light while relaxing to the ground state [27]. This method was reported to measure nitrates in natural waters with flow injection [28].

However, the intensity of emitted light can be easily affected by some environmental factors, so the reproducibility and stability of the CL are relatively poor.

2.2.1.3 Electrochemical detection

The principle of electrochemical detection of nitrites is based on using ion-selective electrodes (ISE). This method is well established and widely used. The electronegativity on nitrite at platinum, gold [29], copper, diamond, glassy carbon (GC) [30] and transition metal oxide electrodes [31] can be used for the determination of nitrites. This method has certain advantages such as independence upon water turbidity or colour. The usage of bare electrodes, however, are limited due to their ability to be affected by interfering substances which cause misreading of the obtained results. In recent years, a variety of modified electrodes applied for nitrite detection has been reported, such as carbon nanotubes (CNTs), metal oxide nanoparticles and nanoclusters, organic and inorganic electro-polymerized films, Chitosan (CS), sol-gel and modifications and combinations of these [6]. As an example, CNTs have become quite promising because of their unique properties such as high electrical conductivity, strong adsorptive ability, and good chemical stability [32]. Owing to these properties, CNTs have been investigated for developing nitrite detectors with high sensitivity [33]. Certain metallic nanoparticles which provide higher catalytic efficiency can be used to increase sensitivity because of their large surface-to-volume ratios [34]. Polyaniline integrated with CNT based nitrite selective electrodes were published by *Gao et al.* with 20 μM to 1.8 mM detection range and 7.8 μM limit of detection (LOD) [35].

Ionophores are chemical entities that are able to reversely bind and transport ions, hence there are ionophores able to reversely bind nitrite ions [36]. Approaches to utilise these for nitrate sensing was published by *Pieterzak et al.* They've examined several porphyrin and salophen complexes with Rh(III) to fabricate nitrite selective polymeric membrane electrodes. The best ionophore material was reported to be an Rh(III)-tetra(t-butylphenylporphyrin), showing a 5 μM LOD in a pH 4.5 buffered sample solution with over two months functioning time [37]. Another example with ionophores embedded in PVC (Polyvinyl chloride) was published by *Néel et al.* In this

work they've used Co(II) tert-butyl-salophen as an ionophore, and reported 2 μ M LOD [38].

Along with the obvious advantages of electrochemical sensors, there are some disadvantages. For instance, relatively low LOD (**Table 2-2**) and interference with other ions.

2.2.1.4 Chromatography

Chromatography is a technique that consists of the separation of a compound from a mixture. Preparative chromatography is used mainly for purification, while analytical uses smaller amounts of material for detection and sensing purposes [39]. Chromatography was invented by Mikhail Tsvet in 1900 [40] and was developed by him later mainly for plant pigment separation. As those pigments had different colours, this caused the name "chromatography".

Nowadays the same principle utilising progress in technology development enables direct sample analysis using high pressure liquid sample carrier called High Performance Liquid Chromatography (HPLC) and ion chromatography techniques [41]. The HPLC method is rapid, selective and sensitive. Recently, several analytical protocols for the separation and detection of nitrites by ion exchange or ion-pair reversed-phase HPLC have been developed [6]. The detection principles of HPLC method includes UV/VIS absorbance, electrochemistry, chemiluminescence, and fluorescence nitrites can be directly measured by their electrical conductivity or by UV absorbance detection at 210–220 nm. However, most of the methods for nitrite sensing require a derivatization procedure, in which nitrites are converted into materials that can absorb light at a specific wavelength or are highly fluorescent [41]. For instance, a nitrite detection method, which involves the post-column Griess reaction have been reported by *Gapper et al.* [41]. The sample first passes through an ion-exchange column, afterwards being mixed with the carrier solution, and passes a copper-coated cadmium reduction column. Afterwards, the eluent is mixed with the Griess reagents and under 60°C forms a diazo-compound, which is then

detected at 540 nm. Hence the UV/VIS spectrometer is the most convenient way to finalize HPLC analysis due to its low cost, wide range and simplicity.

Recent studies also try to make the detection devices portable, for instance, *Nightingale et al.* used a modified Griess method to develop a small droplet microfluidic spectrophotometric sensor for in situ nitrate and nitrite detection in natural waters with low reagent consumption (2.8 ml/day). They've demonstrated a 0.7 μM LOD and 0-600 μM DR [42].

However, the Chromatographic method is quite bulky (requires a column and reagents) and expensive, because it requires a complicated sample preparation procedure [6].

2.2.1.5 Capillary electrophoresis

Capillary electrophoresis (CE) is a method that distinguishes diverse ions upon their electrophoretic mobility (EPM) by using an applied voltage. EPM depends upon the viscosity of the solution, molecules charge and radius of the analyte. CE can be used to analyse anionic, cationic and neutral compounds and has several advantages over other methods such as short time, low sample amount, high separation efficiency.

The detection in CE though, most commonly uses UV/VIS absorbance, fluorometric and electrochemical methods [6]. The most significant disadvantages of CE is low sensitivity due to the restricted capacity of the capillary columns, and numerous preparation stages before measurements.

2.2.1.6 Spectrofluorometric method

The principle of the Spectrofluorimetric (SF) method lies in the fluorescence probes light intensity change under reactions with nitrites. There are two different SF modes, based on Nitrosation, which is a transformation of an organic compound into nitroso derivative and Diazotization, which is converting a primary aromatic amine into a diazo compound [43].

Nowadays there are a lot of nitrosation fluorescence probes that can interact with nitrites under an acidic medium, such as 4-hydroxycoumarin [44] and resorcinol [45]. Also, some other reagents with secondary amides or amines can be used for nitrite determination, several papers were published on nitrosation based SF [9, 46]. Also, several diazotization-based reagents and methods were created in the past years. For instance, 5-aminofluorescein was used to determine nitrites [47].

Both methods rely on measuring the intensity of light emitted as a result of a reaction that correlates with the concentration of nitrites. SF has high selectivity, sensitivity and LOD. It is pretty much effortless and therefore is widely used.

2.2.1.7 Electrochemiluminescence

Electrochemiluminescence (ECL) is a kind of luminescence that occurs during an electrochemical reaction. Liu *et al.* proposed to use this phenomenon for nitrite detection based on quenching ECL emission effect in 2008 [48]. In this case, the nitrite concentration can be determined through the Stern-Volmer equation (Eq. 11). Suppose $X^* + Q \rightarrow X + Q$ or $X^* + Q \rightarrow X + Q^*$, where X is one chemical substance, Q is another, called quencher, and * labels excited state. Then this phenomenon is governed by Stern-Volmer equation:

$$\frac{M^0}{M} = 1 + k_q \tau^0 [Q] \quad (2-2)$$

Where M and M^0 are the fluorescence rates with and without the quencher respectively, k_q quencher rate coefficient, τ^0 is the lifetime of the emissive excited state of X without the quencher, and [49] is the quencher's concentration [50]. A nitrite concentration range of 1-500 μM was given, with LOD 0.1 μM . Later, other analytical ECL detection methods were published with better results [51, 52].

2.2.1.8 Summary of actual nitrite sensing techniques

As a summary of discussed methods, a table of comparisons of sensing techniques is shown, **Table 2-2**.

Table 2-2 Summary of existing nitrite detecting techniques with sensing parameters

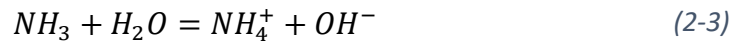
Methods	LOD of NO ₂ ⁻ in water	Range of detection	Ref.
Spectrophotometric method (Griess, SPM, Catalytic-SPM, method) continue to hold a dominant position due to its low cost and easy feasibility.	1.5 nM 1.76 nM 0.7 μM	0-0.1 μM 3.7–65,000 nM 0-600 μM	[16] [23] [42]
Chemiluminescence	0.0002 μM 0.05 μM	0.0002–1.0 μM 0.1–50 μM	[28] [53]
Electrochemistry	0.1 μM 5 μM 2 μM 7.8 μM	0.5–8000 μM 20-1800 μM	[54] [37] [38] [35]
Chromatography	0.2 μM	0–2000 μM	[55]
Capillary electrophoresis	NO ₂ ⁻ 0.2 μM	0–7 μM	[56]
Spectrofluorimetric method	NO ₂ ⁻ 0.01 μM	0.2–50 μM	[57]
Electrochemiluminescence method	0.2 μM	1 μM to 0.5 mM	[51]
Proposed OFS	21.8 μM 36 μM	0-50 μM 0-500 <u>μ</u> M	

2.2.2 Ammonia detection

This section is a brief overview of recently published existing ammonia sensing techniques.

Ammonia/ammonium plays a significant role in clinical, agricultural and biotech industries [58]. Ammonia is also considered as one of the most frequent anaerobic digestion inhibitors, therefore high ammonia concentrations occur at wastewater, animal farms and slaughterhouse sites [59]. Ammonia is toxic for humans, fish and

some aquatic organisms and can cause rapid algae growth, and plays a key role in eutrophication. Ammonia and ammonia salts are soluble in water and can be hazardous and corrosive [60]. In water, ammonia appears in the form of free ammonia, NH_3 and ammonium ion NH_4^+ , with the sum of those two called total ammonia, **Equation (2-3)**.



The form of appearance and their equilibrium depends on pH and temperature and is governed by **Equation (2-4)** [59].

$$NH_3 - N = NH_4^+ - N \left[1 + \frac{10^{-pH}}{10^{-(0.1075 + \frac{2725}{K})}} \right]^{-1} \quad (2-4)$$

The graphical representation of ammonia/ammonium equilibrium for three different temperatures was calculated by *Fernandes et al.*, and can be seen in **Figure 2-2** [59].

One of the conventional methods to detect ammonia in water is to deploy a gas permeable membrane allowing it to diffuse into ammonium chloride, and measure the change in pH [61]. However, nowadays there is a large variety of aqueous ammonia detection methods reported in the literature.

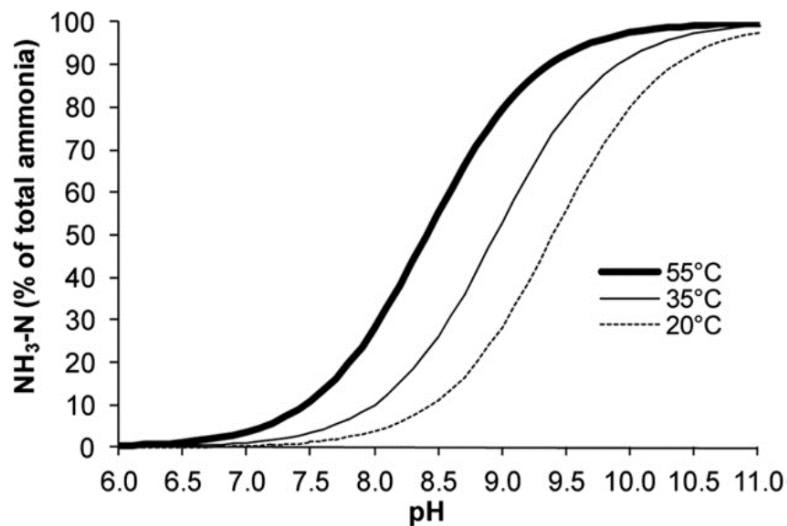


Figure 2-2 Free ammonia (NH_3)/ammonium (NH_4^+) equilibrium at 55, 35 and 20°C [59]

2.2.2.1 Optical detection methods

Optical detection methods of ammonia mainly lay on the tracking of optical properties of participating reagents. Optical methods comprise spectrophotometric, fluorescent, fibre optic, colourimetric and some other methods [60].

2.2.2.1.1 Spectrophotometric detection

The basics for spectrophotometric detection were laid down by Berthelot back in 1859, by reporting a green reaction product as the result of mixing phenol and hypochlorite with ammonia. This becomes known as the Berthelot method [62]. The Berthelot method is in fact ammonia oxidation into chloramine under an alkaline condition with the following reaction between chloramine and phenol resulting in benzoquinone chloramine, which then forms indophenol after reaction with second phenol **Figure 2-3**, [60]. This results in a colour change, the intensity of which depends upon the ammonia concentration. The Berthelot technique is used a lot even nowadays, however as phenol is toxic [63] it was replaced by salicylic acid [64], thymol, 1-naphatnol, o-chlorophenol, o-phenylphenol (OPP) [65], 2-methyl-5-hydroxyquinoline, m-cresol and some others [60].

For instance, *Cogan et al.* utilized microfluidic technology and a light-emitting diode (LED) based optical detection system with modified Berthelot. This automated

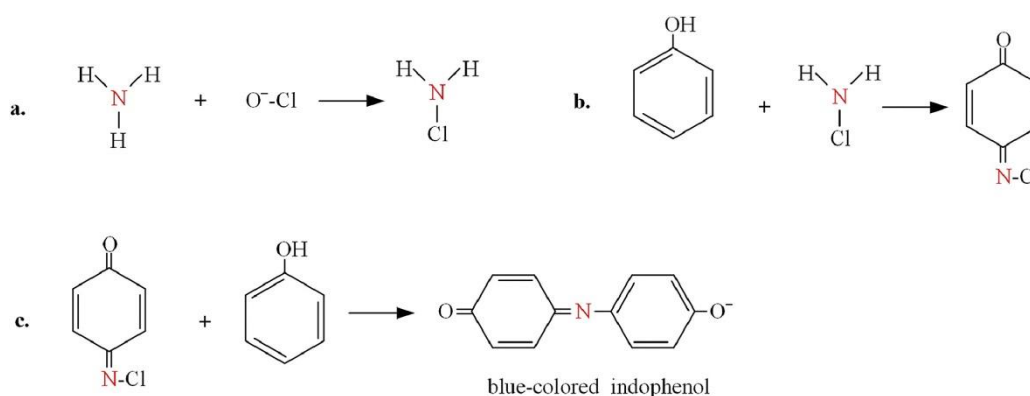


Figure 2-3 Berthelot's reaction for ammonia detection; **a)** ammonia oxidation by hypochloramine into chloramine under high pH; **b)** reaction between chloramine and phenol to form benzoquinone chloramine; **c)** reaction between benzoquinone chloramine with a second phenol to form an indophenol; [60]

method used sodium salicylate, sodium nitroprusside mixed in sodium hydroxide as Reagent 1, and sodium hypochlorite in sodium hydroxide solution. It was applied in fresh and wastewater ammonia detection with a result of 0.88 μM LOD and 0-705.88 μM range [62]. Other work describes an online solid phase extraction into a hydrophilic-lipophilic balance cartridge combined with an automated flow technique system to measure ammonia in seawater. The LOD in optimal conditions was 3.5 nM with 52.4-288.7 nM detection range [66]. Another study used liquid waveguide capillary cells with a segmented flow analysis system combined with a photometric detector that provided a 5 nM LOD and 10–100 nM detection range of ammonia in seawater [67].

The toxic phenol replacement to o-phenylphenol (OPP) was reported by *Hashihama et al.* by utilizing gas segment continuous flow analyser with two types of long path capillary cells demonstrated ammonia detection in seawater with 0-200 nM detection range, however, a non-linear baseline drift was reported as well [65]. OPP was utilized also by *Ma et al.* reported optimization of the reagents which was combined with an integrated automated reverse flow injection analysis system by *Lin et al.*, which achieved 0-50 μM (LOD=0.08 μM) and 0-35 μM (LOD=0.07 μM) detection range in fresh and seawater respectively [68]. A much wider detection range (0-200 mM, LOD=0.15 μM) for seawater OPP based Berthelot's ammonia detection was achieved by a combination of a water analyser with an online filtration station [69].

Overall the Berthelot technique based spectrophotometric detection provides a good detection range and a low limit of detection. One of the main disadvantages is toxicity (which was minimised by substituting the phenol). Among other disadvantages are bulkiness, complexity, the necessity of water sampling and its pre-treatment.

2.2.2.1.2 Fluorescence detection

The fluorescence ammonia detection in water is based on ammonia reaction with o-phthalaldehyde (OPA) and sodium sulphite which results in the formation of a fluorescent compound in a high pH environment. After absorbing external energy, the fluorescent compound emits light with intensity – concentration correlation on a

particular wavelength [70]. In recent years, the fluorescent ammonia detection method benefited from reagent optimisation and substitution, application of modified materials, functional films and matrices, induction flow, separation and gas diffusion techniques and appearance of UV light-emitting diodes (UV-LED), and quantum dots utilization.

To avoid precipitation side effects caused by metal ions, *Hu et al.* used ethylenediaminetetraacetate-NaOH (EDTA-NaOH) as a buffer during the NH_3 -OPA-sulfite reaction. This allowed ultra-trace (0.032-0.5 μM DR and 9.9 nM LOD in natural water) ammonium detection avoiding enrichment necessary in the traditional OPA method [71]. Using 4-methoxyphthalaldehyde (MOPA) reagent instead of OPA, *Liang et al.* reported a 0.025-0.300 μM DR and 5.8 nM LOD for fresh and seawater at room temperature [72]. Further, by utilizing 4,5-dimethoxyphthalaldehyde (M_2OPA) and a single laser, *Zhang et al.* reported a wider detection range, 0-5 μM , in seawater with 6.5 nM LOD and faster response time [73]. By utilizing a dual-life reference technique with polyurethane hydrogel and silicone rubber matrices in combination with Egyptian blue as a reference, *Strobl et al.* reported a 0-17.65 μM DR and 6.47 nM LOD [74].

Implementation of an automated micro pump, UV-LED and reagent optimization in a flow-through or batch analysis method made the detection system reported by *Amornthammarong et al.* more compact and efficient than before, with an adjustable detection range (5-1000 nM; 20-4000 nM; 0-25 nM) with 1 nM LOD [75] in natural and seawater.

Flow injection combined with fluorescence detection is reported to achieve higher collection efficiency, negligible effects of salinity with 100-700 μM DR and 7 μM LOD in natural water by *Liang et al.* [76] and 0-300 nM DR and 2.1 nM LOD in seawater by *Zhu et al.* [77]. Besides the advantages of the flow analysis method such as low sample volumes and simplicity, disadvantages should be mentioned, such as channel and bubble interference [60].

Coupling of separation and gas extraction techniques with fluorescent ammonia detection addresses analyte labelling issues. *Valente et al.* reported a fast single step method via gas diffusion micro-extraction (GDME) with 22.35-368.82 μM DR and 12.94 μM LOD [78]. The simplicity and versatility of the GDME, extraction and

quantification of volatile compounds in highly complex environments can be achieved. *Giakisikli, et al.* improved the detection characteristics in a lab-in-syringe pressure-assisted GDME setup to 8-556 nm DR and 2.8 nM LOD [79].

The fluorescence-based ammonia detection is a promising method, with space for accuracy improvement via reagent optimization and functional films utilization. However, this method is sensitive to temperature and pH and has a limited detection range. Also, it is not really suitable for a long term in situ analysis, and besides the wastefulness of the reagents, it suffers interference to bubbles [60].

2.2.2.1.3 Colourimetric detection

The colourimetric detection of aqueous ammonia is based on the weak alkalinity of the ammonia water solution. Hence, by measuring the pH it is possible to measure the ammonia concentration indirectly.

Utilizing the bromothymol blue pH sensitive dye upon the conversion of ammonium to ammonia in combination with gas diffusion hydrophobic porous membrane, *Segundo et al.* reported a 0.28-5.88 μM detection range with 1.5 μM LOD, for transitional and coastal waters. Also, reagent recovery was reported, which minimize reagent consumption and waste [80]. *O'Connor Šraj et al.* reported a similar gas-diffusion with improved programmable flow control achieving multi range ammonia detection in seawater with 0.028-5.6 μM ; 0.28-13.9 μM ; and 1.4-55.6 μM with 15, 88 and 440 nM LOD respectively [81].

Paper-based devices with bromothymol blue reagent have been reported recently, which benefits from small sample volume and inexpensive strips however the analysers are quite expensive and the method overall is susceptible to human error. However, recent work by *Peters et al.* achieved good multi-stage results with 35.71-214.3 and 142.86-714 μM DR and 22.86 and 33.57 μM LOD respectively [82].

As a pH sensitive probe also hydroxy pyrene trisulfonate (HPTS) was used with 0-1.2 mM DR [83].

As ammonia can be considered as a Lewis base, which is able to provide pair of free electrons, *Zilberman et al.* used zinc tetraphenylporphyrin dye in ion-exchange polymer microspheres to detect ammonia in water [84].

2.2.2.1.4 Other optical detection

Work published by *Eskandari et al.* used the ability of ammonia to reduce to nitrite and nitrate. First, ammonia was oxidized with hypobromite to nitrite, which was then detected via spectrophotometry with sulfabenzamide and N-(1-naphthyl)ethylenediamine. This method achieved good sensitivity with 16.63-332.6 nM DR and 177.4 nM LOD, however, it obviously suffers from interference from nitrites [85].

Other non-direct optical ammonia detection techniques were published recently, such as the conversion of ammonia into volatile chloramine and detection through non-dispersive atomic fluorescence spectrometry [86]. An interesting example of non-direct ammonia detection in water was realised by *Amirjani et al.* using a smartphone instead of a spectrophotometer. This method uses the mechanism of silver nanoparticles manipulation via the formation of $\text{Ag}(\text{NH}_3)_2^+$ complex, which decreased the silver nanoparticles concentration hence the colour intensity as well. The smartphone-based setup (0.59-58.7 mM DR and 11.7 mM LOD) was compared with spectrophotometric setup (LOD 10.6 mM). Both setups showed reliable performance [87]. Few other non-direct ammonia optical detection techniques are developed recently, to eliminate some cross sensitivity issues, they however often create new cross sensitivity elements themselves [60], [1].

Ammonia sensing via fibre optics is possible by detecting the chemical or physical interaction between ammonia and the sensitive coating on the surface of an OFS. The sensing principle of different OFS explained in detail in **Section 0**.

Several examples of ammonia optical fibre sensors are described in **Section 2.3** and **Chapter 5**

2.2.2.2 Electrochemical detection methods

Recent progress of electrochemical determination of ammonia in water is mainly focusing on ammonium ion-selective electrodes and electrodes modified with nanomaterials.

2.2.2.2.1 Potentiometric sensors

Potentiometric ammonia sensors have several advantages such as portability, low cost and user friendliness.

Ionophores able to reversely bind ammonium ions were used to detect ammonium [36]. One of the most commonly used ionophores for ammonium detection in the published literature is nonactin [88]. However, nonactin was first introduced for the detection of potassium, but later it was discovered to be more sensitive towards ammonium. Therefore overcoming the cross sensitivity to K^+ has been a challenge in the case of nonactin utilization. *Humenyuk et al.* polysiloxane polymer containing nonactin as an ionophore with a field transistor, where the binding of nonactin with ammonia influences the carrier's quantity in the source-drain channel, achieving 0.1-10 mM [89]. A similar approach, but using a PVC nonactin embedded membrane was utilised by *Alifragis et al.* with 10-10000 μ M DR and 5.4 μ M LOD [90]. Again a similar approach published by *Karakuş et al.* using PVC+palmitic acid membrane with embedded nonactin resulted in 1 μ M – 100 mM DR and 1 μ M LOD for artificial water samples [91].

2.2.2.2.2 Amperometric sensors

A number of amperometric sensors were published based on the enzymatic conversion of urea and L-arginine, and ammonium and bicarbonate are the final products of such enzymatic conversion. A L-arginine-selective amperometric bi-enzyme biosensor based on recombinant human arginase I placed on a polyaniline–Nafion composite platinum electrode with calcium alginate gel coverage was published by *Stasyuk et al.* The sensor demonstrated a 70 - 600 μ M detection range with 38 μ M LOD. The amperometric sensor demonstrated fast response time (10 s) and good stability [92]. *Dave et al.* presented an amperometric ammonium biosensor based on alanine dehydrogenase of *Bacillus subtilis* and functionalized multiwalled carbon nanotubes. The screen-printed electrodes demonstrated a 0.05-500 mM

detection range and 1 μM limit of detection. The sensor was reported to retain $\sim 90\%$ of its initial output current after 119 days, and showed good selectivity [93].

A non-enzymatic amperometric sensor was presented by *Valentini et al.* using a carbon nanotube (CNT)/copper composite paste electrode. The method is based upon the catalytic action of the CNT/Cu composite material. The sensing setup showed a 3–100 μM detection range and 3.47 μM LOD for ammonia in drinking water. The operational stability of the proposed electrode is reported as reasonable [94].

2.2.2.2.3 Conductometric sensors

Ammonia can be also determined via conductometric transducers with relevant coatings. For instance, *Saipina et al.* presented an approach to reach this aim using calixarene, which is able to capture ammonium and can be determined conductometrically. First, they used calixarene immobilized on planar interdigitated gold electrodes, achieving 0.01–1.5 mM DR and 10 μM LOD in water samples [95]. Later they used a clinoptilolite based sensor prepared by drop-casting of zeolite and Nafion on the gold electrode surface. This sensor demonstrated a similar detection range, 0–1.5 mM and 30 μM LOD. The sensor was tested for interference from competitor ions, and it has shown better performance in comparison to previous electrodes [96]. *Tadi et al.* demonstrated an ultrasensitive detection setup using a fluorographene electrode. This sensing technology is based upon the selective binding of ammonia with fluorine, which was detected conductometrically. The sensor showed an incredible 0.44 pM limit of detection with 1 pM–0.1 μM detection range. Authors claim that different detection limits and ranges can be achieved by adjustment of fluorine doping into graphene [97].

2.2.2.3 Summary of ammonia sensing

This short overview of the existing ammonia sensing techniques lists only a small fraction of all published work by different researchers, showing mainly the latest publications. It can be summarised, however, that spectrophotometric and

fluorescence detection still suffer from pH and temperature interference, as well as lacking user friendliness and usually are bulky and involve reagents preparation, sample pre-treatment and waste. On the other side, the fluorescence detection shows a really low LOD while the spectrophotometric approach shows a reasonable detection range. The colourimetric approach has more modest detection parameters, however, in some cases, it is reported to be more compact and user-friendly.

The electrochemical ionophore based approach showed an impressive detection range and reasonable limit of detection, however still suffers from interference to competitive ions. The Electro biochemical enzymatic approach is a promising field with even broader detection and good selectivity. The conductometric electrochemical sensors are easier and cheaper to develop, however, some interference problems are still present.

As a summary of discussed methods of ammonia/ammonium detection, a table of comparisons of sensing techniques can be shown, **Table 2-3**

***Table 2-3** Summary of existing ammonia detecting techniques with sensing parameters*

Methods	LOD	Range of detection	Ref.
Spectrophotometric detection	0.88 μM	0-705.88 μM	[62]
	3.5 nM	52.4-288.7 nM	[66]
	5 nM	10–100 nM	[67]
	0.07 μM	0-35 μM	[68]
	0.08 μM	0-50 μM	
	0.15 μM	0-200 μM	[69]
Fluorescence detection	9.9 nM	0.032-0.5 μM	[71]
	5.8 nM	0.025-0.300 μM	[72]
	6.5 nM	0-5 μM	[73]
	6.47 nM	0-17.65 μM	[74]
	1 nM	5-1000 nM;	[75]
	1 nM	20-4000 nM;	
	1 nM	0-25 nM	
	7 μM	100-700 μM	[76]

	2.1 nM 12.94 μ M 2.8 nM	0-300 nM 22.35-368.82 μ M 8-556 nm	[77] [78] [79]
Colourimetric detection	1.5 μ M 15 nM 88 nM 440 nM 22.86 μ M 33.57 μ M	0.28-5.88 μ M 0.028-5.6 μ M; 0.28-13.9 μ M; 1.4-55.6 μ M 35.71-214.3 142.86-714 μ M 0-1.2 mM	[80] [81] [82] [83]
Electrochemical ,Potentiometric sensors	5.4 μ M 1 μ M 0.16 μ M 0.22-0.26 μ M	10-10000 μ M 1 μ M – 100 mM 0.77 μ M-10 mM	[90] [91] [98] [99]
Electrochemical – amperometric	38 μ M 1 μ M 3.47 μ M	70 - 600 μ M 0.05-500 mM 3–100 μ M	[92] [93] [94]
Electrochemical – Conductometric	10 μ M 30 μ M 0.44 pM	0.01–1.5 mM 0–1.5 mM 1 pM-0.1 μ M	[95] [96] [97]
Proposed U-shape OFS	3 μ M 25.9 μ M	0-10 μ M 10-200 μ M	
Proposed VIS LPG OFS	3.24 μ M (WL ratio) 4.8 μ M (WL summ)	0-50 μ M 0-50 μ M	

2.2.3 Phosphates detection

This section is a brief overview of the existing phosphate sensing techniques in water published in the literature.

Phosphorus is a vital nutrient for biological cells, although a significant excess can lead to negative consequences, such as a decrease of oxygen concentration, eutrophication, excessive algal growth and as a consequence overall reduction of

water quality [100]. The majority of soluble phosphorus in natural waters are in different forms of orthophosphates in four different species depending on the solution's pH level: H_3PO_4 , H_2PO_4^- , HPO_4^{2-} and PO_4^{3-} , **Figure 2-4** [101].

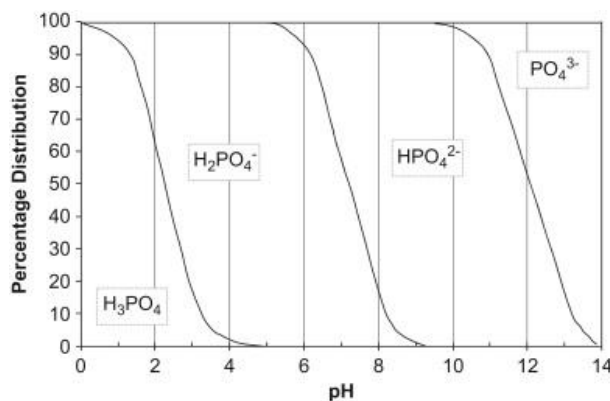
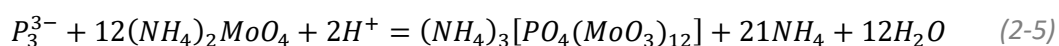


Figure 2-4 pH dependency of soluble orthophosphates aggregation in water [101]

2.2.3.1 Standard colourimetric detection.

Currently, the so-called ammonium molybdate colourimetric method is considered a standard phosphate detection procedure [102]. This method is based on the spectrophotometric determination of the solution of ammonium molybdate in presence of orthophosphates, ascorbic acid and antimony (III). It yields blue colour, and the reaction is as follows:



This is recommended by the Environment Agency [103] as a method for laboratory phosphate detection, however, it suffers from interactions with oxidizing agents, silicates and sulphites, and have some other disadvantages such as refractive index and turbidity errors [104].

2.2.3.2 Synthetic receptors and molecular imprinting

Several ways of molecular recognition techniques based on the structural variety of the phosphate ions were introduced. The phosphate ion has a tetrahedral shape, so receptors were synthesised to match this shape, mainly via the generation of acyclic or macrocyclic molecules [100]. However, sulphate for instance has the

same structure and the sizes of these ions are similar as well, 1.52 Å for phosphate and 1.45 Å for sulphate [105]. Which could potentially lead to limited selectivity and possible interference.

Most of those reported receptors can't function properly in aqueous solutions due to the high hydration energy of phosphates [106].

A similar molecular recognition approach is the so-called Molecular Imprinting method. Mostly performed on polymers while they polymerise in the presence of template molecules. After the polymerisation is done, the template molecule is removed which leaves specifically shaped cavities in the polymer's structure. Then the template molecule or a partially similar molecule can be considered as a target molecule, which rebinds to the cavities through chemical and physical interactions such as, hydrogen bonds, Van der Waals, electrostatic, ionic interactions or even covalent binding, **Figure 2-5**.

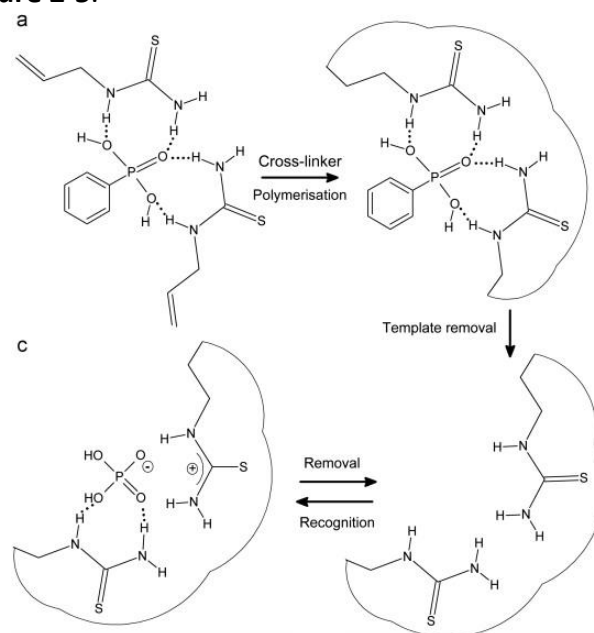


Figure 2-5 Schematic illustration of the thiourea based monomers polymerisation in the presence of a phenol phosphonic acid (PPA) template (a) and its further removal with phosphate shaped cavity and its recognition (c) [107]

Several MIPs able to bind phosphates have been reported, however, not many of them are integrated with transducers for sensing purposes [107].

The ability of thiourea based monomers to create phosphate receptors (**Figure 2-5**) was studied and developed to remove phosphate residues from water. This approach used 1-allyl-2-thiourea monomers to bind phosphates at neutral pH and showed good selectivity compared to nitrates, chlorides and sulphates [108]. Later

the same authors showed selectivity to fluorides, bromides and nitrites [109]. Further development of this approach was realised as a MIP based conductance sensor [107].

In addition to the above mentioned MIP approaches urea based monomers have shown the ability to develop phosphate moieties [110]. Also, a silica sol-gel based non-organic imprinted material was created via 3-trimethoxysilylpropyl-1-guanidinium chloride in combination with the PPA template. After template removal, this substance showed phosphate and phosphonates binding ability in aqueous media in low and neutral pH via ionic and hydrogen bonding [111].

2.2.3.3 Optical detection methods

2.2.3.3.1 Non-standard colourimetric detection

Apart from the standard colourimetric method described above, several other techniques have been presented. For instance, a pyridine based detector showed a $\sim 9.38 \mu\text{M}$ limit of detection (LOD) and $9.38 \mu\text{M}$ linear detection range (DR) for phosphates, with high selectivity toward phosphates among some other ions [112], however, no data were presented about stability, field usage and sensor's lifetime [112]. Also, an ammonium/guanidinium with a copper (II) metalcore method was presented, the sensitivity ($\sim 1.53 \text{ mM}$) was quite low for environmental measurements [113].

Apart from the above mentioned methods, there are several other colourimetric methods with different sensitivity limits from nano to millimolar range. A lot of issues though should be considered such as interference with other anions, fouling from solid particles and limited ability for field usage [100].

2.2.3.3.2 Fluorescence and luminescence detection

Both these methods are based on a change of the particular reagents' emission spectra as a consequence of interaction with phosphates. The change then is

measured optically. Potentially these methods can be integrated with optical devices and tested in field measurements.

The standard phosphomolybdenum method [102] has been modified and luminol was integrated. The luminol generated light when it was oxidised by the phosphomolybdate complex. Competing ions were filtered by a chelating resin, although few ions such as cobalt, copper (II), iron (II) and magnesium interfered as a catalyst for oxidation of luminol. This method can detect phosphates in fresh water with LOD ~ 1 nM with a linear response within the range of 1- 3.26 nM [114]. This is good for environmental measurements, but not suitable for wastewater monitoring where the preferable range is ~ 3 -156 μ M [100].

In a similar way a PVC membrane with an aluminium-morin fluorescence probe was used for phosphate detection, but this method suffered from interaction with zinc and nickel ions as those accelerated the quenching process. This method has a LOD ~ 625.5 pM with a linear response range: 187.6-469.1 nM. The response was reversible but lasts for only 60 measurements, which limits the sensor's suitability [115].

Proteins were used for the detection of inorganic phosphate. One phosphate binding protein was embedded in polyacrylamide nanoparticles combined with two fluorescent proteins, which were creating fluorescent signals. The reported DR lays between 97 and 970 nM. The drawbacks of this sensor are only 6 hours lifetime following rehydration, and lyophilisation storage requirement [116].

The fluorescent detection method of phosphates often utilises so-called synthetic receptors (SR), which includes among others the following chemicals: penta-pyrrolic macrocycles [117], octamethyl calixpyrrole structures with a detection range from 0.281 to 4.85 μ M [118], two quinolines with a cerium chloride with linear range: 0.313 to 4.85 μ M and 25 nM LOD [119]. These numbers are certainly within the range of natural concentrations of phosphates (0-312 nM of P) [120], but the concentrations in wastewater are much higher (3.13-156.37 μ M), so these methods won't be suitable for wastewater measurements [100]. However the ruthenium (II) SR with two pyridine rings multiple amine groups methods could be potentially applied in wastewater measurements as up to ~ 200 micromolar maximum phosphate concentration detection was reported, with selectivity compared to nitrates and

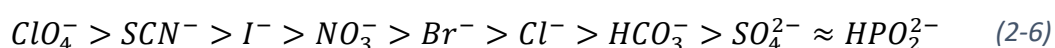
chloride [121]. Also, anthracene together with dipicolylamine and zinc complexes were used as fluorescent SR for phosphates in aqueous media with detection range 1-10 μM [122]. A followed patent from the same researchers states a phosphate detection concentration range from 1 μM to 1.93 mM, but no data for environmental measurements had been shown [123].

2.2.3.4 Electrochemical detection

A lot of electrochemical techniques and electrodes have been reported to detect phosphate ions in water. These methods can be classified into three different groups: potentiometric, voltammetric and amperometric [100].

2.2.3.4.1 Potentiometric method

Due to the large size, the tetrahedral shape of the phosphate anion and dependence on the pH level [124] it is quite difficult to create proper ion-selective membranes and electrodes for detecting phosphate anions without significant limitations [125]. Relatively high hydration energy of phosphate because of the surrounding of the central phosphorus by oxygen atoms (at the end of Hofmeister series, **Equation (2-6)**), is another obstacle for selectivity [126].



All these listed above factors significantly limit the selectivity of possible ion-selective phosphate membranes and ion-selective electrodes [100].

A variety of electrodes based on a polymer membrane with an ion exchange mechanism via ionophores were reported. An ionophore is a chemical that can carry an ion through a membrane due to its reversible binding ability. An electrode with organotin ionophores embedded in a PVC membrane was able to detect phosphates in a range from 5 μM to 32 mM with $\sim 1 \mu\text{M}$ LOD, however with some sensitivity loss after one month [127].

A large variety of ionophores were used within ion selective membranes for phosphate sensing. Among others, macrocyclic ionophores with polyamine groups [128], ionophores based on urea (DR=59 nM-97mM, LOD=19nM) [129] also central metal core ionophores, such as molybdenum (DR=0.1uM-94 mM, LOD=59 nM) [130], ferrocene [131] and tripodal cadmium complex [109]. All these methods have some common disadvantages, such as a limited lifetime of the ion selective membrane. Often they also require pre-treatments, which limits the ability to use them remotely [100].

2.2.3.4.2 Solid state ion selectivity

This detection method has several advantages, such as durability, miniaturisation possibility and no need for reference storage solution [132].

There are variety of sensors based on solid state ion electrodes reported, membranes incorporating a macrocyclic polyamide ionophore (DR=0.97 μ M-96mM, LOD=0.8 μ M) [132], aluminium phosphate and copper powder (DR=0.97 μ M-96mM, LOD=0.97 μ M) [133], carbon paste electrodes (DR=15 μ M-9.7 mM, LOD=12.5 μ M) [134]. All of them responded within a few seconds with one to three month lifetime, however, they suffer from interference to chloride and hydroxide ions. Also, cobalt based electrodes were reported in several papers [100], in one of the latest works cobalt coated microelectrode showed a linear detection range from 9.7 μ M to 9 mM. Interference from dissolved oxygen though required oxygen measurements for reference. However cobalt phosphate precipitation on the active surface somewhat limits the cobalt approach applications [135, 136].

Also, a screen-printed electrode (SPE) has been reported based on bistiurea electrode incorporation. With DR from 9.7 μ M to 9.7 mM and 3.9 μ M LOD. The sensor has 7 second response time and 4-month shelf life. Interferences from acetate, thiocyanate and bicarbonate [137]. Also, a MIP electrochemical sensor with thiourea monomer was reported with 96.95 μ M to 970 μ M calibrated detection range. The MIP was incorporated onto an ion sensitive field transistor [138].

2.2.3.4.3 Amperometric approach

This method measures the current generated by the target at an electrode with constant voltage [100]. Macrolytic, based on the reduction of ferrocene method has shown linear DR from 134.5 μM to 319 μM with a good selectivity, but sensitive to pH extremes [139]. Also an amperometric method based on reduction of phosphomolybdate was introduced with DR from 0.94 μM to 19.4 μM with 281.5 nM LOD [104], and a method based on anodic oxidation of molybdenum with DR from 625.5 nM to 3.13 μM with 115.7 nM LOD [140], were presented. All those methods need additional reagents with safe disposal afterwards. However, a glassy carbon electrode with a modified surface by ammonium molybdate was reported to work without any additional reagents with a linear range from 625.5 nM to 3.13 μM , with interference from silicate [141]. As can be seen, the amperometric method doesn't give the necessary detection range ($\sim 3\text{-}155\text{ }\mu\text{M}$) for wastewater pollution measurements [100].

2.2.3.4.4 Conductometric method

A biosensor based on phosphorylate generated a conductometric response for measuring pre-filtered river water, with a linear range from 0.94 to 387.8 μM with 0.94 μM LOD. Although, it was temperature and pH sensitive with optimal conditions of pH=6 and temperature range from 20 to 50 $^{\circ}\text{C}$. After 3 weeks the sensitivity had fallen by 50% [142].

2.2.3.4.5 Voltammetric approach

This approach is quite limited by oxygen ions surrounding the central phosphorus. Because of that, the voltammetric detection relies on indirect methods, such as reduction of the ammonium phosphomolybdate from a standard phosphomolybdate reaction [100]. In such an approach, via flow injection analysis a LOD of 938 nM was shown. However, a higher concentration of molybdovanadate than in the standard method was needed along with additional reagents to the samples; the glassy carbon electrode required daily cleaning, and interference from some other ions were reported [143]. Another method with the application of flow

injection was reported. A nickel-based electrode in an alkali medium produced a nickel hydroxide film which promoted the absorption of phosphate on the electrode's surface. This phosphate presence inhibited the activity of a glucose detector enzyme electrode. This approach was reported to show a 37.5-969.5 μM detection range with 281.5 nM LOD [144].

2.2.3.5 Summary of phosphate detection

Today the standard methods for phosphate detection still dominate over others. To change this trend a highly selective, sensitive and robust sensor has to be produced.

As can be seen from this short review, none of those methods is suitable for field measurements. The luminescence and fluorescence methods have sufficient detection limits for wastewater testing ($\sim 30 \mu\text{M}$), however, they suffer from significant interferences from competitive ions and haven't been applied to measure real environmental samples. Also, they need additional reagents which require safe disposal.

The electrochemical ways of detection also suffer from a lot of issues. The ISEs have short lifetimes or/and pre-treatment in solution is needed. This naturally limits their ability to function long term in-field measurements. Amperometric and voltammetric methods lack selectivity.

Hence there is a need for a robust, cheap and long-life phosphate sensor development for both environmental and wastewater measurements.

As a summary of discussed methods of phosphate detection, a table of comparisons of sensing techniques is shown below.

Table 2-4 Summary of existing phosphate detecting techniques with sensing parameters

Methods	LOD of P in water	Range of detection	Ref.
Colourometric detection	9.38 μM	9.38 μM	[113]
Fluorescence and luminescence detection	1 nM 625.5 pM 25 nM	1- 3.26 nM 187.6-469.1 nM 0.313 to 4.85 μM	[114] [115] [119]
Electrochemical - Potentiometric method	1 μM 19 nM 59nm	5 μM - 32 mM 59 nM-97mM 0.1uM-94 mM	[127] [129] [130]
Electrochemical – solid state ion selectivity	0.8 μM 0.97 μM 12.5 μM 3.9 μM	0.97 μM -96mM 0.97 μM -96mM 15 μM -9.7 mM 9.7 μM to 9.7 mM 96.95 μM to 970 μM	[132] [133] [134, 137] [137] [138]
Electrochemical – amperometric	281.5 nM 115.7 nm	134.5 μM - 319 μM 0.94 μM to 19.4 μM 625.5 nM - 3.13 μM 625.5 nM - 3.13 μM	[139] [104] [140] [141]
Electrochemical – Conductometric	0.94 μM	0.94 - 387.8 μM	[142]
Electrochemical – voltametric	281.5 nM	37.5-969.5 μM	[144]

2.3 Optical fibre sensors

In recent years, great progress can be observed in the field of optical fibre sensors development. Optical fibres are small, robust, relatively cheap, and do not suffer from electromagnetic and electrical interaction. Due to their size and physical properties, optical fibre-based sensors are perfect candidates for different sensing applications. A large variety of physical, chemical, and biological optical fibre sensors (OFS) have been reported in the literature.

There are several types of optical fibre sensors that are based on different phenomena. However, all of them lay in the detection of light parameters change (in the sensitive area) as a function of the interested target, **Figure 2-6**. Light propagates from the light source and through an optical fibre to the OFS (which is situated in the

sample), afterwards, modified propagates to the detector. Despite the similarities of the setup, the sensing principle of different types of OFS differs.

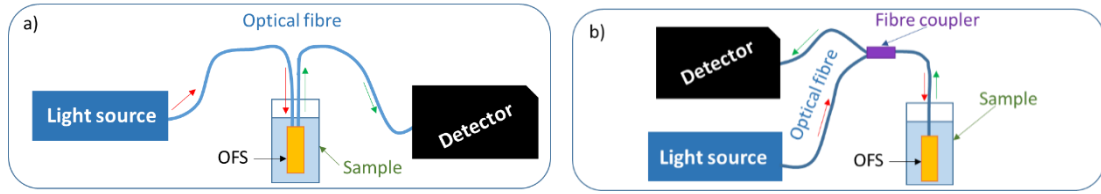


Figure 2-6 Optical fibre sensing setup a) in transmission mode; b) in reflection mode

2.3.1 A brief introduction to the physics of optical fibres

The light propagation through an optical fibre is possible due to the refractive index difference between the core and the cladding, according to Snell's Law [145]:

$$n_1 \sin \theta_1 = n_2 \sin \theta_2 \quad (2-7)$$

or

$$\frac{\sin \theta_1}{\sin \theta_2} = \frac{v_1}{v_2} = \frac{\lambda_1}{\lambda_2} = \frac{n_2}{n_1} \quad (2-8)$$

Where $\theta_{1,2}$ are the incidence and refraction angles, $v_{1,2}$ are the velocities of light in the respective medium, $\lambda_{1,2}$ is the wavelength of light in the respective medium, and $n_{1,2}$ is the RI of the medium.

Suppose, there are two different media named 1 and 2. When $n_2 < n_1$, then $\sin \theta_1 < \sin \theta_2$. Hence there should be some value of θ_1 less than 90° for which θ_2 will be 90° and $\sin \theta_1 = 1$. This means that the reflected beam propagates tangentially to the boundary surface and is called the critical angle. If the angle of incidence θ_1 exceeds the critical angle, the sine of the reflection angle becomes greater than 1, which is impossible. That means that the beam with an incident angle greater than critical cannot pass the boundary and would be totally reflected into the medium with n_1 . This phenomenon is known as total internal reflection and is the functional principle of light propagation inside an optical fibre [146]. **Figure 2-7** shows the schematic diagram of refraction and reflection of light propagating from a medium with a higher RI (medium 2) to a medium with lower RI (medium 1) under different angles. Cases **a)** and **b)** (light and dark orange beams) show light with angles of

incidence θ_1 lower than the critical angle θ_c therefore both refraction to the Medium 1 (under angle θ_2) and reflection back to the Medium 2 occurs splitting the energy of light between each other. The energies proportion depends on the angle of incidence [146]. Case **c)** shows a light (red beams) with an angle of incidence equal to the critical angle, meaning the light will reflect and also propagate alongside the boundary of Medium 1 and 2. Case **d)** shows a light beam with an angle of incidence higher than the critical angle, hence total internal reflection occurs. This is true for any angle larger than the critical angle.

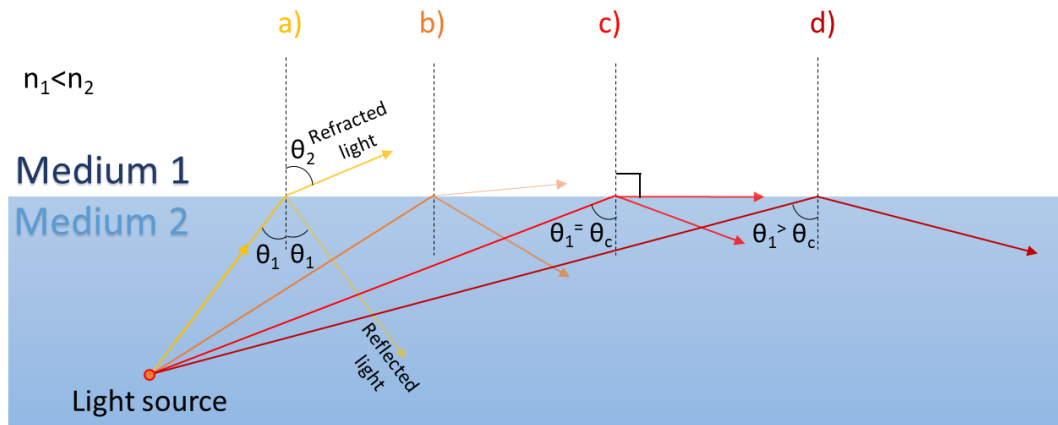


Figure 2-7 Schematic illustration of refraction and reflection of light propagating from a medium with a higher RI (medium 2) to a medium with lower RI (medium 1) under different angles. Cases a) and b) show light beams with angles of incidence $\theta_1 < \theta_c$ therefore refraction to the medium occurs, under an angle denoted as θ_2 ; case c) shows light beam with an angle of incidence equal to the critical angle; and case d) shows a light beam with an angle of incidence higher than the critical angle, hence total internal reflection occur

Hence, the optical fibre materials should obey the rule expressed in **Equation (2-9)**, to achieve total reflection:

$$n_{\text{clad}} < n_{\text{core}} \quad (2-9)$$

Where the n_{clad} (n_2) and n_{core} (n_2 and n_1 in the previous figures and equations) are the refractive indexes of the cladding and core respectively.

Figure 2-8, shows a schematic illustration of the optical fibre. Light orange beam shows an example of incidence angle (between the beam and the normal to core-cladding boundary line) lower than critical (θ_c). In this case, the light will both refract into the core, and reflect into the cladding. Further propagation in such mode will lead to attenuation both in cladding and core. The red beam shows a case of a light's incidence angle equal to the critical angle. In this case, the light will travel

alongside the core-cladding boundary and reflect to the core. However, eventually due to the splitting of the light's energy between the reflection and refraction, and due repetition of this process along with the fibre, the light in this mode will remain only at the core-cladding boundary. Any light with an angle of incidence greater than the critical angle will be totally reflected and will propagate in the fibre core. An example of a beam with an angle of incidence greater than the critical angle is shown by dark orange colour.

The critical angle determines the so-called maximum acceptance angle of the light entering the optical fibre (for propagation in core modes). Such angle α is illustrated in **Figure 2-8** between the axis of the fibre and red beam entering the fibre from the left side. The sine of the acceptance angle is the numerical aperture (NA) of an optical fibre and can be also calculated from the refractive indexes of the core and cladding. The NA is one of the most fundamental quantities of an optical fibre, **Equation (2-10)**, [147]:

$$NA = \sin \alpha = \sqrt{n_{core}^2 - n_{clad}^2} \quad (2-10)$$

Where α is the acceptance angle, n_{clad} and n_{core} are the refractive indexes of the cladding and core respectively.

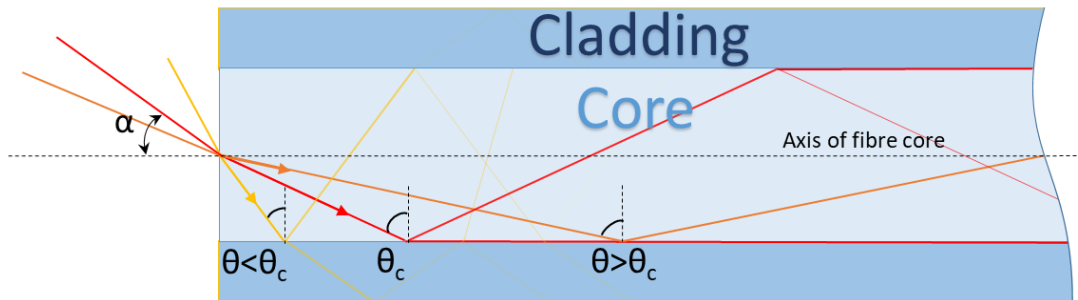


Figure 2-8 Schematic diagram of an optical fibre, α is the acceptance angle. θ_c is the critical angle, θ is the angle of incidence

2.3.2 Absorbance (evanescent field) based sensor

Absorbance based sensors have appeared in scientific literature since the 1970s [148]. Due to the rapid development, they are widely used nowadays. The nature of such sensors lie in the exponentially decaying evanescent field in the lower RI region of a light guide (optical fibre cladding). The material of the cladding can be chosen

and modified in accordance with the desirable sensitivity. As a result, a change in light propagation can be observed. The absorbance is governed by Beer-Lambert's Law and can be expressed by the following **Equation (2-11)** [149]:

$$T = \frac{I}{I_0} = 10^{-\alpha L} = 10^{-\varepsilon CL} \quad (2-11)$$

Where L is the optical path or absorption length, α is the absorption coefficient, ε – molar absorptivity, C – concentration. This can be transformed into absorbance:

$$A = -\log_{10} \left(\frac{I}{I_0} \right) = \alpha L = \varepsilon CL \quad (2-12)$$

Where A is absorbance, or magnitude commonly used in evanescent optical fibre sensors [149].

The light propagation through fibre has two components: guided field in the core, and evanescent field in the medium surrounding this core. With cladding present, the evanescent field remains unreachable for any external impact therefore is not relevant for sensing purposes. However, if the cladding is replaced (or altered in a certain point of the fibre) with physically, chemically or biologically sensitive materials (e.g. chemically sensitive dyes, temperature sensitive dyes, molecularly imprinted polymers, etc.), a so-called functional coating is formed. The light penetrates into the functional coating, which means interaction between the surrounding medium and the evanescent field in the modified cladding (which is selectively sensitive to a target), and changes its optical parameters. This change in the optical parameters can be observed by measuring the propagated light's properties.

Hence, the degree of evanescent field's penetration into a low RI medium plays a key role and is termed penetration depth of the evanescent field, d_p . The definition of the penetration depth in the evanescent field is the perpendicular distance from the core-cladding boundary at which the amplitude of the electric field has become $\frac{1}{e}$ of its value at the boundary. Assume that E_0 represents the amplitude of the electric field at the boundary, then it falls after some distance d , to:

$$E = E_0 e^{-zd_p} \quad (2-13)$$

Where the penetration depth magnitude is defined as:

$$d_p = \frac{\lambda}{2\pi n_{core} \sqrt{\sin^2 \theta - \left(\frac{n_{clad}}{n_{core}}\right)^2}} \quad (2-14)$$

and θ is the incident angle to the normal at the boundary, λ is the wavelength, n_{core} and n_{clad} are the RI values in core and cladding material respectively.

In order to increase the interaction with the evanescent field, the optical fibre can be modified in several ways. Such modifications can be achieved by removing the cladding, tapering or bending of the fibre (**Figure 2-9**).

2.3.2.1 Cladding removed optical fibre sensors

The easiest way to fabricate an optical fibre sensor is to remove the cladding and replace it with a sensitive thin film, a so-called functional coating. There are several approaches to deposit the functional coating. For instance, sol-gel with entrapped sensitive reagent, layer by layer (LBL) electrostatically build film with sensitive reagent [150], spin coating or a Langmuir-Blodgett film.

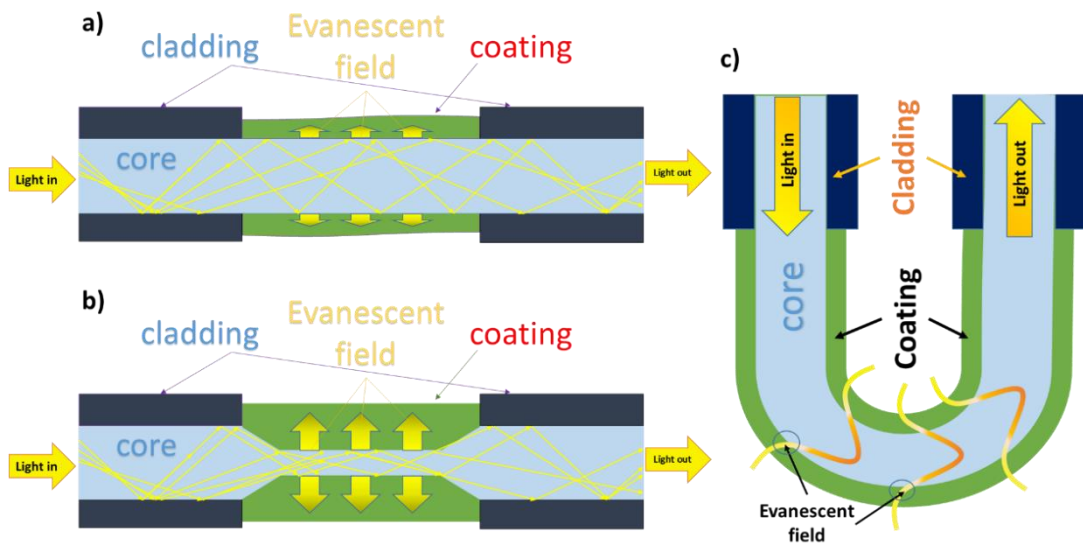


Figure 2-9 Absorbance or evanescent field based optical fibre sensors: a) cladding replaced straight fibre; b) tapered fibre sensor; c) U-shaped or bent cladding replaced fibre

With the replacement of the cladding by a functional coating the penetration depth rises, and the evanescent field interaction increases with the increase of the RI

of the cladding. Embedded into the coating, the optically sensitive reagent interacts with an analyte in the surrounding medium, which causes a change in the propagation of the light and hence transmission spectrum, **Figure 2-9a**. These changes can be observed by photodetectors or spectrometers to compute the presence or the concentration of the target analyte.

2.3.2.2 Bent optical fibre sensors

Bent optical fibre sensors are a particular case of the cladding replaced optical fibre sensors. The cladding removed part of the optical fibre undergoes bending after what is being covered by the functional coating. Sometimes they are called U-shape, or U-shaped optical fibre sensors [151], **Figure 2-9b**. The bending architecture of the sensitive region dramatically increases the evanescent field [152], and as a result, the sensitivity of those types of sensors could be higher [153]. The evanescent field increases in the bent optical fibre due to geometry change. The bending affects the angle of incidence of the light, hence the parameters of total internal reflection change as well [152]. However, it should be mentioned that the U-shape sensor is highly sensitive also to the refractive index change, not only to the absorption change [154].

2.3.2.3 Tapered optical fibre sensors

Tapering the fibre is another option to achieve the exposure of the evanescent field beyond the fibre's core. Thus tapering the fibre, and coating the tapered section with a sensitive coating, one can achieve larger interaction of the light propagating through an optical fibre with the sensitive coating, **Figure 2-9c**. Parameters of the tapered region such as length, waist diameter and refractive indexes affect the evanescent field [155].

Some of the latest tapered based optical fibre sensor publications claim to measure temperature with graphene coating [156], relative humidity utilizing agarose gel coating [157] and ammonia vapours with silica-gel coating [158].

2.3.3 Fibre Bragg Grating sensors

A Fibre Bragg Grating (FBG) is a short period grating (with ~500nm period) inscribed in the core of the single mode fibre, **Figure 2-10**. The field of coupled-mode theory shows that coupling of propagating light in an optical fibre could occur in the same or opposite direction to propagating light [159]. The nature of those types of sensors lays in a periodic disturbance of the fibre core's RI. In the case of FBGs, there is an opposite directional wave coupling, so it acts as a monochromatic mirror reflecting backwards a narrow band. However, that statement could be considered true only in the case of gratings perpendicular to the fibre axis. In the case of tilted gratings, the physics of the coupling is more complicated [160].

The grating period of Fibre Bragg grating sensors is around 0.5 micrometres. This type of grating acts like a dichroic mirror, which means they reflect backwards at a certain wavelength value λ_B . This phenomenon is governed by **Equation (2-15)**:

$$\lambda_B = 2n_{eff}\Lambda \quad (2-15)$$

Where n_{eff} is the effective RI of the fibre core and Λ is the grating period. The effective refractive index of the waveguide is defined as a ratio of the propagation constant to the wavenumber (or the ratio of the speed of the wave in vacuum to the speed of the wave in the waveguide in the given mode). In other words, the effective refractive index is a measure of the overall delay of a wave in the given mode.

Hence the reflected wavelength λ_B depends on internal factors that can influence the period or the core's refractive index which is the reason why an FBG is an excellent instrument to measure temperature and strain. The temperature would affect n_{eff} because of thermo-optic effect, and longitudinal deformation – both, n_{eff} and Λ because of photo-elastic and mechanical (elongation) effects respectively [161]. The shift in the reflected wavelength peak is depicted in **Figure 2-10** by solid and dashed red curves and marked as λ and λ' . The light in FBG does not interact with the external medium and is confined only inside the fibre core. Thus the FBGs are not really suitable for chemical sensing, because **Equation (2-15)** does not depend on the claddings RI.

However, once the FBG is modified (cladding altering, grating tilt), they become sensitive to the surrounding RI, hence can be deployed as chemical or biochemical sensors with appropriate sensitive materials. FBGs are relatively small (typically ~3-5 mm long) and are realized on single mode thin fibres (core diameter ~8-11 μm , cladding diameter ~125 μm , buffer ~250 μm). Several FBGs with different gratings can be (and often are) placed on one fibre in series which means a possibility of relatively cheap and simple multi-parameter measurement systems.

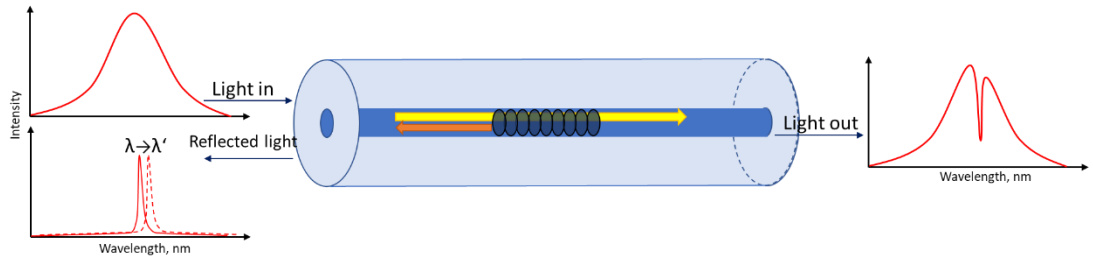


Figure 2-10 Schematic illustration of the FBG and reflected/propagated light

2.3.4 Long Period Grating sensors

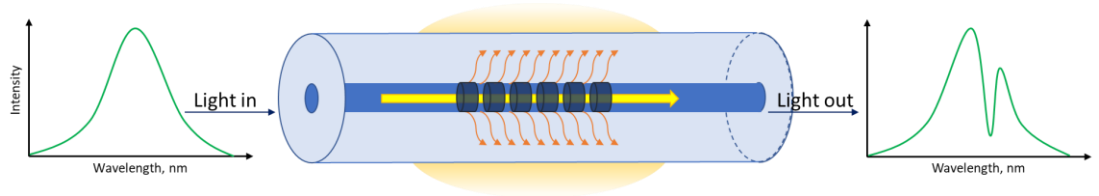


Figure 2-11 Schematic illustration of the LPG and the propagated light

Long period gratings (LPG), often called a Long Period Bragg Grating, are single mode fibres with periodically altered RI of the fibre's core whose grating period is larger (100-500 μm). The long period grating coupling phenomena involves the cladding mode propagation in the grating area, this causes coupling of the light between core and cladding modes (**Figure 2-11**), thus the central wavelength of the coupled light, λ_{cw} depends on the cladding's effective RI (thus it's dependent on the surrounding refractive index) [162] and is governed by **Equation (2-16)** [163]:

$$\lambda_{cw} = \Lambda(n_{eff\ core} - n_{eff\ clad}^i) \quad (2-16)$$

Where Λ is the grating period, $n_{eff\ core}$ and $n_{eff\ clad}^i$ are the effective RI of the fibre's core and i -th mode of the cladding respectively.

The minimal transmission value T_i at the given mode can be expressed in **Equation (2-17)** [163]:

$$T_i = 1 - \sin^2(k_i L) \quad (2-17)$$

Where L is the length of the grating and k_i is the coupling coefficient for i -th cladding mode, which can be determined by the mode propagation constant periodic modulation amplitude and by the overlap integral of the cladding and core mode. However, only with large values of overlap integral effective coupling could be observed which means, the electric field profiles should be similar [164]. The coupling occurs between the core and circularly symmetric cladding modes, because the amplitude of the even order modes electric field profiles is low inside the core, the odd modes, however, have peaks within the core [164].

Figure 2-12 shows the theoretical calculations of coupling wavelength done by James and Tatam [163] using the method described by Patrick *et al.* [162].

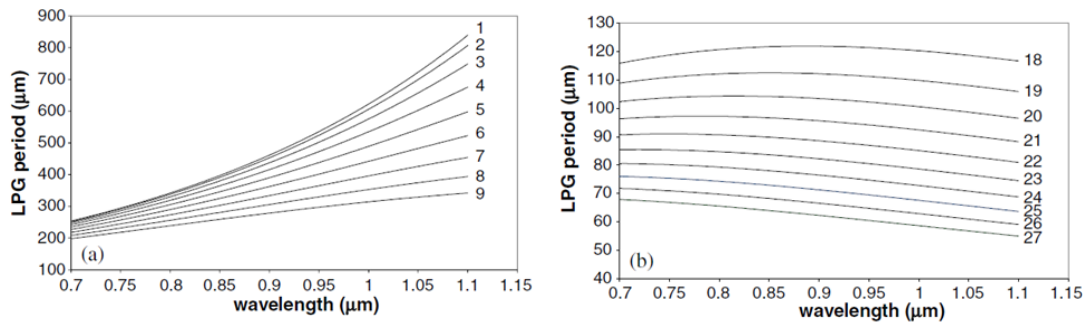


Figure 2-12 a) lower order and b) higher order cladding modes phase matching curves [163]

As can be seen from **Figure 2-12**, the higher order cladding modes phase matching curves have a turning point at a certain value of the LPG period. This point is called the phase matching turning point (PMTP). That phenomenon means that two attenuation bands with different central wavelengths can be observed on one resonance mode if the LPG is fabricated with a grating period close to one of the PMTPs. This is called dual resonance [165]. LPG sensors fabricated to operate at PMTP are extremely sensitive and were used to measure physical parameters such as strain, temperature and refractive index [166]. Also with appropriate

modifications, those kinds of LPG-s were used as chemical sensors e.g. ammonia [167] and methanol [168].

2.3.5 Fabry–Pérot interferometer (FPI) OFS

A Fabry-Perot Interferometer consists of a set of parallel partial mirrors. When light enters the mirror cavity, it's partially reflected back and forth causing interference [147]. Interference between the reflected beams will allow only certain angles to be transmitted (constructive interference) while angles between the constructively interfering exit angles are suppressed in amplitude (dark). This appears in form of fringes of the signal. FPIs on optical fibres are usually made on the fibre's tip by depositing a thin film on the tip's surface. Thus, the obtained optical system is composed of three different materials in terms of refractive index: the optical fibre's core, the deposited film, and the surrounding medium. When the transmitted light passes through the structure, the two media interfaces (fibre-coating and coating-air) act as partial mirrors, where part of the optical power is transmitted, and the other part is reflected (**Figure 2-13**). The reflected spectrum depends on the RI of the three materials and the thickness of the coating. However, the resonance wavelength itself depends on the effective refractive index of the cavity and its thickness, which should be equal to an integer number of the value of the wavelength, **Equation (2-18)** [147]

$$\lambda_N = 2Ln_{cav}/N \quad (2-18)$$

where λ_N is the resonance wavelength, L - the thickness of the cavity, n_{cav} - effective refractive index of the cavity and N is a positive integer.

Hence by applying sensitive films on the fibre tip, the desired analyte can be detected.

There are other interferometric optical fibre sensors, such as the Michelson interferometers (MI), Mach-Zehnder interferometers (MZI) and Sagnac interferometers, all of which work on interferometric bases [169].

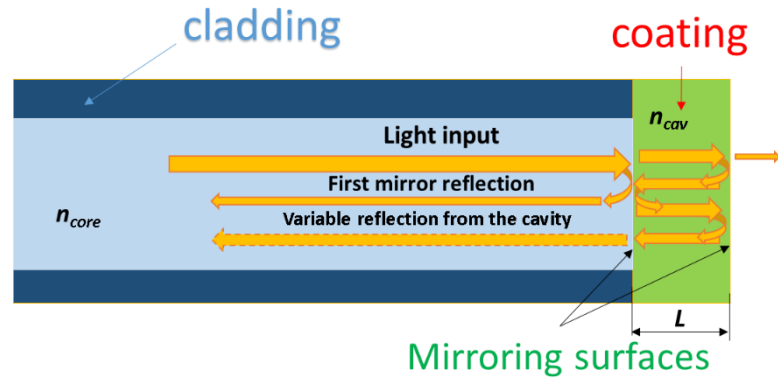


Figure 2-13 Schematic illustration of the Fábry-Perrot interferometric sensor

2.3.6 Extrinsic OFS

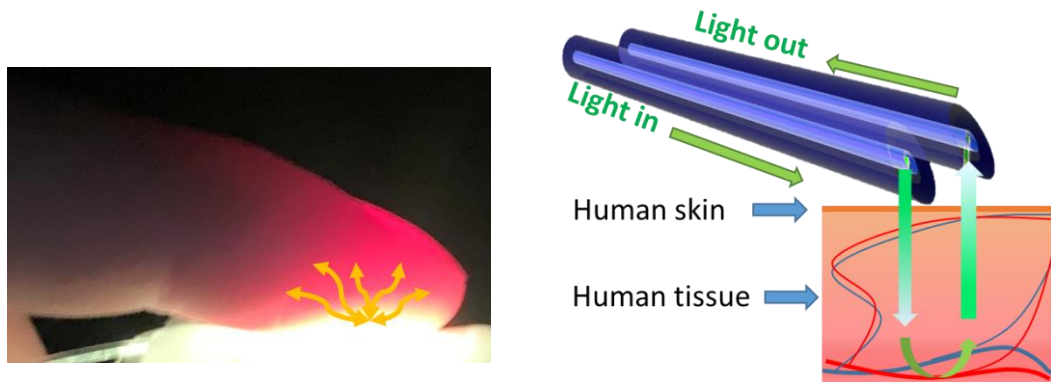


Figure 2-14 Pulse Oximetry principle of operation

Extrinsic optical fibre sensors are such devices, where the light leaves the fibre, undergoes modulation in an external medium and returns to the fibre. In those sensors, the optical fibre is used just as a waveguide and is not a part of the sensing mechanism [170]. The signal modulator can be a miniature optical component, or the surrounding medium itself. The major advantage of optical fibres deployment in such applications is that optical fibres can reach places otherwise difficult to reach, for instance in compact wearables or medical devices. One example of an extrinsic OFS is in pulse oximetry, where optical fibre deployment is beneficial especially in integrated medical devices due to compactness, safety reasons and immunity to electromagnetic fields of optical fibres.

Pulse oximetry is a simple, relatively cheap and non-invasive technique to monitor oxygenation. It monitors the percentage of haemoglobin that is oxygen-saturated. Oxygen saturation should always be above 95%, although in those with long-standing respiratory disease or cyanotic congenital heart disease, it may be lower, corresponding to disease severity [171]. Oximeters work by the principles of spectrophotometry: the relative absorption of red (absorbed by deoxygenated blood) and infrared (absorbed by oxygenated blood) light of the systolic component of the absorption waveform correlates to arterial blood oxygen saturation. Measurements of relative light absorption are made multiple times every second and these are processed by the machine to give a new reading every 0.5-1 seconds that average out the readings over the last three seconds.

This approach is well known and widely used, not only in medical devices but also in modern wearable fitness trackers and smartwatches. On those devices, the light-emitting and collecting are being done by LEDs. However, that is difficult to perform in some cases. Therefore, it is sensible to deploy optical fibres, both for emitting and collecting the signal. Several such solutions were presented recently to measure capillary refill and contact pressure [172, 173], SpO₂ measurements with embedded in textile optical fibres during MRI [174, 175], SpO₂ measurement in the oesophagus [176], combined pressure and SpO₂ measurement in the trachea with OFS incorporated into an endotracheal tube [177].

2.3.7 Requirements for the candidate sensors

Taking into account the above literature review, some required parameters for the candidate sensors can be established. Sensing parameters for detecting nitrites in water depends on the different targets in different environments. For instance, the concentration values differ for natural, drinking, and waste waters. **Table 2-5** shows the required limits of detections and detection ranges in different waters for nitrite, ammonia and phosphate sensors. The lowest concentrations can be found in the open ocean or sea, hence a sensor operating there should be able to detect at least 0.1 nM of nitrites, 0.05 μ M of ammonia and 0.02 μ M of phosphates. These are pretty

low concentrations, and the seawater itself makes it even more challenging. Usually, they are targeted with large (10-50 kg), lab-based complicated analyzers [178].

Table 2-5 *Desired sensitivity parameters (LD and DR) of new nutrient sensors operating in different environments, μM unless stated otherwise.*

	Sea/ocean water		Natural coastal waters		Drinking/tap water		Waste water	
	LOD	DR	LOD	DR	LOD	DR	LOD	DR
Nitrite	0.0001	0-0.3	0.1	0.1-5	10	10-100	70	70-200
Ammonia	0.05	0.05-2	5	5-30	15	5-2000	500	0-12mM
Phosphate	0.02	0-0.3	5	5-30	7	2-100	30	0-2 mM

A sensor for natural coastal waters should be able to detect at least 0.1 μM nitrites, 5 μM ammonia and phosphates. The proposed ammonia sensors with their LOD $\sim 3\text{-}4 \mu\text{M}$ could be suitable for such a task.

For tap and drinking the regulations differ across countries, however, if summarised the LODs should be at least as low as 10 μM for nitrites, 15 μM for ammonia and 7 μM for phosphates [179]. The proposed ammonia sensor has a lower LOD, so it can be suitable for drinking water monitoring.

Waste water contains the highest concentration of nutrients with a bottom limit of, 70 μM for nitrites, 500 μM for ammonia and 30 μM for phosphates. Both proposed ammonia ($\sim 3\text{-}4 \mu\text{M}$ LOD) and nitrite ($\sim 22 \mu\text{M}$ LOD) can be suitable for monitoring waste water quality.

The proposed platforms based on optical fibres offer a possibility to develop a compact handheld device that can measure ammonia and nitrites concentrations in water. It would be a huge improvement compared to other sensing platforms. Although the detection limits are not low enough to use the proposed sensors in sea water, wastewater monitoring can be achievable for both, nitrites and ammonia sensors. Drinking water targets allow deployment of the ammonia sensor and the nitrite sensor is close to the target. Coastal natural waters can be measured for ammonia with the proposed sensor.

2.3.8 OFS for detecting nutrients in water

Only a few nitrite sensitive OFS were reported in the recent literature.

A combination of flow spectroscopy with optical fibres used an optical fibre as a probe to take the colourimetric for instance reported by *Xlong et al* [180], and *Yang et al* [181], however, these methods are not purely OFS based.

A glass slide coated with cobalt(II) tetrakis(o-aminophenyl)porphyrin film, as the sensitive element, placed on the tip of an optical fibre bundle is reported by *Yang et al*. The paper claims that the sensor has a LOD of 6 μM , reversible response and long lifetime.

By utilizing UV spectroscopy, OFS with filtered light source and photodiode was reported by *Moo et al*. Taking the 356 nm absorbance wavelength, authors achieved 109 μM nitrites in water quantification limit [182].

In recent years there were quite a few OFS for ammonia detection in water reported in the literature. The most promising among them will be listed below.

Bent optical fibre sensor coated with bromocresol purple dye incorporated in sol-gel was reported to be sensitive to aqueous ammonia by *Tao et al*, with the lowest experimentally detected concentration of 29.4 μM and 10 minutes response time. The sensor response was recoverable, [183].

By utilizing a sensitive SnO_2 film on a tip of the fibre in combination with FBG as a temperature compensator, *Pisco et al* reported an aqueous ammonia detector with 117 μM LOD and a few minutes response time, [184].

Using cladding replaced plastic OFS with Oxazine 170 Perchlorate dip coating *Jalal et al*, published an aqueous ammonia sensor with $\sim 82 \mu\text{M}$ LOD with DR up to 15 mM and ~ 10 sec response time and 99% reversibility [185].

Strobl et al, reported a fluorescent OFS based on BF_2 -chelated tetraarylazadipyrromethene dye physically entrapped in polyurethane hydrogels, dispersed in rubber with Egyptian blue as reference material. The LOD is $\sim 6.5 \mu\text{M}$ and the DR is 0-17 mM, [74].

Tiwari et al, reported a TSPP incorporated into TiO_2 matrix with tapered OFS with ammonia in water LOD $\sim 5.9 \mu\text{M}$ [186], and *Korposh et al* used an LPG OFS coated with silica nanoparticles with TSPP. Ammonia detection LOD in water was reported

to be $\sim 8.24 \mu\text{M}$, [150]. These sensors however were not reversible because of the weak bondage of the TSPP to the fibre. The possibility to use a covalent binding with DAR resin played a key role in choosing TSPP based OFS in this research.

To the best of my knowledge, only one attempt of phosphate sensor based on OFS was attempted. *Verma et al.*, described an OFS for detection of phosphate ions in the soil, utilizing surface plasmon resonance with cobalt oxide coating over gold-coated fibre core. The sensor's LOD is claimed to be $51 \mu\text{M}$, with 0-1.03 mM DR. The sensor is not reversible [187].

2.3.9 Conclusion to the optical fibre sensors

Using optical fibre sensing techniques, a large variety of chemical, biological, and physical sensors and their combinations were reported in the literature, some of them are commercialized. Some of those chemical and biological sensors are briefly described in the tables below at the end of this section with literature references. It should be noted that those tables are only a small part of the existing research and that those most relevant to this thesis have been selected.

By deploying a relevant functionalisation, almost any chemical can be detected provided their concentration is above the limit of detection. For instance, by using molecular imprinting (which creates cavities in the polymer matrix with affinity to a chosen "template" target molecule), functional coating for almost any bigger organic molecule (i.e. drugs, hormones,) can be developed and tailored to exclude cross-sensitivity.

Sensitive dyes were successfully applied in combination with most sensor types to measure pH, nitrites, ammonia, ethanol, methanol, acetone, CO_2 and temperature.

Fibre Bragg Grating and Pulse oximetry OFS were successfully incorporated into bandages and wearables to measure Blood pressure, the photoplethysmogram (PPG) and Pulse Transit Time (PTT) in situ.

This list of OFS sensors presented below is far from complete but provides examples of currently developed OFS prototypes most relevant to this thesis.

Table 2-6 Gas phase fibre optic chemical sensors

Sensing platform	Modification (sensing principal)	Target analyte	Detection Range	LOD	source
FPI	Au/ZnO (oxidation temp change tracking)	Carbon Monoxide (CO)	0-4%		[188]
Dual FPI	Co ₃ O ₄ /graphene oxide membrane	Carbon Monoxide (CO)	0–60 ppm		[189]
LPG	ZIF-8 metal organic framework	Acetone vapour Ethanol vapour Methanol vapour	49-543 ppm 62-666 ppm	6.67 ppm 5.56 ppm	[190]
Attenuation	Au-Pd film	Hydrogen	0.8-4.6%		[191]
micro-mirror	Pd/WO ₃ film		0.5-3%		[192]
E-FBG and SP-FBG	Pd/WO ₃ and PD/Ag film		1-8%		[193]

Table 2-7 Aqueous Fibre optic chemical sensors

Sensing platform	Modification (sensing principle)	Target	Range	LOD	source
fluorescence quenching core fibre sensor	CdTe/CdS Quantum dots	Nitric Oxide (NO)	10 pM 10 mM	10 pM	[194]
37 concentric fibre tips	CdSe quantum dots/cellulose acetate film		0.1-1 µM	0.01µM	[195]
multimode fibre tip	Sol-gel immobilized ruthenium(II) complex		0-60 µM		[196]
D-FBG	Polydopamine immobilized concanavalin A	Glucose	1-100 µM	0.1 µM	[197]
FPI	Partially-reduced graphene oxide film (Rh6G fluorescence)	Dopamine Nicotine ssDNA	0-10 mM 0-1.24 µM 0-100 nM		[198]
	iron(II) phthalocyanine (FePc) fluorescence	Adrenalin	2-9 µM	0.4 µM	[199]
Absorption fibre sensor	Enzyme immobilization	Glutamate	0-10 mM	0.36 mM	[200]
Tip based sensor	Molecularly imprinted polymer (MIP) - Fluorescent	Cocaine	0 -500 µM	2 µM	[201]
LPG	MIP Nanoparticles - RI	Vancomycin	0.01 -700 µM	2.5 pM	[202]

Tip based sensor	D-70 Dye - Fluorescent	Adriamycin	0.01-10 $\mu\text{g/mL}$	0.057 $\mu\text{g/ml}$	[203]
Tip based sensor	MIP - Fluorescent	Propofol	0.1-15 mg/mL		[204]

Table 2-8 Biological optical fibre sensors

Sensing platform	Modification (sensing principal)	Target or interaction	Range	LOD	Source
D-FBG	Graphene film	Erythrocyte	0 to 10 000 ppm		[205]
Microfibre FBG	poly-l-lysine - ssDNA	DNA hybridization	0.5–20 μM	0.5 μM	[206]
TFBG	Immobilised BSA	BSA/anti-BSA	1–100 $\text{mg}\cdot\text{L}^{-1}$	0.086 $\text{mg}\cdot\text{L}^{-1}$	[207]
TFBG	gold nanospheres-Avidin	Avidin/Biotin	10 pM–100 μM	8 pM	[208]
81° TFBG	Enzyme immobilization	Glucose	0–3 $\text{g}\cdot\text{L}^{-1}$	0.1 $\text{g}\cdot\text{L}^{-1}$	[209]
SPR-based TFBG	Gold- aptamer	Aptamer/Thrombin	0.1–5 μM	22.6 nM	[210]
SPR-based TFBG	monoclonal antibodies	Single cell and membrane proteins	0.5–5 $\times 10^6$ cells $\cdot\text{mL}^{-1}$	2 $\times 10^6$ cells $\cdot\text{mL}^{-1}$	[211]
LPG	Goat anti-human IgG (antibody) immobilization	IgG/anti-IgG	2–50 $\text{mg}\cdot\text{L}^{-1}$	2 $\text{mg}\cdot\text{L}^{-1}$	[212]
TAP LPG	Immunoglobulin G (IgG) deposition	Biotin/Streptavidin	0–100 $\text{mg}\cdot\text{L}^{-1}$	12.5 $\text{mg}\cdot\text{L}^{-1}$	[213]
LPG	Immunoglobulin G (IgG) deposition	IgG/anti-IgG	0.1–500 $\text{mg}\cdot\text{L}^{-1}$	0.5 $\text{mL}^{-1}/3.3$ 3 nM (buffer)	[214]
TAP LPG	Immunoglobulin G (IgG) deposition	IgG/anti-IgG	0.001–100 $\text{mg}\cdot\text{L}^{-1}$	70 $\mu\text{g}\cdot\text{L}^{-1}/460$ pM (serum)	[215]
LPG	Sol-gel – Polymer – IgG deposition	IgG/anti-IgG	0.001–100 $\text{mg}\cdot\text{L}^{-1}$	8 $\mu\text{g}\cdot\text{L}^{-1}/53$ pM (serum)	[216]
TAP LPG	Oligonucleotides immobilization	DNA hybridization	0–5 μM	4 nM	[217]
LPG	ssDNA immobilization via PLL	DNA hybridization	-	62 nM	[218]
Michelson-based LPG	AuNP doped sol-gel with QDNA	Aptamer/Adenosine triphosphate	0–2.5 mM	400 μM	[219]

LPG	ECA–EcOMPs immobilization	Aptamer/E. coli outer proteins	0.1–30 nM	1 nM	[220]
LPG	T4 phages immobilization	Bacteriophages/E. coli	0–10 ⁹ cfu·mL ⁻¹	10 ³ cfu·mL ⁻¹	[221]
LPG	Enzyme immobilization	Glucose/enzymatic	0.1–3 g L ⁻¹	-	[222]
LPG	Lypas enzyme immobilization	Triacylglyceride/enzymatic	0–7 mM	0.5 mM	[223]
BLI	SA-dsDNA-RepA protein interaction	Testosterone		2.13–136.63 mg/ml	[224]

Abbreviations used in Tables 2-9, 2-10, 2-11:

FBG – Fibre Bragg Grating

LPG – Long Period Grating

OFS – Optical fibre Sensor

TFBG - Tilted Fibre Bragg Grating

BSA - Bovine serum albumin

FPI - Fabry–Perot interferometer

BLI – biolayer interferometer

PPG - photoplethysmogram

PTT - Pulse Transit Time (PTT)

Chapter 3 Experimental techniques and methodology

3.1. Introduction

This chapter provides an overview of the techniques used in this PhD research to fabricate absorbance based and LPG, and explains the fabrication procedures of the OFS. Also, a description of the optical fibre coatings, such as sol-gel, layer-by-layer and silanization and their fabrication deposition is provided.

3.2. Optical fibre sensors

Overview of the optical fibre sensors principle of operation and literature review is given in **Chapter 2 section 2.3**. In this section, the fabrication process of the OFS used in this PhD research will be given.

3.2.1. Straight absorbance based optical fibre sensors fabrication

As described in **section 2.3.2**, to fabricate an evanescent field sensor, it is necessary to remove the fibre's cladding and buffer. Several techniques to achieve this were reported in the literature, depending on the type of fibre. For instance, in the case of plastic or silica optical fibre, both the core and the cladding are made from the same material with minor differences in their (core and cladding) structures and chemical composition, achieved by doping an additional material component. In the case of silica optical fibre, chemical etching can be applied via hydrofluoric acid to remove the cladding and gain access to the evanescent field. [225, 226]. In the case of plastic (poly(methyl methacrylate)) optical fibre, acetone can be used as an etching agent to remove the cladding [49]. To achieve the desired etching depth (e.g. to remove the cladding but to save the core) strict etching timing is applied.

In this PhD research, FT200UMT TECS multimode optical fibre was used to fabricate an evanescent field OFS. An FT200UMT TECS multimode fibre manufactured by Thorlabs with silica $200\pm5\ \mu\text{m}$ diameter core, TECS Hard Fluoropolymer cladding ($d=225\pm5\ \mu\text{m}$), and tetrafluoroethylene (Tefzel) ($d=500\pm30\ \mu\text{m}$) jacket was used. The FT200UMT optical fibre is transparent in 300 - 1200 nm optical range and has a numerical aperture of 0.39 [227]. This fibre was chosen because of its plastic cladding and glass core, which makes the modification process of cladding removal easy via burning the cladding.

In the case of the straight cladding removed (replaced) absorbance based OFS, the fabrication procedure was as follows. The section of the cladding to be removed was measured and marked with a permanent marker. The fibre was fixed and straightened out by weak tension force applied using spring as shown in **Figure 3-1**. Using a hand gas burner (2200 VersaFlame, Dremel, Netherlands), the cladding of the FT200UMT fibre was burned off by moving the burner from one end of the marked part of the fibre to the other, **Figure 3-1**.

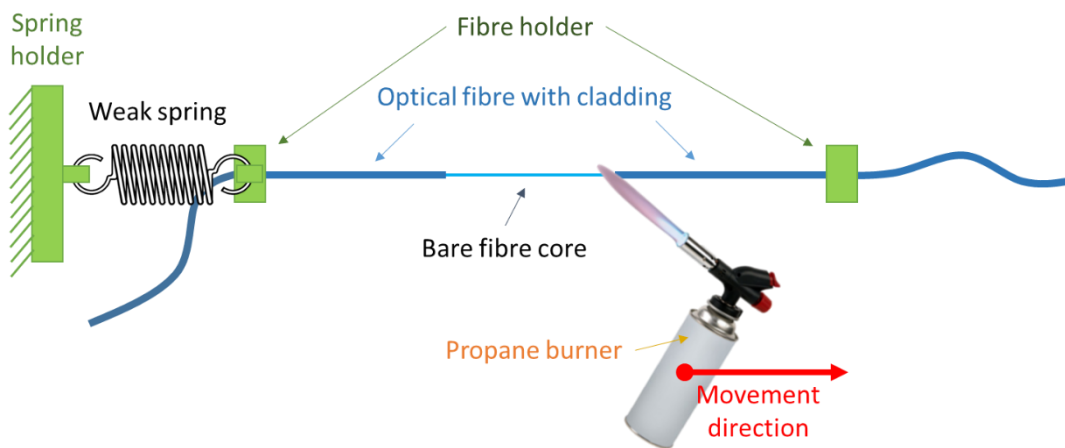


Figure 3-1 Setup for fabrication of the cladding remover straight OFS from FT200UMT fibre via cladding burning off

3.2.2. U-shape and absorbance based optical fibre sensors fabrication

The U-shape OFS (principle explained in **section 2.3.2.2**) in this PhD research was fabricated out of the same FT200UMT TECS multimode fibre. Hence, in the case of the U-shaped sensor fabrication, the applied heat via gas burner was also a

reasonable way [228]. This fibre was chosen because of the ease of U-shape sensor fabrication via burning. Together with the burning of the plastic buffer and cladding the silica core was melted and shaped to form a U-shape. The setup of the U-shape fabrication used in this research is shown in **Figure 3-2**. This method and setup allow the regulation of the desired bending radius by adjusting the size of the flame. The resulting U-shape bending radius was chosen to be ~ 0.5 mm, as an optimal with the largest RI sensitivity [228]. The bending radius plays a key role in the evanescent field increase, and the bigger is the bending radius, the larger is the evanescent field on the outer surface of a U-shape and hence the sensitivity of the U-shape, [229]. In his thesis, D.Gomez has shown experimentally a comparison between the RI sensitivities of U-shapes with bending radii from 1.5 to 0.5 mm for the same FT200UMT fibre and found the 0.5mm bending radius U-shape the most sensitive [228]. As the smallest achievable bending radius via this setup is 0.5 mm, it was chosen to be the optimal bending radius.

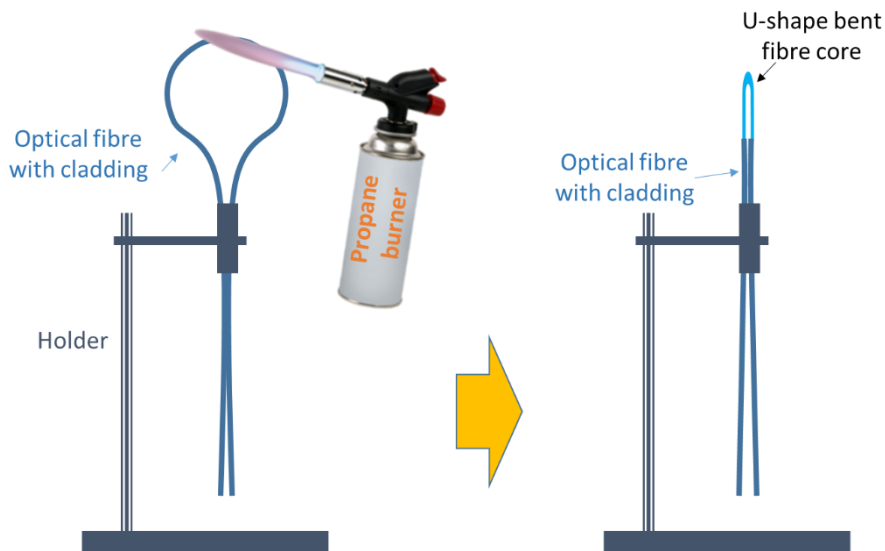


Figure 3-2 Setup for fabrication of the cladding remover U-shape OFS from FT200UMT fibre via cladding burning off

The procedure of the U-shape fabrication was as follows. The FT200 UMT was bent over to 180° to form a loop and placed into a holder. The position of the fibre was adjusted to achieve the desired bending radius. Using a hand gas burner (2200 VersaFlame, Dremel, Netherlands), the cladding of the FT200UMT fibre was burned on the top of the fibre loop. The temperature of the flame was $\sim 2800^\circ\text{C}$, the duration of the exposure was determined empirically by observing the burning procedure

(until the plastic cladding and jacket burned out) and lasted for a few seconds. As a result of the extensive heat applied, together with the burning of the cladding the silica core would melt and form a U-shape. Photographs of the U-shape fabrication evolution at different stages via the burning procedure can be seen in **Figure 3-3**.



***Figure 3-3** U-shape optical fibre sensor fabrication process via burning, photographs from left to right. Fiber shaping and fixation, burner on, burning procedure start, shaping the fibre under the heat exposure, red U-shape with scaling ruler*

3.2.3. Long period grating optical fibre sensors fabrication

A brief overview of the operational principle of the LPG OFS was given in **Section 2.3.4**.

To cause the periodic RI perturbation inside an optical fibre a periodical grating should be inscribed on its core or cladding [230]. To achieve such perturbation different techniques have been used. Heat induced by an electric arc is a low cost and relatively simple and flexible technique to create LPGs. In this method, the heat modifies the viscosity, as a result the RI symmetry changes as well [231]. This method is limited, however, to gratings with periods above 200 μm [232]. Another way to fabricate an LPG is by applying mechanical stress. This can be done via a sandwich squeezer with inscribed periodic ridges and gaps [233]. However, the most common technique to produce LPGs is to use local exposure of the fibre to laser radiation. Several different lasers were used, high-power UV laser [230], CO_2 laser [234] and infrared femtosecond laser [235]. The localization of the beam can be achieved either by point by point fabrication of each RI disturbance perturbation with a certain period

along with the fibre or by using an amplitude mask placed between the laser and the fibre which creates a light-shadow projection onto the core of optical fibre [168].

LPG's for the research described in this thesis were fabricated using frequency quadrupled Nd:YAG laser emitting at 266 nm light with amplitude mask setup [168]. The output power of the laser beam was ~6-8 mW (measured with laser power meter from Coherent Ultima Labmaster). The near infrared range LPGs were fabricated on photosensitive boron–germanium co-doped optical fibre (Fibercore SM750), the visible range LPGs were fabricated on hydrogen loaded visible range fibres SM450 and SM600, (Thorlabs). The hydrogen loading procedure and LPG fabrication on hydrogen loaded fibres process are described in **Section 5.5.2** as an integral part of **Chapter 5**. The SM750 fibre has high photosensitivity toward UV light because of co-doped germanium core, hence there is no need for any pre-treatment with H₂ of the fibre for LPG fabrication. The amplitude masks were chosen empirically based on their grating period to achieve PMTP at a higher cladding mode, where the RI sensitivity is largest [236]. The transmission spectra during the fabrication were observed and recorded using a Halogen Light source (HL-2000, Ocean Optics) and a spectrometer (Ocean Optics USB2000+) connected to a PC.

The LPG fabrication setup is shown in **Figure 3-5**. The setup consists of a frequency-quadrupled Nd:YAG laser (Continuum Minilite-10, USA) operating at a wavelength of 266 nm, A 100 mm focal length cylindrical lens (oriented orthogonally to the beam and parallel to the fibre in a way that it focuses the light of the laser in a short strip alongside the amplitude mask), a 1µm resolution moving stage (Physik Instrumente, Germany) with magnetic fibre holders, a grating pattern projection amplitude mask, Halogen Light source (HL-2000, Ocean Optics) and a spectrometer (Ocean Optics USB2000+).

The fabrication procedure was as follows. First, the region of the fibre allocated for LPG fabrication was stripped from acrylate coating by a mechanical stripper and cleaned with isopropyl alcohol (IPA), ~3 cm for LPGs operating in a transmission mode and 1-1.5 cm for LPGs operating in the reflection mode. The fibre was carefully placed into the V-grooves on the moving stage and secured with neodymium magnets. Then the fibre was connected for each side to a light source and spectrometer. The

translation stage was set for 0.02 mm/sec. velocity. Afterwards, laser irradiation was initiated simultaneously with the moving of the translation stage.

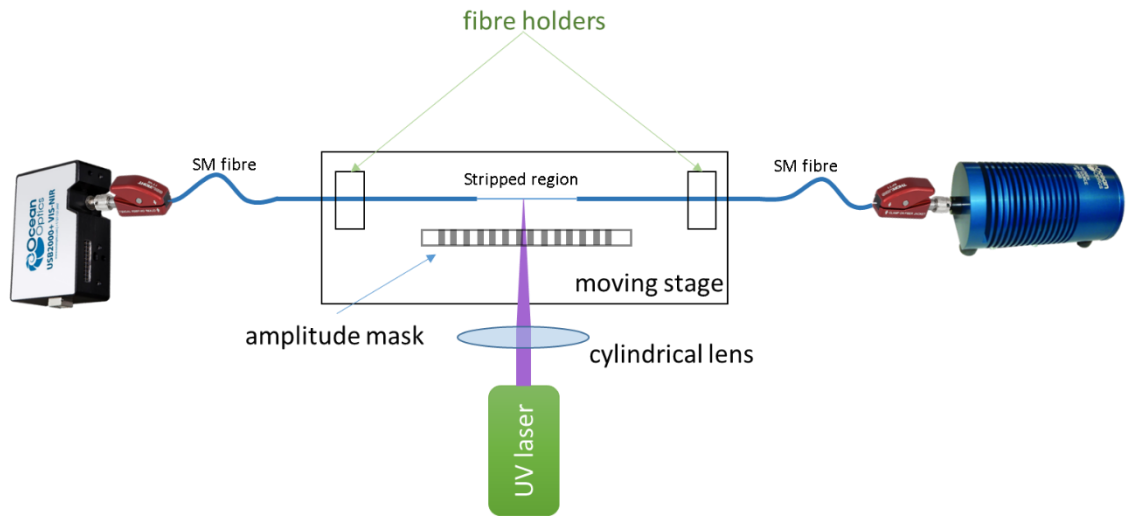


Figure 3-5 LPG fabrication set-up SM fibres using YAG frequency quadrupled UV laser and translation stage with amplitude mask

An example of an LPG fabrication with PMTP around 675-775 nm can be seen in **Figure 3-4**. Here hydrogen loaded SM600 fibre was used with a 110 μm amplitude mask between the cylindrical lens and the fibre. Each transmission spectrum corresponds to the distance made by the translation stage (from ~ 4 mm to 30 mm, black and light blue colours respectively), hence correlates with the length of the LPG.

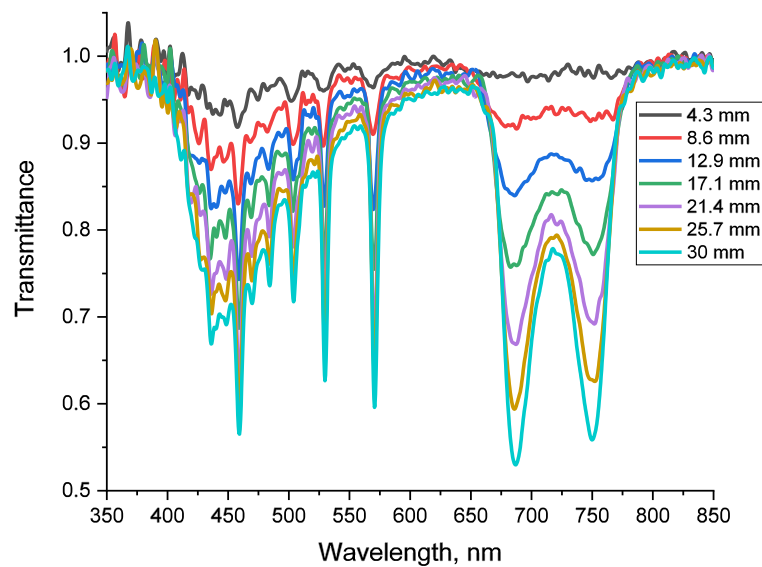


Figure 3-4 Transmission spectra taken during an LPG fabrication of the SM600 hydrogen loaded fibre with 110.5 μm grating period

As one can see, with the increase of the LPG's length, the attenuations become deeper, and the PMTP first appears as a wide single attenuation (red curve, 8.6 mm) but further evolves to a W-shape attenuation which deepens and splits stronger with the grating's elongation. This is due to the full width of half maximum (FWHM) of each band reducing with LPG length, and the spectrometer becomes able to resolve the two bands better while the LPG becomes longer.

By applying amplitude masks with different grating periods to different fibres, it is possible to alter the shape of the transmission spectra to achieve the desired configuration, such as PMTP or attenuations at certain wavelengths.

3.3. Thin film coatings preparation and deposition.

In this section, a brief overview of the functional coatings used in this PhD research will be described, such as sol-gel, along with their deposition methods such as layer-by-layer, dip coating and silanization on glass optical fibre.

3.3.1 Sol-gel matrix, formation and properties.

Sol-gel is a way to produce a solid material out of inorganic monomers. The chemistry of the sol-gel can be described as a silica formation from molecular precursor through hydrolysis and condensation [237].

Briefly, the formation of sol-gel from the most common components can be explained as follows. Tetraortosilicanes such as Tetraethyl orthosilicate $\text{Si}(\text{OC}_2\text{H}_5)_4$ (TEOS) and Tetramethyl orthosilicate $\text{Si}(\text{OCH}_3)_4$ (TMOS) undergoes polymerization in

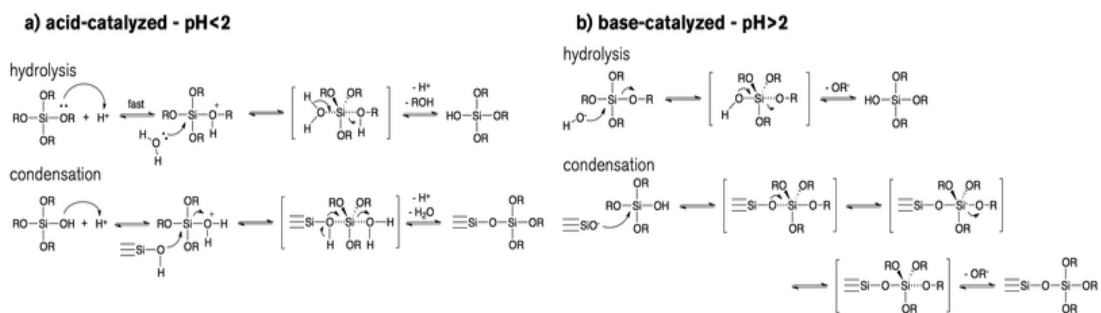


Figure 3-6 Schematic of the acid (a) and base (b) catalysed sol gel formation [227]

ethanol and water (metal alkoxide solution). The chemical transformation involves hydrolysis and condensation which results in a Si-O-Si chemically linked network. When a tetraortosilicane and water are dissolved in a mutual solvent, e.g. ethanol or methanol. This reaction results in a siloxane group (Si-O-Si) formation. The reactions occur with acidic ($\text{pH} < 2$) or base ($\text{pH} > 2$) catalysts (Fig 13) [238]. At pH levels close to 2 the reaction velocity is extremely low because this is the silica's isoelectric point which means its electric mobility level is close to zero [238].

The schematic reaction of typical sol-gel hydrolysis and condensation reactions are shown in **Figure 3-6a** with acid and **Figure 3-6b** with base catalysts. Base catalysed hydrolysis and condensation is faster than those catalysed by acid. That difference leads to different aerogel formations under different reaction conditions. Acid catalysed aerogel is more linear and dense, while base one is more porous and clustered. The reaction rate also could be regulated by pH, nature of alkyl group and water amount. If the drying occurs under natural conditions, aerogel occurs, if under critical, - xerogel, which is extremely porous [238].

It is possible to entrap water or ethanol soluble reagents (sensitive molecules) in aerogel. In case a reagent had been mixed into the sol gel solution before the hydrolysis and condensation occurs. Then, after aerogel formation, the sensitive molecules will be entrapped within the silica matrix [239] which provides a simple way to build the functional coating. A lot of chemically sensitive coatings based on the sol gel entrapment were reported recently [240-242].

Sol-gel was chosen as an entrapping matrix because of the convenience of usage, and optimal material for the requirements. In this PhD research, sol-gel based matrices were used and described in Chapters 4 and 6. Each particular sol-gel synthesis and its components are described in the relevant sections.

3.3.2 Deposition of thin films on a substrate or fibre's surface

There are several techniques to deposit a thin film coating on fibre glass surface, such as layer by layer (LBL) method, dip coating, electrospun deposition [243, 244], direct covalent binding via silanization [245], chemical vapour deposition [246]. In this PhD research three approaches were used among those mentioned

earlier: dip coating, layer by layer and direct covalent binding via silanization. These three techniques will be described below

3.3.2.1 Dip coating

The dip coating works by dipping and raising the substrate in and out from a liquid with constant velocity. The dip coating technique is widely used in different areas of modern fabrications and industrial processing, such as painting, rubber and polymer goods fabrication, coating, etc. In the area of OFS, dip-coating is among the most common deposition techniques.

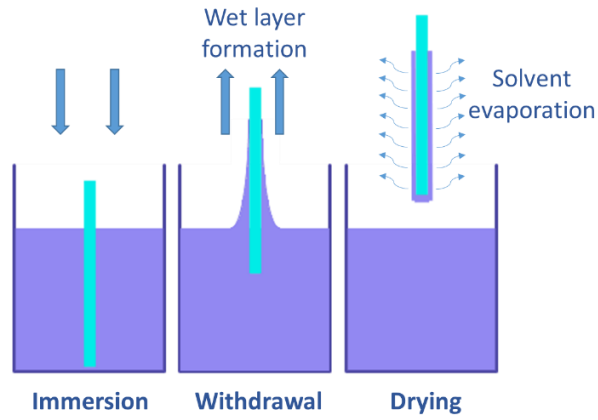


Figure 3-7 Schematic of the dip coating deposition process

The thickness of the dry film deposited on the substrate is determined by a variety of forces and processes during the deposition process, such as capillarity, drag, gravity, drainage, evaporation and viscosity [247]. If simplified, theoretically the thickness of the dry film in the so called “constant thickness zone” (**Figure 3-9**) is determined by a complex equation which includes the Landau-Levich Equation (3-1) [248], and capillarity regime [247],[249] and yields,

$$h_{0_dry} = k \left(\frac{E}{LV} + c \frac{(\eta V)^{2/3}}{\gamma^{1/6} \sqrt{\rho g}} \right) \quad (3-1)$$

Where $h_{(0_dry)}$ is the dry film thickness within the constant film thickness zone, k is the material proportion constant, E is the evaporation rate, L is the width of the coated film, V is the withdrawal speed, c is a constant related to the curvature of the dynamic meniscus (which yields ~ 0.8 for most Newtonian liquids), η is the viscosity,

γ - is the surface tension of the liquid at the meniscus' position, ρ is the liquid's density, and g is the gravitational constant $\sim 9.81 \text{ m/s}^2$.

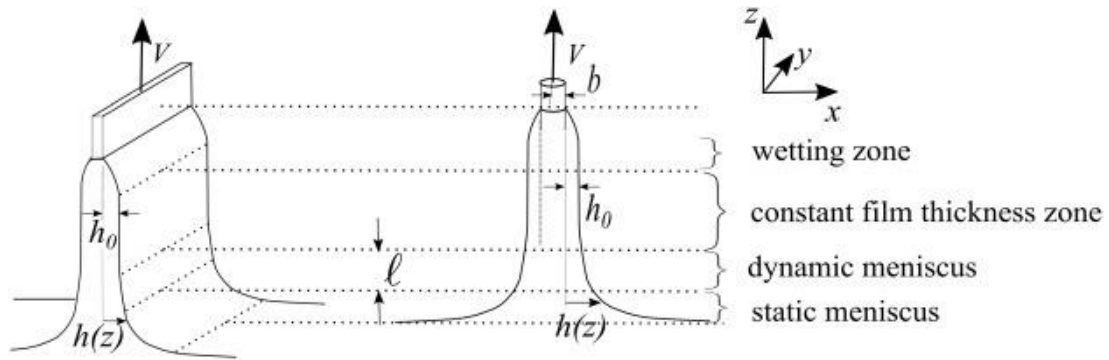


Figure 3-9 Dip coating of a substrate (left) and a fibre (right) by a liquid with constant velocity V . At the bottom is the static meniscus, above it is the dynamic meniscus, then comes the constant film thickness zone, and the upper wetting zone[236].

As can be estimated from the equation, the capillarity (first term of the bracket) contributes to the thickness only in case of low withdrawal velocities ($\sim V < 0.1 \text{ mm/s}$), while the drainage (second term of the bracket) would contribute to the thickness in case of higher velocities ($\sim V > 0.25$). At some point x between 0.1 and 0.25 mm/sec, there would be a minimum thickness of the dip coated film which grows in both directions from it, **Figure 3-8**. In this work, 0.25 mm/s was the minimal used withdrawal speed, which means the greater the speed, the thicker the coating (**Equation (3-1)**, **Figure 3-8**), this was backed by empirical evidence.

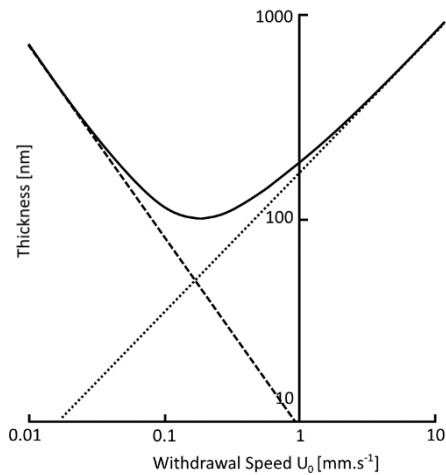


Figure 3-8 Dip coated thin film thickness dependence from withdrawal speed, estimation, [238];

The dip coating technique was chosen as suitable and easily applicable for glass substrates and optical fibres [250]. Dip-coating thin layers deposition of sol-gel was used in Chapter 4 and sol-gel and polymer dip coating deposition was used in Chapter 6, using the sol gel. In each deposition case, the relevant sol-gel and polymer composites and deposition parameters were described.

3.3.2.2 Layer by layer deposition

LBL nano-assembly technique is a diverse, flexible and easily controlled deposition technique. The first usage of the LBL technique dates back to the XIX century [251]. With the growth of the field of optical fibre sensors, LBL becomes one of the leading approaches to deposit sensitive nano-assembled coatings. LBL can be created from a large number of materials such as polyelectrolytes, nano-clays, metallic or ceramic nanoparticles, carbon nanotubes, semiconductor quantum dots and chromophores. Also, LBL can be used to embed materials. The properties of the LBL deposition can be regulated via temperature, concentration and some other features of the deposition components [252]. LBL deposition approach has been applied on OFS numerous times in recent years to detect chemical, biological and physical parameters [251]. Among those were: pH sensing [253-255], volatile organic compounds [256-258], label-free DNA sequence detection [259], immunoglobulins [260, 261], C-reactive protein [262] and many others [251].

In this PhD research, the ionic interaction combined with the covalent binding LBL approach was applied in Chapter 5. The procedure of the ionic LBL deposition is as follows. First, the glass needs to be hydroxylated, create a crosslink between the SiO_2 and the first layer, to do so it is dipped into a specific solution, in this case, it was KOH, to form a negatively charged surface with OH group. Afterwards, the glass is washed and dried and immersed into a cationic solution for a certain time interval. This forces the negatively charged surface to bond electrostatically with the cations in the solution. After the glass surface is washed and dried again. Then the glass is immersed into an anionic solution for a certain time interval. This forces the cations in the solution to bind with the positively charged previous layer. Then the glass is washed and dried. Each such layer has a certain thickness which depends on the parameters of the solutions and the immersion time (until saturation is achieved). The procedure of the polycationic/polyanionic immersions can be repeated to achieve the desired thickness of the LBL film, **Figure 3-10**.

The specific materials and solutions used in LBL deposition are described in the relevant chapters.

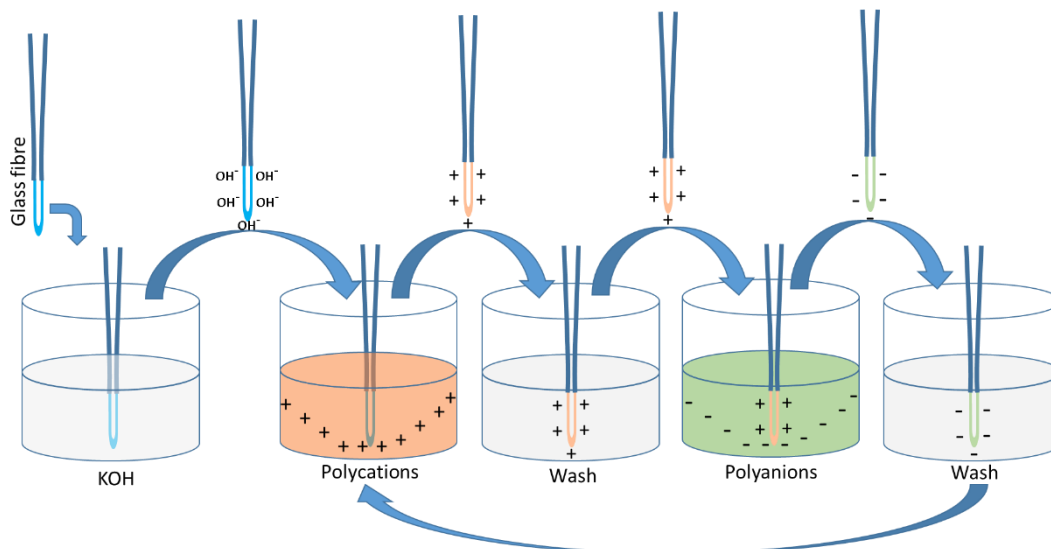


Figure 3-10 Schematic of LBL polyelectrolytes deposition on a glass U-shape fibre

3.3.2.3 Silanization deposition

Silanization is a process to create a durable crosslink between inorganic and organic materials. Silane coupling agents act at the interface between inorganic and organic surfaces. This method is extremely useful when a polymer or any other organic coating needs to be deposited on a glass surface [263]. This is possible due to the chemical properties of Silicon, although in the Periodic Table Silicon is the same group as Carbon, similar silicon and carbon components have significant differences in their chemical reactivity. Silicon, as well as carbon, is able to bond to substituents, however, due to silicon's larger electro-positivity it does not form stable double bonds, and it is capable to have both organic and inorganic substituents [263]. Thus silane chemistry is a perfect tool to create coupling agents and adhesion promoters. The majority of widely used organosilanes have one organic substituent (for bounding to an organic material) and three hydrolyzable substituents (for bonding to the inorganic surface and between each other). Structurally the same is the silane

agent used in this PhD research, 3-Aminopropyltriethoxysilane (APTES). Besides the three hydrolyzable substituents, is the fourth substituent with an amino group in the end. A schematic of Aminopropyltriethoxysilane functionalization of glass can be seen in **Figure 3-11**. The procedure of such a process is as follows. 2% APTES methanol (or acetone) solution is prepared, yielding a diverse mixture of free silanes and silanols. A hydroxylated glass immersed in the APTES solution for a few minutes caused the silanols to physisorb to hydroxyl groups via hydrogen bonding. After removal of the glass, it is rinsed thoroughly in DI water. Then the glass is placed in an oven set to 110°C for ~ an hour. The heat supplies energy for the formation of siloxane bonds between the physisorbed silanes/silanols and the glass surface and removes condensation products (water and ethanol) by evaporation [264].

Once the APTES silanization is completed, the glass becomes functionalised with amino groups, **Figure 3-11**. Now the glass surface by its main group can be covalently attached to polymers such as acrylic, nylon, epoxy, phenolics, PVC, urethanes, melamines, nitrile rubber via amino groups, such as acrylic, nylon, epoxy, phenolics, PVC, urethanes, melamines, nitrile rubber [263]. If any other polymer needs to be bonded, an additional cross linker can be introduced, which will create a crosslink between the existing amino groups with the functional groups of that polymer.

Each particular silanization procedure and mechanism used in this PhD (**Chapter 6**) is explained in detail in the relevant section.

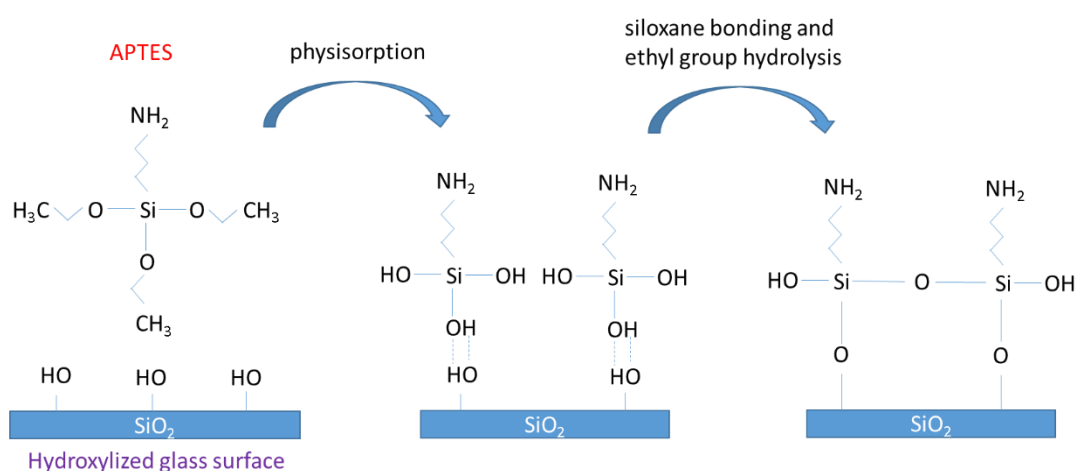


Figure 3-11 Amino functionalization of glass using APTES

3.4. Spectrophotometer

When spectral information of the functional layer or an optical fibre device, such as an LPG, is needed in a range of 200-1000 nm one of the most convenient and commonly used devices are spectrophotometers. Modern spectrometers are based on Charge-coupled devices (CCDs). A CCD is an integrated circuit of a coupled array of metal-oxide semiconductor capacitors [265]. CCD converts the incoming photons to an electronic signal which allows wide range detection across the spectral range by applying a dispersion of the light into spectral components.

A schematic illustration of an Ocean Optics spectrometer can be seen in **Figure 3-12**, [266]. The optical fibre is connected to the connector (1), where light enters through a slit (2) which acts as the entrance aperture and a bandwidth limiting filter (3). Afterwards, the light reflects from the collimating mirrors (4), reflects from diffraction at the grating (5) with a periodic structure that splits and diffracts light into several beams travelling in different directions. The grating acts as a prism and dismantles the light signal into its spectral components. Now each spectral component of the light is dispersed under slightly varying angles. Then the light is focused by the focusing concave mirror (6) and finally reaches the CCD detector (7) where each wavelength will be assigned to an electronic signal. Then the signal is transformed and sent to a PC where it appears in the form of two dimensional information, wavelength and intensity.

Depending on the required performance characteristics such as light sensitivity, signal-to-noise ratio, wavelength resolution, different types of spectrometers exist [266]. There are several types of noise sources that affect the signal to noise ratio. Thermal noise is caused by the random movement of electrons in the detector. The thermal noise is often reproducible, therefore can be subtracted with after processing. Counting noise, due to the statistical nature of the counting, depends on the maximum number of electrons needed to fill a single well. The counting noise is reducible via statistical methods, e.g. averaging. Pixel to pixel variations, when the neighbouring pixels in the photodiode array are illuminated with comparable light levels and produces a similar signal. Reduction of this type of noise can be addressed via averaging, however, it heavily depends on the pixel resolution of the

spectrometer. In general, the signal to noise ratio of a spectrometer can be reduced with the increase of the input light intensity, integration time increase, and by limiting the incoming spectrum only to the wavelengths of interest.

The spectral resolution of a dispersive array spectrometer is determined by three factors. The detector determines the maximum size and the number of digitized discrete points. The slit determines the minimum formed in the detector plane image size. The diffraction grating determines the total wavelength range.

The exact type of spectrometer used in each experiment is mentioned in the relevant sections.

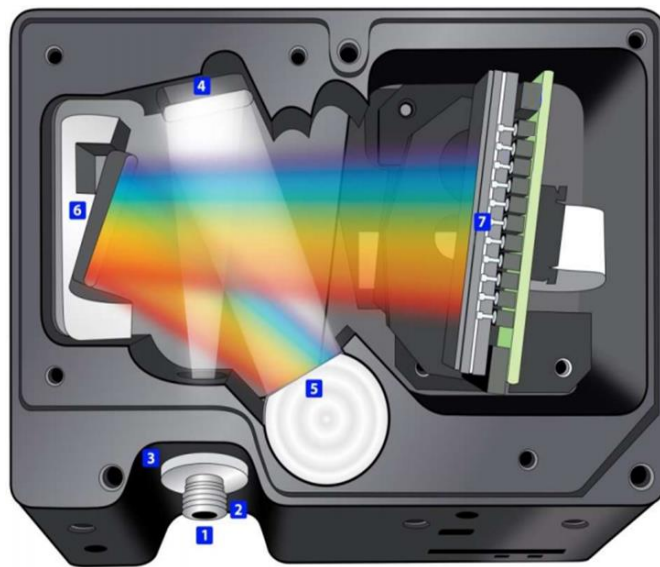


Figure 3-12 Schematic illustration of an Ocean Optics spectrometer from Ocean Insight website. 1 - connector; 2 - slit; 3 - filter; 4 - collimating mirror; 5 - diffraction grating; 6 - focusing mirror; 7 - detector array [255]

Chapter 4 Optical fibre sensor for detection of nitrites

4.1 Introduction

Nitrite (NO_2^-) is a symmetric anion and a nitrous acid salt. In the environment, Nitrite is part of the nutrient nitrogen cycle and appears in nature mainly due to conversion from Ammonium (NH_4^+) by soil bacteria. It appears also due to human activity, mainly as a result of transportation and industrial pollution. Nitrites can also be reduced to ammonia or oxidized to nitrates. The importance of nitrite monitoring in natural waters is crucial, because high levels of nutrients may cause plant overgrowth, increase bacterial activity and decrease the dissolved oxygen level in waters. High concentrations of nitrites and nitrates could be hazardous for human health as well. Hence the detection and monitoring of nutrients are crucial for environmental and health issues [267].

The nitrite levels in water are regulated by authorities. **Table 4-1** shows the allowed concentrations of bottled, tap, waste and natural waters in the EU/UK and USA. The aim of the described below research was to target at least some of the needed concentration levels mentioned in **Table 4-1**.

In this chapter, the development of a nitrite sensor based on the nitrite sensitive dye compound, BCB (Brilliant cresyl blue), is described. To begin with, the BCB was dissolved in an aqueous solution and exposed to nitrite to determine its sensitivity. This was followed by the deposition of a sol-gel coating containing BCB on a glass substrate to determine the optical response of the BCB thin film to nitrite. This helped to establish the fabrication parameters for thin films that were later used to coat optical fibres. Absorbance measurements on glass substrates were useful to find the optimal procedures such as component concentration ratios, withdrawal speed, number of layers and drying conditions for the sol-gel coating. The measurements were also used for examining the quality of the coating such as adhesion, optical

Table 4-1 Some Nitrate/nitrite concentration regulative allowances and some average values in water (molar concentration shown as used in this dissertation, for NO_2^- ion)

	EU/UK		USA	
	Nitrate NO_3^-	Nitrite NO_2^-	Nitrate NO_3^-	Nitrite NO_2^-
Bottled water, max			714 μM (10 mg/L as N) [268]	71.4 μM (1 mg/L as N) [268]
Tap water, max allowed conc.	806.45 μM (50 mg/L) [269]	10.87 μM (0.5 mg/L) [269]	714 μM (10 mg/L as N) [270]	71.4 μM (1 mg/L as N) [270]
Ground water	EU states and candidates Southeast & west $\sim 370 \mu\text{M}$ East & South $\sim 210 \mu\text{M}$ North $\sim 16 \mu\text{M}$ [271]		<143 μM natural [272] 12 - 665 μM (average) [271]	
Waste water	0.7-1.4 mM, total Nitrogen (10-20 mg/l N) [273]			
Rivers/lakes Unpolluted. Good qual.	<57.16 μM [274] <128.6 μM [274]	<3.56 μM (0.5 mg/L as N) [274]		
Sea/ocean	~ 5 -245 μM , European and Baltic Sea coastal waters (<1.5-61 μM as N) [275]			

transparency and uniformity. For the purpose of nitrite sensing, sodium nitrite (NaNO_2) was chosen. Once it dissolves in water, it dissociates to Na^+ and NO_2^- ions.

Once the substrate tests were completed, the same coating technique was applied to two types of optical fibre sensors. U-shape and straight cladding replaced optical fibre sensors.

The sensitivity target value of the sensor should allow for the measurement and monitoring of critical levels of nitrites in drinking and waste waters (LOD ~ 11 -70 μM).

4.2 Nitrite sensitive dye investigation

4.2.1 Introduction to the sensitive dye investigation

Before applying the sensitive dye onto an optical fibre sensor it is reasonable to study the Nitrite response of the sensitive dye in an aqueous solution. For this

purpose, spectroscopic Nitrite sensitivity and repeatability experiments were conducted on the BCB aqueous solution. The BCB dye in a diluted state was found to be sensitive to nitrites in a low pH environment, however, the response was not fully repeatable.

The BCB dye was chosen as a nitrite reagent, as it showed some promising results in the works presented by *Ensafi et al*, where an immobilized BCB was used to spectrophotometrically detect nitrites in an aqueous media [20, 23].

4.2.2 BCB description

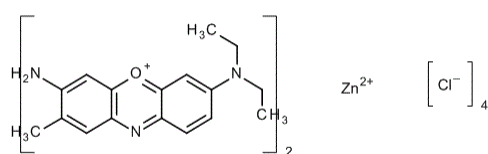


Figure 4-1 Molecular structure of BCB

Brilliant cresyl blue is a zinc chloride double salt and an oxazine dye which is positively charged in neutral solutions, and it has planar molecular structure. BCB can donate and accept hydrogen bonds, although the nature of the hydrogen bond

formation between its monomeric units is still unclear [276]. The molecular structure of BCB is shown in **Figure 4-1**. Recently a similar dye with the same name (BCB) was used to sense nitrites through catalytic oxidation reaction with bromate in solution [277] and in immobilized condition [23]. It should be mentioned that BCB is available commercially as a hemi zinc chloride (MW= 2x385.96, **Figure 4-1**) and as chloride (MW=317.8) [267]. The cations of BCB exist in four different forms, however, only two of them are widely used [278]. This fact causes some confusion in the scientific literature. Luckily the two versions of BCB differ only in the location of the methyl

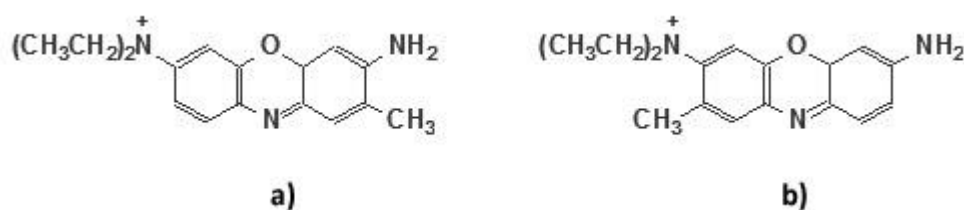


Figure 4-2 a) more often used nowadays Brilliant cresyl blue (ALD) contains a 2-methyl modification on the phenoxazine core, used in this thesis; **b)** Brilliant cresyl blue used previously and also by *Ensafi et. al*. [266] contains a 8-methyl modification on the phenoxazine core

group, **Figure 4-2** and their properties are similar. In this thesis, the hemi zinc chloride BCB (ALD) were used (MW= 2x385.96), as shown in **Figure 4-1** and **Figure 4-2a**

4.2.3 Materials and Method for dissolved BCB investigation

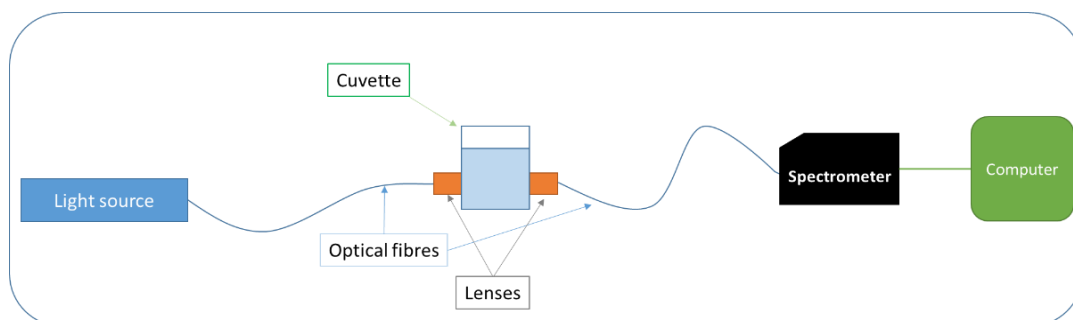


Figure 4-3. Schematic illustration of the absorption measurements setup for liquids

BCB interacts with sodium nitrite only in an acidic environment [277]. To examine this interaction the pH of the 50 μM BCB solutions was adjusted using sulphuric acid to the pH level of 2.0. The pH was measured in situ by HANNA HI-98100 pH tester. 1.4 ml of this bulk solution was then placed into several vials. Afterwards, the appropriate amount of high concentration sodium nitrite (Pure sodium nitrite was purchased from Sigma-Aldrich and dissolved in DI water) stock solution was added to each of those vials in order to set the desired concentration. For instance, 14.14 μL of $1000\text{mM}\cdot\text{L}^{-1}$ was added to 1.4 ml 50 μM BCB pH~2.0 solution to achieve $10\text{mM}\cdot\text{L}^{-1}$ NaNO_2 concentration. In the same way other concentrations, from 1 μM to 10 mM, were achieved. Then the vials were left for one hour, to complete the reaction. The one hour time interval was determined empirically as the time after which the absorption spectra do not change.

The absorption spectrum of each solution was measured using the set-up shown in **Figure 4-3**. The setup consists out of the Halogen light source (HL-2000 Ocean Optics) connected via Thorlab's 1 meter M92L01 SMA-SMA Fibre Patch Cable to Ocean Optics' CUV-UV Fibre Optic Cuvette Holder. The cuvette holder then is connected by a same 1 meter M92L01 SMA-SMA Fibre Patch Cable with Ocean Optics' HR-4000 spectrometer, which is connected via USB interface with a PC with Ocean Optics Spectra Suite software. In the cuvette holder, a standard parallel-sided 12.55 x 12.65 x 44.5mm disposable plastic cuvette with a 10 mm optical path (10x10

mm inner dimensions of the square sides) was placed. The examined liquids were poured into the cuvette.

4.2.4 Results of the dissolved BCB investigation

Figure 4-4 shows the absorbance response of 50 μM BCB pH $\sim$ 2 aqueous solution to sodium nitrite in 1x1 cm, plastic cuvette. (As can be seen from **Figure 4-4b**, there is a clearly distinguishable response of the BCB solution with pH 2.0 to concentrations of sodium nitrite as low as 1 μM . The absorbance of $\sim$ 620 nm peak decreases with concentration increase. With higher concentrations, from 50 μM (magenta line), of sodium nitrite, the $\sim$ 620 nm absorbance peak decreases even more and a new peak at $\sim$ 510 nm starts to form around $\sim$ 500 μM , which becomes dominant in **Figure 4-4b** for 1 mM (green curve) and even more for 10 mM sodium nitrite. Also, the $\sim$ 620 nm peak along with the significant decrease in intensity shifts towards the higher wavelength and reaches $\sim$ 660 nm.

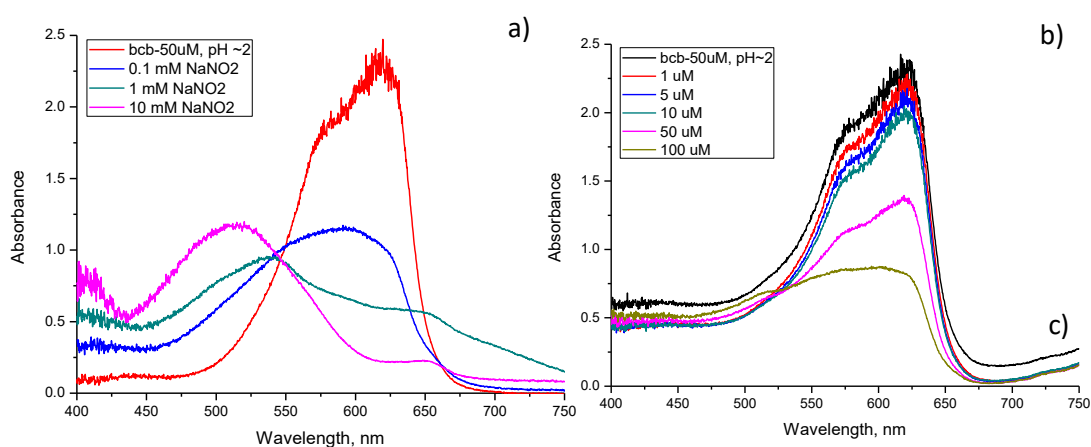


Figure 4-4 a) Absorbance spectra of BCB aqueous solution measured at different concentrations of Sodium nitrite; Red curve shows the absorbance of 50 μM BCB in water (pH 2.0), blue, green and magenta, 0.1, 1 and 10 mM sodium nitrite in 50 μM BCB solution, respectively; b) concentrations from 1 to 100 μM .) Black curve shows the absorbance of 50 μM BCB in water, next curves, - the absorption with addition of: red – 1 μM , blue – 5 μM , green – 10 μM , magenta – 50 μM ; sodium nitrite c) Absorbance values at 510 nm (blue line) and 620 nm (red line) against NaNO_2 concentration

Figure 4-4c shows the absorbance values at 510 and 620 nm (blue and red lines respectively) of the absorbance spectra of BCB aqueous solution plotted against the concentration of NaNO_2 from 0 to 100 μM .

Figure 4-5 shows the dynamic response of the pH~2 50 μM BCB aqueous solution to 1 mM sodium nitrite concentration with natural diffusion, without stirring. The spectra were recorded in situ, using the setup described in **Figure 4-3**. The 50 μM BCB with pH 2.0 in 1x1 cm plastic cuvette was placed into the setup. After the data acquisition began sodium nitrite of 1 mM was added into the cuvette.

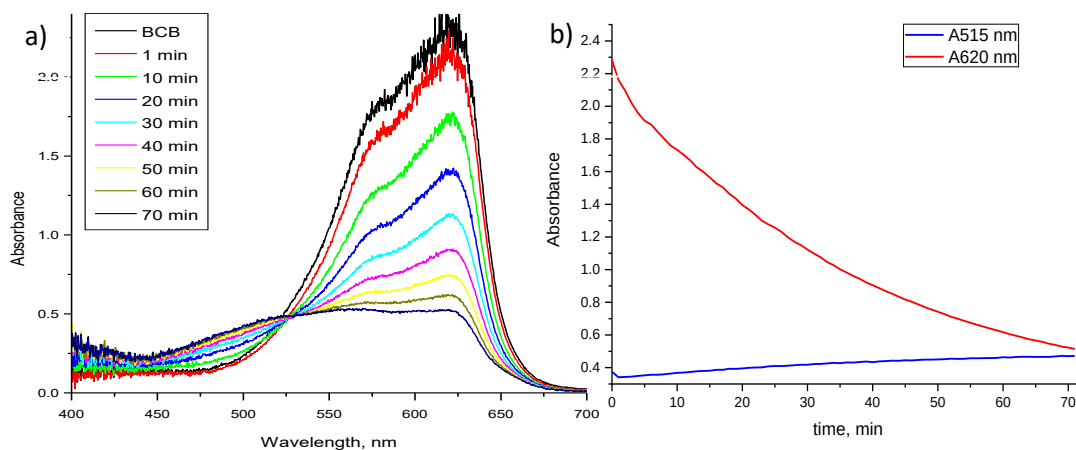


Figure 4-5 Dynamic response of the absorption spectrum of the pH~2 BCB aqueous solution to 1 mM sodium nitrite concentration, 1 cm optical path. **a)** Full spectra from 1 to 70 min. Black curve, 50 μM BCB, red curve, 1 minute after addition of 1 mM NaNO_2 . Green curve, after 10 minutes, and all the rest were taken with 10 minute interval until 70 minutes. **b)** Dynamic response of absorbance over time at 515 nm (blue line) and 620 nm (red line).

4.2.5 Repeatability of BCB response in low pH aqueous solution

A 50 μM BCB solution with pH altered by H_2SO_4 to ~ pH 1.45 (a lower pH was used in order to increase the rate of the reaction [277]) was tested with different multiple concentrations of NaNO_2 . A bulk pH ~1.45 50 μM BCB solution was made. Then NaNO_2 was added to the bulk BCB solution which resulted in a solution with 6 different sodium nitrite concentrations of 1, 2, 6.25, 12.5, 25 and 50 mM. Absorption spectra of those were taken after the reaction's completion, approximately 30 min.

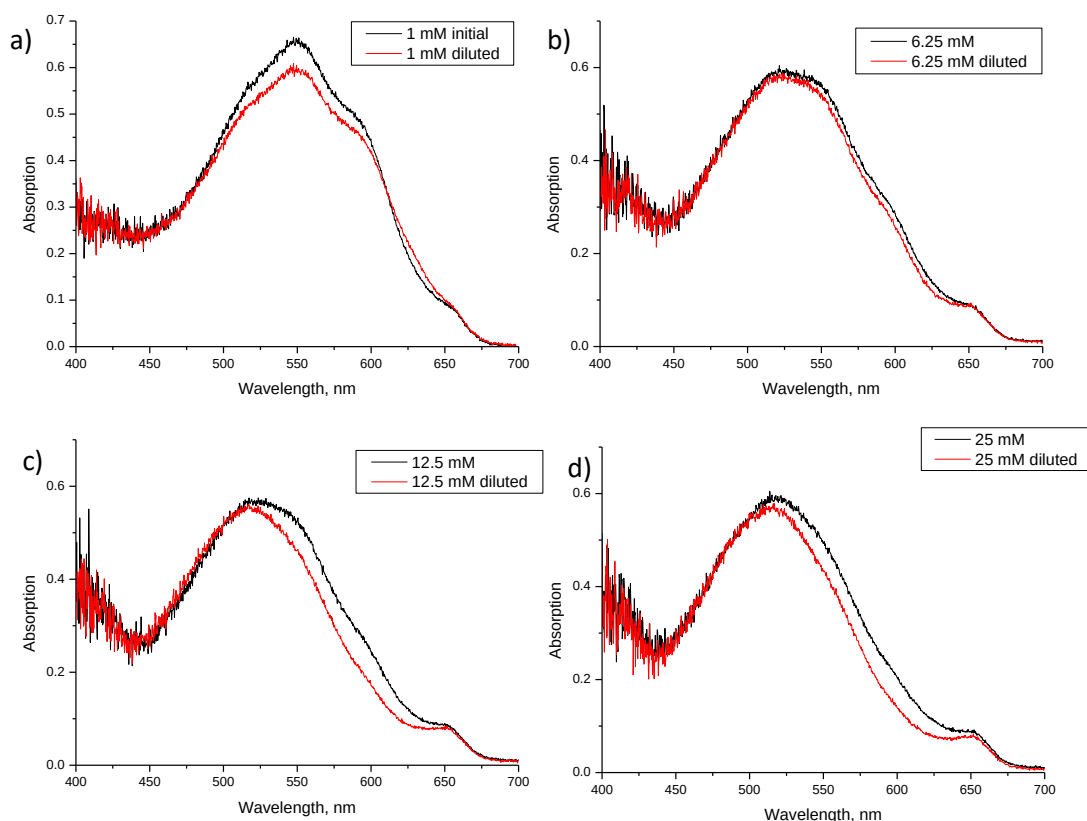


Figure 4-6 Absorption comparison of initial and diluted sodium nitrite concentrations in $\sim$ pH $\sim$ 1.45 50 μ M BCB solution; black curve is the absorbance of the initial, red of the diluted solutions for: a) 1 mM NaNO₂ b) 6.25 mM NaNO₂ c) 12.5 mM NaNO₂ d) 25 mM NaNO₂

Afterwards, the 1 ml of each solution was diluted by 1 ml of the bulk pH $\sim$ 1.45 50 μ M BCB solution, so the NaNO₂ concentration decreased two times. So 50 mM became 25 mM, 25 mM became 12.5 mM and so on. The absorption spectrum was taken after 30 min to compare each diluted solution with the “initial”, **Figure 4-6**

4.2.6 Discussion

According to *Enzafi et al.* [277], the BCB undergoes an oxidation reaction in a low pH environment in the presence of Nitrites that act as a catalyst, however, *Enzafi et al.* [277] bromate was also used as the oxidant. It is possible, that oxidation of BCB could occur in acidic aqueous solution by either oxygen dissolved in water or by oxygen atoms in the water molecule in the presence of nitrites. Because there is no Bromate involved as described by *Enzafi et al.* [277], but a similar to oxidation response can be clearly observed. The other potential cause of colour change could

be the monomer-dimer equilibrium change, or exciton excitation distribution change in the presence of nitrites, which will be discussed below.

As can be seen from **Figure 4-6** black initial lines' absorbance is higher than the red, diluted ones within certain ranges. With the dilution procedure described above, the concentration of the sodium nitrite is being lowered twice, but the absorption spectra do not fully return to the same position as for the same initial concentrations. This means the BCB in low pH aqueous solution is not fully reversible in terms of nitrite sensing. However, the BCB dye still can be reversible in the bonded or entrapped state.

4.2.7 Conclusion to the BCB sensitive dye investigation

The spectrophotometric experimental data conducted on BCB aqueous solution showed its sensitivity to Nitrites in an acidic environment. The response was found to be not repeatable. However, the BCB can still be repeatable if immobilized. Therefore, sol-gel was chosen as the BCB's host matrix for deposition on the slide/fibre.

4.3 Investigation of nitrate sensitive coating on slides

4.3.1 Introduction

In order to investigate the BCB dye in the entrapped state, the sol-gel matrix was used. The sol-gel formation and fabrication itself are described in **Chapter 3, section 3.3.1**. To deposit a thin layer on a glass slide, the dip coating technique was used, which is described in **section 3.3.2.1**

As explained in detail in **section 3.3.1** in order to conclude the sol-gel reaction rapidly, a catalyst is needed. It could be either an acid or a base. The structure and properties of sol-gel heavily depend upon the chosen catalyst. Both variations were tested in various concentration ratios.

Before applying a functional sol-gel onto a fibre coating, it is worthwhile testing it on a glass substrate. Several types of BCB embedded sol-gel fabrications were

conducted and evaluated. A Series of experiments were carried out in order to investigate the BCB embedded sol gel coating's Nitrite sensitivity and repeatability. It was proven that the BCB embedded acid catalysed sol-gel is repeatedly sensitive to Nitrites in water. A sensing mechanism hypothesis was discussed.

4.3.2 Materials and method for BCB embedded sol-gel coating on a glass substrate

Sol gels with different acid and base catalysts and dye concentrations (**Table 4-2**, **Table 4-3** and **Table 4-4**) were tested.



Figure 4-7 Oven treatment of the sol gel coated glass substrates

For sol-gel deposition, the cuvette containing a liquid sol-gel mixture was placed under a PI translation stage, Model: M-126.2S1, which was operated through a computer. A glass substrate was held in the translation stage and then immersed into the sol-gel solution. Then the substrate was

lifted from the liquid with constant velocity (velocities described in **Table 4-2**, **Table 4-3** and **Table 4-4**) and air-dried for ~10 minutes between each layer and overnight before usage, If more than one layer is needed, the procedure can be repeated **Figure 4-7**.

After the desired number of layers are deposited (the number of layers varies from 1 to 3), the substrate was left for drying overnight at room temperature. For a more robust coating, the coated substrates were temperature treated in an oven at 70°C over 1 hour followed by an hour of natural cooling within the oven, **Figure 4-7**. After the deposited substrates were examined for light absorbance.

Firstly an acid catalyst was used, Hydrochloric acid:

Table 4-2 Sol-gel solution ratios, number of layers and withdrawal speed tested on glass substrates with hydrochloric acid as a catalyst

samples	TEOS	Water	Ethanol	HCl	BCB conc in SG, mM.	Layers x Rising velocity
1	3.0	1	2.25	0.03	0.107	3 mm/sec

2	3.0	0.5	2.35	0.03	0.113	2x0.5 mm/sec
4	3.0	0.5	2.35	0.03	0.113	1.5 mm/sec
5	3	0.5	3.1	0.01	7.564	2x0.5 mm/sec
7	3	0.5	3.1	0.01	7.564	3x0.5 mm/sec
8	3	0.5	3.1	0.03	7.541	3x0.5 mm/sec
9	3	0.5	3.1	0.03	7.541	2 mm/sec

Table 4-3 Sol-gel solution ratios and withdrawal speed tested on glass substrates with base catalysts

samples	TEOS	Ethanol	Water	1 % KOH Aqueous solution	1 % NaOH Aqueous solution	BCB conc in SG, mM.	Rising velocities, mm/sec
1	3	0.5	3.1	0.1	-	4.627	0.5-2
2	3	0.5	3.1	-	0.1	4.627	0.5-2

Although the BCB embedded sol-gel prepared using acid catalyst forms a smooth, uniform, transparent and strong film, it showed no response to nitrites.

Next, a sol-gel matrix with a base catalyst was tested. Because of the dependence of the sol-gel structure on catalyst type [279], the consistency of sol-gel with basic catalyst turned out not suitable for dip coating deposition, because of its lumpy consistency the film was highly non-uniform and in some cases, there was no film at all. The ratios of those are shown in **Table 4-3**. Therefore a sol-gel recipe with one more silane component, Triethoxymethylsilane $\text{CH}_3\text{Si}(\text{OC}_2\text{H}_5)_3$ (TEMS) were tested [280]. The tested ratios are shown in **Table 4-4** below:

Table 4-4 Sol-gel solution ratios and withdrawal speed tested on glass substrates with TEMS component and base catalysts (Sample N4 highlighted by a yellow colour, denotes the most common coating for substrates)

samples	TEMS	TEOS	Ethanol	Methanol	Water	TMAOH, 10% aqueous	NaOH, 1% aqueous	BCB conc in SG mM.	Rising velocities, mm/sec
1	1.8	0.2	6	-	2	0.3	-	0.971	3-6
2	1.8	0.2	-	6	1	-	1.3	4.854	3-6
3	1.8	0.2	-	6	2	-	0.3	4.854	1-6
4	1.8	0.2	6	-	2	0.3	-	4.854	1-6

The consistency of sol-gel with both TEMS and TEOS components catalysed by alkali (**Table 4-4**) turned out to be far more suitable than the sol-gel with TEOS only (Table 4-3). The consistency of the mixture itself was uniform, and the deposited film was smooth, uniform, and transparent with strong adhesion to the substrate.

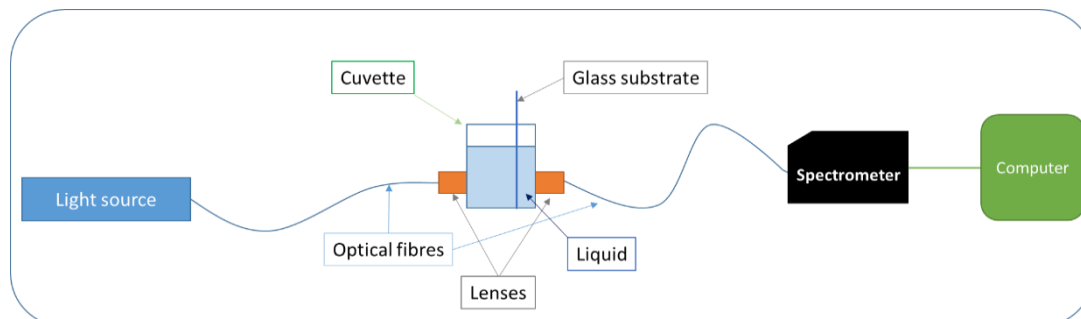


Figure 4-8 Absorption measurements setup for substrates submerged in liquids consists from of the HL-2000 Ocean Optic Halogen light source connected via Thorlab's 1 meter M92L01 SMA-SMA Fibre Patch Cable to Ocean Optics' CUV-UV Fibre Optic Cuvette Holder

The setup for measuring absorbance on the glass substrate while immersed in different aqueous solutions is shown in **Figure 4-8**. The setup consists from of the HL-2000 Ocean Optic Halogen light source connected via Thorlab's 1 meter M92L01 SMA-SMA Fibre Patch Cable to Ocean Optics' CUV-UV Fibre Optic Cuvette Holder. The Cuvette holder then is connected by the same 1 meter M92L01 SMA-SMA Fibre Patch Cable with Ocean Optics' HR-4000 spectrometer, which is connected via USB interface with a PC with Ocean Optics Spectra Suite software. In the cuvette holder, a parallel-sided homemade plastic cuvette with a slide holder was placed. Water with appropriate chemicals concentrations was poured into the cuvette. So the light propagates through the slide immersed in water.

4.3.3 BCB embedded sol-gel coated substrate fabrication results

Glass substrates coated by BCB embedded sol gel No 1 and 4 described in **Table 4-2** were tested for a high concentration of nitrites (100 mM), **Figure 4-9**. Absorbance spectra obtained via the setup shown in **Figure 4-8**. As can be seen in **Figure 4-9**, there is no sign of shifting of the spectra in response to the presence of 100 mM sodium nitrite in DI water in the case of sol gel catalysed with hydrochloric acid. The reason behind these could be due to the highly dense aggregation of the acid catalysed sol-gel [279] (as described in **section 3.3.1**). The possible sensing

mechanisms will be described below. The absence of response was observed in several other acid catalysed sol-gel coatings described in **Table 4-2** as well.

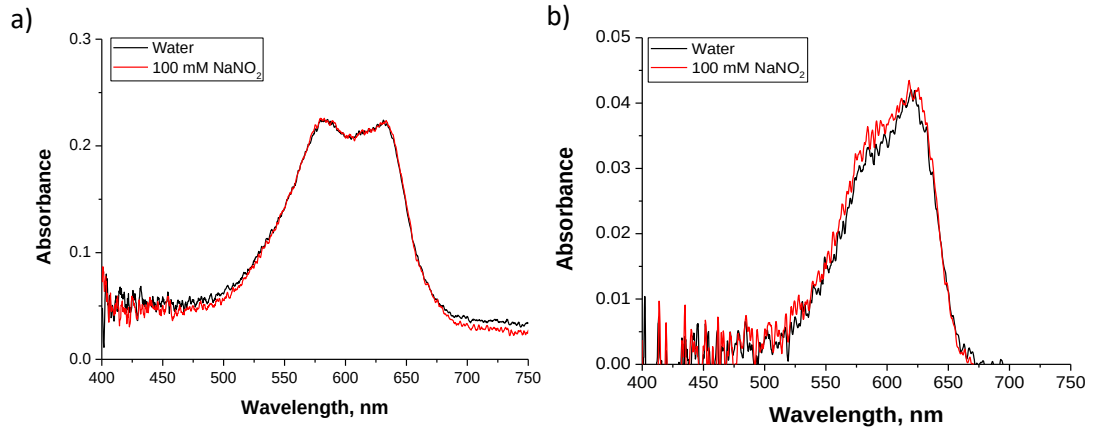


Figure 4-9 a and b - Absorbance spectra obtained through a setup described in **Figure 4-8** for glass substrates coated by sol gel with ratios No 1 and 4 shown on **Table 4-5** respectively. Black line shows absorption spectra of the substrate immersed in water and red, - in 100 mM NaNO₂ solution for 20 minutes, for both a and b figures.

Next, the base catalysed sol-gel with an additional silane component was tested, as mentioned above in **section 4.3.2**.

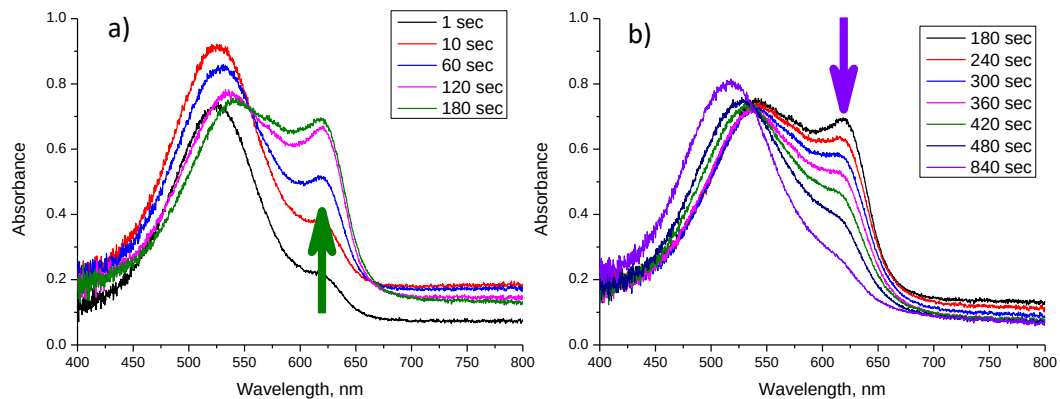


Figure 4-10 evolution of the absorption spectra of glass substrate in air coated by sol gel containing BCB described in section 4 (highlighted by yellow) of **Table 4-4** with 6 mm/sec withdrawal speed, immediately after deposition; a) The absorbance from 1 to 180 sec consecutively with green arrow pointing in the direction of ~625 nm peak movement over time; b) The absorbance from 180 to 840 sec with violet arrow pointing in the direction of ~625 nm peak displacement over time

It was observed that the base catalyst sol-gel changes its absorption spectra drastically during the gel drying procedure immediately after fabrication. Absorption spectra of this phenomenon were recorded, **Figure 4-10**. As one can see, in the first 180 seconds (**Figure 4-10a**), the spectra's ~ 620 nm peak absorbance rises dramatically from ~ 0.21 in the first second (black line) to ~ 0.69 (olive green line), this tendency is emphasized in the graph by the green arrow. On the other hand, the ~ 520 nm peak rises in the first 10 seconds (red curve), then starts to fall back (blue, 60 sec) with some shift of the peak into the longer wavelength range and returns roughly to the same absorption values (magenta, - 120 sec and olive green, -180 sec) but with a shift from ~ 527 nm initially (black curve) to ~ 541 nm in 180 sec (olive green). After 180 sec, the tendency begins to go in the opposite direction, **Figure 4-10b**. The 620 nm absorbance peak starts to decrease from ~ 0.69 at 180 sec (black curve) and onwards (the remaining curves) down to 0.24 at 840 sec (violet curve). This tendency is emphasized in the graph by the violet arrow. At the same time, the ~ 541 nm absorbance peak remains at the same absorption value from 180 to 480 seconds (black to dark blue curves) with a shift to the left from ~ 541 to ~ 530 nm, and forward increases from 0.75 to 0.8 at 840 sec with further left-hand shifting slightly beyond the initial value to 517 nm.

4.3.4 Base catalysed sol-gel coated substrate in water

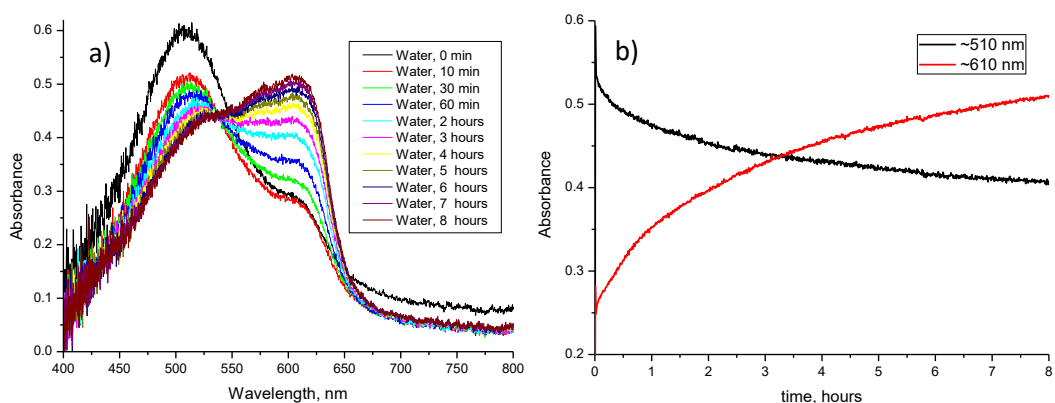


Figure 4-11 Absorption spectra of BCB embedded sol gel coated glass substrate in water, fabricated according to Table 4-4, sample 4 (highlighted by yellow) with 6 mm/sec withdrawal speed; **a)** shows the absorbance spectra in water immediately after drying from 0 to 8 hours; **b)** shows the 510 (black curve) and 610 (red curve) nm absorbance peaks in water immediately after drying from 0 to 8 hours

As the aim of this research was to develop an aqueous nitrite sensor it was necessary to study the sensitive coating's behaviour in water. **Figure 4-11** shows the absorbance of the BCB embedded sol gel coated glass substrate, fabricated as described in **Table 4-4** sample N4 (highlighted by yellow) with 6 mm/sec withdrawal speed.

Figure 4-11a shows the whole absorbance spectra behaviour of the substrate in water immediately after the drying was completed. **Figure 4-11a** starts with black curve denoting the absorbance immediately after submerging in water, the remaining curves show the absorbance within a particular time interval from, red – 10 min, green - 30 min, blue - 60 min and all the next curves were taken every hour concluding with the burgundy colour after 8 hours. The direction of the change is very similar to what we have seen in **Figure 4-10a** and, the speed of change is decreasing over time. **Figure 4-11b** shows the 510 (black line) and 610 (red line) nm absorbance peaks over time in water for 8 hours immediately after the drying was completed.

Figure 4-12 shows the same substrate the next day for 6 hours. While **Figure 4-12a** shows the whole absorbance spectra change, **Figure 4-12b** shows the 510 (black line) and 610 (red line) nm absorbance peaks overtime for 6 hours in water. It is clear from **Figure 4-12b** that the rate and amount of change are much lower, and the coating is more stable when the coating is measured the next day.

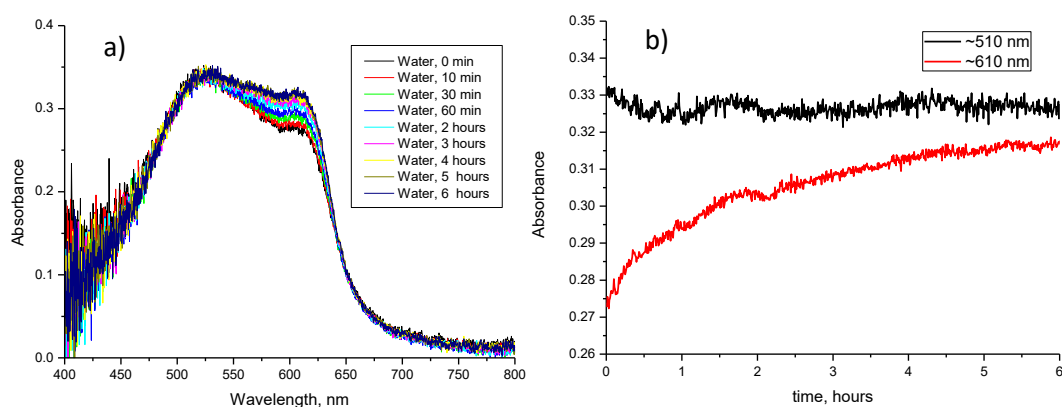


Figure 4-12 Absorption spectra of BCB embedded sol gel coated glass substrate in water, fabricated according to Table 4-4, sample No 4 (highlighted by yellow) with 6 mm/sec withdrawal speed; **a)** shows the absorbance spectra in water next day from 0 to 6 hours; **b)** shows the 510 (black curve) and 610 (red curve) nm absorbance peaks in water next day from 0 to 6 hours

A similar substrate with thinner sol gel coating was tested for sodium nitrite response, **Figure 4-13a**, but before the test, it was placed in water for 40 minutes, **Figure 4-13b**.

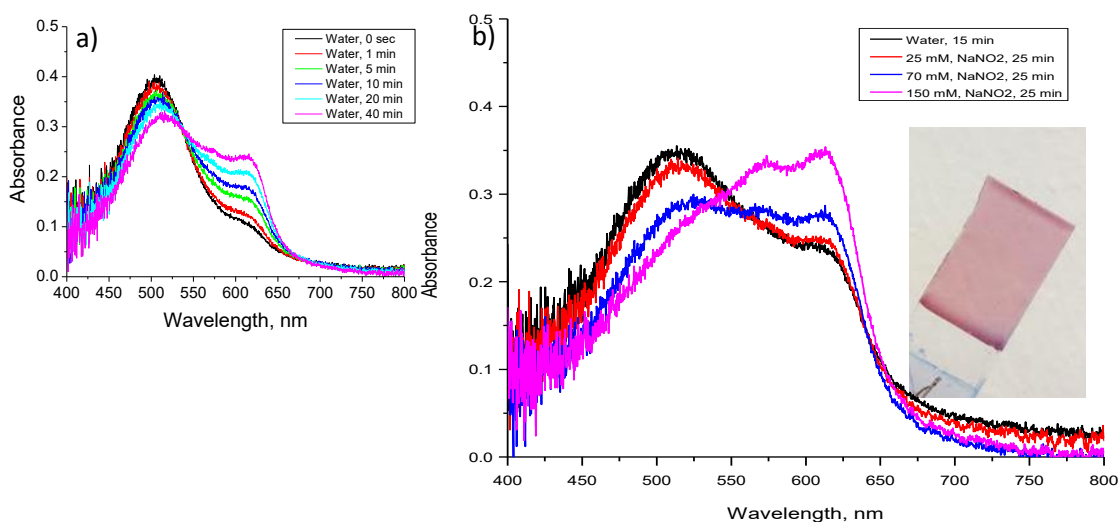


Figure 4-13 a) Absorption spectra of glass substrate dip coated by alkali catalysed sol gel described in section 4 of **Table 4-4** (yellow highlighted) with 1 mm/sec withdrawal speed in water; **b)** Absorption spectra of glass substrate dip coated by alkali catalysed sol gel described in section 4 of **Table 4-4** with 1 mm/sec withdrawal speed in aqueous media. The spectra were taken in different NaNO_2 aqueous solutions. Black line shows the absorbance in DI water after 15 minutes. Red, blue and magenta curves show the absorption after 25 minutes in 25, 70 and 150 mM sodium nitrite solution respectively. The inset on the right side of this figure is the actual coated substrate.

Figure 4-13a shows the 1 mm/sec (dip coat withdrawal velocity) base catalysed sol gel substrate (**Table 4-4**, sample No 4, highlighted by yellow) behaviour in water. The pattern is the same as we have seen in **Figure 4-11a**, i.e. an increase in the intensity of $\sim 610\text{nm}$ absorption peak, and a decrease in the intensity of $\sim 510\text{nm}$ absorption peak. After the substrate was elevated from water and immersed again for nitrite test, **Figure 4-13b**. The substrate was left in DI water for 15 minutes, black curve, then it was exposed to 25 mM NaNO_2 for 25 minutes, red curve after the substrate was exposed for 70 mM NaNO_2 for 25 min, blue curve, and finally to 150 mM, magenta curve. The $\sim 510\text{nm}$ absorption peak has decreased with sodium nitrite concentration increase from ~ 0.345 in case of water to ~ 0.248 in case of 150 mM NaNO_2 while on the contrary, the $\sim 610\text{ nm}$ peak has increased from 0.237 in water to ~ 0.35 in 150 NaNO_2 aqueous solution. This difference is not the same and much

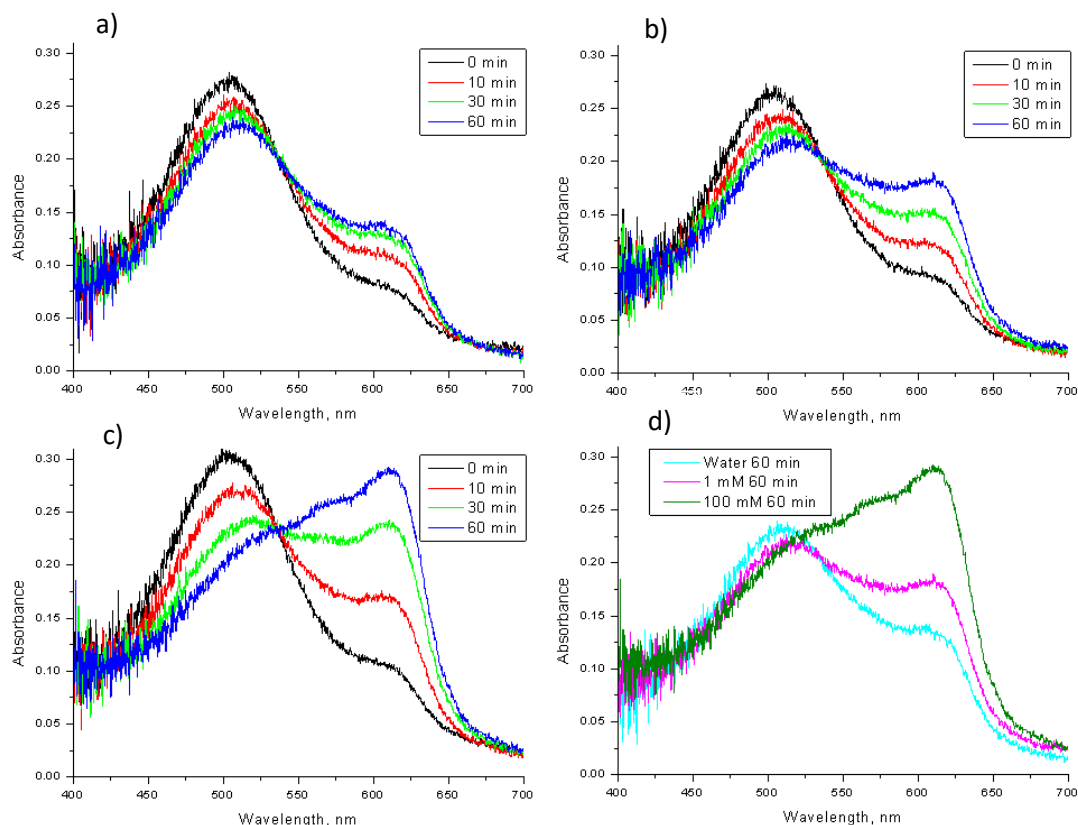


Figure 4-14 Absorbance of sol gel coated substrate as per Table 4-4 sample No 4 (highlighted by yellow), 2 mm/sec for 60 minutes in: **a)** water, **b)** 1 mM NaNO_2 , **c)** 100 mM NaNO_2 . In the each of mentioned graphs black curve shows the absorption in the respective medium immediately after immersion, red curve – after 10 minutes from immersion, green – 30 minutes, blue, 60 minutes. **d)** is the 60's minutes of each test, cyan, 60 min in water, magenta in 1 mM NaNO_2 solution, olive green in 100 mM NaNO_2 solution.

bigger compared to the submersion in water alone. In order to compare those differences properly, three identical substrates were made by the sol gel recipe described in Table 4-4 sample No 4, highlighted by yellow with 2 mm/sec withdrawal speed. Each of them was immersed for 60 minutes, one was tested in water, the other two in 1 mM and 100 mM NaNO_2 solution respectively, **Figure 4-14**.

From **Figure 4-14** it can be seen that the amount and rate of spectral change significantly differ in cases of DI water, 1 and 100 mM NaNO_2 solution. **Figure 4-14d** shows absorption spectra in each solution after 60 minutes at the end of the measurement. The cyan colour represents water, magenta 1 mM sodium nitrite and olive green – 100 mM. One can see the huge difference in the region of ~ 610 nm and a smaller difference with the opposite trend in the region of ~ 510 nm. To summarize and analyse the data presented in **Figure 4-14** one can see that the ~ 510 nm differs

in the case of higher concentrations of sodium nitrite, while the ~610 absorption peak differs significantly in each case.

The rate of these changes can be shown in **Figure 4-15**. On the left side, in **Figure 4-15a** one can see the absorbance values of ~510 (solid line) nm and ~610 (dash line) nm peaks over time for each immersion. Water – cyan curves, 1mM NaNO₂ – magenta, 100 mM NaNO₂ – olive green curves. On the right side, in **Figure 4-15b** the difference between each point of ~510 and ~610 nm absorbance measurement and the first measurement of those is shown. Water – cyan curves, 1mM NaNO₂ – magenta, 100 mM NaNO₂ – olive green curves. As can be seen in the case of higher concentration (100 mM, olive green curves) of sodium nitrite the rate of change is faster and the change is bigger as compared with water (cyan curves) at both observed absorption peaks. In the case of lower concentration (1 mM, magenta curve) at 510 nm, the overall change is barely distinguishable with those observed for water, but the rate is somewhat higher. At 610 nm though (dashed line), both the rate and absolute value of change can be clearly seen.

As can be observed in **Figure 4-10**, **Figure 4-11**, **Figure 4-13**, **Figure 4-14**, two

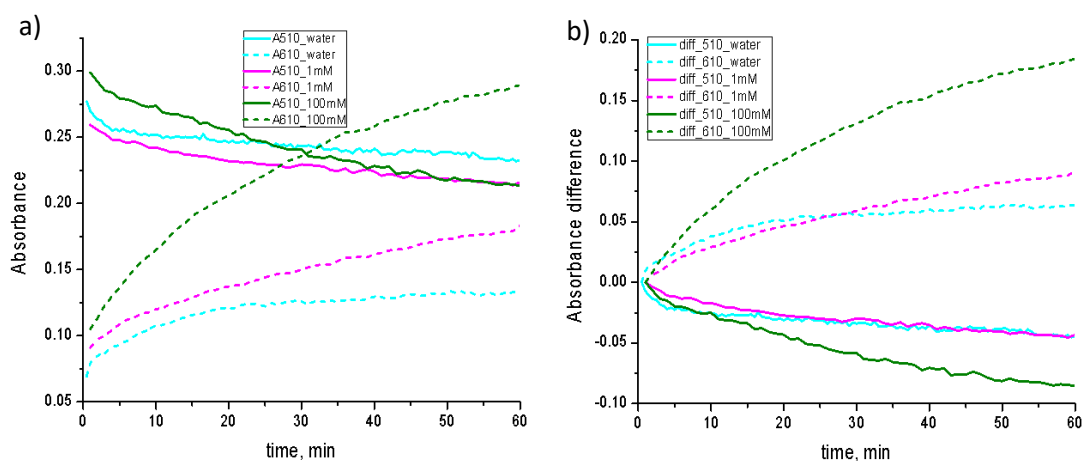


Figure 4-15a) Absorption of ~510 (solid line) and ~610 (dash line) nm peaks over time for water – cyan curves, 1 mM NaNO₂ – magenta curves and 100 mM NaNO₂ – olive green curves; **b)** difference of each measurement in time from the first after immersion measurement of ~510 (solid line) and ~610 (dash line) nm peaks, cyan curves, 1 mM NaNO₂ – magenta curves and 100 mM NaNO₂ – olive green curves;

absorption peaks (~near 510 and near 610 nm) are changing in different directions from each other. Therefore it is reasonable to track the ratio of those values, which makes the comprehension easier, the LOD lower and the data normalized, **Figure 4-16**.

In **Figure 4-16** the cyan curve represents the 610 nm/510 nm absorbance ratio for water, the magenta curve represents 1 mM NaNO_2 and the olive green 100 mM NaNO_2 aqueous solution. The rate of change of water's ratio is slowing down after 20 minutes, while the other two curves continue to rise more rapidly and clearly correlate with the concentration. Therefore the absorbance or intensity ratios will be used to evaluate the sensing response in the following results.

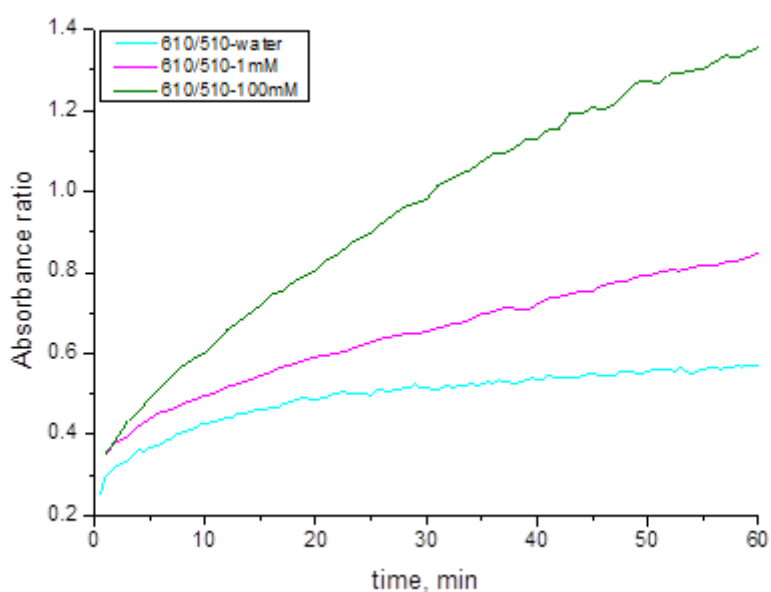


Figure 4-16 Absorbance ratios between 610 and 510 nm values over time of sol gel coated substrates as per Table 4-4 sample No 4 (highlighted by yellow), 2 mm/sec elevation speed, immersed in water (cyan curve), 1 mM NaNO_2 (magenta) and 100mM NaNO_2 (olive green) for 60 minutes

4.3.5 Results for BCB embedded sol gel film repeatability

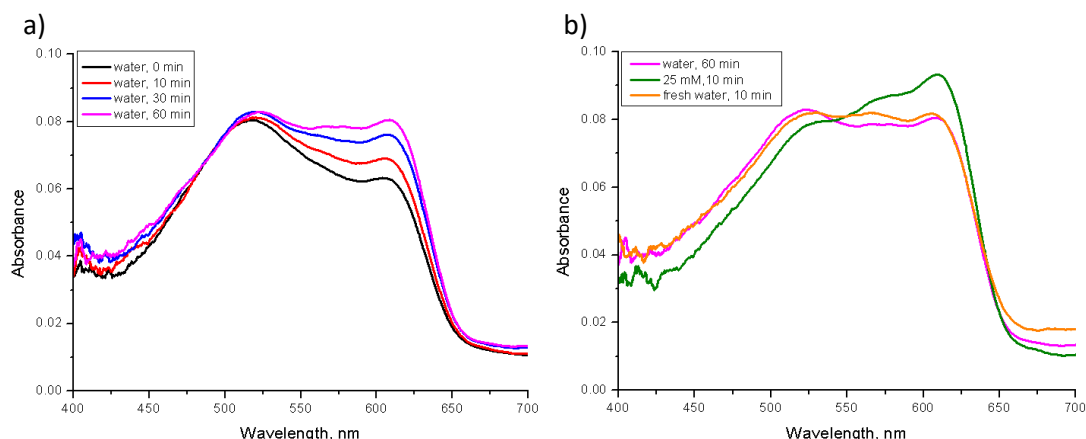


Figure 4-17 Absorbance of BCB embedded sol gel coated substrate as described in Table 4-4 sample No 4 (highlighted by yellow), 0.25 mm/sec **a)** over time in water for 60 minutes, black curve – 0 min, red curve – 10 min, blue – 30 min, magenta – 60 min; **b)** in water after 60 min- magenta, in 25 mM NaNO₂ for 10 min – olive green, in water again for 10 minutes – orange curve; In both graphs the Savitzky-Golay smoothing algorithm was applied with 300 points of window and 5th polynomial order

To examine the repeatability of the base catalysed BCB embedded sol gel response to sodium nitrite, a glass substrate with thin coating were tested, **Figure 4-17**. The coating composition is described in Table 4-4 sample No 4, highlighted by yellow, withdrawal speed - 0.25 mm/sec. The rising velocity of the substrate was much lower compared to previous examples, and as described in **Chapter 3 section 3.3.2.1** and Equation (3-1) lower withdrawal speed (down to ~0.25) yields a thinner dip coat film. This makes the light absorption lower, as there is a smaller optical path through the BCB embedded sol gel, however, the response is faster because the aqueous solution penetrates throughout the thinner film at a higher rate. In 10 minutes the spectra restores back after 25 mM NaNO₂, whereas even with lower concentrations of NaNO₂ the recovery took longer in the case of thicker coating.

During the DI water immersion of the substrate in water, it can be seen that the absorption peak at wavelength ~515 nm remains relatively constant, and the ~610's peak is increasing rapidly in the first ~35 minutes and starts to slow down after 40 minutes.

After adding sodium nitrite, the 515 peak drops significantly and fast; concurrently the 615 nm peak is rising dramatically within the observed 15 minutes.

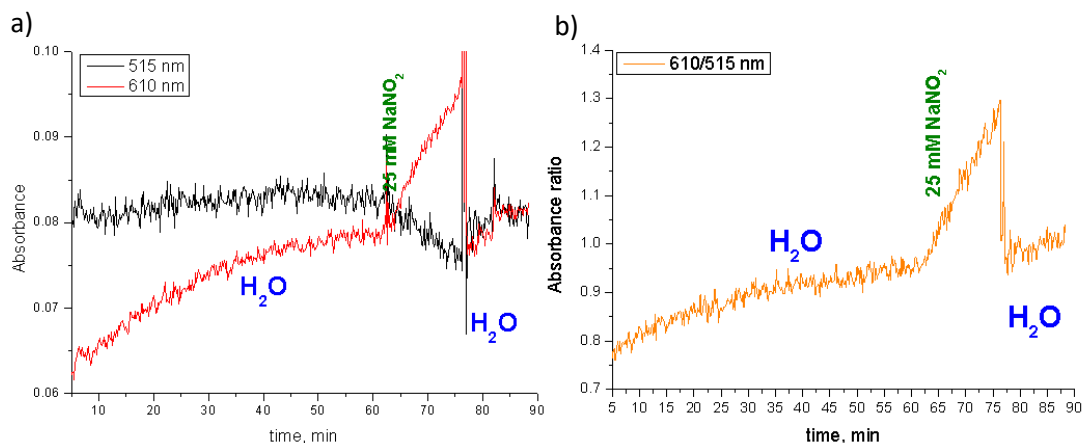


Figure 4-18 a) 515 (black) and 610 (red) nm absorption peaks values over time in water, 25 mM NaNO₂ and water again of the substrate shown in **Figure 4-17**; **b)** ratio of 610 and 515 nm absorbance values over time plotted on **Figure 4-18a** in water, 25 mM NaNO₂ and water again.

Next, the solution was replaced by fresh DI water and the 515 nm peak rises back to the previous position, as well as the 615's decreasing back to its pre nitrite values.

It can be concluded that some recovery of the sensitive substrate was observed, however, an overall drift in direction of absorbance rise can be seen. **Figure 4-18b** shows the ratio between 610/515 nm absorption values described in **Figure 4-18a**.

4.3.6 Comparisons of free and entrapped BCB responses

To understand the BCB's response to NaNO₂ it is reasonable to compare the response of free BCB dye in water with BCB entrapped into sol gel matrix.

It is easy to observe from **Figure 4-5** and **Figure 4-14** that the change of the absorption spectra in cases of low pH BCB aqueous solution and alkali catalysed sol-gel occurs in a different direction, meaning that the two absorption peaks (near 500 and 600 nm) tend to change their absorption values in opposite directions. **Figure 4-19a** shows the absorption peaks comparison between the base catalysed sol gel coated glass substrate fabricated according to Table 4-4 sample No 4 (highlighted by yellow), with 2 mm/sec elevation speed, and a pH~2 50 μM BCB aqueous solution, while both were exposed to 1 mM NaNO₂ over 70 minutes. Left-hand vertical axes

are the substrate's absorbance, right-hand vertical axes are the solution's absorbance. Solid lines represent the substrate, dashed lines – the aqueous solution. Green curves are the absorption values of ~515 nm peak, orange the 610 (620 nm for solution) nm peak. **Figure 4-19b** shows the ratios between those absorbance peaks over time, 610/515 nm for substrate (magenta curve) and 620/515 nm for aqueous solution (blue curve). Left-hand vertical axes are the substrate's absorbance, right-hand vertical axes are the solution's absorbance. Although the absolute ratio values differ by about an order, it is clear that the direction of change is opposite.

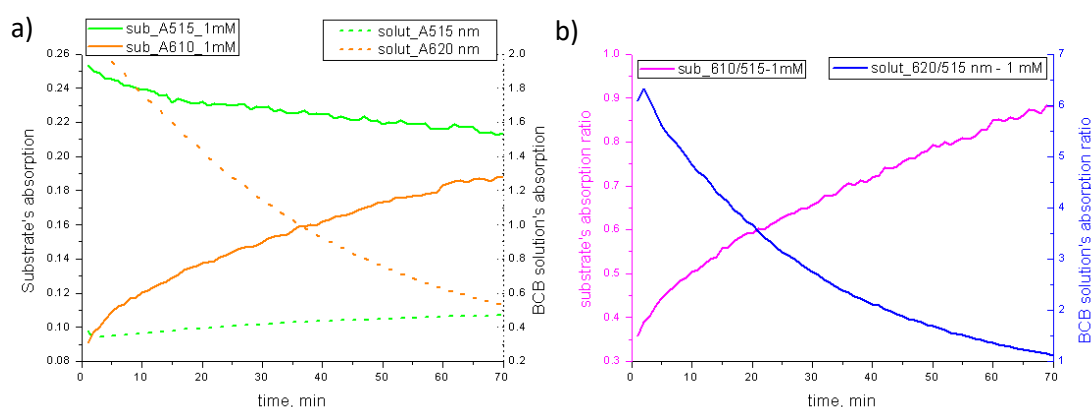


Figure 4-19 a) Absorbance of ~515 (green curves) and ~610(620) (orange curves) nm values comparison of the substrate described in **Figure 4-14b** (continuous lines) and 50 μ M pH~2 BCB solution plotted on **Figure 4-5** (dashed lines); **b)** Absorbance value ratios of 610/515 nm of substrate (magenta curve) and 620/515nm of low pH BCB solution.

This fact together with the reversibility of the base catalysed BCB embedded sol-gel's response to sodium nitrite described in section 4.3.5 and irreversibility of the low pH BCB aqueous solution's response to sodium nitrite proven in section 4.2.5, suggests that different mechanisms are involved in those responses.

4.3.7 pH response comparison

The BCB dye is pH sensitive [281], and sodium nitrite can be considered as a weak base [282] which will alter the pH of a solution. Therefore, a pH evaluation should be conducted. **Figure 4-20a** shows the response of BCB embedded base catalysed sol gel coating to pH~9 KOH solution. **Figure 4-20b** shows the response of

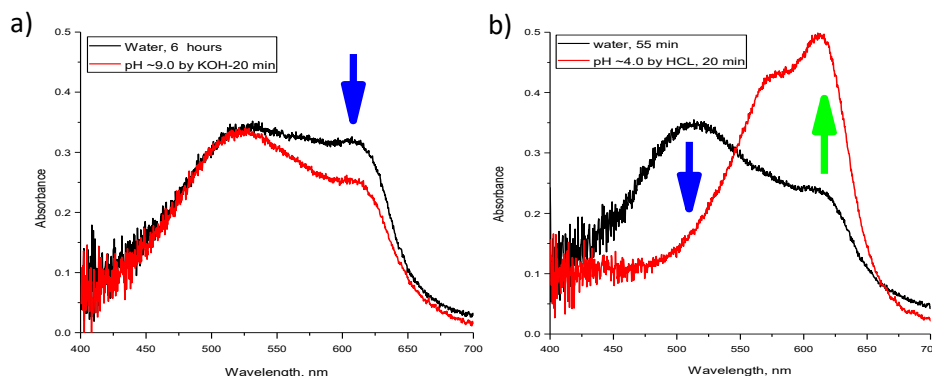


Figure 4-20 Comparison of basic and acidic pH response of the BCB embedded base catalysed sol gel coated substrate and blue green arrows are pointing in the direction of change; **a)** water (black curve) and pH~9 KOH solution (red curve); **b)** water (black curve) and pH~4 HCL solution (red curve);

BCB embedded base catalysed sol gel coating to pH~4 HCL solution. One can see that in the case of basic condition (pH~9) the ~620nm peak absorption value decreases, while the ~510 nm remains relatively stable. On the other hand, in the case of an acidic environment (pH~4) the ~620 nm absorption value increases significantly, while the ~510nm drops dramatically. However, in the case of NaNO₂ aqueous solution, the response is rather similar to the acidic one (620 goes up, 510 slightly down). With NaNO₂ being a weak base it would increase the pH value of the solution, so it can be concluded that the BCB embedded sol gel response to sodium nitrite is not due to the pH change. However, because there is a response to pH change, for real-time measurements a pH reference sensor should be used.

4.3.8 Discussion

The observed and described above patterns are very similar to that published by *Gilani et. al.* [276] work, about aggregate formation in BCB aqueous solution, **Figure 4-21**. From **Figure 4-21a**, one can clearly see the dependence of the absorption peaks and their positions upon the concentration of the BCB in water. The authors explain this shift because of the monomer-dimer aggregation. **Figure 4-21b** shows the calculated dimer (violet curve) and monomer (blue curve) molecular absorptivity. The BCB in solutions with lower concentrations appears mainly in monomer-dimer aggregates, and for concentrations below 50 μ M only monomeric form occurs [276]. Hence, for concentrations higher than 50 μ M the absorption spectra have two

absorption bands. As can be seen in **Figure 4-21b**, the J band is significantly lower than the H band for dimers, which explains the experimental spectral behaviour through increasing concentration shown in **Figure 4-21a**. At 2 μM of BCB, there is one clear peak, and while the concentration is growing, two absorption peaks can be observed, **Figure 4-21a**.

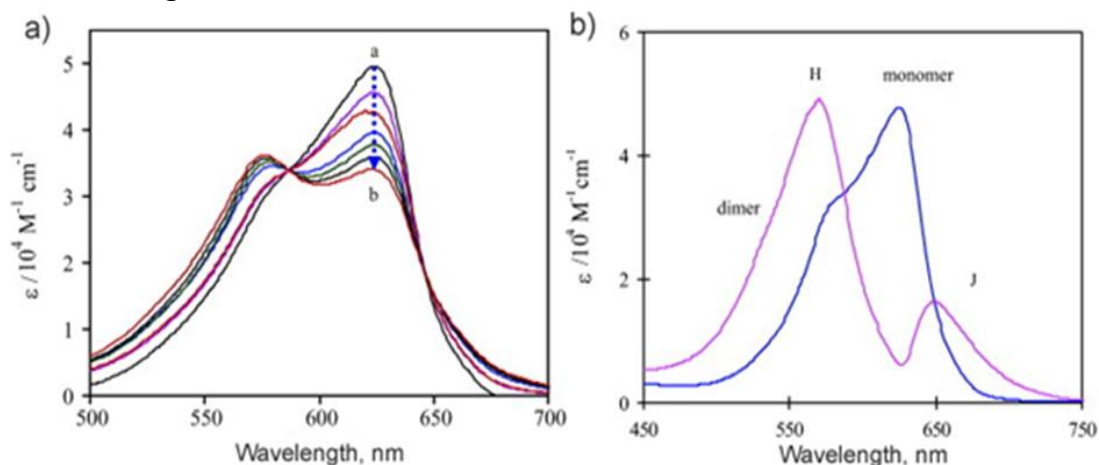


Figure 4-21 a) Molecular absorptivity of BCB in water 2-1000 μM (from a-b); b) Theoretical calculations of monomer and dimer molecular absorptivity [272]

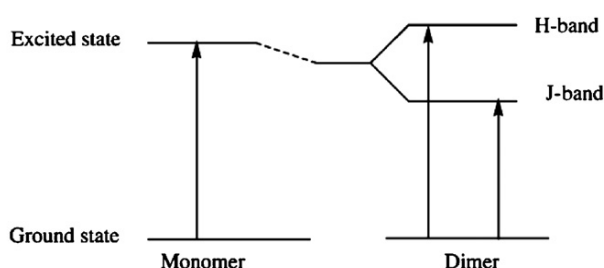


Figure 4-22 Sketch of the energy levels of a molecular dimer [272]

The interaction nature between pairs in oxazine dyes was explained via the excitonic model [283]. The dye's molecules aggregation results in strong coupling between the molecular transition dipoles. The interaction between the chromophores'

molecular transition dipoles splits energy levels of the molecular excited states in the dimer, while the ground state of the dimer remains doubly degenerated **Figure 4-22**. Only two geometric models are accepted for dye aggregates, according to the molecular exciton theory. For model 1 (H-aggregate), the chromophores of the involved molecules are in parallel planes, while in model 2 (J-aggregate) they appear to be in the same plane. In the reality though, dimers tend to form both H- and J-aggregates, which results in both H- and J-bands in the dimer spectrum.

Above in **section 4.2.6** it was suggested that oxidation of BCB could occur in acidic aqueous solution by both, oxygen dissolved in water or as a part of the water

molecule, in presence of nitrites which causes the colour change. However, it was shown in **section 4.2.5** that this process is not fully reversible, and responds oppositely, **section 4.3.6**.

The pure pH-based charge distribution change also cannot be the cause of the colour change, as it occurs in opposite direction, as it was shown in **section 4.3.7**.

It can be suggested that the potential colour change cause could be the monomer-dimer equilibrium change or the exciton excitation distribution change of the BCB in presence of nitrites. It is reasonable to assume this because the response of the entrapped BCB is similar to that of the changes in the absorption spectrum of BCB when the concentration is changed, **Figure 4-21** [276] as a consequence of monomer-dimer equilibrium change which causes the excitation distribution change of the BCB [283]. The other reason to suggest the exciton excitation distribution change is the reversibility of the response in the case of entrapped BCB, which is not true for the low pH free BCB solution as shown in section 4.2.5 and mentioned by *Ensafi et. al* [277].

4.3.9 Conclusion

The BCB embedded sol-gel coated glass substrates fabrication, evaluation and testing were described. It has been shown that the BCB embedded base catalysed sol-gel film is responsive to the presence of Nitrites in an aqueous solution and such a response is repeatable. A sensing mechanism hypothesis was discussed. The proven to be sensitive sol-gel coating deployment on an optical fibre sensor is described in the following sections of this Chapter below.

4.4 Optical fibre sensing for Nitrites

4.4.1 Introduction to optical fibre Nitrite sensing

After the sensitive base catalysed BCB embedded sol gel film was tested and studied on a glass substrate, it was applied to an optical fibre sensor. Two types of evanescent wave sensors were fabricated and studied empirically; cladding replaced straight fibre sensor and cladding replaced U-shape fibre sensor. The principle of operation of those was described in **section 2.3.2**.

4.4.2 Method for fabrication, characterisation and measurement

The fabrication of the U-shape optical fibre sensor is described in **Chapter 3 section 3.2.2**.

When the cladding is removed and part of the fibre core is bare, it can be coated by dip coating in the same way as the glass substrate. For the U-shape fibre, the coating procedure is easier, as we have to immerse only one end of the fibre, **Figure 4-23**. For a straight fibre though, the bare part is in the middle of the fibre. So a setup that enabled to coat the middle part of the fibre, was constructed in house, **Figure 4-24**. Here a vertically fixed syringe is filled with sol gel solution and attached through the needle hole to a thin plastic tube, which is bent around in such a way that the end of the tube reaches above the liquid surface in the syringe. Then the fibre is immersed into the syringe through the tube in such a way that the bare part could be raised and lowered by the translation stage.

The deposition process was observed and recorded by an Ocean Optics HR 4000 spectrometer (**Figure 4-24**) and an Ocean Optics HL-2000 halogen light source. When the light propagates through the bare part of the fibre it is modulated by interaction with the sensitive layer during the coating procedure.

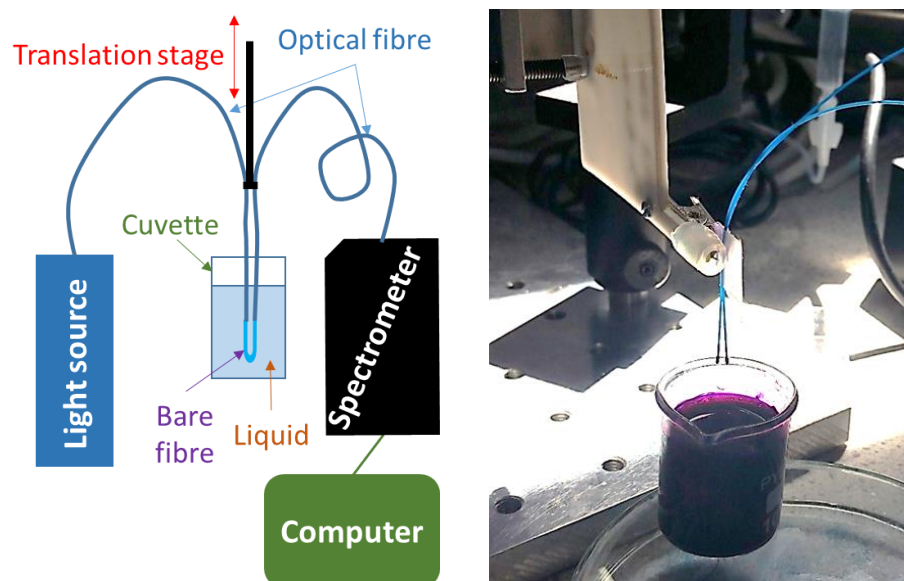


Figure 4-23 Schematic and photo of the U-shape fibre dip coating set up

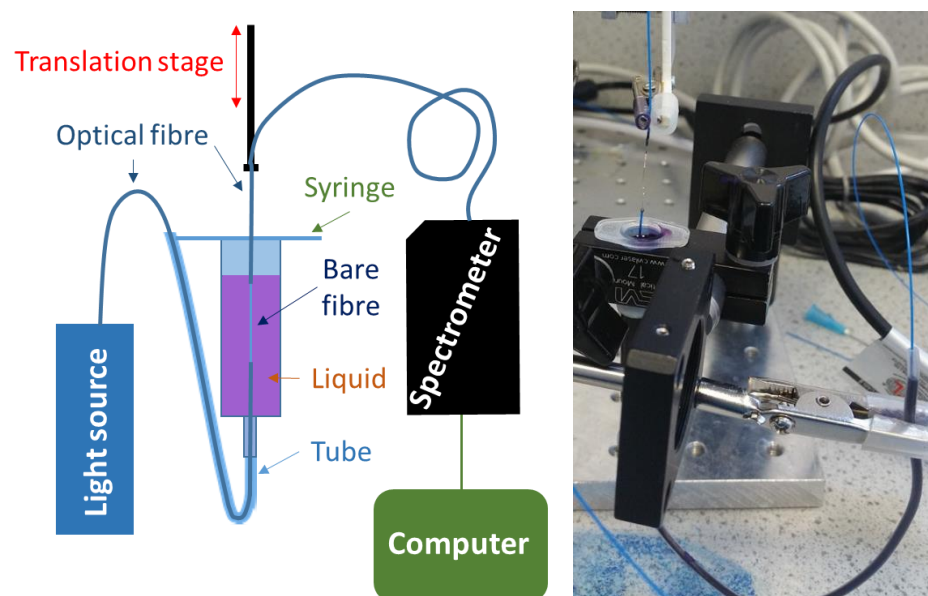


Figure 4-24 Schematic and photo of the straight fibre dip coating set up.

After the desired number of layers are deposited 1-3, depending on the particular fabrication, the fibre was left to dry overnight at room temperature. For a more robust and stable coating, the fibres were temperature treated in an oven at 70°C for over 1 hour followed by an hour of natural cooling within the oven.

4.4.3 Method for optical fibre measurements

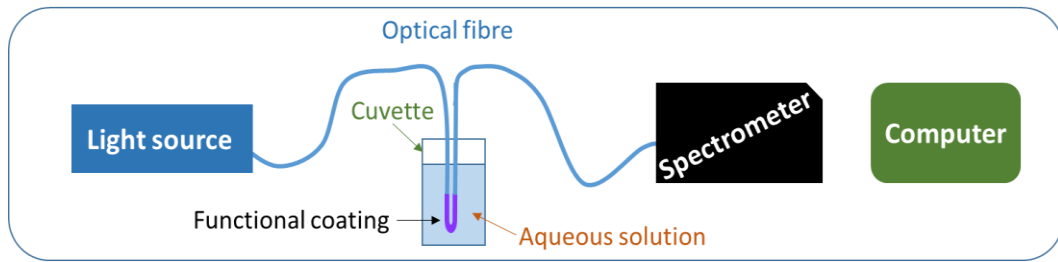


Figure 4-25 Set up of the U-shape optical fibre sensor testing

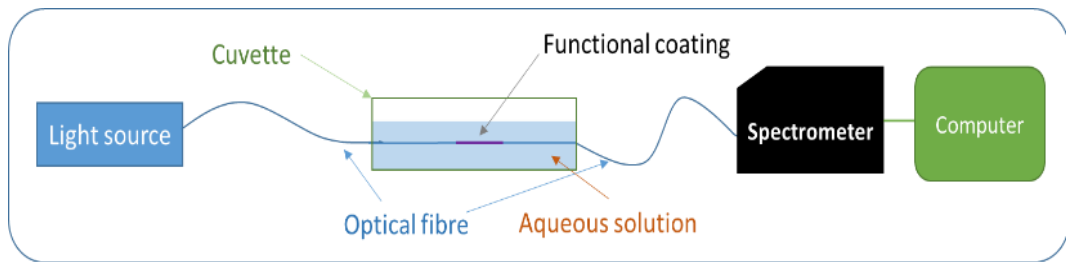


Figure 4-26 Set up of the straight absorbance based optical fibre sensor testing

The setup for nitrite measurements with a U-shape fibre sensor and straight evanescent fibre sensor is shown in **Figure 4-25** and **Figure 4-26**, respectively. From one side, the fibre multimode FT200UMT fibre was connected to the Ocean Optics HL-2000 halogen light source which gives us a wide VIS/IR spectrum. The coated part of the fibre was immersed in a vessel where different solutions with tested concentrations were changed. The other end of the fibre was connected to the Ocean Optics HR 4000 spectrometer which was connected to a PC for signal processing.

The limit of detection (LOD) of the nitrite optical fibre sensor was determined according to Equation (4-1) below [284]:

$$\text{LOD} = 3 \frac{s}{m} \quad (4-1)$$

Where s is the standard deviation of the response and m is the slope of the calibration curve.

4.4.4 Deposition results

Figure 4-27 shows the results from the deposition of two layers of base catalysed sol gel described in **Table 4-4** sample No 4 (highlighted by yellow), with 12 mm/sec withdrawal speed. A higher withdrawal speed was used on the fibre than on the substrate in order to achieve a thicker coating, thus making the absorbance higher. The necessity of this was determined empirically during coating trials.

$$A = -\log_{10} \left(\frac{I}{I_0} \right) \quad (4-2)$$

Where A is the absorbance, I is the light intensity transmitted after the coating, I_0 is the light intensity transmitted before the coating, through the bare fibre.

Figure 4-27a shows the transmission spectra of the fibre before the deposition (black curve), after 60 seconds of first elevation (red line) and after 60 seconds of

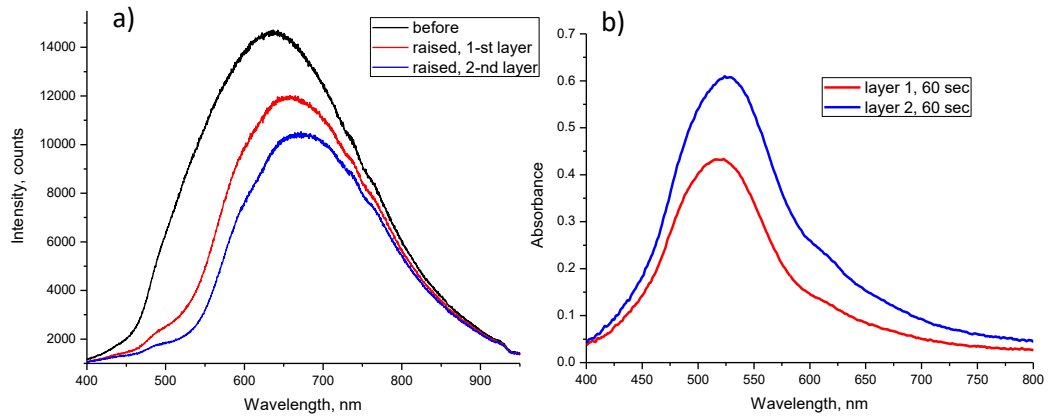


Figure 4-27 a) intensity spectra of the straight cladding replaced optical fibre coated by base catalysed sol gel described in Table 4-4 sample No 4 (highlighted by yellow), withdrawal speed - 12 mm/sec, 2 layers. Black curve shows the transmission spectra of the fibre before the deposition, red one shows the transmission after 1 layer was deposited (60 sec after elevation); blue curve shows the transmission after second layer was deposited (60 sec after elevation); **b)** illustrates the absorbance after 60 seconds of first (red) and second (blue) deposition;

second elevation (blue curve). **Figure 4-27b** shows the same procedure but in terms of absorbance, which was calculated from the transmission spectra shown in **Figure 4-27a** using **Equation 4-2**, with the spectra before the deposition as a reference I_0 and smoothed via Matlab “smooth” command, which is a method of reducing the noise using moving average filter within a data set [285]. The red curve corresponds to the first deposited layer (after 60 seconds from elevation), and blue, - the second deposited layer (after 60 seconds from elevation). Both in the first and second layers’

absorption spectra one strong and one weak absorbance peak can be seen, around ~515 and 610 nm respectively.

Figure 4-28a shows the absorbance spectra behaviour from 1st to 60th second

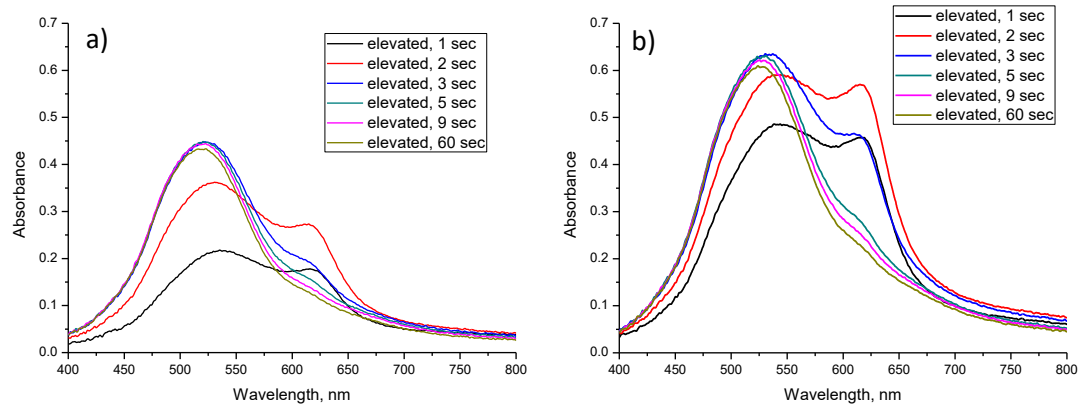


Figure 4-28 a) absorbance spectra taken from 1st to 60th second of the first elevation; **b)** absorbance spectra taken from 1st to 60th second of the second elevation;

after the first elevation. **Figure 4-28b** shows the absorbance spectra behaviour from 1st to 60th second after the second elevation. The same phenomenon could be observed as on the glass substrate, as seen in **Figure 4-10** but with a different velocity.

4.4.5 Results for the U-shape sensor

This subsection describes results for the U-shape sensors coated by two layers of base catalysed BCB embedded sol gel with mixture ratios described in section 4 of Table 4-4. Samples were prepared with 6 mm/sec elevation velocity.

Figure 4-29a shows the whole spectra of the U-shape optical fibre sensor coated with two layers of base catalysed BCB embedded sol-gel (sample No 4 of Table 4-4 highlighted by yellow) when immersed twice into the water and 100mM nitrite. As can be seen from the graph, in the range from ~ 475 to 625 nm the intensity shifts in the opposite directions in water and nitrite aqueous solution. Since the highest rate change was measured at 515 nm and 610 nm, therefore it is sensible to take the ratio of those points for sensing purposes. In the same way, as it was done for the substrates (**Figure 4-10b**, **Figure 4-12b**, **Figure 4-18b**, **Figure 4-19b**). **Figure 4-29b** shows the dynamic change of transmission ratio between two observed intensity peaks – 610/515 nm through the whole testing time. The measurements start with

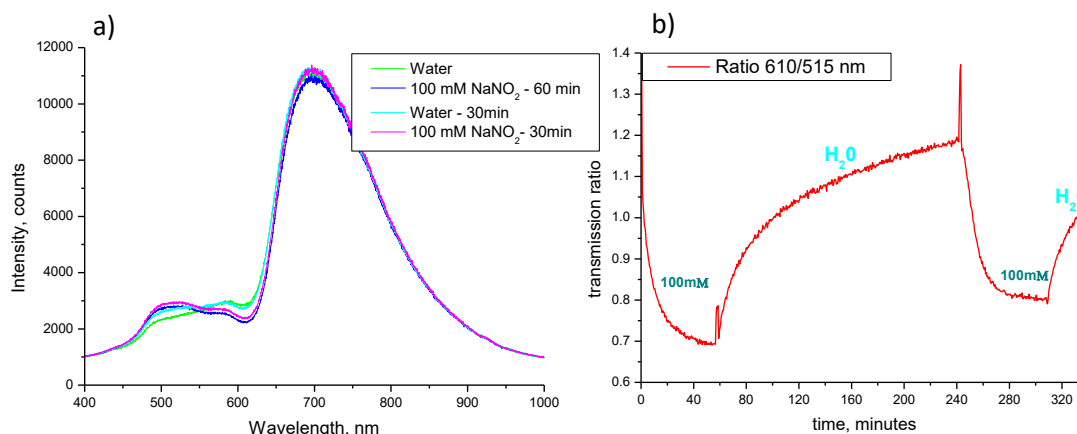


Figure 4-29 U-shape fibre sensor response to 100 mM of NaNO₂ aqueous solution.

a) Whole range spectra. Green and light blue curves represent the spectra obtained in DI water, while the dark blue and purple, - 100 mM NaNO₂ aqueous solution; b) 610/515nm intensities transmission ratio

immersing the U-shape fibre sensor into 100 mM NaNO₂ aqueous solution, which results in a steep decline of the 610/515 nm intensity ratio in the first 20 minutes from ~1.07 to ~0.744, then the decline rate slows down becoming a moderate slope and reaches ~0.689 in the 60th minute. Afterwards, the NaNO₂ solution was substituted with Di water for 180 minutes. The 610/515 nm ratio grows rapidly in the first 20 minutes from ~0.715 to ~0.934, then the growth rate slows down and continues almost linearly with a slight slowdown of the growth from the 60th (~1.065) to the 120th minute, where it reaches a value of 1.22. After the U-shape was exposed to 100 mM NaNO₂ concentration again for 60 minutes, this time the decline was even more rapid, especially in the first 20 minutes (from 1.215 to 0.88), which can be the consequence of the fibre being in the water for 2 hours previously. In the next 40 minutes the ratio's decline slows down, but still can be observed and reaches a value of ~0.82. After the U-shape was immersed in the water again, for 20 minutes, which resulted in a rapid restoring tendency of the ratio from 0.82 to 1.06.

4.4.6 Results for the straight absorption based fibre sensor

Several coatings with different mixture ratios were tested on a straight fibre sensor in order to find the optimal sensing parameters. The presented results are for 2 cm long cladding replaced straight absorbance fibre sensor coated by BCB

embedded NaOH catalysed sol gel with mixture ratio described in section 3 of Table 4-4, and with a 12 mm/sec elevation speed:

Figure 4-30 a) and b) show the ratio between transmission intensities of 515 and 610 nm of different sensors for higher (100 and 500 μM) and lower (25 and 50 μM)

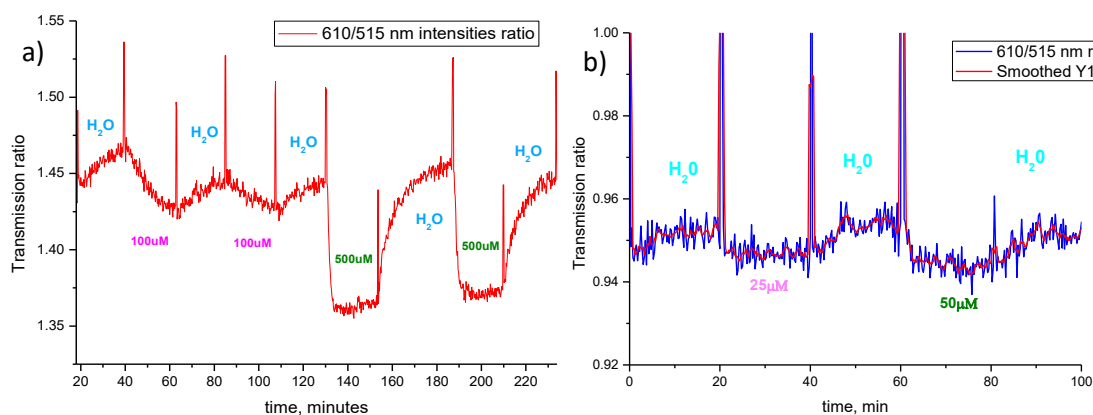


Figure 4-30 BCB embedded sol gel nitrite sensitive optical fibres 610/515 nm transmission intensities ratios for: a) Higher, 100 and 500 μM sodium nitrite concentrations in DI water; b) lower, 25 and 50 μM sodium nitrite concentrations in DI water

μM) sodium nitrite concentrations respectively. As can be seen in the figure, the response of the sensor is repeatable. In the case of **Figure 4-30a**, one can see that after immersion in DI water, the ratio starts to rise from ~ 1.44 (in the same way as previous results on substrates and U-shape fibre sensors) and reaches ~ 1.46 after 20 minutes. Then, the liquid was changed for 100 μM of nitrite. The ratio starts to fall gradually from 1.46 and reaches 1.43 after 22 minutes. During the next immersion in water and a recovering process can be observed. The ratio goes from ~ 1.42 to ~ 1.45 in 20 minutes. There is still no equilibrium, but as can be seen from **Figure 4-29b**, the biggest ratio change happens within the first 20 minutes. The next two immersions in water and 100 μM NaNO_2 for ~ 20 minutes each, follows the same trend: ~ 1.45 to ~ 1.43 , and ~ 1.43 to ~ 1.45 respectively. Then the DI water was replaced by 500 μM NaNO_2 which resulted in a rapid decrease of the ratio from ~ 1.45 to ~ 1.36 during the first 4 minutes of the immersion and stayed roughly constant during the following 18 minutes. In water afterwards, the ratio recovers rapidly from ~ 1.36 to 1.41 during the first 4 minutes, then slower with a decreasing pattern showing the approach of the equilibrium, reaching ~ 1.45 in the next 18 minutes. The next two immersions show

the same pattern with a bit smaller difference. From 1.45 to ~1.37 in 4 minutes in 500 μM NaNO_2 , which seems to be the saturation point, as it stays relatively stable during the following 18 minutes of this immersion. Then recovers in water with the same pattern as previously, reaching ~1.45 after 22 minutes.

In the case of lower concentrations (25-50 μM), **Figure 4-30b**, the change is smaller, therefore an Origin Adjacent-Averaging algorithm with 4 points of the window without boundary conditions curve was used (red) alongside the real ratio (blue). The ratio in water starts at ~0.946 and rises to ~0.952 in the first 5 minutes and stays roughly there with some fluctuations. Afterwards, when exposed to 25 μM NaNO_2 solution, the ratio falls rapidly within one minute from ~0.952 to ~0.946 and remains at this value with some fluctuations (related to the noise of the spectrometer and light source throughout) the next 19 minutes of the immersion. Then in water, the ratio tends to recover and rises from ~0.947 to ~0.955 in 7 minutes, then in the next 7 minutes slightly drops to ~0.952 and recovers back to ~0.955 where it remains with fluctuations down to ~0.953 for the rest 6 minutes of this immersion. Afterwards, the sensor was exposed to 50 μM of sodium nitrite, the ratio drops rapidly from ~0.953 to ~0.945 within 2 minutes, and through fluctuations continues to decrease steadily to ~0.942 in the next 12 minutes and returns to ~0.945 until the end of the immersion. After that, in water, the ratio recovers with similar trends as previously from ~0.944 to ~0.953 in 8 minutes, in the following 12 minutes the ratio settles at ~0.951 indicating recovery of the sensor.

Table 4-5 Integrated ratio values at the first and last minute of each measurement shown in **Figure 4-30a**, their errors and differences.

	Ratio - beginning	Standard deviation	Ratio - end	Standard deviation	Difference
H₂O -1	1.4457	0.0027	1.4651	0.0030	0.0194
100 μM -1	1.4668	0.0020	1.4282	0.0018	-0.0386
H₂O -2	1.4238	0.0036	1.4437	0.0032	0.0199
100 μM -2	1.4469	0.0051	1.4278	0.0022	-0.0190
H₂O -3	1.4289	0.0019	1.4440	0.0022	0.0151
500 μM -1	1.4261	0.0171	1.3652	0.0030	-0.0610
H₂O -4	1.3775	0.0091	1.4562	0.0019	0.0786
500 μM -2	1.4317	0.0152	1.3725	0.0031	-0.0593
H₂O -5	1.3819	0.0053	1.4458	0.0020	0.0639

Table 4-5 shows the integrated ratio values of the first and last measurement, their errors and differences.

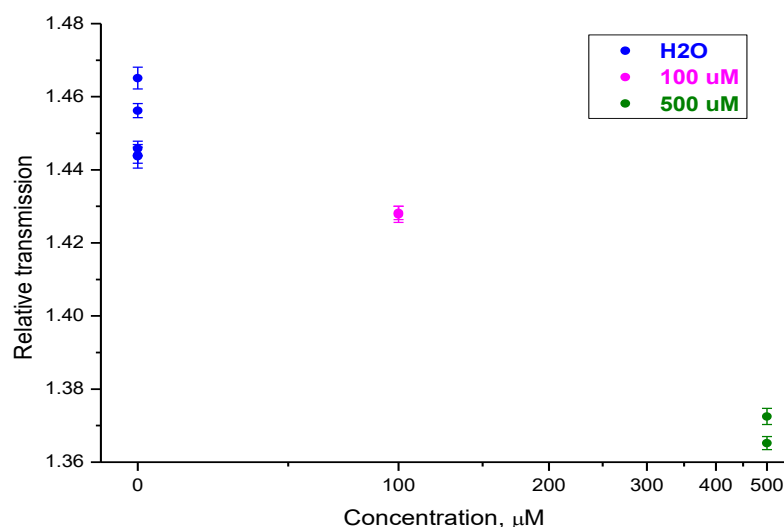


Figure 4-31 Integrated ratio values of the last minute of each measurement and their standard deviation errors as a function of NaNO_2 concentration. Blue dots represent the ratio value in DI water, magenta and olive green, - 100 μM and 500 μM respectively.

Figure 4-31 shows the integrated ratio values for each last minute measurement and their error bars. Blue dots represent the 610/515 nm ratio at the end of the water measurement. They are scattered from ~ 1.443 to ~ 1.465 , however, three of them among five are concentrated near ~ 1.445 and lay within their error ranges. The magenta dots represent two sensors' ratio values in the last minute of 100 μM sodium nitrite aqueous solution with ~ 1.428 for both within errors (standard deviations calculated from the given minute measurement points). The olive green dots represent the sensors response for 500 μM sodium nitrite in water. These two ratios are ~ 1.365 and ~ 1.372 .

In **Figure 4-32** one can see the differences in ratio values between the end of each immersion. The magenta coloured bars represent the differences between the end of water and 100 μM sodium nitrite aqueous solution. These ratio differences yield -0.0369 and -0.0159 for the first and second exposure respectively. The cyan bars show the restoring trend after 100 μM nitrite exposure in water. Olive green bars are the difference between the end of water and the end of 500 μM NaNO_2 measurements. The first and second exposure gives -0.0788 and -0.0837 respectively. And finally, the blue bars show the restoration of the ratio. In this case, the

subtractions from an end value of 500 μM for the end of the water value are 0.091 and 0.0733 for each immersion respectively.

Overall the sensor shows recognisable sensitivity down to 25 μM . These numbers are acceptable at urban waste water effluence monitoring (from 70 μM) [273],[286] and in some cases even for drinking water monitoring ($\sim 11\mu\text{M}$ for EU/UK and 71.4 μM for the USA) [270], [268] (**Table 2-5, Table 4-1**).

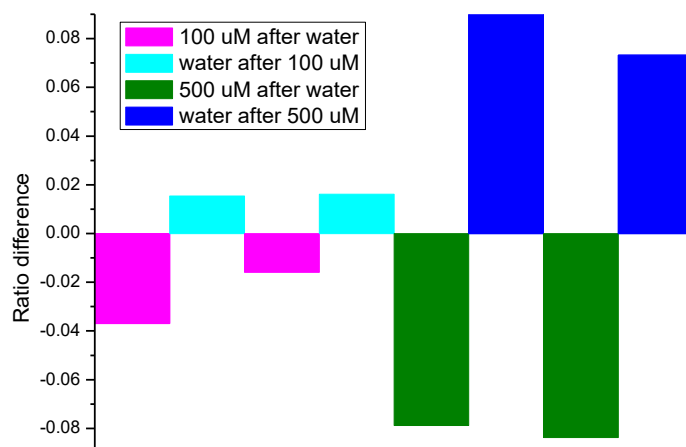


Figure 4-32 Ratio differences between the last minutes of each measurement. The Magenta bars shows the difference between ratio values of 100 μM after water, cyan the restoring trend in water water after 100 μM . The Olive green shows the difference between the ratio values of 500 μM after water, and the blue shows the restoring trend in water.

The LOD was calculated using **Equation (4-1)**. The data shown in **Figure 4-30b** were used to create a linear fit. For the data points, the average value of the last four minutes of each measurement was used, and their standard deviation was

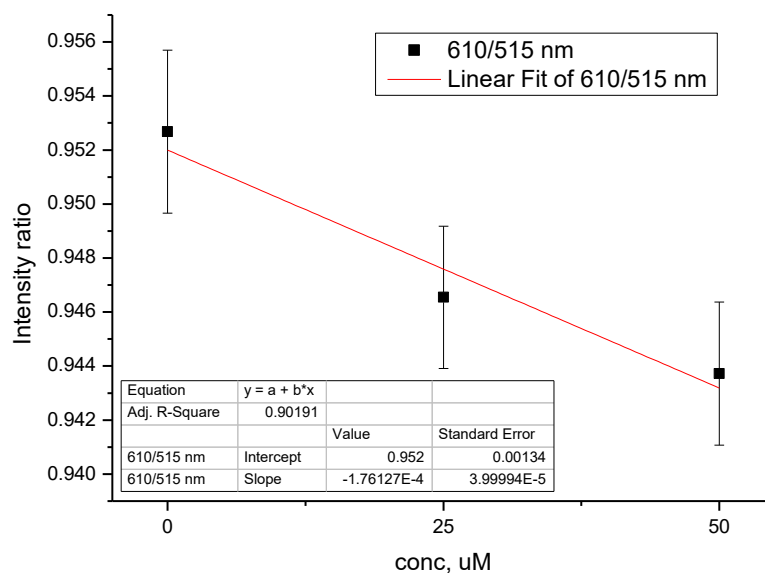


Figure 4-33 Linear fitting of the last four minutes measurement shown in calculated. The limit of detection calculated according to equation (4-1), taking the intercept's error as a standard deviation gives us 22.81 μM LOD

Or

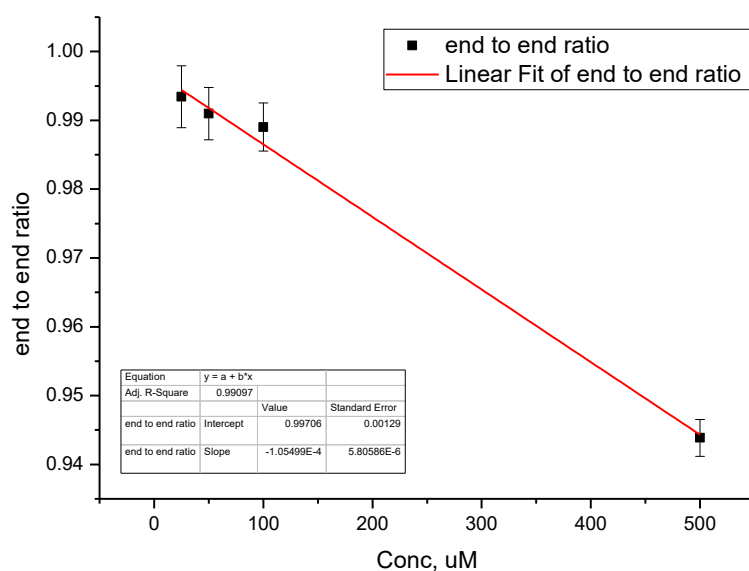


Figure 4-34 Amount of change between water and 25, 50, 100 and 500 μM NaNO_2 respectively. Their SD and linear fit.

Taking the amount of change between measurements of the last two minutes in water and last two minutes of respective sodium nitrite solutions from the data shown in **Figure 4-30a** and **Figure 4-30b** thus allows the tracking of the amount of change of each concentration after 20 minutes immersion. The plots with their standard deviations and linear fit are shown in **Figure 4-34**.

The limit of detection calculated according to equation (4-1), taking the intercept's error as a standard deviation gives us 36.68 μM LOD, which is bigger than one can clearly see in **Figure 4-30b**.

4.4.7 Discussion

Besides the advantages of the U-shape OFS, like easy fabrication, convenient handling, high sensitivity, the U-shape fibre sensor has a few disadvantages. For example, it is difficult to repeat exactly the same shape during the fabrication procedure without automation. However, the main obstacle is the dip-coating process of the U-shape fibre sensors. Despite the simplicity of the dipping procedure, the resulting coating turns out slightly different and non-uniform, because of the capillarity of the sol gel liquid and gravitation. Therefore, it is more reasonable to deploy the straight cladding replaced OFS, the results of which was shown in **section 4.4.6**.

In the development of this optical nitrite sensor, the main focus was on finding and observing a possible repeatable nitrite sensing response, rather than lowering the LOD and establishing robust sensor performance. Consequently, only a few concentration points are studied. Nevertheless, results suggest measurements down to 25 μM NaNO_2 concentration in water are possible. However, it was found that the sensitivity of these sensors decrease with time and become completely insensitive after 7 days when stored both in water and air. The sensitivity loss could be a result of several processes related to the BCB dye itself, to the base catalysed sol gel matrix, or to the complex they've formed. Such as photobleaching, sol gel ageing-related processes: drying, stiffening, matrix shrinkage, or some other processes [287]. Attempts to tackle this issue and prologue the sensor's lifetime are shown in **Appendix 1**.

4.5 Conclusion

This chapter shows the research and development of the BCB sol gel incorporated optical fibre nitrite sensor for aqueous media. First, the BCB dye in an aqueous medium was studied. After different types of sol gel matrices were used, studied and tested on glass substrates. A sol gel with optimal BCB concentration was found, which is described in Table 4-4, samples 3 and 4. The coating procedure and optimisation of glass substrates and two types of optical fibre sensors were described in sections 4.3.2 and 4.4.2. Also, a difference between the response of aqueous and embedded BCB was observed, studied and described. In section 4.2.5 the irreversible response of the aqueous BCB solution was shown, compared to the reversible behaviour of the base catalysed BCB embedded sol gel coating. Also, different response patterns of aqueous and entrapped BCB oxazine dye were observed and described in section 4.3.6. Opposite pH response behaviour compared to the pH change caused by slightly alkali NaNO_2 was described in section 4.3.7. A monomer-dimer and/or exciton charge distribution based response in presence of Nitrites was proposed as a sensing hypothesis for the entrapped BCB.

Afterwards, a transfer from glass substrates to optical fibre sensor was completed. A cladding replaced straight optical fibre was chosen as an optimal solution. A repeatable and strong response to 100 and 500 μM and a weaker but distinguishable response down to 25 μM sodium nitrite concentration was shown. This is sufficient sensitivity to use for some environmental applications. A relatively short lifetime (a few days) of the sensor was shown which gradually decreases over time with almost complete insensitivity in 7 days. However, storage in Nitrogen improves the lifetime of the sensor up to at least 7 days, with the same level of response. This can serve as a starting point for further research in the direction of improvement of the sensors shelf time storage. Once this issue is resolved, the presented nitrite sensor has a potential application in monitoring critical levels of ground and waste waters with the potential to be applied for tap and drinking water monitoring, as can be seen from **Table 4-1**. The next chapter focuses on the ammonia aqueous sensor development

Chapter 5 Optical fibre sensor for ammonia detection

5.1 Introduction

This chapter describes the fabrication of ammonia sensors based on U-shape and LPG optical fibre sensors. First, the evaluation of the tetrakis-(4-sulfophenyl) porphyrin (TSPP) dye itself, afterwards, the diazonium resin (DAR) synthesis and the DAR/TSPP coating deposition on a glass substrate are described. Then the coating was applied to an absorbance-based U-shape OFS. Next, the procedure of the visible range LPG fabrication and its advantages are shown. A novel conception of the visible range LPG incorporated with sensitive dye is demonstrated empirically with two different sensing mechanisms. Based on the complex nature of the refractive index, a hypothesis of the visible LPG response is proposed.

Ammonia is a Nitrogen trihydride compound with the chemical formula NH_3 . Ammonia is a colourless gas with a specific caustic odour. It easily dissolves in water and forms Ammonium hydroxide, NH_4OH [288]. When diluted in water, ammonia tends to deprotonate yielding ammonium ion and hydroxide:



From the ecological point of view, ammonia is one of the nutrients upon which the quality of the aquatic ecological system heavily depends. Ammonia naturally occurs in the environment as a result of the bacterial decomposition of organic matter. Ammonia is involved in the nitrogen cycle and it is a huge factor in phytoplankton growth [289]. Nowadays higher ammonia concentration occurs in Nature because of human activity from fertilizers and waste [290].

The range of concentrations strongly varies in natural waters. For instance, the lowest concentrations of NH_3 can be found in the open ocean and unpolluted freshwaters in the range of tenth and hundreds of nano moles $\cdot\text{L}^{-1}$ [291]. In coastal and continental waters, the range increases up to single units of micromoles and in some places reaches millimolar levels due to extreme bioactivity or pollution [292, 293]. High levels of ammonia are toxic to fish [294] and it also increases the bacteria

population which means lower levels of dissolved oxygen. This leads to a decrease in species diversity [290].

There is a demand for a low cost and simple ammonia sensor with the ability to detect ammonia in coastal waters with LOD around $5\mu\text{M}$, in drinking water with LOD $\sim 15\mu\text{M}$ or at least for waste water management with LOD $\sim 500\mu\text{M}$. For developing such a sensor a dye called tetrakis-(4-sulfophenyl) porphyrine (TSPP) was chosen as it showed some encouraging results on an optical fibre previously as an ammonia gas sensor in absorbance based [295],[296] and LPG [297, 298] OFS configurations. A TSPP based ammonia in water sensor was demonstrated using a tapered OFS [186] and on an LPG [150]. It should be noted that these two sensors operated in distilled water only and operating in the seawater would need to overcome several challenges, such as the effect of salinity, pH and the presence of other ions. In addition, the sensor reported by *Tiwari et al.* [186] is not reversible without achieving acid-base equilibria, or by removing the TSPP from the TiO_2 matrix and reinfusing. In the sensor reported by *Korposh et al.* [150], during the sensing procedure the TSPP diffuses from the silica nanoparticle matrix, however, it is possible to reinfuse the TSPP for new measurements.

This chapter will examine TSPP dye in DI and seawater, describe DAR/TSPP interaction and deposition, the sensitivity of the DAR/TSPP film to absorption and a RI sensitive OFS.

5.2 TSPP dye examination

5.2.1 TSPP dye examination, introduction

The TSPP dye is a solid water-soluble compound with chemical formula 5,10,15,20-tetrakis(4-sulfophenyl) porphine, $\text{H}_2\text{P}(\text{PhSO}_3\text{H})_4$. The TSPP molecule comprises a hydrophobic porphyrin chromophore H_2P and four hydrophilic sulfophenyl substitutes, PhSO_3H_3 on the peripheries. The combination of these yields the unique properties of TSPP [299]. TSPP is also known for changing its optical properties in response to ammonia [300].

5.2.2 Materials and methods of the TSPP dye examination

To understand the TSPP's response to Ammonia, it was tested spectrophotometrically in DI, salt and seawater. DI water was obtained from a deioniser (PURELAB Option S/R, ELGA). Salt water was fabricated by adding a 3.5 weight % of pure NaCl (purchased from Sigma-Aldrich). Plymouth Marine Laboratory donated the fresh seawater samples. To perform the tests, a bulk solution of 50 μM of TSPP dye was made in each type of water sample. A stock solution of 10 and 100 mM Ammonium hydroxide was prepared by diluting with DI water from 28% Ammonium hydroxide solution (purchased from Sigma-Aldrich).

For the spectrophotometric examination of TSPP solutions, the setup shown in **Figure 5-1** was used. The setup consists of the DH-MINI Ocean Optics UV-VIS-NIR Fibre Optic Light Source connected directly to SMA-to-SM1 Fibre Adapter with $\varnothing 1/2$ " UV Fused Silica Plano-Convex Uncoated Lens, which is mounted into a Thorlabs' CVH100/M cuvette Holder. The cuvette holder is connected via an identical SMA-SM1

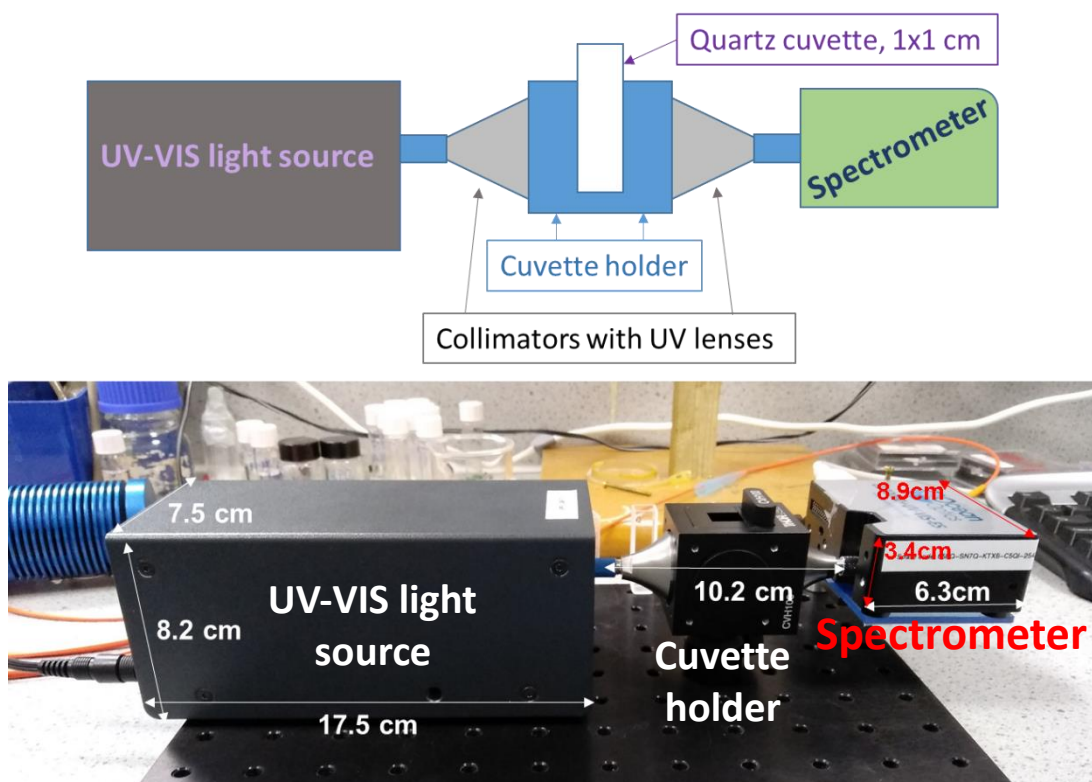


Figure 5-1 UV-VIS spectrophotometry set-up and photo

connector to Ocean Optics' USB-2000 spectrometer, which is connected via USB interface to a PC with Ocean Optics Spectra Suite software. In the cuvette holder, a 3500 μL parallel-sided 12.55 x 12.65 x 44.5mm Quartz Macro cuvette with a 10 mm optical path (10x10 mm inner dimensions of the square sides), is placed.

For each measurement, 1.4 ml of the examined TSPP solution was placed into the cuvette, and the absorption spectra were taken. Afterwards, the appropriate amount of higher concentration Ammonium Hydroxide stock solution was added to each TSPP solution in order to achieve the desired NH_4OH concentration. For instance, 14.14 μL of 10mM stock was added to 1.4 ml 50 μM TSPP in DI water solution to achieve 100 μM NH_4OH concentration. The absorption spectrum of the resulting substance was acquired after 1 hour. Then using the 100 mM stock, the concentration of the ammonium hydroxide was similarly increased to 1 mM. Again, the cuvette was left for one hour for the reaction to complete and the absorption spectrum was acquired. The one hour time interval was determined empirically as the time after which the absorption spectra do not change.

5.2.3 TSPP dye examination results

The absorption spectra of TSPP solution in DI water, salt water and seawater, exposed to 0.1 and 1mM ammonium hydroxide are shown in **Figure 5-2**. The largest response to ammonia can be observed in DI water, while there is no response of the TSPP in seawater, moreover the pure TSPP's spectra in seawater coincides with that in salt water exposed to 1 mM ammonium hydroxide.

In the case of DI water, **Figure 5-2a**, the pure TSPP (black curve) has a strong absorption peak reaching 1.79 at 645 nm, the second strongest peak with ~ 1.5 absorbance can be seen below 450 nm. Some weaker peaks can be observed at 594, 50 and 490 nm with 0.5, 0.18 and 0.22 absorbance respectively. After the exposure to 100 μM of ammonium hydroxide (red curve), a significant reduction of the main peak (at 694 nm) can be seen from 1.79 to 1.62. The 490nm peak shrinks as well, from 0.22 to 0.16. In the area around 515 nm a new peak arises increasing from 0.09 to 0.11. After exposure to 1 mM ammonium hydroxide (black line), the spectral picture differs significantly. The 645 nm peak disappears almost completely and shifts toward

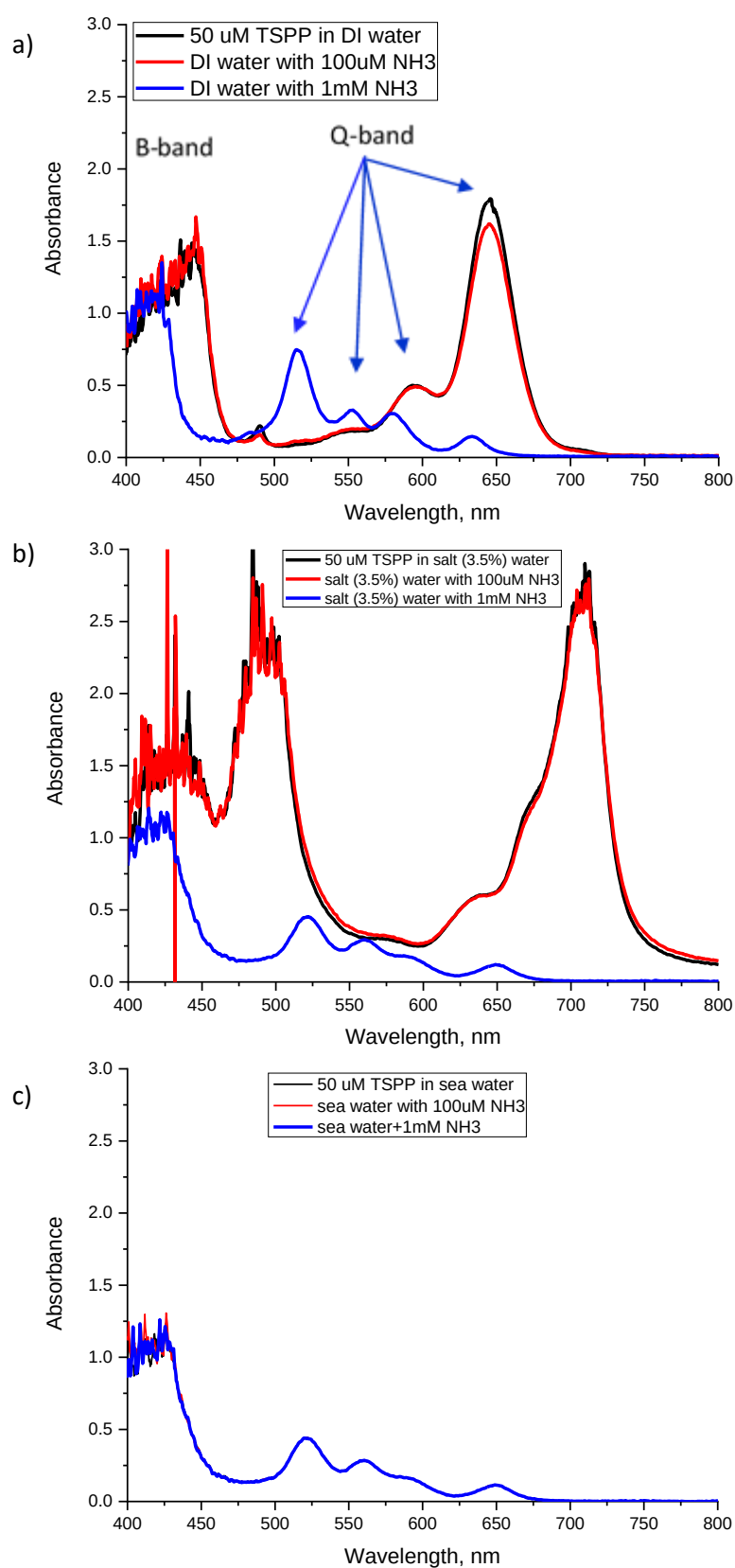


Figure 5-2 Absorption spectra of the 50 μM TSPP solution exposed to 100 μM and 1 mM ammonium hydroxide in: a) DI water; b) 3.5% salt water; c) seawater. Where the black curve represents the pure 50 μM TSPP in each medium, red and blue curves represent the 100 μM and 1 mM NH_4OH concentrations respectively.

the lower wavelengths, while the 515 nm peak rises to 0.75. The 595 nm peak shows a similar trend, by shrinking and shifting to the left. In addition, a clear 550 nm peak appears with a 0.33 absorbance value. The peak below 450 nm shifts even further towards the UV range.

The TSPP's response in salt water follows similar trends, however, the initial TSPP peaks are located differently, and the absorbance overall is much higher. The response to the 100 μM ammonium hydroxide concentration is significantly weaker, but still can be observed. The initial salt water TSPP peaks of ~ 709 and ~ 490 nm both slightly fall, while the absorption in the range from 515 to 625 nm increases. In the case of 1 mM NH_4OH concentration, the two main absorption peaks almost completely disappear, while the 560 nm peak remains on the same absorbance value, forming however a small peak which wasn't seen in two previous curves, due to high absorption levels. Three more peaks can be seen, the strongest one at 520 nm with 0.45 and two weaker ones, at ~ 590 and ~ 650 nm with 0.17 and 0.12 absorbance values respectively.

In the case of seawater, there is no significant difference between all three curves and the 50 μM TSPP with 1 mM ammonium hydroxide in salt water.

5.2.4 TSPP dye examination discussion

The intensity and colour of porphyrins depend on their highly conjugated π -electron system [301]. There are three main regions on their electronic spectra, Q, B (also known as Soret) and N bands. The Q band occurs mainly in the visible range. B and N bands are close to each other and occur closer to the UV region [301].

The absorption spectrum of porphyrins is successfully explained in terms of the so-called "four-orbital" model by Martin Gouterman [302]. Two highest (B) occupied and two lowest (Q) unoccupied orbitals, π and π^* respectively. The vibration of those Q and B transition dipoles give the Q band (645, 594 and 550 nm **Figure 5-2a**, black and red curves) and the B band (~ 443 nm black and red curves, ~ 420 nm blue curve) in the UV region.

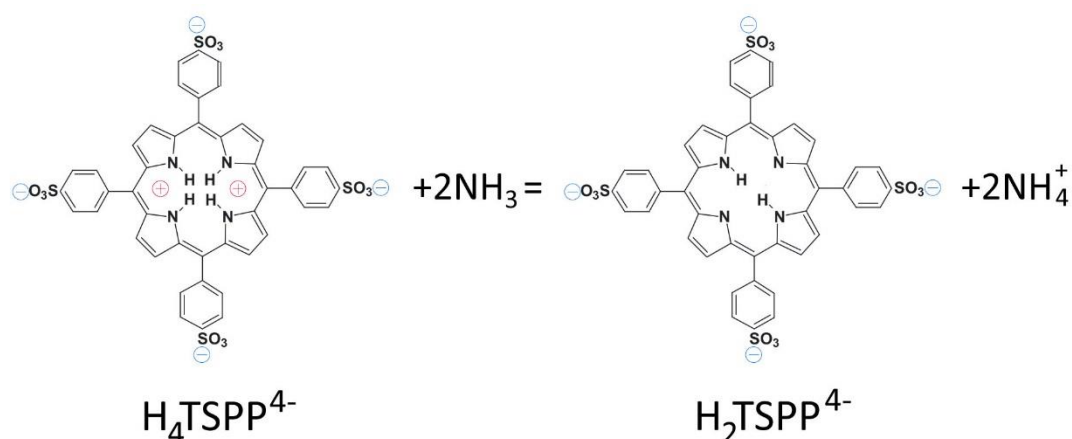


Figure 5-3 TSPP – ammonia interaction schematic

Several things can influence the transition dipoles, which would affect the absorption spectra of the TSPP. These are the solvent effect, redox reaction, protonation of the core nitrogen atoms' metal anion, π - π electron interaction, structural changes and mutual interaction among the porphyrin molecules (aggregation) [303]. The nature of the Ammonia sensing by the TSPP can be explained by deprotonation of the pyrrole ring by NH_3 molecule and formation of ammonium ions, **Figure 5-3**, which disrupts the *J*-aggregation and can be observed as a spectral change [303, 304]. As can be seen from **Figure 5-2a**, in the case of a higher concentration of Ammonia (1mM – blue line) the Q band transforms overall, and the weakest absorption peak of the Q band in the case of DI water (which was barely seen), becomes the strongest at 515 nm in the case of 1 mM NH_3 concentration, - blue curve. The blue curve clearly shows 4 peaks of the Q band. The TSPP spectral change correlates well with the study of *Sheinin et al.* [303], which describes 4 coupling equilibria of TSPP depending on the pH. Thus the TSPP's light absorption depends on the resulting type of protonation centres in water, $[\text{H}_4\text{P}^{++}](\text{H}_2\text{O})_2$; H_4P^{++} and H_3P^+ and the aggregation of the TSPP as a result [303].

Figure 5-4a shows the visible absorption spectrum of the TSPP (Q band) solution in water, from deprotonated to fully protonated states (in pH range 2 - 8). Thus, the TSPP is highly pH-dependent, which could be the reason why it is not sensitive to ammonia in seawater (**Figure 5-2c**), which is well buffered at pH ~ 8 . As free ammonium, being a base, affects the pH this may raise concerns of TSPP usage as a

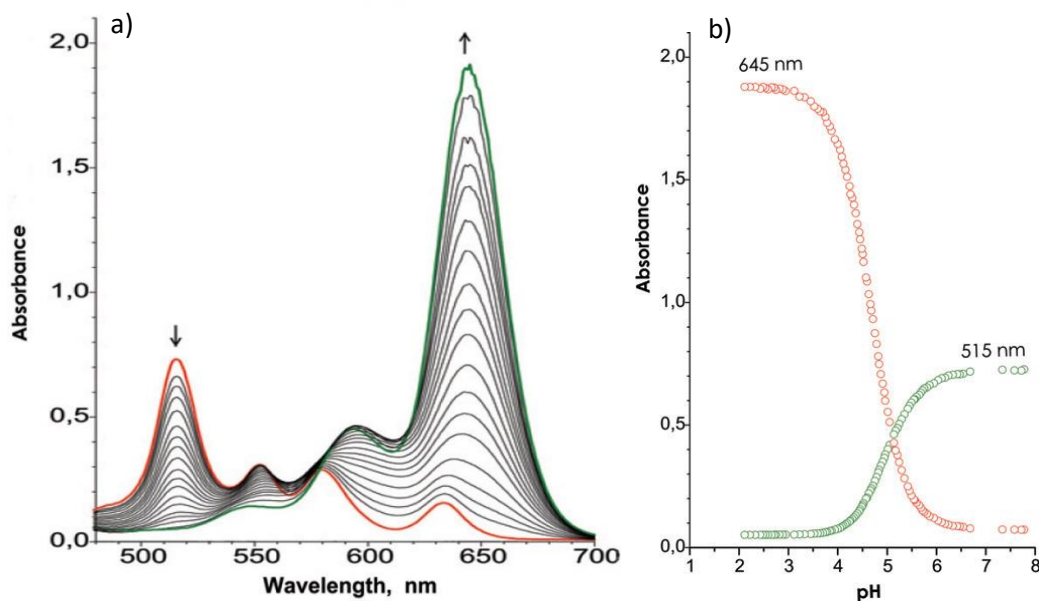


Figure 5-4 **a)** Visible spectra of 1.23 mM TSPP in water with 2-8 pH range, $\downarrow$ $H_2P(PhSO_3^-)_4$, $\uparrow$ $[H_4P_2+(PhSO_3^-)_4](H_2O)_2$; **b)** 645 and 515 nm absorbance values in the pH range from 2 to 8. [293]

sensitive dye, however, the TSPP response to ammonia is stronger than those to the pH only, especially in the bounded state, which was shown by Korposh et al. [150].

As can be seen from the TSPP sensing results described in **Figure 5-2 a** and **b** and the spectral behaviour of the TSPP's protonation, shown in **Figure 5-4** a reasonable way to track the sensitivity of the TSPP will be the ratio of the lowest and highest absorption peaks of the Q band (645/515 nm in case aqueous TSPP), as they move in opposite directions.

From the results presented in **Figure 5-2c**, it is clear that using TSPP as a sensing compound for ammonia in seawater would be challenging. Some properties of the seawater clearly interfere with the TSPP's ability to respond.

5.2.5 TSPP dye examination conclusion

The TSPP dye examination in aqueous solutions has shown its sensitivity to ammonia in DI and salt water, however, it was found to be not sensitive to ammonia in seawater. Moreover, the absorption spectra of the TSPP dye in case of seawater and salt water with 1 mM NH_3 concentration appears to be fairly similar. This makes a seawater sensor development based on TSPP more complicated and requires additional efforts to overcome this issue.

5.3 Immobilized TSPP on a glass surface

5.3.1 Introduction to the immobilized TSPP

The immobilized TSPP would differ from that in a free solution due to factors, such as bonded parts of the molecule, limited mobility, and reactivity.

The sensitivity of TSPP to ammonia has been used on optical fibre sensors modified with TSPP, to detect ammonia concentration in air [295-298] and in water [150, 186]. However, in the case of water sensing, the repeatability and coating stability was poor. For instance in work presented by Korposh et al. [150] TSPP being washed out from the sensitive coating in the presence of ammonia in water is described. The layer-by-layer electrostatic interaction deposition did not provide strong enough binding of TSPP to the fibre's surface. To solve this issue a new approach was proposed by collaborator in Japan, Prof. Seung-Woo Lee from the University of Kitakyushu, Kitakyushu, Japan. Prof Lee suggested using a DAR to generate covalent bonding between the resin and TSPP.

5.3.2 Materials and methods of DAR/TSPP on a glass substrate

The DAR was synthesized as described by Son Y-A et al [305]. Briefly, as follows: a 20ml glass vial was cooled in an ice-water bath, 1 g of 4-diazodiphenylamine sulphate (purchased from Sigma-Aldrich) was added into the 20ml flask. Then 6ml of concentrated sulfuric acid (95-97%, from Sigma-Aldrich) was added dropwise with stirring. Afterwards, 0.6g of paraformaldehyde (purchased from Sigma-Aldrich) was added in batches at a temperature of 0-5 °C. The reaction was continued for 3 h with gentle stirring via a magnetic stirrer, the temperature was monitored by a thermocouple and kept within 0-5 °C throughout the whole duration of the reaction. After 3 hours, the 20 ml vial content was poured carefully into 30ml of the ice water (in a 50ml flask). Then 1g of zinc chloride (from Sigma-Aldrich) was added to precipitate the diazonium resin as a $1/2 \text{ ZnCl}_2$ Complex. The diazo resin with HSO_4^- as

a counteranion is water-soluble; its complex with ZnCl_2 is insoluble in water. After the precipitation was filtered and the prepared diazonium resin was dried in a vacuum and kept in the dark.

As the DAR is positively charged and the TSPP is negatively charged, it is possible to deposit them via the layer-by-layer technique using the ionic interaction between them. DAR/TSPP multilayers were deposited on a glass substrate as follows. For deposition purposes, 1 wt% of DAR and 1 mM TSPP solution aqueous solutions were prepared. First, the substrate was immersed in 1% KOH water/ethanol solution for 20 min in order to create negatively charged hydroxyl groups on the surface of the glass. Then it was washed by DI water, dried by nitrogen flow and immersed in 1%

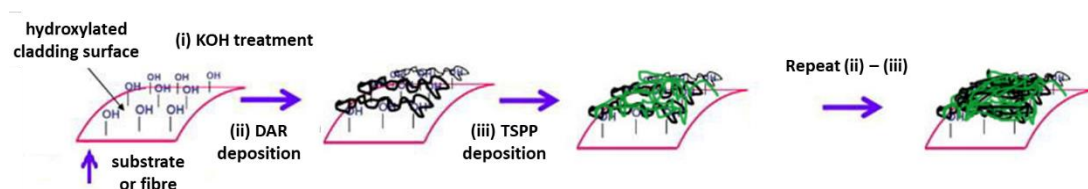


Figure 5-5 DAR/TSPP deposition schematic illustration

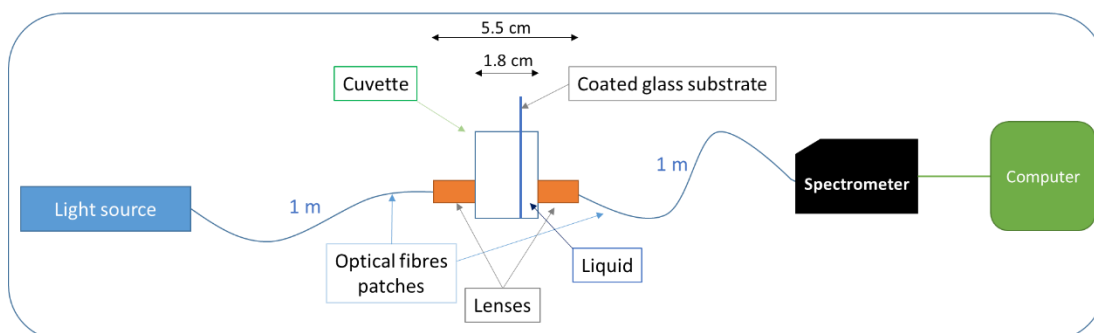


Figure 5-6 Glass substrate absorbance measurement setup.

DAR for 12 minutes. Positively charged DAR interacts with the hydroxyl group depositing a thin film layer onto the glass surface. This was followed by washing and drying. After, a TSPP layer deposition followed by immersion in 1 mM TSPP with $\text{pH} \sim 1.5-2$ (lowered by H_2SO_4 to enhance the electronegativity of the TSPP) for 12 min. Steps 2 and 3 were repeated to achieve the desired number of layers, in this case, five, **Figure 5-5**. The number of layers was chosen empirically by observation of the transmission spectra, taking into account the absorbance value.

After the nitrogen flow drying, followed by each layer, deposition and absorption spectra were taken using a setup shown in **Figure 5-6**. The setup consists of the HL-2000 Ocean Optic Halogen light source connected via Thorlabs' 1 meter M92L01 SMA-SMA Fibre Patch Cable to Ocean Optics' CUV-UV Fibre Optic cuvette Holder (which was used without the cuvette in this case). The cuvette holder is then connected by the same 1 meter M92L01 SMA-SMA Fibre Patch Cable with Ocean Optics' HR-4000 spectrometer, which is connected via USB interface to a PC with Ocean Optics Spectra Suite software. In the cuvette holder a homemade slide holder was placed in which the coated quartz slide was placed, so the light propagates through the slide.

The 5 bilayer DAR/TSPP substrate was tested for ammonia response both in air and in water. The response to ammonia concentration in air was tested using the setup shown in **Figure 5-7**. The setup is the same as was used for deposition absorbance spectra (**Figure 5-6**), with the only difference that the cuvette-substrate holder was confined in a sealed vessel (vessel's volume $\sim 3\text{e-}3\text{ m}^3$) with two valves on the top of the vessel. To create an ammonia vapour inside the sealed container a 0.01% ammonium hydroxide aqueous solution droplet was dropped through the valve. Immediately after that, the valve was sealed.

For the DAR/TSPP substrate's absorbance measurements in aqueous solutions, the same setup, as described in **Chapter 4, Figure 4-8** was used.

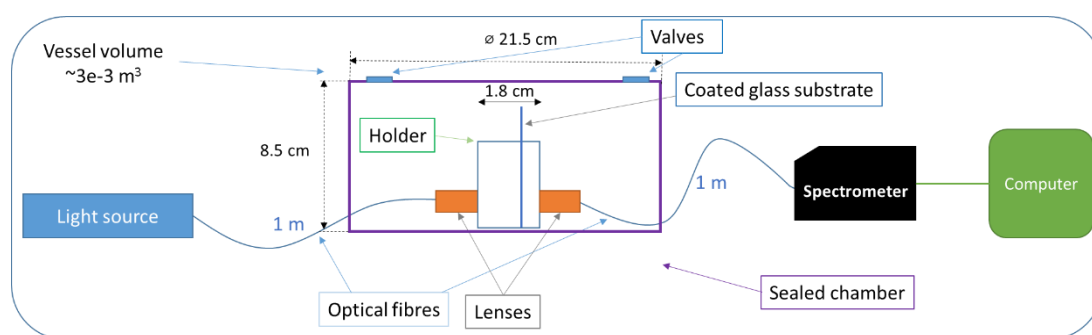


Figure 5-7 setup of the DAR/TSPP substrate response to gas phase ammonia

5.3.3 Results and discussion of the DAR/TSPP glass substrate in air

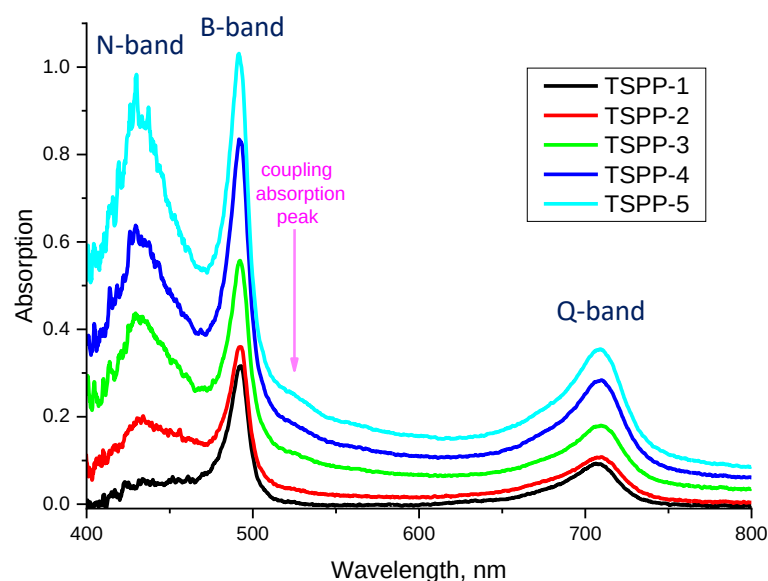


Figure 5-8 Absorption spectra of a glass substrate in air after each DAR/TSPP layer deposition;

The absorption spectra after each deposition cycle of DAR and TSPP are presented in **Figure 5-8**. The black curve corresponds to the first bilayer, red to second, yellow to third, dark blue to fourth and finally, the light blue line shows the absorption of the fifth deposited layer. As can be seen from the described above **Figure 5-8**, with each deposited layer the absorbance overall is growing and there are three clear absorbance peaks. At the last TSPP layer, the absorbance peaks are as follows: the weakest can be seen at ~ 708 nm with an absorbance of ~ 0.35 , the strongest at ~ 491 nm with ~ 1.0 and around 430 nm with ~ 0.9 absorbance value. The weakest one at ~ 708 nm corresponds to the Q band, the ~ 491 nm and ~ 430 nm to B and N bands respectively.

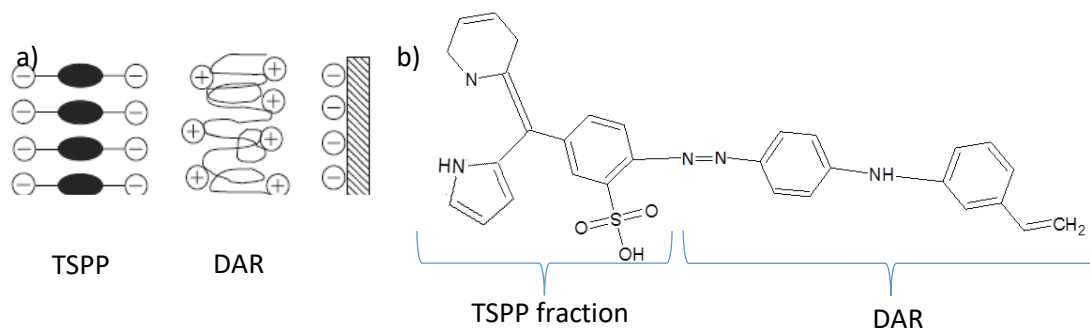


Figure 5-9 a) DAR/TSPP deposition schematic; b) alleged diazo covalent coupling between the DAR and TSPP

During the electrostatic interaction of the DAR and TSPP, a covalent diazo phenol coupling bond may occur between the phenyl ring of the TSPP and DAR, **Figure 5-9**, in the case of which an absorption peak occurs around 520-570 nm [305]. Exactly such a peak is clearly seen in **Figure 5-8**, in the form of a shoulder, around 525 nm (magenta arrow points on it), on the 4th and 5th TSPP layers' absorption spectra (dark blue and the light blue lines respectively). Thus, it can be speculated that diazo binding occurs between the DAR and TSPP, **Figure 5-9b**.

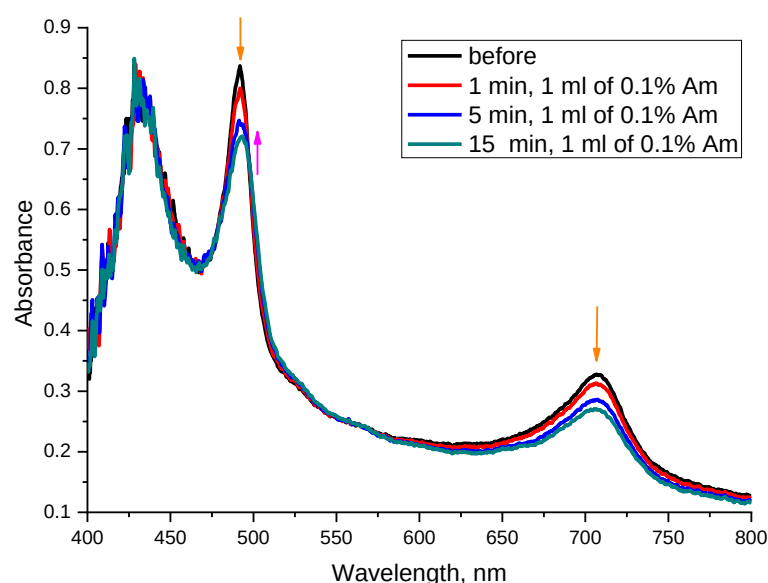


Figure 5-10 Spectrophotometric response of the 5-bilayer DAR/TSPP glass substrate to a 1 ml droplet of a 0.1% NH_3 aqueous solution into a 3.3l cylindrical gas chamber

The response of the 5-bilayer DAR/TSPP glass substrate to a 1 ml droplet of a 0.1% NH_3 aqueous solution placed into a 3.3l cylindrical gas chamber can be observed in **Figure 5-10** where the black curve represents the absorbance of the 5-bilayer DAR/TSPP substrate in air before the 1ml of 0.1% NH_3 droplet; the red curve represents the absorbance of the substrate 1 minute after the droplet; blue and green lines show the absorbance after 5 and 15 minutes respectively; orange and magenta arrows show the direction of absorbance change. As can be seen, the 708 and 491 nm absorbance peaks decrease significantly, however, the absorbance values around 500 nm increase after interaction with ammonia in the air.

By taking the ratio of each absorbance spectrum after the droplet to the previous absorbance spectrum, one can see the amount and direction of change

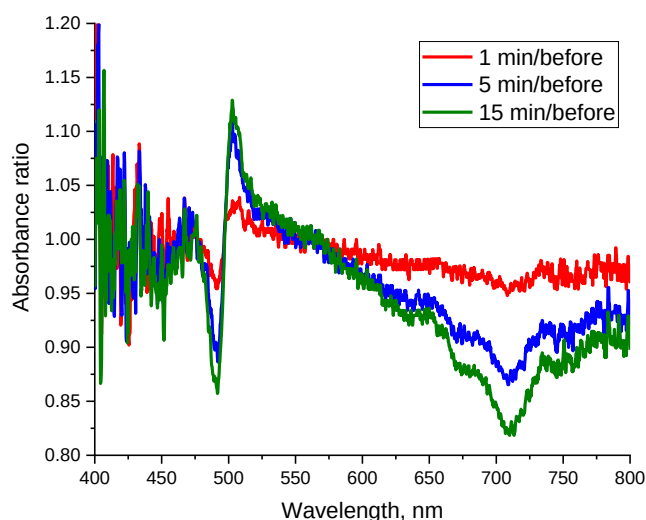


Figure 5-11 Ratio of the absorbance spectra taken after 1, 5 and 15 minutes after the 1 ml 0.1% NH_3 droplet to the absorbance spectrum before.

across the spectrum, **Figure 5-11** where the red line represents the ratio between absorbance spectra after 1 minute of the 1ml 0.1% NH_3 droplet and before the droplet. Blue and green lines show such ratios after 5 and 15 minutes respectively. As can be clearly seen, the biggest decline of the absorbance value happens at ~ 708 - 712 nm, followed by a decline at ~ 491 nm. On the other hand, a significant increase of the absorbance value occurs at ~ 502 nm. Hence, the best way to monitor the ammonia response in the gas phase would be the ratio between ~ 708 and 502 nm.

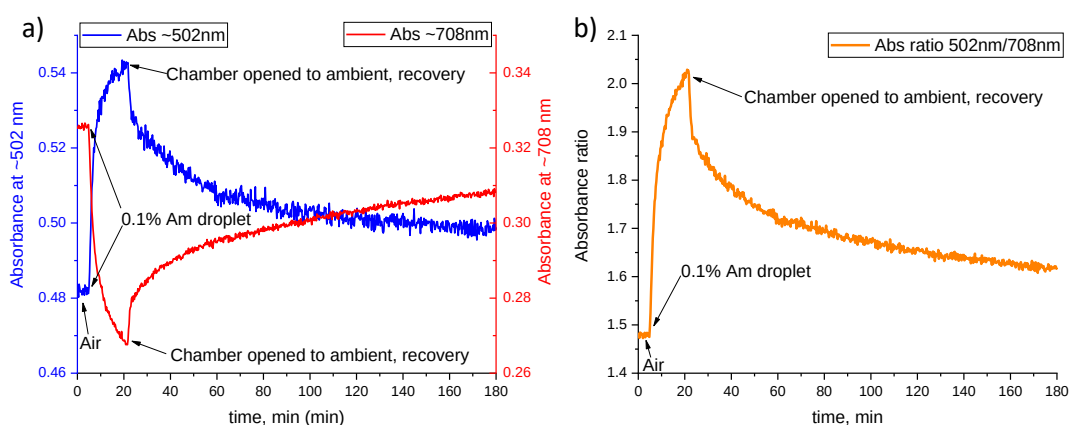


Figure 5-12 a) Absorbance over time at ~ 502 nm (blue curve) and ~ 708 nm (red curve), of the 5-bilayer DAR/TSPP substrate during its exposure to ammonia and recovery in ambient air; b) 502nm and 708 nm absorbance values ratio over time, of the 5-bilayer DAR/TSPP substrate during its exposure to ammonia and recovery in ambient air.

The recovery of the substrate's absorbance properties is much slower than the response, as can be seen in **Figure 5-13**, where the black curve represents the absorbance spectrum immediately after the chamber was opened to the ambient. Red, blue and green curves – absorbance after 10, 60 and 160 minutes in ambient respectively. **Figure 5-12a** shows the change of the absorbance values at ~502 nm (blue line) and ~708nm (red line) over time. As can be seen, immediately after the 0.1% ammonia solution droplet entered the chamber, absorbance at ~502 nm increases while absorbance at ~708 nm decreases significantly. Then the change rate slows down exponentially. At the 20th minute, the lid from the top of the gas chamber was removed, exposing the substrate to the ambient. An immediate change in the opposite direction can be observed on both wavelengths' absorbance, followed by a very steady recovery. The whole recorded recovery time is ~160 minutes, to not fully undo the response after 20 minutes only. The slowness of the recovery in the air can be explained by the “stickiness” of ammonia due to the polar nature of the ammonia molecule [306]. Therefore, in the case of water measurements, the picture can be different.

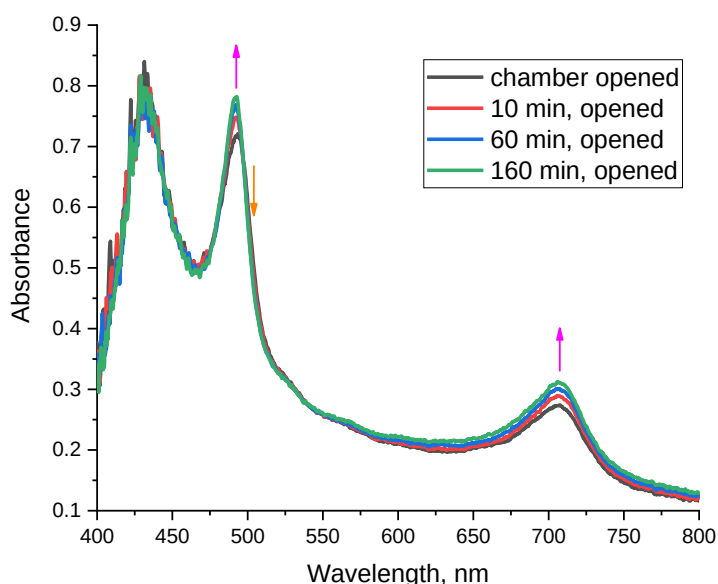


Figure 5-13 5-bilayer DAR/TSPP glass substrate recovery after the gas chamber's top was opened to the ambient. Where the black curve represents the absorbance spectra immediately after the chamber was opened to ambient. Red, blue and green – absorbance after 10, 60 and 160 minutes in ambient.

5.3.4 Results and discussion of the DAR/TSPP glass substrate in water

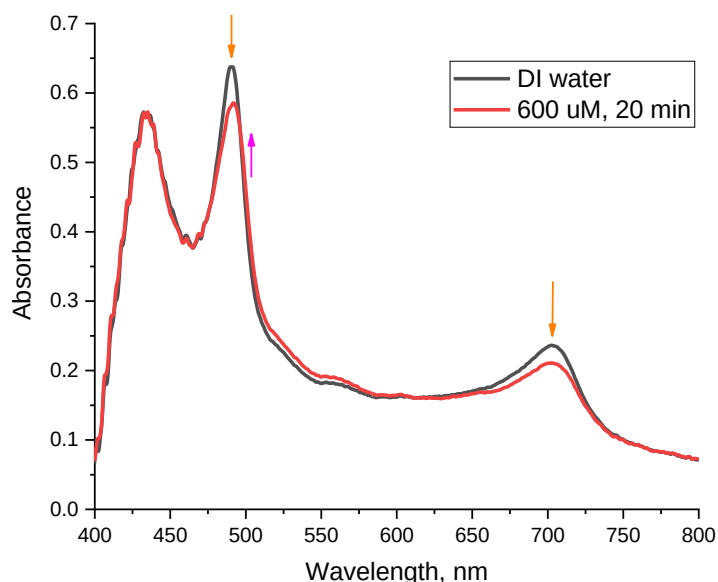


Figure 5-14 absorbance spectra of 5-bilayer DAR/TSPP glass substrate in DI water (black curve) and in 600 μM NH_4OH solution after 20 min of immersion (red curve).

The same 5-bilayer DAR/TSPP substrate was tested in water for response to ammonium hydroxide, **Figure 5-14**, where the black curve represents the absorbance spectrum of the substrate in DI water; the red curve is the absorbance spectrum in 600 μM ammonium hydroxide solution 30 minutes after immersion. As can be seen, the response trend is the same as it was in the case of ammonia in the air. However, some differences can be noted, such as a slight shift of the absorbance peaks, ~ 705 nm instead of 708 nm, and 490 nm instead of 491 nm. The other distinction is a difference in a bigger spectral range in absorption after 500 nm.

By taking the ratio between the absorbance spectrum in water and 600 μM ammonium hydroxide solution, one can observe the changes across the spectrum, **Figure 5-15**. **Figure 5-15** shows that the biggest decrease across the spectrum occurs at ca. 705 nm, followed by ca. 490 nm. The biggest increase of the absorbance occurs at ca. 504 nm, however, the amplitude of the increasing part of the spectrum in water is wider than the gas phase response. Hence, the best way to monitor the response

of DAR/TSPP coating to ammonia in water would be the ratio between ~ 705 and 504 nm.

5.3.5 DAR/TSPP glass substrate, conclusion

DAR/TSPP coated glass substrate was examined for ammonia response in DI water. The best absorption wavelengths to detect the DAR/TSPP response to ammonia were determined. The biggest absorbance decrease was detected at ca. 705 nm, while the biggest absorbance increase at ca. 504 nm. The usage of ratio between absorption or intensity values at ca. 705 and ca. 504 nm was proposed for ammonia detection in DI water.

5.4 DAR/TSPP on a U-shape optical fibre sensor

5.4.1 Introduction to the DAR/TSPP coated U-shape OFS

A U-shape optical fibre sensor was chosen as a platform for aqueous ammonia sensing. The U-shape fibre is extremely sensitive, as it has a strong evanescent field, it is easy to be deployed repeatedly for layer by layer deposition, and is convenient

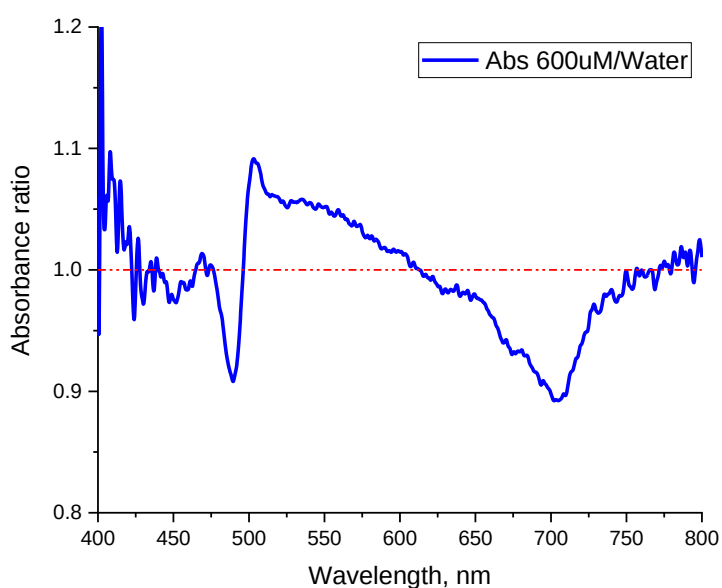


Figure 5-15 ratio of the absorbance spectra taken in DI water and 30 min in $600 \mu\text{M}$ ammonium hydroxide aqueous solution

to handle in real-time application. The fabrication of the U-shape sensor is described in detail in **Chapter 3 section 3.2.2 [307]**.

5.4.2 Materials and method for DAR/TSPP coated U-shape OFS

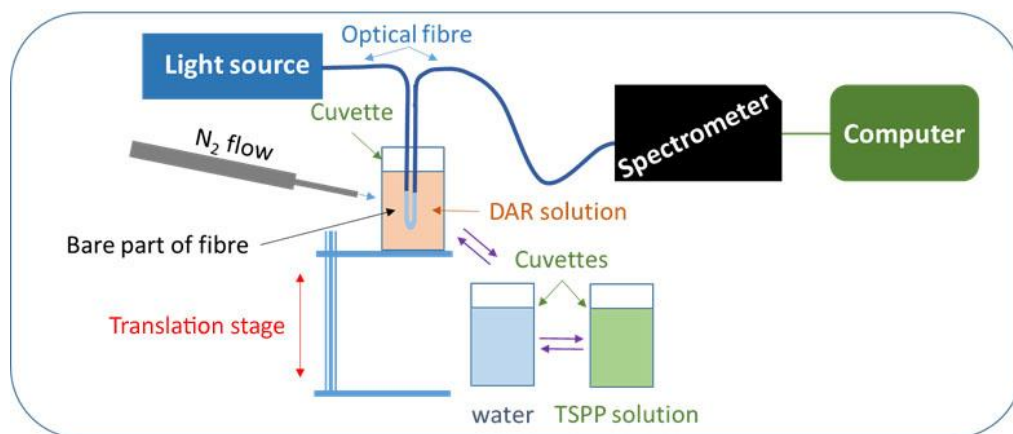


Figure 5-16 Setup of the layer-by-layer deposition on a U-shape optical fibre sensor

The DAR/TSPP coating on a U-shape optical fibre sensor was deposited via layer-by-layer deposition method, in the same way, with the same chemicals' concentrations as on the glass substrate and is described in **section 5.3.2**. The only difference is the number of layers, which will be mentioned in each specific case.

The DAR/TSPP deposition setup on a U-shape OFS is shown in **Figure 5-16**. The setup consists of a Halogen Light source (HL-2000, Ocean Optics) which feeds the U-shape OFS made out of multimode optical fibre (FT200UMT TECS, Thorlabs). The other end of the fibre is connected to an Ocean Optics HR 4000 spectrometer, which is connected to a computer. The bare part of the fibre is suspended over a moving stage, which accommodates the cuvette in such a way, that the bare part of the fibre is completely immersed into the liquid when the translation stage is up, while completely out of the cuvette when the stage is down. The fibre is first immersed into 1% KOH water/ethanol solution for 20 min in order to create negatively charged hydroxyl groups on the surface of the glass. After the stage is moved down, the fibre is rinsed in DI water, and dried by N₂ flow. Then a cuvette with 1% DAR solution is placed onto the translation stage and lifted up to cover the bare part of the fibre for

12 minutes. Afterwards, the stage goes down, the fibre is rinsed in water and dried by N_2 flow. In the next step, a cuvette with 1 mM TSPP solution was placed on to the translation stage, which was lifted to cover the bare part of the fibre for 12 minutes. After the fibre was rinsed in DI water and dried by N_2 flow. The DAR and TSPP steps were repeated until the required number of bilayers was achieved. Fibre bending losses due to stage movements are negligible, this was observed empirically during the fabrication procedure.

For testing of the DAR/TSPP coated U-shape fibre a setup shown in **Figure 5-17** was used. From the one side multimode fibre (FT200UMT, Thorlabs) was connected to the Ocean Optics HL-2000 halogen light source which gives a wide VIS/IR spectra.

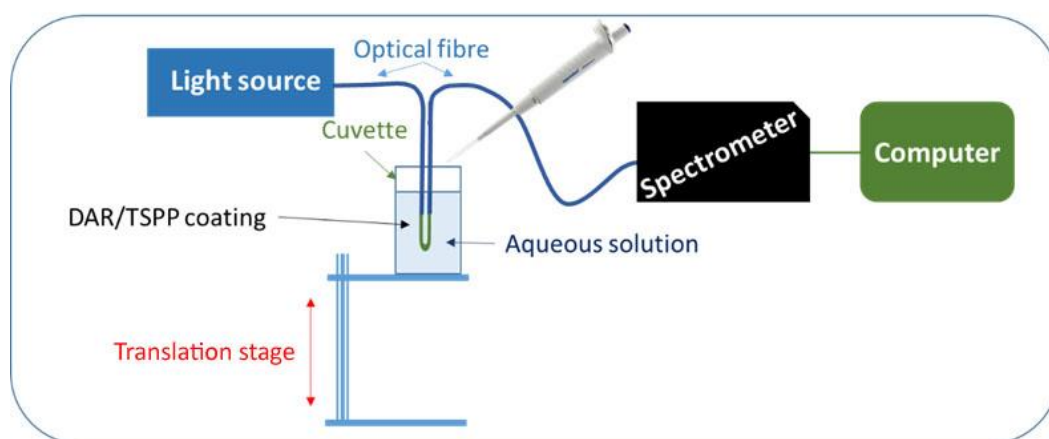


Figure 5-17 DAR/TSPP coated U-shape fibre testing setup

The coated part of the fibre was immersed in a vessel, which was situated on a translation stage. The rising concentrations of NH_4OH were achieved by dropping an appropriate amount of higher stock concentration. For instance, if the amount of water in the testing cuvette is 10 mL, and the required concentration of NH_4OH is 10 μM , then adding 101 μL of 1 mM NH_4OH stock solution, would yield a resulting solution of 10 μM . In cases when the solution was changed back to DI water, the translation stage was lowered and the cuvette with ammonia solution replaced with cuvette with DI water, and the stage was lifted back to the same height. The other end of the optical fibre was connected to an Ocean Optics HR 4000 spectrometer, which was connected to a computer for data acquisition.

5.4.3 Results and discussion of the DAR/TSPP coated U-shape OFS

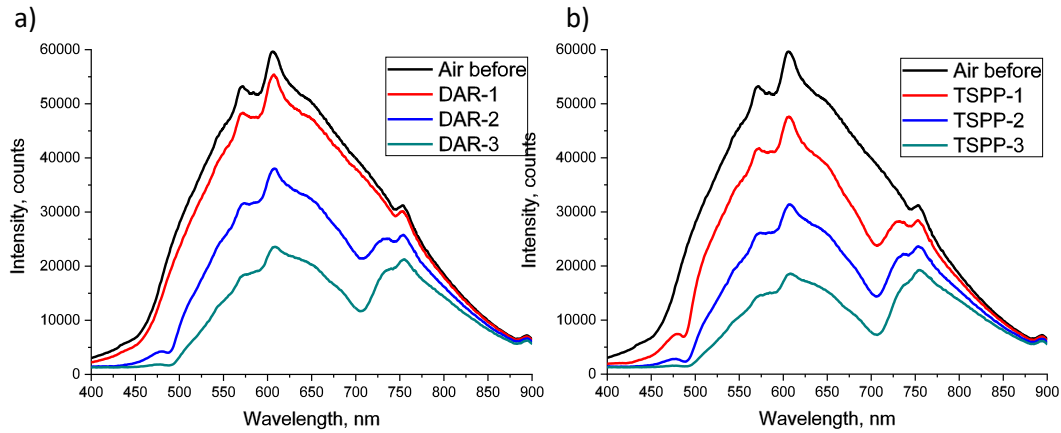


Figure 5-18 a) transmission spectra of U-shape OFS taken in air after each deposited DAR layer; b) transmission spectra of U-shape OFS taken in air after each deposited TSPP layer;

The transmission spectra of a 3-bilayer DAR/TSPP U-shape OFS during the deposition procedure, taken after each deposition cycle of DAR and TSPP in air, are presented in **Figure 5-18 a** and **b** respectively. The transmission spectra were then converted to absorption using **Equation 4-2** taking the black curve (transmission in the air) as a reference, **Figure 5-19**. Where the black line represents the absorbance after the first layer of DAR (**Figure 5-19a**) and TSPP (**Figure 5-19b**) and the red and blue lines represent the second and third layers respectively. As one can see the deposition procedure is similar to that on a substrate (**Figure 5-8**), the first layer of DAR gives a wide, but weak absorption peak around 400 nm. After the first TSPP layer deposition (black curve on **Figure 5-19b**), three absorption peaks can be clearly seen, at ~431 nm (0.34 units), ~488 nm (0.5 units) and ~705 nm (0.21 units), corresponding to B and Q bands respectively. The next two deposited bilayers increase the absorption across the spectrum, however, in the regions of the mentioned peaks, the absorbance adds up much more significantly. After the final, third TSPP layer these three absorbance peaks reach 0.65 units for N-band (now ~438 nm), 1.2 units for B-band (now ~491 nm) and 0.72 units for Q band (~705 nm).

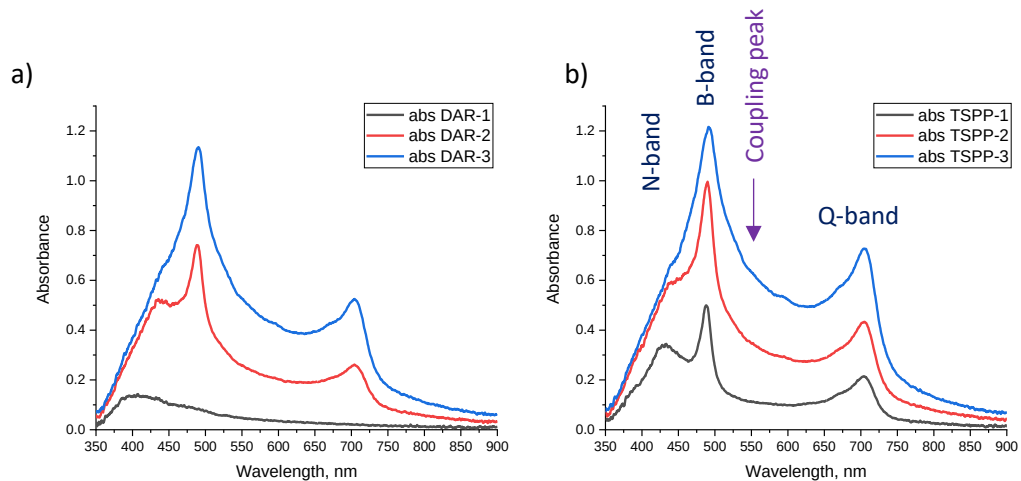


Figure 5-19 a) Absorbance spectra of U-shape OFS in air after each deposited DAR layer; b) Absorbance spectra of U-shape OFS in air after each deposited TSPP layer;

However the N and B peaks are merged, but still distinguishable. Also, the alleged DAR+TSPP coupling peak starts to emerge around 530 nm, but not as strong as observed on the substrate, which could be because of fewer deposited layers.

To detect NH_4OH using DAR/TSPP coated fibre the same wavelength ratio was applied as for the DAR/TSPP coated glass substrate, intensities value ratio at 706 and 504 nm. In **Figure 5-20** continuous ammonia response measurements with increasing

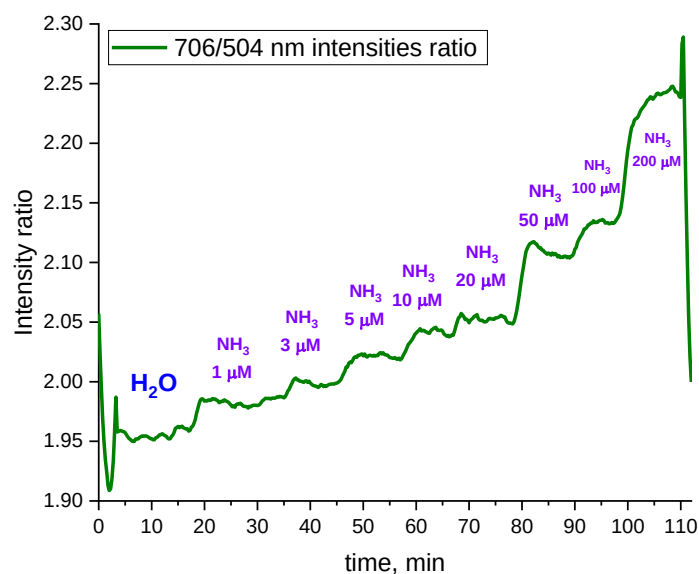


Figure 5-20 Ratio of intensity values of the DAR/TSPP coated U-shape fibre between 706 nm and 504 nm during steady increase of ammonia concentration from 0 to 200 μM

concentrations from 0 to 200 μM NH_4OH can be seen. After adding ammonia (each concentration), the ratio grows rapidly reaching equilibrium after ~ 5 min.

To perform linear fit from the data shown in **Figure 5-20**, the average and standard deviations of data were taken from each ammonia concentration level from the 4th minute until the next concentration was introduced. As the graph is not linear in the whole range, it was divided into two sections, 0-10 μM and 10-200 μM . A linear fit was performed on those, and LOD was calculated using **Equation (4-1)**, taking the intercept's error as a standard deviation. LOD yielding 3.01 μM and 25.94 μM respectively, **Figure 5-21**.

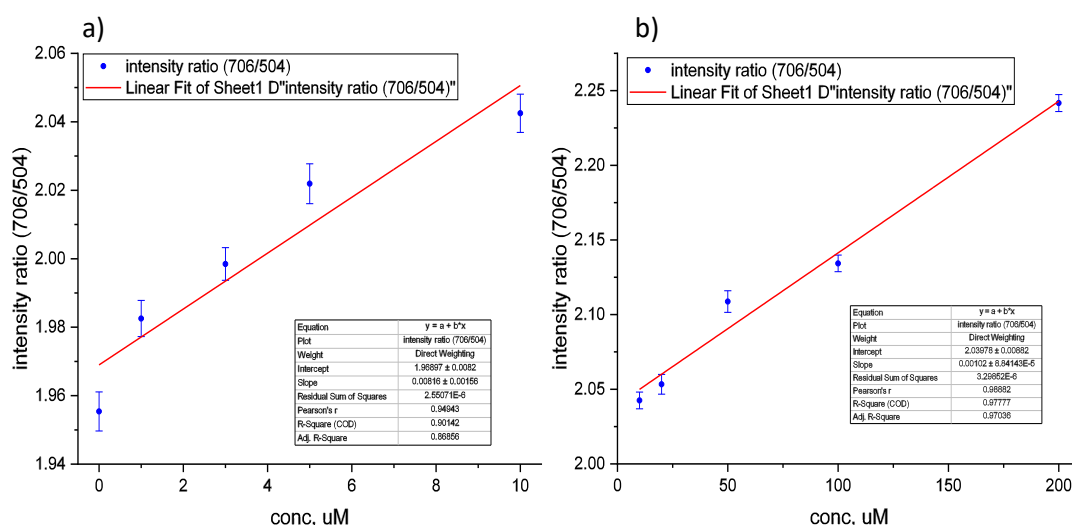


Figure 5-21 Linear fit of the DAR/TSPP U-shape OFS' performance shown in **Figure 5-20**, a) DR=0-10 μM ; b) DR=10-200 μM

The repeatability of the 3-bilayer DAR/TSPP U-shape OFS is shown in **Figure 5-22**. The ratio of the 706 and 504 nm intensity values grows in the water slowly, and immediately after the ammonia concentration is raised to 50 μM the ratio grows rapidly from ~ 1.87 to ~ 2.05 in ~ 8 minutes, then stabilises there for the rest of the measurement. Then, immersed in DI water, the ratio drops rapidly from 2.05 to 1.92 in ~ 5 minutes, and grows to 1.93 in the next 15 minutes. Exposed to 50 μM again, the ratio grows sharply to 2.05 again in ~ 4 minutes and stabilises there for the rest of the measurement. After water immersion, the ratio drops immediately to ~ 1.94 . Thus, the DAR/TSPP coating's response on a U-shape OFS is reversible and repeatable.

The 3 bilayers DAR/TSPP coated U-shape has been tested in seawater, however, it does not work as the attenuation bands disappear **Figure 5-23**. In **Figure 5-23a** one can see how the shape of the transmission spectrum changes and the attenuation bands disappear. In **Figure 5-23b** the 707 nm wavelength's intensity is plotted, while the fibre was immersed alternately in water, seawater and exposed to 200 μM of ammonia in the sea and DI water. The intensity value stabilizes in DI water, however, after immersion in seawater, the 707 nm intensity value rises rapidly, which means the attenuation band is getting weaker. After, it stabilizes in DI water but continues to grow in seawater in the same manner as previously, then stabilizes in DI water again. The next exposure to seawater continues the rising trend, at 110 minutes 500 μM of ammonia was added into the seawater, but with no sign of response. Then in DI water, the intensity value stabilizes, and after 10 minutes 200 μM ammonia was added. The response to that result is a small increase of the value, however much weaker than expected, before the seawater impacts on the sensor.

Hence the seawater has an irreversible effect on the DAR/TSPP coating. To overcome this issue a barrier coating should be added on top of the sensor, to prevent the seawater ions from reaching the sensitive coating.

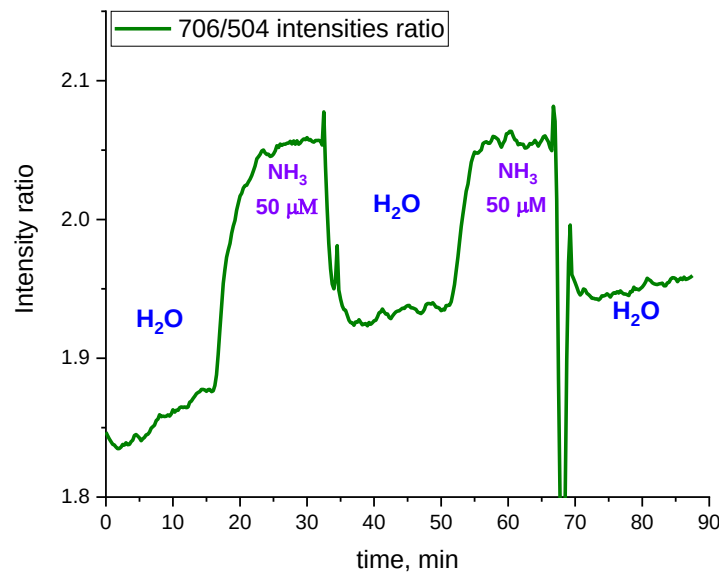


Figure 5-22 Repeatability of the 3-bilayer DAR/TSPP coated U-shape OFS

5.4.4 Conclusion of the DAR/TSPP coated U-shape OFS

In this section, the TSPP dye properties were studied in aqueous solution. A difficulty of the TSPP working in seawater was observed.

DAR synthesis is described, and a DAR/TSPP coating is performed and studied on a glass substrate. The DAR/TSPP coated substrate ammonia sensitivity were investigated both in air, DI water and seawater.

The DAR/TSPP coating was applied to a U-shape OFS. The sensitivity and repeatability to ammonia detection, of such a sensor were investigated in DI water, and in seawater. The seawater application requires further improvement to overcome the destructive effect of the seawater on the DAR/TSPP film.

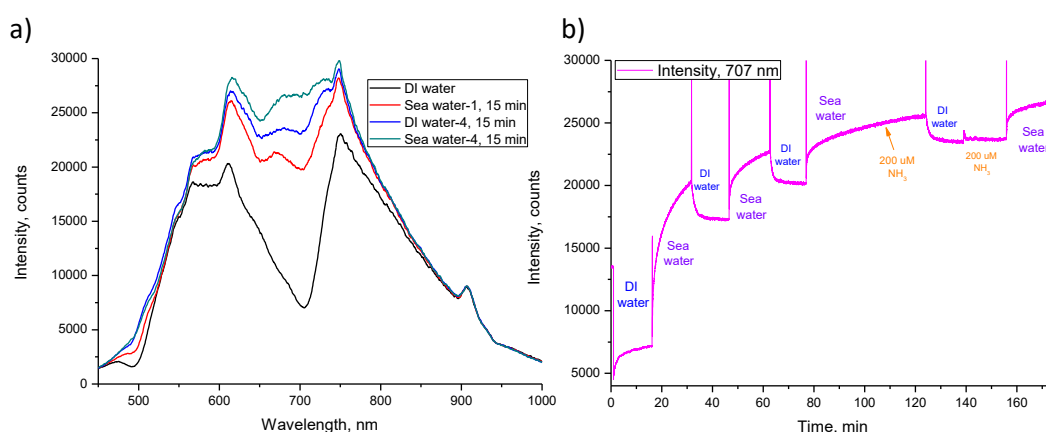


Figure 5-23 a) Transmission spectra of a three bilayer DAR/TSPP U-shape OFS tested in seawater, black curve shows the transmission spectra in DI water, red curve, - in seawater after 15 minutes, blue curve, - in DI water again, for 15 minutes, and the green curve shows the transmission spectra in seawater again after 15 minutes; b) The 707 nm intensity value over time, while immersed consequently in DI water and seawater.

5.5 DAR/TSPP Ammonia sensor for aqueous environment based on Long Period Grating Fibre operating in visible range

5.5.1 Introduction

The response LPG sensors are usually not intensity based in contrast to absorbance based OFS. This means LPG OFS does not suffer from intensity-based interferences, such as light source drift, water turbidity or bending losses. Therefore the usage of LPG instead of absorbance based sensors was possible is justifiable.

Long period grating sensors usually operate in the near-infrared and infrared regions. There were visible LPGs reported previously as a sensor in combination with surface plasmon resonance (SPR) [308, 309] but to the best of my knowledge not in combination with a sensitive dye.

The advantages of using a VIS LPG instead of an absorption band sensor:

- a) Light bleaching problem elimination. The coupled-out light into the sensitive coating is much less intense and is a narrow band (the range is limited to the attenuation modes). This makes the sensor more stable over time.
- b) VIS LPG offers the combination of RI and Absorbance as a sensing response instead of just Absorbance itself. Furthermore, the Absorbance band is narrow and tuneable which gives selectivity advantages.

As described in detail in **Sections 2.3.4** LPGs are sensitive to the surrounding refractive index, and this sensitivity is governed by Equation (5-2), [162]

$$\lambda_{cw} = \Lambda(n_{eff\ core} - n_{eff\ clad}^i) \quad (5-2)$$

Where λ_{cw} is the attenuation's central wavelength, Λ is the grating period, $n_{eff\ core}$ and $n_{eff\ clad}^i$ are the effective RI of the fibre's core of i -th cladding mode respectively.

The refractive index n is a complex value:

$$n = n_{Re} + ik_{Im} \quad (5-3)$$

Where n_{Re} , k_{Im} are real and imaginary components of the complex RI. The real part is responsible for phase change, the imaginary part is responsible for absorption. In other words, the imaginary part of the refractive index describes the attenuation of the electromagnetic wave in the medium [310];

The refractive index also depends on absorbance or extinction coefficient, which is governed by Kramers-Kronig relation [311]:

$$n(\omega) = 1 + \frac{2}{\pi} P \int_0^{\infty} \frac{\Omega k(\Omega)}{\Omega^2 - \omega^2} d\Omega \quad (5-4)$$

Where $k(\Omega)$ is the extinction coefficient, Ω is angular frequency variable over the whole integration range, $\omega=2\pi c/\lambda$ is the angular frequency, P is the Cauchy principal value.

Hence, the refractive index of a medium can be calculated from absorbance using **Equation**

(5-4). Knowledge of the RI of a particular coating can help to enhance the sensitivity of the LPG with the PMTP in the visible range.

5.5.2 Fabrication of the LPG with PMTP in the visible range

5.5.2.1 Background for visible LPG fabrication

The standard boron–germanium co-doped photosensitive SM750 optical fibre (from Fibercore) is ideal for creating LPGs with a PMTP in the near IR region, however, due to their properties, they are not suitable for achieving a PMTP in the visible range.

In order to fabricate an LPG with a PMTP in the visible range, a fibre with a different operating wavelength range should be used. For instance, single mode fibres SM450 and SM600 both with germanium co-doped core and pure silica

cladding [227], were purchased from Thorlabs. Some characteristics of the mentioned above fibres can be seen in Table 5-1.

Table 5-1 Some properties of SM750, SM600 and SM450 single mode optical fibres

Fibre	Operating Wavelength, nm	Cut-Off Wavelength, nm	Numerical Aperture	Cladding, Diameter, μm	Coated Fibre Diameter, μm
SM750	780-980	610-750	0.12-0.14	125 $\pm$ 1	245 $\pm$ 7
SM600	500-600	633-780	0.10-0.14	125 $\pm$ 1	245 $\pm$ 15
SM450	350-470	488-633	0.10-0.14	125 $\pm$ 1	245 $\pm$ 15

However, the SM450 and SM600 optical fibres are not photosensitive. This means it is impossible to fabricate an LPG via a YAG Laser as described in **Section 3.2.3** because the 266 wavelength laser's beam won't affect the fibre core. It is possible to make them photosensitive by hydrogenating their silica structure. In order to make the fibre photosensitive, so-called "hydrogen loading" was applied. When hydrogen is diffused into the silica optical fibre it increases photosensitivity of the fibre and a grating can be inscribed [312]. It is believed to be related to hydroxyl groups formation in GeO₂ fibres and interaction of the OH with the Silica molecules under UV irradiation [313]. In pure silica glass, a hydroxyl formation also occurs alongside with hydride formation [314]. However, in the GeO₂ co-doped silica the hydroxyl concentration is higher because of the weaker Ge-O bond compared to the Si-O bond [315].

To make the Hydrogen molecules diffuse into the silica fibre, it needs to be placed under high pressure Hydrogen for several days [316]. The saturation time and concentration of the hydrogen in the fibre's core depends on pressure, time and temperature [317]. *Swart et al* showed the theoretical calculations and experimental evaluation of the hydrogen concentration in the core. The experimental evaluation was shown through refractive index change of a fibre Bragg grating sensor while loaded and unloaded with hydrogen, **Figure 5-24** [316, 317].

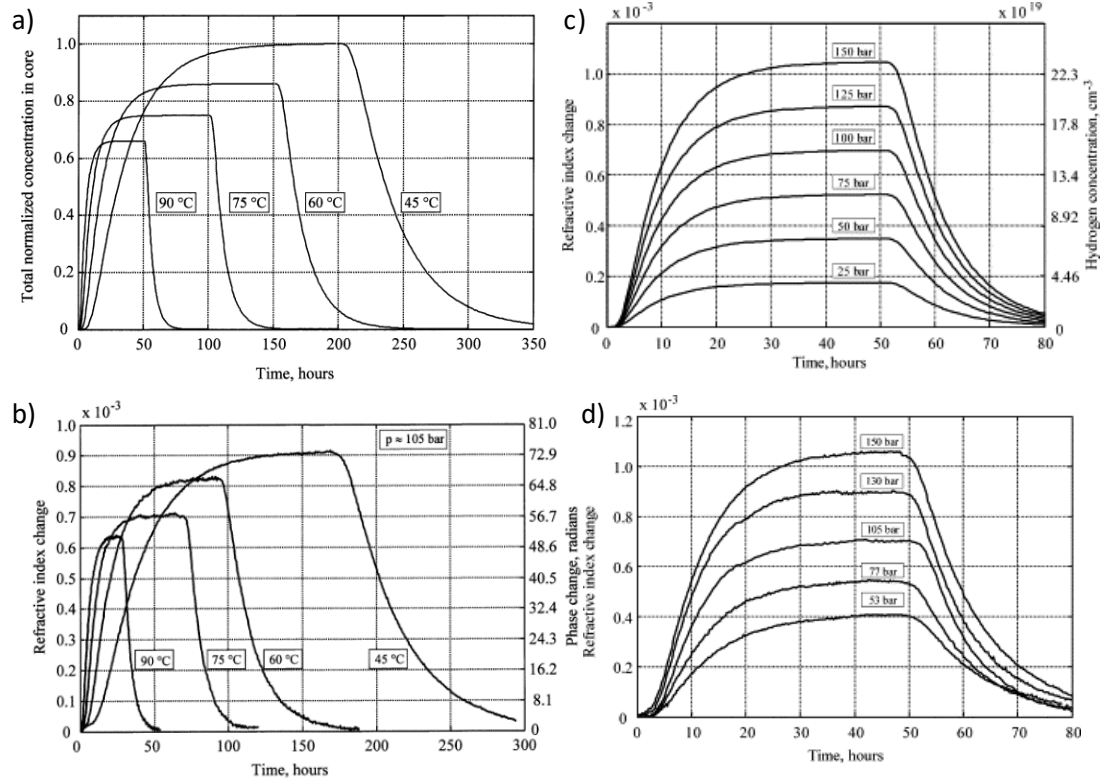


Figure 5-24 **a)** Theoretical time and temperature dependence of the total normalized hydrogen concentration in the core of an optical fibre at 105 bar [316,317]; **b)** Empirical phase change and corresponding RI change in the optical fibre measured during isothermal loading and unloading of hydrogen in the optical fibre at 105 bar [317]; **c)** Theoretical time and pressure dependence of the refractive index change and the hydrogen concentration in the core of an optical fibre at 75 °C, [316]; **d)** Experimental RI change pressure and time dependence in the optical fibre during isothermal loading and unloading of hydrogen at 75 °C [317];

Figure 5-24 a) and b) show the theoretical and experimental RI change/H₂ concentration in the optical fibre during hydrogen loading and unloading under 105 bar pressure at 90, 75, 60 and 45 °C. After the pressurization, the hydrogen concentration at each curve rises over time in a logarithmic manner reaching a saturation point, the highest concentration can be seen at the lowest temperature (45 °C), while at the highest temperature (90 °C) the hydrogen concentration reaches its saturation point faster. After depressurization, the hydrogen leaves the fibre rapidly, faster at higher temperatures, and the hydrogen concentration decreases exponentially. **Figure 5-24 c) and d)** show the theoretical and experimental RI change/H₂ concentration in the optical fibre during hydrogen loading at 75 °C under 50, 75, 100, 125 and 150 bar. After pressurization, the hydrogen concentration at each curve rises over time in a logarithmic manner reaching a saturation point

roughly at the same time. As the pressure is higher, so is the hydrogen concentration. After depressurization, the hydrogen leaves the fibre rapidly and exponentially.

5.5.2.2 Materials and methods for visible LPG fabrication

A Sievert's apparatus 2 system was used (**Figure 5-25**) for hydrogen loading.

The optical fibre was placed into the sample holder, which is marked in **Figure 5-25** in order to expose it to hydrogen. However, the maximum pressure available for the hydrogen was ~ 75 bar and the sample holder had a geometrical limitation, therefore the maximum length of the loaded fibre was limited to 9 cm only. The schematic illustration of the sample holder with fibre offcuts is illustrated in **Figure 5-26**. Most of the hydrogen loadings were conducted under ~ 70 bar pressure, and 45°C temperature, because of the higher hydrogen saturation concentration for 6-7 days. As can be seen from **Figure 5-24** this amount of time at 45°C temperature and 70 bar pressure should be enough to reach the maximum saturation for the given parameters [316, 317].

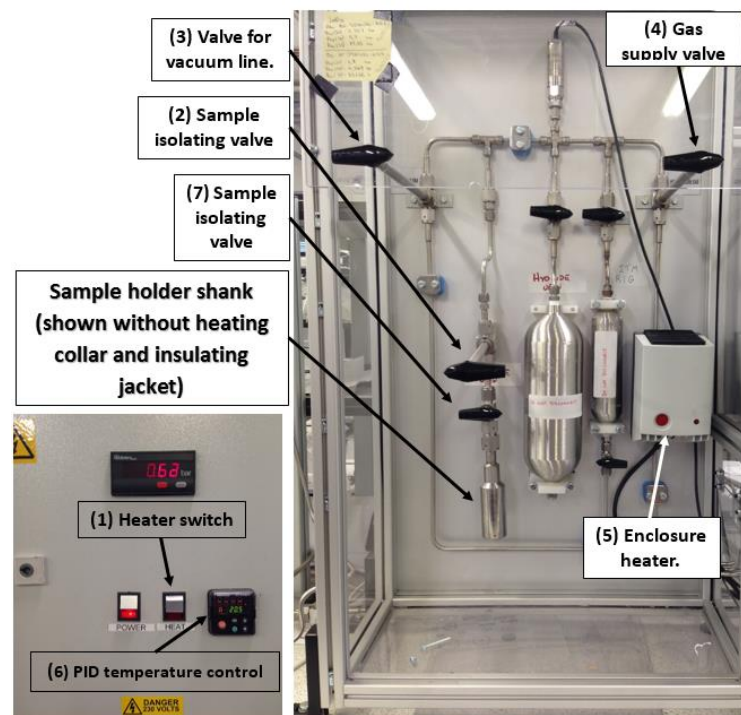


Figure 5-25 Sievert's apparatus2 hydrogen loading setup

(Approx. 90x40x40cm)

After the pressure was relieved, the fibres were removed and placed under low temperature ($-8-0^{\circ}\text{C}$). Then the 9 cm hydrogenated fibre pieces were spliced via Fujicura ARC Master FSM-100P fibre splicer from both sides with longer pieces of the same types of optical fibre, in order to make the grating writing possible, **Figure 5-27**

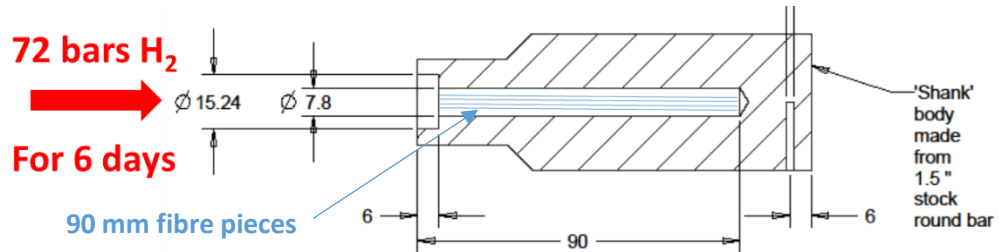


Figure 5-26 Hydrogen loading set up schematic (all dimensions in mm)

After splicing, an LPG was written on the hydrogen loaded piece of fibre, via a frequency quadrupled UV laser in the same way as described in **Section 3.2.3**, **Figure 5-28**.

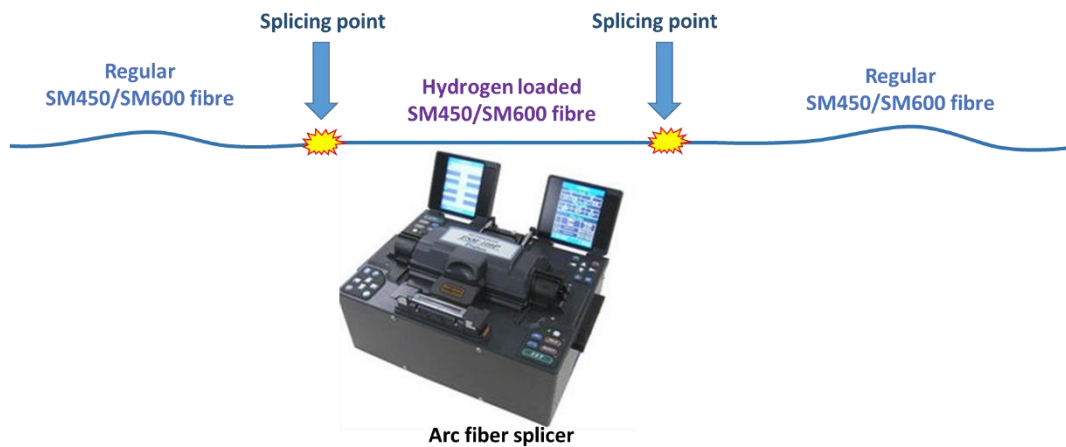


Figure 5-27 Schematic of the 9 cm hydrogen loaded SM450 and SM600 optical fibre splicing with regular longer fibres of the same type

Once the LPG fabrication was completed, the fibres were placed in an oven for 2 hours under 120°C for temperature treatment in order to diffuse the hydrogen from the fibre. The transmission spectra during the fabrication and after the temperature treatment were recorded using a Halogen Light source (HL-2000, Ocean Optics) and a spectrometer (Ocean Optics USB2000+) connected to a PC.

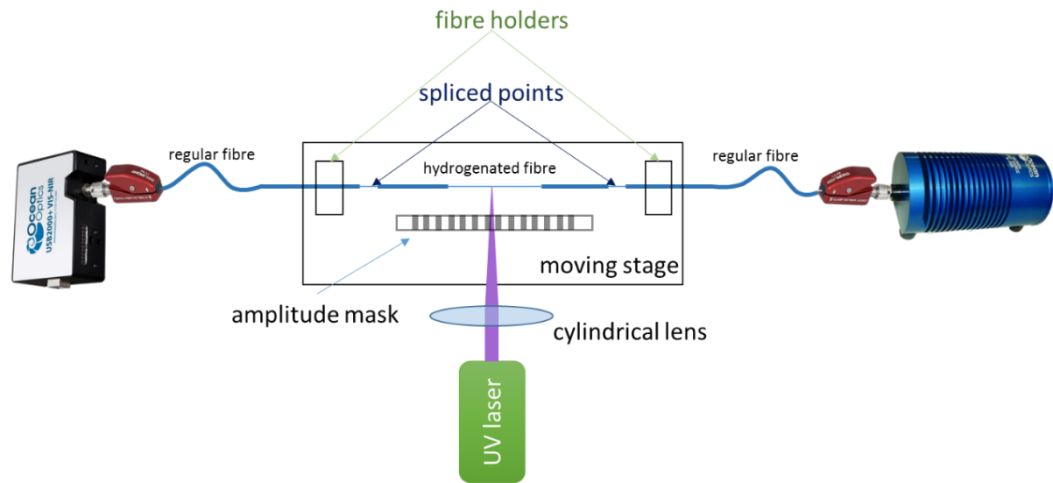


Figure 5-28 LPG fabrication set-up on hydrogen loaded SM450 and SM600 fibres using YAG frequency quadrupled UV laser

5.5.2.3 Results and discussion of the visible LPG fabrication

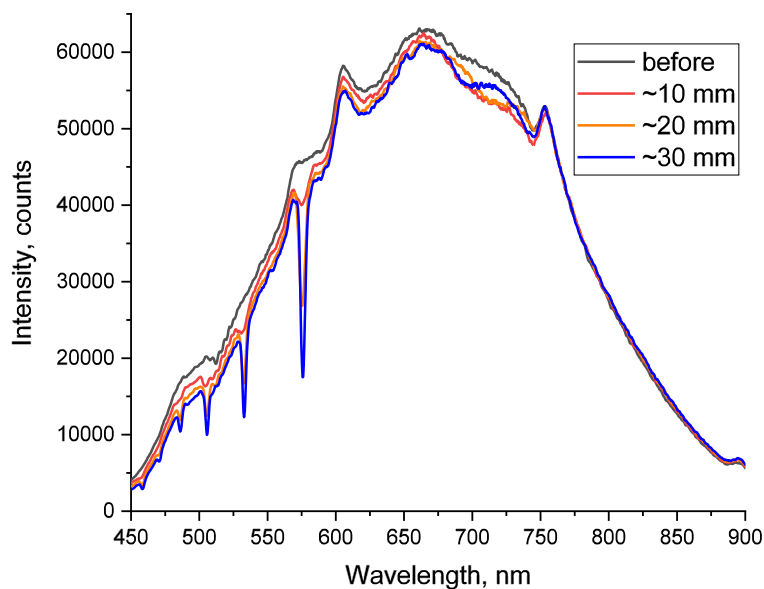


Figure 5-29 30 mm long LPG with 111.5 μm grating period fabrication on a hydrogen loaded SM600 fibre. Black curve represents the spectrum before the start of the fabrication, red curve shows the transmission spectrum at the point when 10 mm of the grating were written, orange, - 20 mm, and blue – 30 mm

The visible LPGs fabrication aimed to have a PMTP of the LPGs in water, and preferably in the region of the TSPP's biggest absorption change. However, the LPG attenuations before and after hydrogen removal from the fibre would differ a lot because of the difference in the effective refractive index of the core and the cladding [316, 317]. Therefore, LPGs with different grating periods ranging from 105.0 up to 114.5 μm were fabricated and examined. Only a few of them will be shown here, those which resonate with our aims.

Transmission spectra in **Figure 5-29** show the fabrication process of the hydrogen loaded SM600 fibre. One can see a gradual formation of four attenuation bands at ~ 576 nm, 533, 505 and 486 nm, followed by the spectrum with two smaller attenuation bands. However, by looking at **Figure 5-30**, one can see the transmission spectra of the same LPG but after the temperature treatment, which has forced the hydrogen to leave the fibre, one can see a different picture. In **Figure 5-30** two transmission spectra can be seen, the red curve is when the LPG is in air, and blue when it is immersed in water. Now a PMTP can be seen split in air transmission at 686 and 762 nm. Another two attenuation bands can be seen at 568 and 527 nm respectively. This means a major shift of the attenuation bands occurred after the

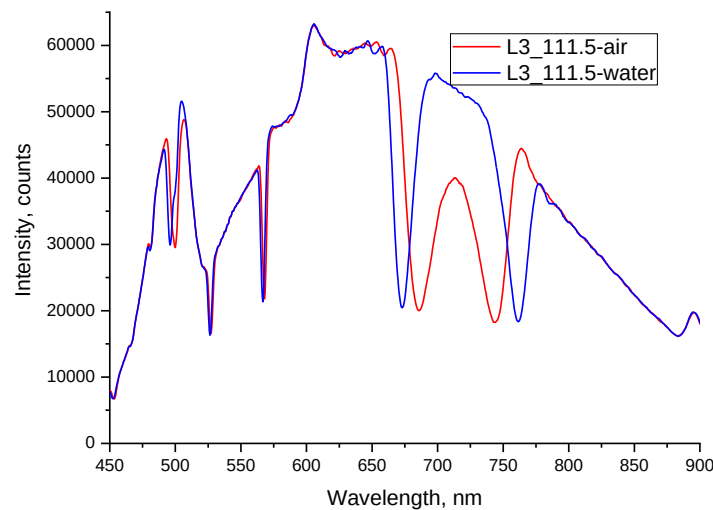


Figure 5-30 transmission spectra of the 30 mm long LPG with 111.5 μm grating period on the SM600 fibre, after temperature treatment (when the hydrogen has left the fibre). Red line represents transmission of the LPG is in air, blue - in water;

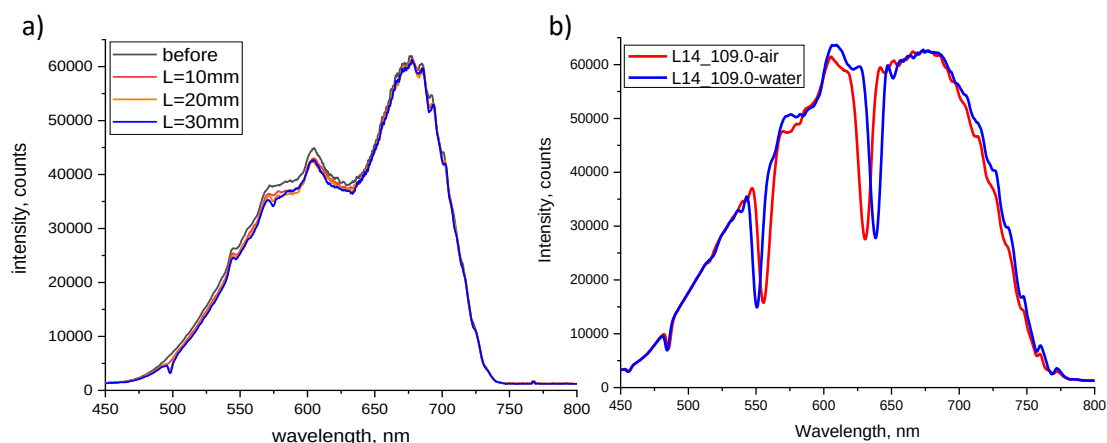


Figure 5-31 a) fabrication of the 109.0 μm grating period, 30 mm long LPG on a hydrogenated SM450 fibre. Black curve represents the spectrum before the start of the fabrication, red curve shows the transmission spectrum at the point when 10 mm of the grating were written, orange, - 20 mm, and blue – 30 mm; **b)** Same 109.0 μm LPG on SM450 fibre after the temperature treatment, when the hydrogen has left the fibre. Red line represent transmission of the LPG is in air, blue - in water;

hydrogen was removed from the fibre. The blue, water curve, shows the same PMTP splits even further from each other, to 673 and 762 nm, with slight shifts to the left of the other attenuations. Thus, the visible ranged LPG behaves similarly to the regular LPG in response to the change of the surrounding medium.

With SM450 fibre, the PMTP can be brought even lower to the visible range. **Figure 5-31a)** shows the fabrication process of the 30 mm long, hydrogen loaded, LPG with a 109.0 μm grating period on an SM450 fibre. As one can see, only one attenuation band near 498 nm. However, as can be seen in **Figure 5-31b)** after the hydrogen out-diffusion, the 498 nm attenuation band shifts to the left, to roughly 484 nm, and a split PMTP appears at 555 and 630 nm in air - red line. In water, the PMTP splits further to 550 and 638 nm respectively - blue line.

Moving the grating period up to 110.0 μm , on hydrogen loaded SM450, we can see a similar picture during the fabrication. **Figure 5-32a)** shows the fabrication process of the 30 mm long, hydrogen loaded, LPG with a 110.0 μm grating period on an SM450 fibre. Again, only one clear attenuation band can be observed at $\sim 505\text{nm}$. However, after the hydrogen left the fibre, **Figure 5-32b)** where the red line shows the transmission of the 110.0 μm LPG in air, blue – in water, one can observe that the 19th mode attenuation is now split further between 492 and 755 nm in air, 491 and 759 nm in water respectively. This is one mode because the attenuation difference is

growing from air to water, as the RI grows. Also in water, a wide and deep PMTP attenuation appears which presumably corresponds to the 18th cladding mode.

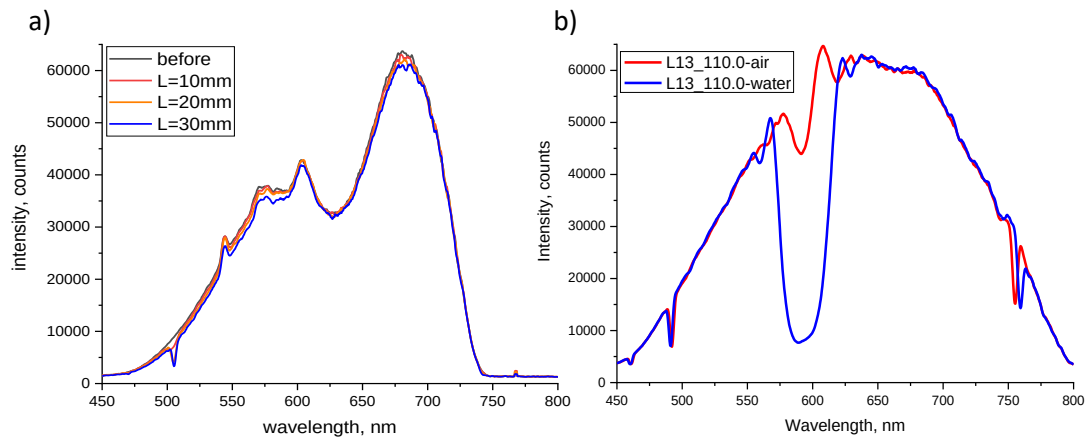


Figure 5-32 a) fabrication of the 110.0 μm gating period, 30 mm long LPG on a hydrogenated SM450 fibre. Black curve represents the spectrum before the start of the fabrication, red curve shows the transmission spectra at the point when 10 mm of the grating was written, orange, - 20 mm, and blue – 30 mm; **b)** Same 110.0 μm LPG on SM450 fibre after the temperature treatment, when the hydrogen has left the fibre. Red line represents transmission of the LPG is in air, blue - in water;

5.5.2.4 Conclusion for the visible range LPG fabrication

It has been shown that is possible to fabricate long period gratings in the visible range within our University's facilities. The grating periods for creating PMTPs on SM450 and SM600 fibres were selected empirically.

5.5.3 DAR/TSPP on the visible range LPG

5.5.3.1 Introduction to the DAR/TSPP coated LPG operating in the visible range

To the best of my knowledge, the response of the LPGs operating in the visible range to sensitive dyes has not been studied before. So apart from the pure scientific research interest it is unknown whether this kind of OFS will have advantage over the absorbance OFS.

5.5.3.2 Materials and method for DAR/TSPP coated LPG operating in the visible range

The layer by layer deposition of the DAR/TSPP film on LPG inscribed on SM600 and SM750 fibres were conducted in a very similar way as it was done for glass substrate and U-shape OFS described in **Sections 5.3.2** and **5.4.2** respectively. The solution concentrations were the same, the only difference was, that in case of LPGs, the fibre was fixed with the vessel. Instead of the vessel replacement described for U-shape OFS coating (**section 5.4.2**), the liquids were changed in the vessel via suction (using Flaem Suction Pump) and pouring (using Eppendorf Reference® 2, single-channel variable pipettes). The LPG was always in the middle of the vessel, **Figure 5-33**.

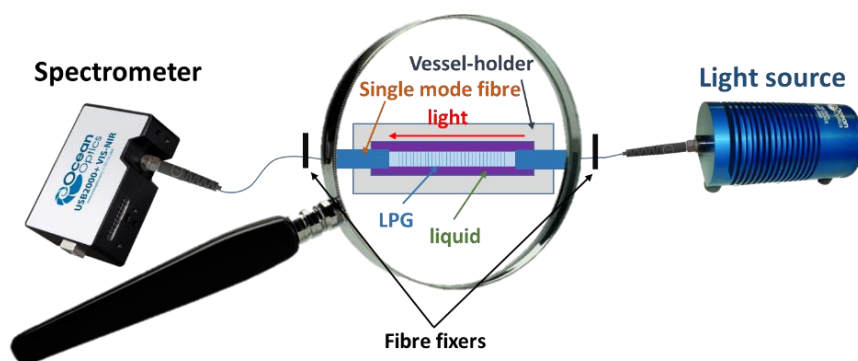


Figure 5-33 Deposition and experimental set-up for LPG optical fibre sensors

The same setup (**Figure 5-33**), approach and method were used for testing the response of the DAR/TSPP coated LPG to ammonia. The testing was conducted in the same way as described for the DAR/TSPP functionalised U-shape OFS described in **section 5.4.2**.

5.5.3.3 Method for the refractive index calculation

The Kramers-Kronig (KK) relation ties the complex refractive to absorbance, Equation (5-4). Hence, it is possible to calculate the RI across a wavelength range from an absorption spectrum. To do so, a Hilbert transform computational approximation of the KK relation was used, for easier and faster calculations [318].

In terms of frequency dependant susceptibility, the KK relation can be written:

$$\chi_{Re}(\omega) = \frac{2}{\pi} \int_0^{\infty} \frac{\Omega \chi_{Im}(\Omega)}{\Omega^2 - \omega^2} d\Omega \quad (5-5)$$

Where $\chi_{Re}(\omega)$ is the real part of the frequency dependent susceptibility, and $\chi_{Im}(\Omega)$ is the imaginary part.

Any causal function $h(t)$ can be expressed as the sum of even $h_e(t)$ and odd $h_o(t)$ functions, the sum of which equals zero when $t < 0$.

The Fourier transform (FT) of $h_e(t)$ is purely real, while the FT of $h_o(t)$ is purely imaginary. Any odd function can be expressed through even function using and vice-versa, using the signum function, $sgn(t) = \frac{t}{|t|}$

$$h(t) = h_o(t) + h_e(t) = h_o(t) + sgn(t)h_o(t) \quad (5-6)$$

Hence, the FT of a function $h(t)$, marked H , becomes:

$$H = \mathcal{F}\{h(t)\} = \mathcal{F}\{h_o(t)\} + \mathcal{F}\{sgn(t)\} * \mathcal{F}\{h_o(t)\} \quad (5-7)$$

Where the $*$ sign denotes convolution. As the FT of a signum function is $-i/\pi\omega$, the second part of the Equation (5-7) becomes a well-known Hilbert transform[319], and can be written as:

$$\hat{H}(\omega) = \frac{1}{\pi} \int_{-\infty}^{\infty} \frac{H(\Omega)}{\omega - \Omega} d\Omega \quad (5-8)$$

Where $\hat{H}$ denotes a Hilbert transform of H .

Hence now the $H(\omega)$ can be expressed as

$$H(\omega) = H_o(\omega) - i\hat{H}_o(\omega) \quad (5-9)$$

Using Cauchy residual theorem, the Equation (5-5) can be expressed as:

$$\chi_{Re}(\omega) = \hat{\chi}_{Im}(\omega) \quad (5-10)$$

Using Equation (5-10) instead of (5-5) is much easier and faster as the Hilbert transform is a built-in function in MATLAB. The data was processed according to *Whittaker et al* [318]. The following mathematical procedure was applied:

The experimental transmission spectra were converted to imaginary susceptibility through the whole spectral range, using:

$$\chi_{Im} = -\frac{\ln T}{kL} \quad (5-11)$$

Where $k=2\pi/\lambda$ is the wave vector, and L is the length of the absorption path.

A Hilbert transform of χ_{Im} is taken to determine the χ_{Re} , as shown in Equation (5-10) and the complex refractive index is:

$$n = \sqrt{\chi_{Re} + 1}. \quad (5-12)$$

The complex, real and imaginary parts of the calculated RI were extracted via MATLAB.

5.5.3.4 Results and discussions for DAR/TSPP coated LPG operating in the visible range

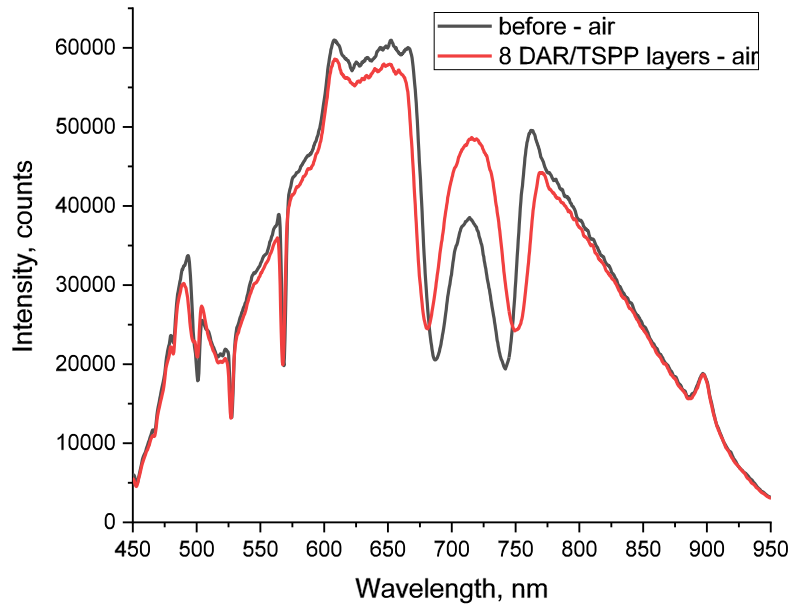


Figure 5-34 30mm long LPG with grating period of $111.5 \mu\text{m}$ on SM600 fibre coated with 8 bilayers of DAR/TSPP film. The black curve represents the transmission spectrum of the LPG before the deposition in air, the red curve, after 8 bilayers of DAR/TSPP film deposited

The 30 mm $111.5 \mu\text{m}$ period LPG fabricated on a hydrogenated SM600, described in **Figure 5-30** was coated with 8 bilayers of DAR/TSPP coating. **Figure 5-34** shows the transmission spectra of the $111.5 \mu\text{m}$ LPG in air, before the deposition, -

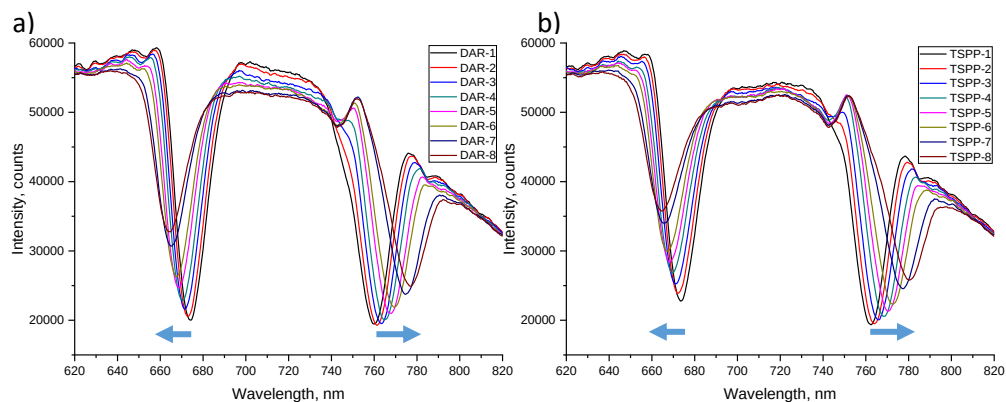


Figure 5-35 Response of the SM600, $111.5 \mu\text{m}$ LPG's PMTP to layer by layer deposition. Spectral reading taken in solutions in the end of each deposition cycle. First layer is illustrated by black curve, last eight layer, - by brown; **a)** DAR solution; **b)** TSPP solution;

black curve; and after the deposition of eight bilayers of DAR/TSPP film, - red curve. As can be seen, because of the RI growth associated with the deposited film, the gap of the split PMTP has widened from 687 and 742 nm (black curve) to 680 and 750 nm respectively (red curve). The rest of the attenuations on the shorter side of the spectrum moved to the left slightly.

Figure 5-35 shows the PMTP response to the layer by layer deposition of the DAR and TSPP layers in solutions, at the end of each deposition cycle. Here one can see a steady step-by-step growth of the gap between the two attenuation bands of the PMTP. Following **Figure 5-35b**), each layer deposited moves the left attenuation

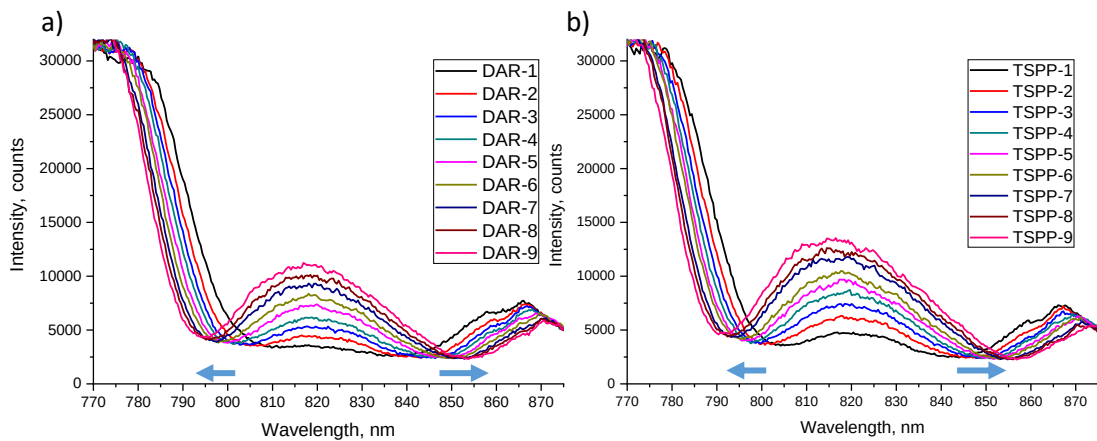


Figure 5-36 Response of the SM750, 112.0 μm LPG's PMTP to layer by layer deposition. Spectral reading taken in solutions at the end of each deposition cycle. First layer is illustrated by black curve, last eight layer, - by brown; **a)** DAR solution; **b)** TSPP solution;

from 673.7 nm (1st layer) to 664.4 nm (8th layer), with an average wavelength shift per deposited layer $\sim 1.32\text{nm}$. The right attenuation band moved from 762 nm (1st layer) to 780.4 nm (8th layer), with an average wavelength shift per deposited layer $\sim 2.62\text{ nm}$.

For comparison, **Figure 5-36** shows a similar, but 9 bilayer DAR/TSPP deposition on a “regular” 112.0 μm LPG inscribed on SM750 fibre with PMTP in the near IR region. Following **Figure 5-36b**) each layer deposited moves the left attenuation band from 804.2 (1st layer) to 791 nm (9th layer), with an average wavelength shift per

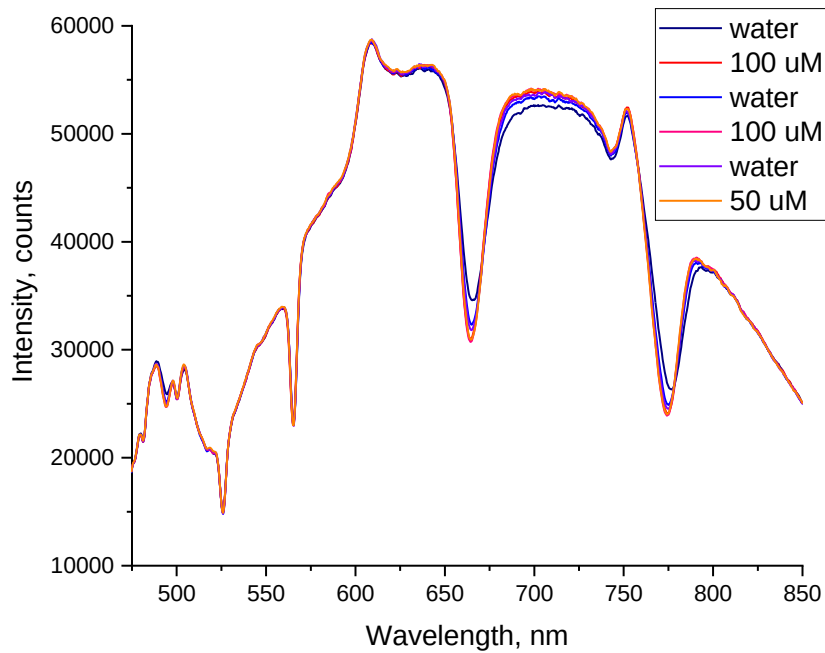


Figure 5-37 Transmission spectra of 111.5 μm LPG on SM600 fibre while immersed alternately for 15 minutes in DI water (navy curve), 100 μM NH_3 (red curve), DI water (blue curve), 100 μM NH_3 (pink curve), DI water (violet curve) and 50 μM NH_3 (orange curve)

deposited layer $\sim 1.65\text{nm}$. The right attenuation band moves from 843.9 (1st layer) to 856.7 nm (9th layer), with an average wavelength shift per deposited layer $\sim 1.6\text{nm}$. As one can see from **Figure 5-36** and **Figure 5-35**, the response of the PMTP on the 112.0 μm LPG on SM750 fibre to DAR/TSPP deposition is similar to that of the 111.5 μm LPG on SM600.

The testing of the response to the ammonia of the LPGs was made in the same way as for the U-shape OFS described in **Section 5.4.2**. The response of the eight bilayers DAR/TSPP coated “visible LPG” to Ammonia in water can be seen **Figure 5-37**. **Figure 5-37** shows the transmission spectra of the eight bilayers DAR/TSPP coated 111.5 μm LPG on SM600 fibre, while consequently immersed in water (navy line), and then twice in 100 μM NH_3 aqueous solution (red and pink curves), 50 μM NH_3 aqueous solution (orange curve) for ~ 15 minutes each, separated by immersions in DI water

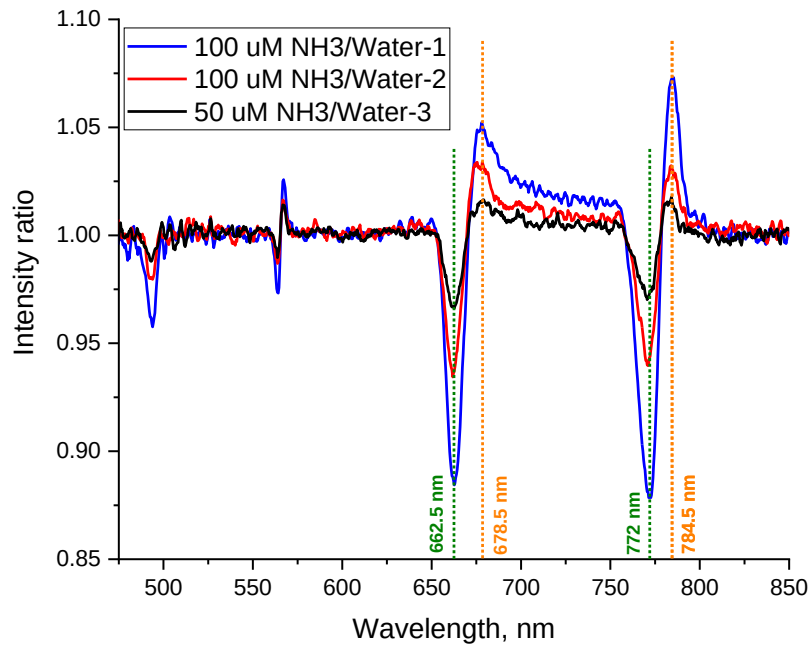


Figure 5-38 Ratios taken between transmissions of each DI water and subsequent ammonia solution immersions, of the 111.5 μm LPG on SM600 fibre. First 100 μM /water, -blue curve, second 100 μM /water – red line, 50 μM /water, - black line.

(blue and violet curves) for the same duration. One can see that the intensity values of the PMTP change with ammonia concentration, to find out in which part of spectra the biggest change happens, the ratio was taken between the transmission spectra of each DI water and subsequent ammonia concentration immersions, **Figure 5-38**. From this figure, clear absorbance change peaks can be seen. Two peaks are higher than 1 (which means the absorbance here is rising), at 678.5 nm and 784.5 nm, where absorbance increases with ammonia presence, with the latter being stronger during the first immersion, but becomes equal to 678.5 nm in the following immersions. In addition, two valleys are lower than 1, (which means the absorbance here falls), at 662.5 nm and 772 nm with the second valley showing greater absorbance change during the first immersion, but becoming equal with the 662.5 nm change in the following immersions. Therefore, a ratio of absorbance values at 678.5 and 662.5 nm or 784.5 and 772 nm or 678.5 and 772 nm or 784.5 and 662.5 nm can be taken to detect the ammonia concentration. The most efficient ratio in terms of response

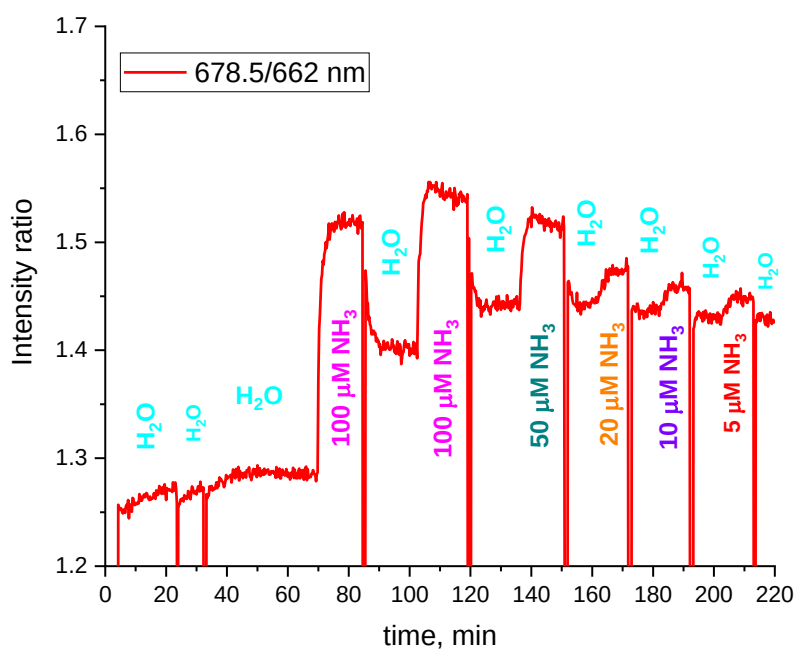


Figure 5-39 Intensity values ratio taken continuously at 678.5 and 662 nm, of the light propagating through 8 bilayer DAR/TSPP coated 111.5 μm LPG inscribed on SM600 fibre, during consequent immersion of the LPG in DI water and 100, 50, 20, 10 and 5 μM NH_3 aqueous solution.

change appears to be between the 678.5 and 662.5 nm intensity values and is shown in **Figure 5-39**. From the 678.5/662 nm intensity ratio shown in **Figure 5-39**, one can see a clear continuous intensity-based response of the DAR/TSPP coating on an LPG with PMTP operating in the visible range. The ratio starts with ~ 1.25 value immediately upon the first immersion, then grows slightly and at 42 minutes on the third DI water immersion stabilises at ~ 1.28 . Immediately after the exposure to 100 μM NH_3 solution, the ratio rises dramatically to ~ 1.50 and with slight saturation, growth remains around that value. When immersed in the water again, the ratio drops rapidly and stabilises after 4 minutes, however stays much higher, compared to the previous water value, at ~ 1.40 . Upon the second exposure to the 100 μM NH_3 solution, the ratio rises slightly higher than before to ~ 1.54 and drops to ~ 1.44 in water. Immersion to 50 μM gives rise to the ratio to ~ 1.51 , and immersion to water drops the ratio back to ~ 1.44 . 20 μM of ammonia concentration gives a ~ 1.47 ratio value, after which water gives ~ 1.435 . After immersion to 10 μM ammonia solution the ratio is ~ 1.45 , while in water goes back to ~ 1.433 , and 5 μM gives ~ 1.445 ratio and falls back in the water to ~ 1.3 .

According to **Figure 5-39**, the 5 μM NH_3 concentration is clearly detectable by DAR/TSPP coated LPG operating in the visible range. In comparison, an LPG with PMTP in near-infrared, coating of which is shown in **Figure 5-36** does not respond to even higher NH_3 concentration, **Figure 5-40**. As can be seen from the PMTP spectra of the 9 bilayers DAR/TSPP 112.0 μm LPG on SM750 while immersed in water and ammonia solutions for ~ 15 min, **Figure 5-40a**. Navy and blue curves show the first and third immersions in water for ~ 15 minutes respectively, followed by immersion in 200 μM ammonia solution, -red curve and 500 μM , - pink curve. The difference between them is indistinguishable. The ratio between each ammonia and previous water transmission is shown in **Figure 5-40b**, on the same scale as shown in **Figure 5-38** for the “visible” LPG. In the case of 200 μM (black), the change is negligible, and in the case of 500 μM the change is very small for such a high ammonia concentration. Hence, it is clear that these two LPGs coated with similar DAR/TSPP coatings respond significantly differently to ammonia.

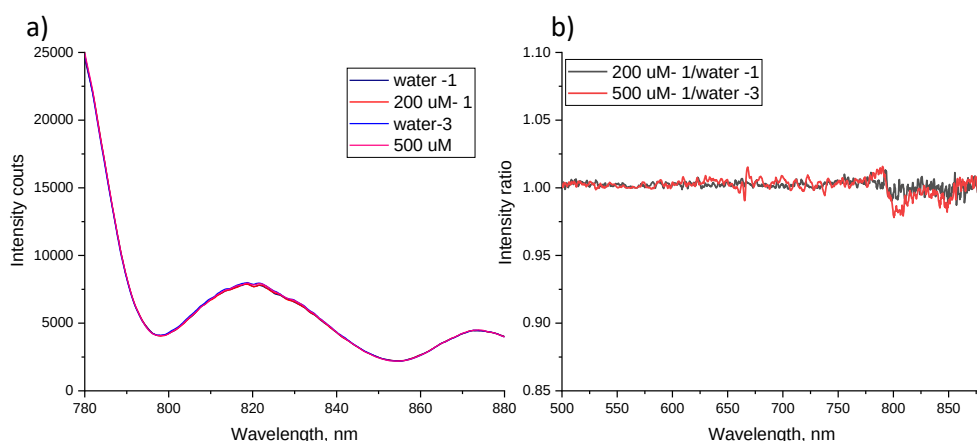


Figure 5-40 a) transmission spectra of the PMTP of the 9 bilayer DAR/TSPP 112.0 μm LPG on SM750 while immersed in water (navy curve), 200 μM NH_3 aqueous solution (red curve), water again (blue curve) and 500 μM (pink curve); **b)** Ratio between 200 μM NH_3 solution to water, and 500 μM to water of the transmission spectra of the DAR/TSPP 112.0 μm LPG on SM750;

5.5.3.5 Results and discussions for DAR/TSPP coated visible LPG's refractive index response

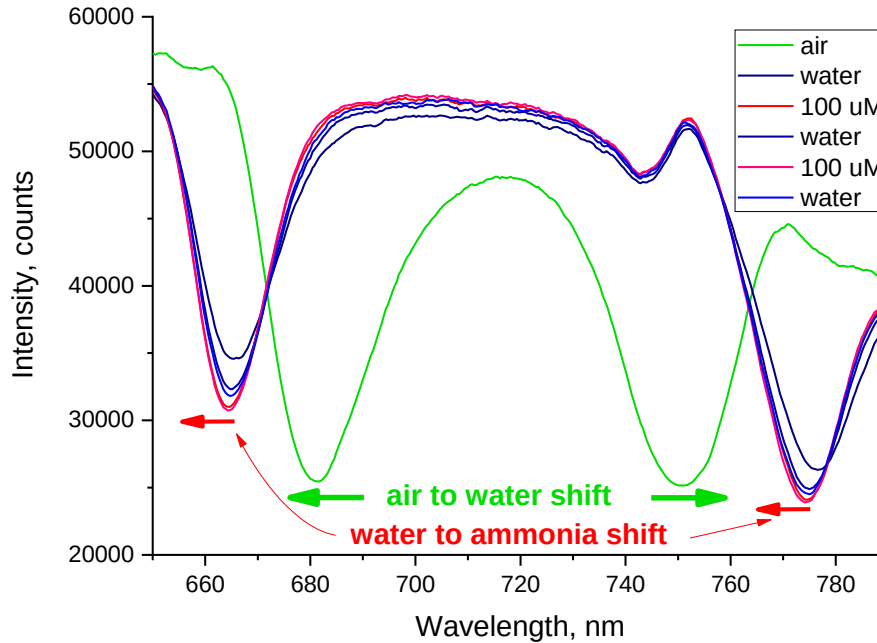


Figure 5-41 Transmission spectra at the PMTP of the 111.5 μm LPG on SM600 fibre coated with 8 bilayers of DAR/TSPP. While being in air (green curve), then immersed alternately for 15 minutes in DI water (navy curve), 100 μM NH_3 (red curve), DI water again (royal blue curve), 100 μM NH_3 again (pink curve), DI water again (blue curve)

By having a closer look at the visible LPG's PMTP **Figure 5-41** one can observe an interesting phenomenon. In **Figure 5-41** the transmission spectrum in the air is represented by the green curve, the three water immersions are shown by navy, royal blue and blue curves, and the two exposures of the LPG to 100 μM ammonia solutions are pictured by red and pink curves. The immersion length for each liquid was ~ 15 minutes. As one can see, when the fibre moves from air to water, the PMTP's attenuation bands widen significantly in opposite directions. The left attenuation band moves to the left from ~ 681 (air) to ~ 665 (water) nm and the right attenuation band moves to the right from ~ 750 (air) to 776.5 (water) nm, as the RI grows from ~ 1.0 in the air to ~ 1.333 in DI water. However, in the case of exposure of the DAR/TSPP coated visible LPG to ammonia, both attenuation bands shift in the same direction, to the left. The strongest shift occurs after the first exposure to 100 μM ammonia solution, from ~ 665.7 to ~ 664.6 nm for the left attenuation band, and from ~ 774.5 to 774.6 nm for the right attenuation band. Then in water, it recovers but not

completely to ~ 665.2 and ~ 775 nm respectively. In 100 μM again the attenuation bands shift to ~ 664.5 and 774.2 nm respectively and in subsequent water immersion, it shifts back to the right. The continuous wavelength shifts of the PMTP during consequent exposures to 100, 50, 20, 10 and 5 μM ammonia aqueous solutions separated by immersions in DI water can be seen in **Figure 5-43**.

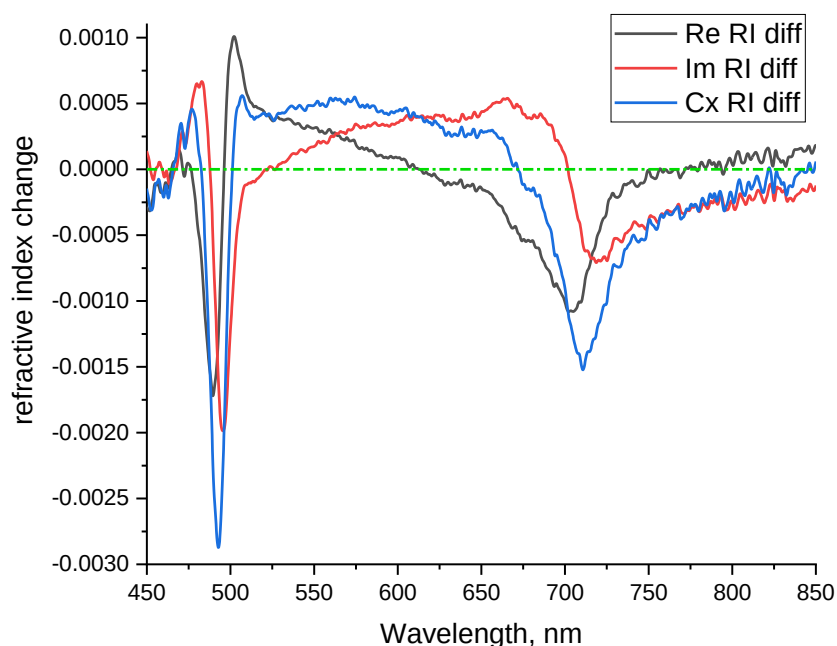


Figure 5-42 Calculated refractive index change of a 5 bilayer DAR/TSPP coated glass substrate immersed in water and 600 μM aqueous ammonia solution, Black curve, - difference between real parts of the RI, red curve, - difference between imaginary parts of the RI, blue curve, - difference between complex refractive indexes. Green dotted line shows the zero point, meaning, - above the green line RI is growing, below the green line, the RI is declining

Putting aside the ammonia sensitivity, an interesting question to ask would be: “Why do the PMTP attenuation bands shift in the same direction as a reaction of the DAR/TSPP film?”. If the attenuation shifts happen due to RI change, then the RI behaviour in the PMTP’s wavelength range is not linear, otherwise, the PMTP attenuation bands should shift in the opposite direction [280], this can be also seen when this LPG goes from air to water, **Figure 5-41**, **Figure 5-43**. To check this assumption, the RI of the DAR/TSPP film was calculated based on the absorption spectra obtained on a 5 bilayer DAR/TSPP coated glass substrate immersed in water and 600 μM aqueous ammonia solution **Figure 5-14**. The complex RI for each

absorbance curve was calculated following the Hilbert transform approximation explained in **Section 5.5.3.3**, then the difference between the refractive indexes was calculated by subtraction of the “substrate in water” RI from the “substrate in ammonia solution” RI, **Figure 5-42**. The black curve shows the real part of the RI change, the red curve – the imaginary part, and the blue shows the combined complex RI change. The green dotted line shows the zero point in the difference, meaning, everything above it can be attributed to a rise of the RI, everything below, a decline of the RI. In the wavelength region of the PMTP of the 111.5 μm period LPG on SM600 fibre ($\sim 650\text{--}790\text{nm}$), both complex and imaginary parts of the RI crosses the zero line, at 671 and 702 nm respectively. Taking into account the whole complex RI change, the RI below 671 nm grows, while above, declines. This can explain why the PMTP attenuation bands both shift to the blue. As in the region of the left-hand attenuation band ($\sim 665\text{ nm}$), the RI index is growing in the case of ammonia-TSPP interaction, while in the region where the right-hand attenuation occurs ($\sim 775\text{ nm}$), the RI is declining, thus following the opposite trends and they move in the same direction.

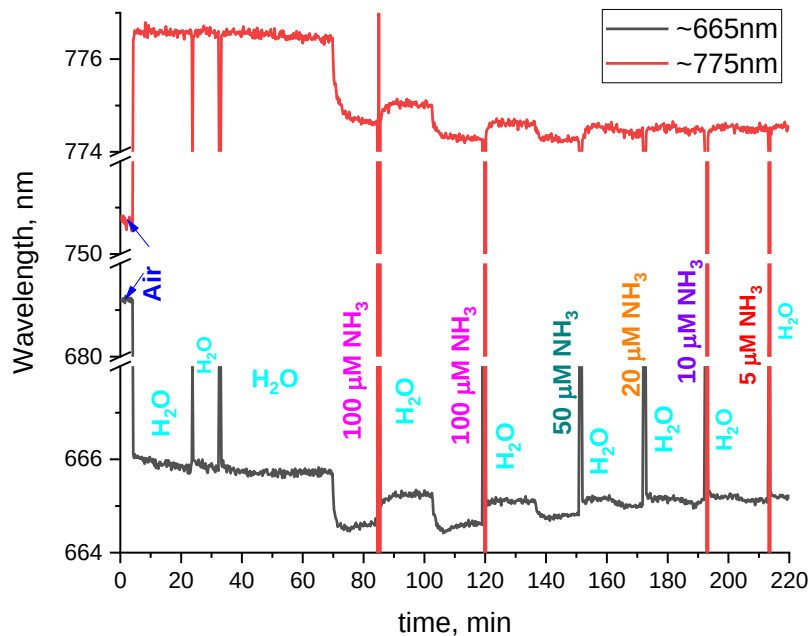


Figure 5-43 PMTP attenuations of the 8 bilayer DAR/TSPP coated 111.5 μm LPG inscribed on SM600 fibre, during consequent immersion of the LPG in DI water and 100, 50, 20, 10 and 5 μM NH₃ aqueous solution.

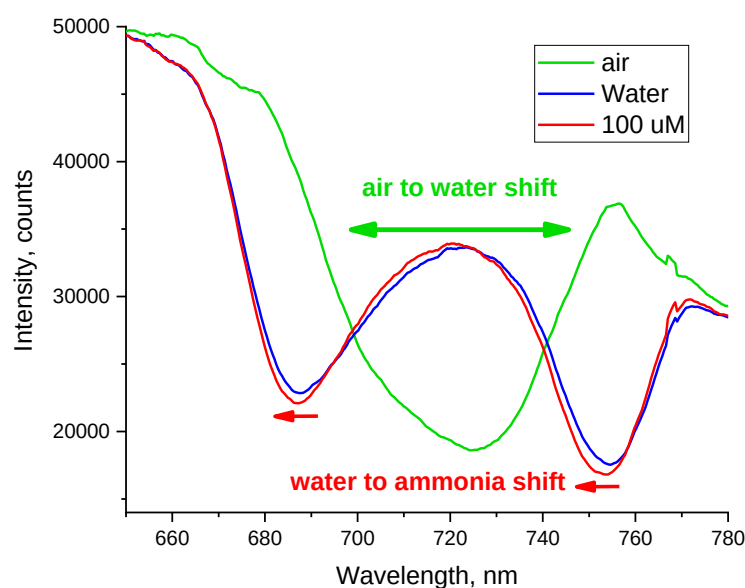


Figure 5-44 Transmission spectra at the PMTP of the 112.0 μm LPG on SM600 fibre (coated with 7 bilayers of DAR/TSPP). While being in air (green curve), then immersed alternately for 15 minutes in DI water (navy curve), 100 μM NH_3 (red curve), DI water again (royal blue curve), 100 μM NH_3 again (pink curve), DI water again (blue curve)

The non-linear RI change in the range of the PMTP of the VIS LPG is likely to be the reason for the phenomenon depicted in **Figure 5-38**, where ratios of the transmission spectra of the DAR/TSPP coated 111.5 μm LPG on SM600 fibre in water and ammonia solutions are shown. The form of the ratio curves in **Figure 5-38** is probably due to the shift of the resonance band wavelength and the width of the bands in response to the RI change.

For calculation purposes in **Equation (5-11)** the optical path length was estimated to be ~ 300 nm, taken from the assumption of 1 bilayer thickness ~ 30 nm, thus 5 bilayers from both sides give us 300 nm. However, this figure does not match the experiment, it can be due to the RI calculations are not precise numerically, but they give an idea of the behavioural trends. Possibly a fit of the model to the experimental data might allow a better estimate of the RI change and layer thickness. In addition, the number of layers on the LPG and substrate differ, and the thickness affects the RI [320], this also is clearly seen from the deposition process, **Figure 5-35**, **Figure 5-36**. This factor can affect the precise turning point of the RI, as another LPG on SM600, but with a 112.0 μm period, coated with 7 bilayer DAR/TSPP, has a narrower PMTP attenuation band and shows a similar trend, **Figure 5-44**. As can be

seen, immediately after the 7 bilayers DAR/TSPP coated 112.0 μm period LPG on SM600 fibre is immersed in water, the wide PMTP attenuation band splits into two attenuation bands with valleys at ~ 687.5 and ~ 754.5 nm. After being exposed to 100 μM ammonia solution, the left-hand attenuation bands shifts to ~ 687 nm, the right-hand attenuation band shifts to ~ 753.5 nm, so both shift to the blue. If the attenuation shift happens because of the RI change, it can be assumed that in this particular coating, the complex RI change curve crosses the zero somewhere between 685 and 755 nm.

Overall, this phenomenon will be studied more deeply and systematically in the near future, with more precise data. Several sensitive dyes will be applied to different grating periods on different types of fibre, to match the PMTP and the biggest complex RI change of the particular film.

5.5.3.6 LOD for the 8 bilayers DAR/TSPP 111.5 μm period LPG on SM600

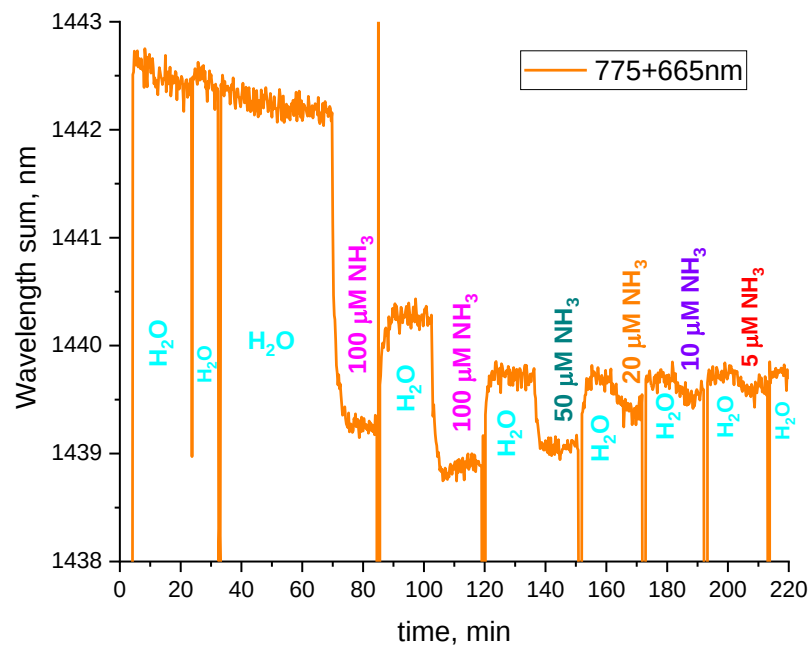


Figure 5-45 Sum of the PMTP's attenuation bands wavelength, on the 8 bilayer DAR/TSPP coated 111.5 μm LPG inscribed on SM600 fibre, during consequent immersion of the LPG in DI water and 100, 50, 20, 10 and 5 μM NH_3 aqueous solution.

A reasonable way to detect the response to ammonia concentration of the DAR/TSPP coated 111.5 μm period LPG, would be to take the sum of the PMTP wavelength during the immersions, **Figure 5-45**. Following the water immersion, the wavelength sum declines steadily from ~ 1442.7 to 1442.2 nm after around 50 minutes and stabilises there for the next 20 minutes. First exposure to $100 \mu\text{M}$ ammonia, the sum falls dramatically in the first four minutes, and then slowly stabilises at 1439.2 nm. In water, it rapidly recovers partially to ~ 1440.3 nm and falls again in $100 \mu\text{M}$ ammonia solution to ~ 1438.9 nm. After it rises in water to ~ 1439.7 nm and in $50 \mu\text{M}$ ammonia solution falls to ~ 1439.0 nm, then in water, it recovers to previous value ~ 1439.7 nm and falls again in $20 \mu\text{M}$ ammonia to ~ 1439.4 nm. Then in water recovers back to ~ 1439.7 nm and will recover to this position in water at the end of the experiment. Then, the next two ammonia readings give ~ 1439.5 and ~ 1439.6 nm for 10 and $5 \mu\text{M}$ respectively.

Now we can compare the two ways to use the 8 bilayers DAR/TSPP coated $111.5 \mu\text{m}$ LPG inscribed on an SM600 fibre as a sensor. The first way is the tracking of the Intensity ratio taken at 678.5 and 662 nm, shown in **Figure 5-39**, the other by tracking the wavelength change sum of the attenuation bands wavelength around

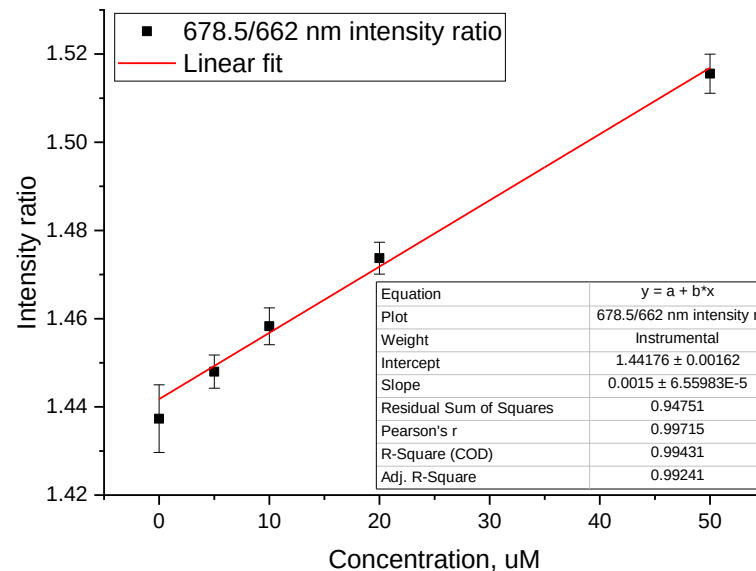


Figure 5-46 Average intensity ratio (678.5 and 662 nm) points of the last 5 minutes of each immersion from 0 to $50 \mu\text{M}$ ammonia, the DI water immersion corresponds to $0 \mu\text{M}$ concentration, taken from all 7 immersions before, in-between and after ammonia solutions, - black plots with standard deviation as Y-errors; Linear fit, - red line;

the PMTP shown in **Figure 5-45**. To do so, both datasets were plotted as follows. The average data points of the last 5 minutes of each immersion from 0 to 50 μM ammonia is plotted, while the DI water is taken from all 7 immersions before, in-between and after ammonia solutions, then a linear fit was performed on both datasets, **Figure 5-46**, - for intensity ratio and **Figure 5-47** for PMTP attenuation wavelength shift. The Limit of detection was calculated using Equation (4-1). With taking the intercept's error as a standard deviation (SD).

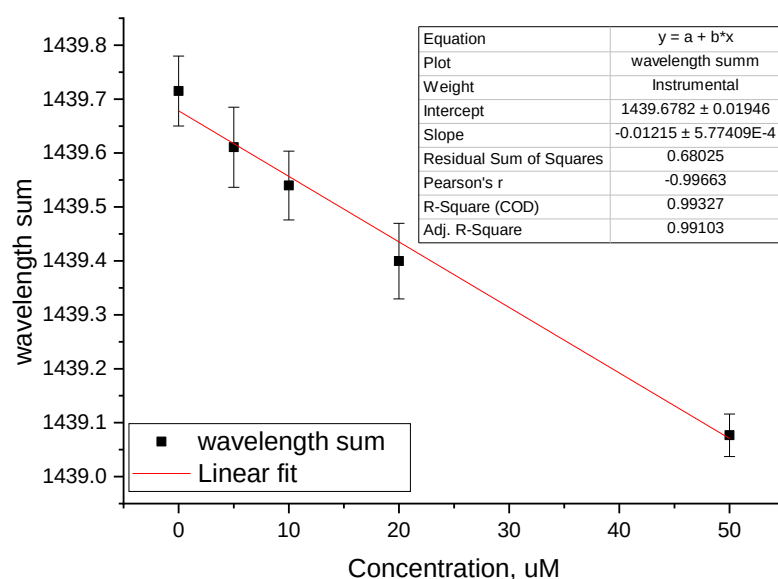


Figure 5-47 Average PMTP attenuations (~ 665 and 775 nm) wavelength sum points of the last 5 minutes of each immersion from 0 to 50 μM ammonia, the DI water immersion corresponds to 0 μM concentration, taken from all 7 immersions before, in-between and after ammonia solutions, - black plots with standard deviation as Y-errors; Linear fit, - red line;

In the case of the intensity ratio (**Figure 5-46**), the SD is 0.00162, and the slope of calibration is 0.0015 which yields a LOD ~ 3.24 μM (55 ppb).

In the case of the PMTP wavelength sum shift (**Figure 5-47**), the SD is 0.01946, and the slope of calibration is 0.01215 which yields a LOD ~ 4.81 μM (82 ppb).

However, it is reasonable to assume, that with optimising the parameters of the DAR/TSPP coated LPG with PMTP operating in the visible range, a much lower LOD can be reached.

5.5.4 Conclusion to the DAR/TSPP coated LPG with PMTP operating in the visible range

In this section the fabrication of LPGs, operating in the visible spectral range, on non-photosensitive optical fibres via hydrogen loading was described. DAR/TSPP coating applied on visible range LPG, its sensitivity to ammonia in water were shown, investigated and compared to an LPG operating in the near infrared region. The calculation of the complex refractive index via the Hilbert transform was shown and performed. Two different ways of ammonia sensing, using the visible range LPG with DAR/TSPP coating were shown: transmission based, using intensity ratio; and refractive index change based, using the PMTP attenuation bands shift.

To the best of the author's knowledge, this is the first time that sensitive dye was used in combination with an LPG operating in the visible range. Overall, the visible range LPG requires deeper research. The role of the complex refractive index of sensitive dyes on such LPG's behaviour needs to be further investigated.

5.6 Conclusion

In this chapter, an ammonia TSPP based aqueous sensor development was shown based on two different OFS.

In the first half of this chapter, the TSPP dye's response to ammonia was examined in an aqueous solution in DI, salt and seawater, the latter proved to be non-functional. The DAR synthesis process was explained. The DAR/TSPP layer-by-layer deposition was shown on a glass substrate, U-shape and LPG OFS. The DAR/TSPP coated U-shape OFS was tested in DI water for response to ammonia concentration from 1 μM to 200 μM concentration, and also a repeatable response was shown from 0 to 50 μM NH_4OH . The DAR/TSPP coating in seawater was shown to be non-functional, presumably because of the non-reversible effect of seawater on the DAR/TSPP film.

In the second half of this chapter, an LPG OFS operating in the visible range was used in combination with DAR/TSPP coating for the ammonia sensor. First, the fabrication of the LPG operating in the visible range on SM450 and SM600 single mode fibres via hydrogen loading was shown. The deposition of the DAR/TSPP was compared between a “visible” LPG and a “regular” near-infrared (NIR) LPG on SM750 single mode fibre. The response to ammonia in DI water of VIS and NIR LPG was compared and shown that such response can be observed only in the case of VIS LPG. Two different response measurement mechanisms were proposed on the VIS DAR/TSPP coated LPG, one via intensity ratios in the PMTP range (LOD=3.24 μ M) the other via wavelength shift sum of the central wavelength on the PMTP attenuation (LOD=4.81 μ M). The first method is assumed to be working mostly on principles of absorbance change of the TSPP in the range of the PMTP, the second is assumed to work on principles of the RI change in the range of the PMTP.

The sensitivity of the DAR/TSPP coated VIS LPG is not very high, however, to the best of the author’s knowledge the usage of sensitive dyes in combination with VIS LPGs were never used before in general, and not with TSPP for ammonia sensing in particular. This approach requires further research and in-depth study of the assumed RI change phenomenon.

The next chapter describes the attempt to develop an aqueous phosphate sensitive OFS.

Chapter 6 Optical fibre sensor for phosphates detection

6.1 Introduction

Phosphorus is a vital nutrient for biological cells, although a significant excess can lead to negative consequences, such as a decrease of oxygen concentration, eutrophication, excessive algal growth and as a consequence overall reduction of water quality [100]. The majority of soluble phosphorus in natural water are in different forms of orthophosphates in four different species depending on the solution's pH level: H_3PO_4 , H_2PO_4^- , HPO_4^{2-} and PO_4^{3-} , **Figure 2-4** [101]

This chapter describes research into the development of optical fibre sensors for measuring orthophosphates in water using (i) an OFS coated with a molecularly imprinted polymer [107], and (ii) an OFS with deposited phosphate imprinted nanoparticles provided by MIP Diagnostics Ltd under collaboration with Gill R&D.

Molecular imprinting is a technique that allows to shape precise cavities during the polymerization of functional monomers in presence of the template molecules. First, the monomers are polymerized with the presence of target molecules, template, then the target molecules are removed, and the polymer yields a porous structure, where pores retain the precise shape and some properties of the target molecules [321]. If simplified, the MIP process is analogous to a process of imprinting a ridged geometrical figure into a piece of plaster. For instance, if a coin is pressed onto a piece of Blu-tack plaster, and then the coin is removed, the Blu-tack will retain the precise imprint cavity of the coin.

There are two different molecular imprinting methods, covalent and non-covalent [321]. It depends on the nature of the interaction between the template and the polymer. Covalent molecular imprinting means a covalent interaction between the template and the functional monomer, hence non-covalent – other interactions, such as hydrogen bonds, electrostatic interaction and coordination bonds. Each method has its advantages and disadvantages. The covalent bonding is in general more sensitive and selective, but the removal of the template is more difficult. Also, the binding/release time is longer, and it is less reversible (which is crucial for sensing)

compared to non-covalent MIP. The non-covalent MIP is less “clear cut”, potentially leading to a reduction in selectivity; nevertheless, it is easier to achieve and manage, there are more options to choose from in comparison with chemical bonding, the template removal is much easier, binding/releasing procedure is faster [321].

6.2 MIP coated LPG fibre sensor

6.2.1 Introduction to MIP coated LPG

In this subsection, the non-covalent N-allylthiourea MIP for phosphate sensing [107] fabrication and evaluation is described. The deposition of the N-allylthiourea MIP film on an LPG surface is explained and analysed, as well as the phosphate sensing test performed.

6.2.2 Materials and method for phosphate MIP evaluation

As a first step, the evaluation of the imprinted and non-imprinted polymers with different solvents was prepared to investigate which one is washed out best in the fabricated MIP. Two different solvents were used Dimethylformamide (DMF), and Acetonitrile (ACN). Both DMF and ACN are polar aprotic organic solvents, however, the difference in some of their parameters, such as density, evaporation rate, and relative polarity, affects the properties of the resulting solution and influences the chemical processes. As a result, the resulting polymers differ to a certain extent in their porosity, ability to form hydrogen bonds and mechanical properties [321]. N-allylthiourea and Ethylene glycol dimethacrylate (EGDMA) were used as functional monomer and crosslinker respectively. 2,2'-Azobis(2-methylpropionitrile) (Azobis) was used as a UV initiator and phenylphosphonic acid (PPA), Figure 6-2a, as a template. The PPA is an organic acid, which consists of a phenyl ring, conjugated with a phosphate group [322], the imprint of this phosphate group serves as a phosphate recognition cavity in the MIP [107], **Figure 6-1b**. PPA was used instead of inorganic

phosphate, because of solubility and ease of template removal. All the chemicals were purchased from Sigma-Aldrich.

The ratios for MIP preparations were as follows:

Table 6-1 materials ratio for phosphate imprinted MIP preparation in ACN and DMF solvents respectively

ACN	1 ml
EGDMA	943 ml
N-allylthiourea	116.2 mg
Azobis	10 mg
PPA	39.75 mg

DMF	0.5 ml
EGDMA	943 ml
N-allylthiourea	116.2 mg
Azobis	10 mg
PPA	79.5 mg

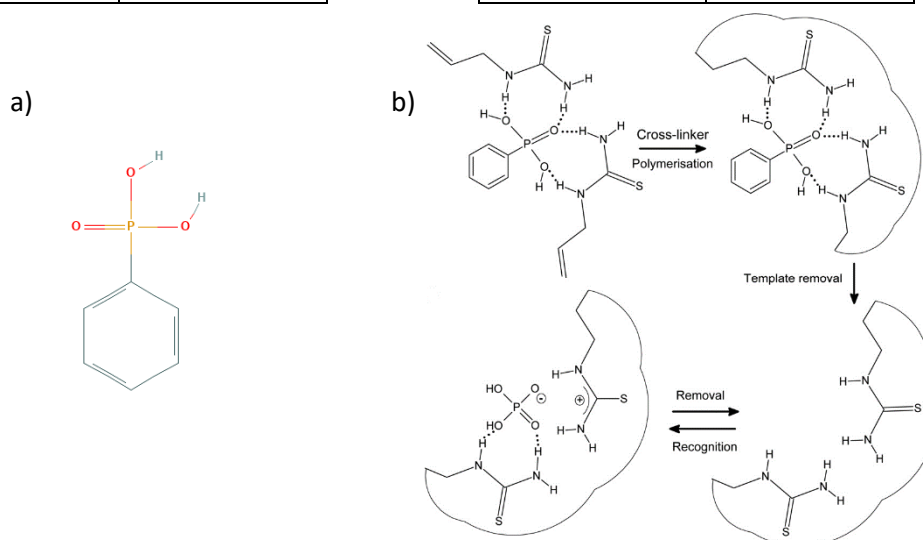


Figure 6-1 a) chemical structure of the Phenylphosphonic acid [322] ; **b)** Diagram of the phosphate imprinting procedure in thiourea based polymer by phenylphosphonic acid as a template [107]

Alongside the imprinted polymer, a non-imprinted polymer (NIP) with the same components, except for the PPA target molecule, was produced. This is necessary for comparison reasons between the MIP and NIP. In order to evaluate the properties of the resulting polymer, a certain amount of each MIP and NIPs were created as follows. The materials were mixed together in a vial with a magnetic stirrer in proportions given in **Table 6-1** with (MIP) and without (NIP) PPA. After the components were mixed and dissolved, the content was poured into a glass vial, and

nitrogen gas was bubbled through the liquid in order to remove any oxygen, as it reacts with free radicals and prevents the polymerization process. After, the vial was sealed and placed under a 2x18W mercury UV-light source (T18W mercury Sylvania double lamp) for 30 minutes. The UV light acts to initiate the crosslinking via the Azobis UV initiator. Then, those polymers were ground separately in two stages to a fine powder using a Retsch PM100 ball grinder. After, 100 mg of each powder was stirred in 10 ml methanol for 10 hours, in order to remove the template molecule (PPA) from the polymer. After the methanol-powder mixture was filtered, with 0.1 um pore sizes filter via a Whatman vacuum filtering funnel (Whatman 1950-002 3 Piece Filter Funnel). Then the filtered methanol was diluted 1 to 200 in order to achieve acceptable UV transparency. UV transmission spectra of those were taken by a UV_VIS Spectrophotometry set-up **Figure 5-1**. Then, the noise of the spectrometer was subtracted from each polymer's spectra. Because the MIPs and NIPs tend to dilute slightly differently, presumably because of the different structure and porosity, the MIP spectrum was normalised to achieve the same absorbance value at 245 nm peak, where the polymer's second absorption peak can be found but the PPA's absorbance is small, Equation (6-1). The total PPA in each MIP was calculated, and the difference spectra compared with the spectra of pure PPA dissolved in methanol taken via the same setup **Figure 5-1**.

$$I_{MIP_M} = \left(\frac{I_{NIP_{245}}}{I_{MIP_{245}}} \right) * I_{MIP} \quad (6-1)$$

6.2.3 Results and discussion for phosphate MIP evaluation

The UV spectra of several concentrations of the PPA dissolved in methanol were taken with a pure methanol UV spectrum as a reference, **Figure 6-2**. As can be seen PPA has one strong and one weaker absorbance band when dissolved in methanol. The stronger band is located around 210-215 nm and gives ~1.2 units at 25 µM concentration, ~0.6 at 10 µM, ~0.3 at 5 µM, 1.5 at 2.5 µM and ~0.5 at 1 µM. The weaker absorption band is broader and peaks at ~263 nm, with value of ~0.12 units at 25 µM, 0.05 at 10 uM 0.025 at 5 uM, 0.013 at 2.5 uM and is close to 0 at 1 uM.

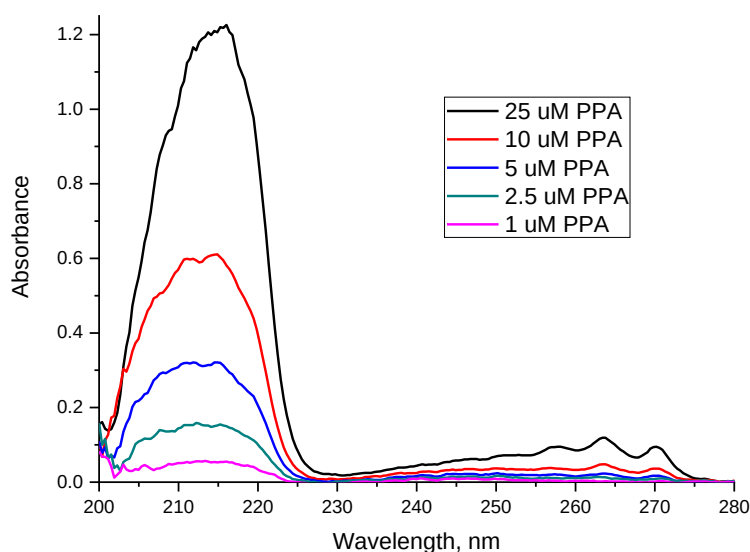


Figure 6-2 UV transmission spectra of PPA diluted in methanol from 1 to 25 μM . Black line represents 25 μM concentration, red – 10 μM , blue – 5 μM , green 2.5 μM and pink 1 μM .

The methanol from each, MIP and NIP, stirring was filtered through 0.1 μm filter paper, diluted 1 to 200 and UV spectra of each were taken, **Figure 6-3** for polymers created in ACN solvent, and Figure 6-5 for polymers created in DMF solvent. **Figure 6-3a** shows the absorption spectra of washing methanol, diluted 1 to 200, ACN MIP (red curve) and NIP (black curve) polymers, with pure methanol as a reference spectrum. As one can see, both curves have two absorption peaks, one at ~210-215

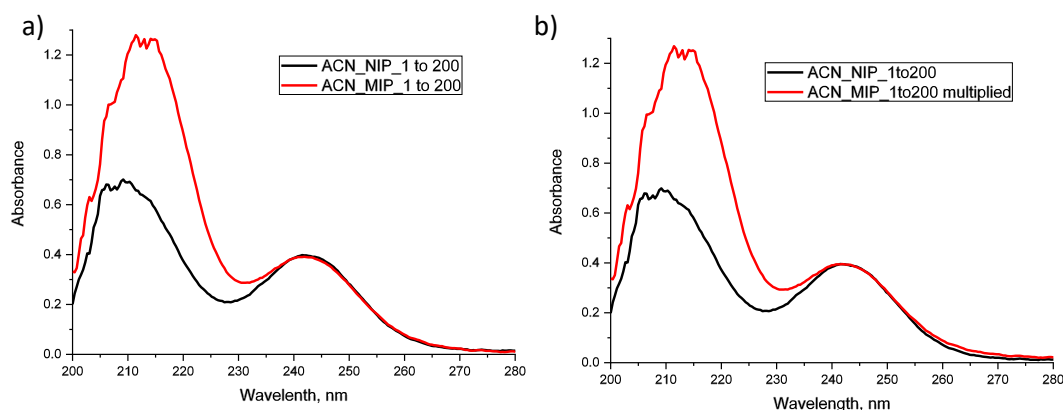


Figure 6-3 a) Absorption spectra of the methanol stirred with 100 mg ACN polymers, diluted 1 to 200, for NIP (black curve) and MIP. **b)** Absorption spectra of the methanol stirred with 100 mg DMF polymers, diluted 1 to 200, for NIP (black curve) and MIP multiplied spectra to achieve the same value at 245 nm as the NIP spectra

which is stronger in both cases, however, much stronger in the case of MIP, ~ 1.25 against ~ 0.7 for NIP, also the last is narrower with a shift to the blue and peaks at 206-211 nm. It is reasonable to assume that the highest absorbance in the range of 210-215 nm is caused by the PPA washed away from the polymer matrix. The weaker peak has a maximum at ~ 242 nm with ~ 0.39 absorbance units for both NIP and MIP ACN, however, the NIP absorption is lower in this case. This can be caused by differences in the polymers' structures and thus, the solubility of the polymer residues or their unreacted components in methanol. As an attempt to equate the absorbance values, the intensity ratio at 245 nm was taken, as the PPA's absorbance has small values at 245 nm, hence the PPA being the only significant difference between those two polymers it does not affect the absorbance. The MIP spectra then were multiplied by the value of the ratio, **Equation (6-1)**, **Figure 6-3b**. After, the difference between MIP multiplied and NIP was taken and plotted together with pure PPA in methanol spectra, **Figure 6-5a**. The difference is taken as a suggestion that it should yield the PPA absorption, as it's the only significant difference between the MIPs and NIPs. As one can see from the **Figure 6-5a** the ~ 215 nm absorption peak of the washing methanol of ACN MIP-NIP difference (red curve) has a value of ~ 0.48 , which is in-between the 5 and 10 μM PPA in methanol absorption spectra (blue and green curves), with ~ 0.32 and ~ 0.61 μM respectively. The full content of the diluted washing methanol should yield ~ 6.5 μM of PPA if all the PPA was removed from the polymer by washing.

The same steps were done for the DMF based polymer, the absorption of the diluted 1 to 200 washing methanol of DMF based NIP and MIP can be seen in **Figure 6-4a** and the version has gone through **Equation (6-1)** for MIP spectra, which can be seen on **Figure 6-4b**. The difference between DMF based MIP multiplied and NIP is plotted together with pure 5 μM PPA in methanol spectra, **Figure 6-5b**. The total PPA in the diluted 1 to 200 washing methanol DMF based MIP is ~ 15 μM if all of the PPA would be washed out from the MIP. As one can see from **Figure 6-5b** the peak value of the 5 μM PPA absorbance spectrum (green curve) is ~ 0.3 , while the peak value of the MIP-NIP difference is ~ 0.32 , the difference is minimal. Hence, if the assumption that the spectral difference of MIP and NIP yields mainly the PPA absorbance, which

should be $\sim 15 \mu\text{M}$ in this case, it can be assumed that roughly less than one-third of the PPA has been removed from the DMF based MIP.

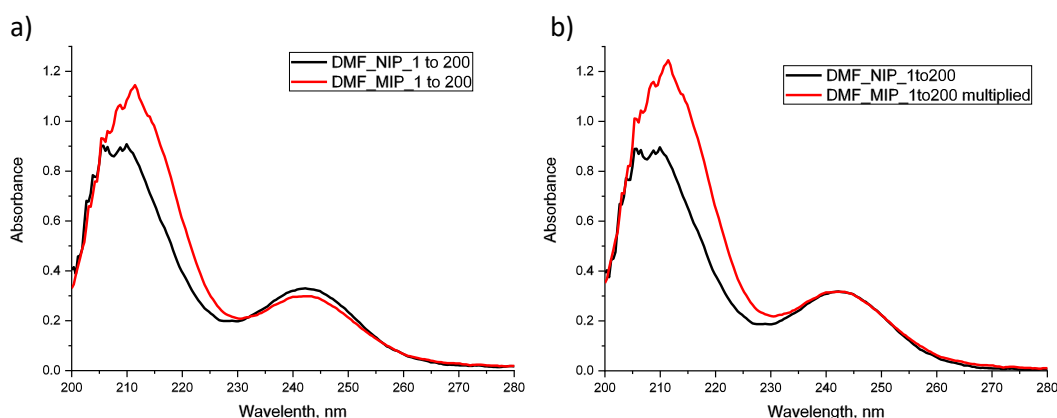


Figure 6-4 a) Absorption spectra of the methanol stirred with 100 mg DMF polymers, diluted 1 to 200, for NIP (black curve) and MIP. **b)** Absorption spectra of the methanol stirred with 100 mg DMF polymers, diluted 1 to 200, for NIP (black curve) and MIP multiplied spectra to achieve the same value at 245 nm as the NIP spectra;

The results and method for evaluation of the template removal are not 100% accurate as of the shape of the spectral difference and the pure PPA spectra are not identical. Nevertheless, it gives an approximate understanding of which type of solvent, ACN or DMF based, is better to use for the fibre coating. As the DMF based polymer with an initially higher concentration of the PPA appears to be even lower in the washed out PPA than the ACN, then it is reasonable to proceed with the ACN based MIP.

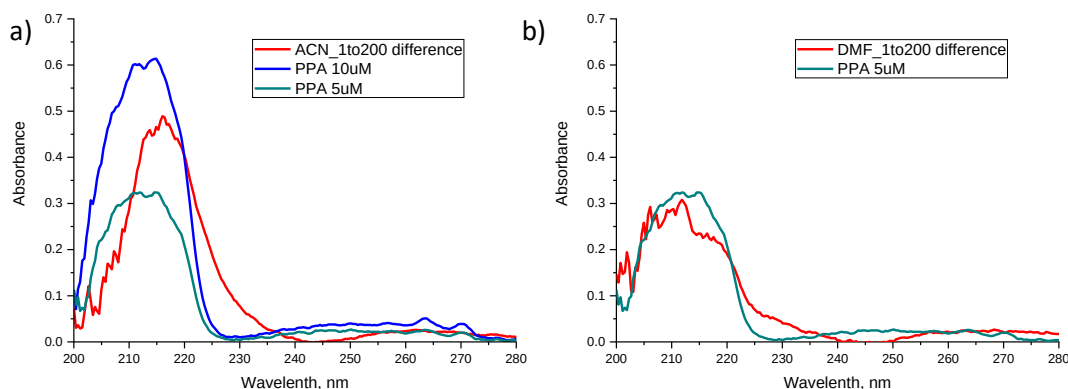


Figure 6-5 a) Absorption spectra difference between the washing methanol of ACN MIP with $\sim 6.5 \mu\text{M}$ PPA content and NIP (red curve) compared to absorption spectra of $10 \mu\text{M}$ (blue curve) and $5 \mu\text{M}$ PPA (olive curve) in methanol. **b)** Absorption spectra difference between the washing methanol of DMF MIP with $\sim 15 \mu\text{M}$ PPA content and NIP (red curve) compared to absorption spectra of $5 \mu\text{M}$ PPA (olive curve) in methanol;

6.2.4 Materials and method for phosphate MIP coated LPG fibre

Deposition of our EGDMA + N-allylthiourea organic polymer on an inorganic silica glass surface has two main challenges. Firstly, it must bind strongly to the silica to withstand the template removal procedure by a solvent, and to be robust enough for long usage in water. Secondly, it should be done strictly under an oxygen-free environment to prevent the free radical – oxygen interaction [321].

The first issue was resolved by using the silanization procedure, which is a covalent linkage between inorganic silica with one of the organic functional groups of the material we want to bind [323]. In our case, it is a linkage between silica and the amino group of N-allylthiourea. The protocol for the glass–amino group were adapted from Nicholas et al. [264]. The procedure contains 5 steps:

1. Functionalization of the glass surface with hydroxyl groups. The fibre/substrate is dipped into 1% KOH ethanol/water (3/2 respectively) for 20 min. After the fibre/substrate is removed from KOH solution, washed in DI water and dried;
2. Functionalization of the silica with amino groups. The fibre/substrate is immersed and stirred in 2% APTES solution in methanol for 10 minutes, with proper washing in DI water and 70 min heating in an oven under 110°C afterwards;
3. Binding the silanes' amino group to glutaraldehyde. Immersing and stirring the fibre/substrate in 8% glutaric aldehyde aqueous solution for 30 minutes, with the washing in DI water and air drying after;
4. Binding the glutaraldehyde's again to the Allylthiourea's amino group. MIP/NIP dip coating deposition under nitrogen atmosphere.
5. Removing the template molecule from the coating by washing the fibre in methanol for ~2 hours.

One of the most challenging parts of the procedure described above is the MIP/NIP deposition via dip coating. As mentioned previously, the polymer mixture

should be completely deoxygenated. The simplest way to do it is to bubble nitrogen in the polymer mixture, substituting the oxygen in the liquid with nitrogen. However, our experimental attempts have shown that the oxygen should be removed from the surrounding atmosphere as well, as it penetrates back into the polymer mixture, inhibiting the polymerisation reaction. Moreover, it happens if the polymer is spread as a thin film on the surface of the fibre/substrate during the polymerisation process. In order to achieve this condition, a sealed dip coating setup was designed and built, **Figure 6-6a**. Here, confined in a glass jar, a vial with the polymer mixture is equipped with a thin tubing on the bottom, to allow the nitrogen gas to propagate through the polymer mixture. On top of the vial with the polymer mixture, a hydraulic moving arm was constructed, for the fibre's dip coating. The fibre was fixed to the moving arm, in a way that allows one end of the fibre (with the LPG and mirror layer on the end of the fibre and outer edge of the LPG) to be dipped and raised into the polymer mixture. The other end of the fibre exits the sealed jar and is connected to a light source and spectrometer via a fibre coupler. The other obstacle was in the LPG sensor itself, as it normally operates in transmission mode, which means the fibre's physical end would be far from the LPG itself, and it would be even more difficult to apply the dip coating technique. To overcome this issue, before the fibre's silanization procedure, a silver mirror layer (using silver nitrate reduction Tollens' approach) was

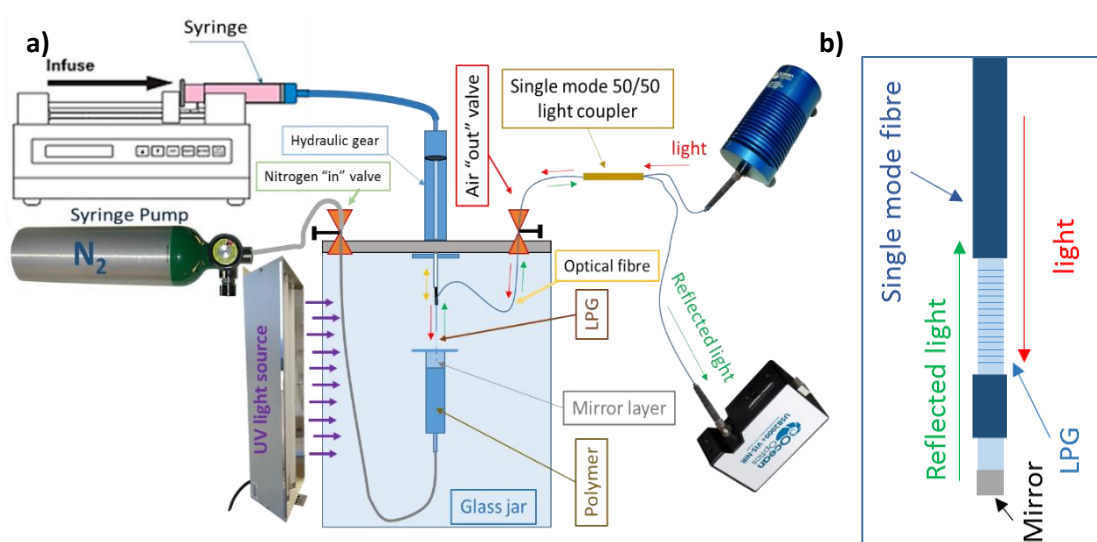


Figure 6-6 a) Schematic illustration of polymer dip coating deposition setup on a mirrored fibre under Nitrogen atmosphere; **b)** schematic illustration of an LPG in reflection mode

coated on the tip of the fibre [280, 324], approximately 1 cm further from the edge of the LPG. This allows the LPG to work in reflection mode, which doubles the light propagation through the grating region, **Figure 6-6b**. The fact that light goes forward and back in the LPG allows us to make it twice as shorter, 1.5 cm instead of 3 cm, and keep the same attenuation values [280]. This approach has many advantages, such as miniaturisation, one long feeding/receiving fibre (instead of two in transmission mode) which makes it much easier to use especially when applying of dip coating technique.

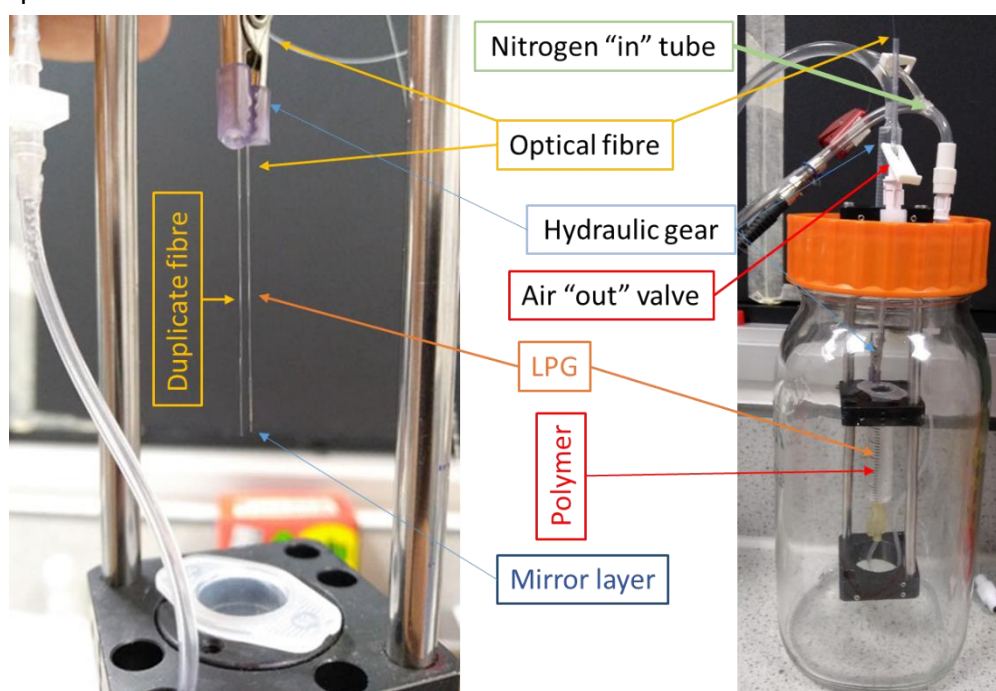


Figure 6-7 Photographic illustrations of the MIP dip coating deposition setup under Nitrogen atmosphere.

The deposition of the MIP was performed simultaneously on two fibres **Figure 6-7**, the actual LPG and a piece of the same fibre with removed cladding, functionalised in the same way as the LPG. This was done in order to examine the coating on SEM, before and after washing.

The deposition procedure:

1. Two fibres, apart from each other, were attached to the clips in the lower end of the hydraulic pulling gear;
2. The jar was sealed, and the air was sucked from the jar, using a vacuum pump, then immediately replaced by Nitrogen to atmospheric pressure.

3. The MIP mixture was delivered to the deposition vessel via a plastic pipe attached to the bottom of the deposition vessel, using a pipette;
4. The fibres were immersed into the polymer mixture using the NE-300 syringe driver via hydraulic gear.
5. Nitrogen was bubbled gently through the same plastic pipe for 30 minutes, thus removing any oxygen from the polymer and substituting the oxygen residue in the jar with incoming nitrogen (using a lab oxygen meter inside the jar, it was shown that the oxygen level drops below 3% after 10 minutes of bubbling);
6. The nitrogen flow was stopped, the valves were closed. Then UV light was switched on and the hydraulic gear pulled up the fibre with a velocity of ~ 1.6 mm/sec. The UV light remained on for 30 minutes.
7. The fibres were extracted from the deposition setup. A piece of the dummy fibre was cut and sealed for SEM examination. The remaining part, together with the LPG fibre was immersed in methanol for gentle stirring over 2 hours.

Transmission/reflection spectra of the LPG's MIP dip coating deposition and washing were recorded in situ using a spectrometer (Ocean Optics USB2000+) connected to a PC, a coupler (Thorlabs), and feeding light by a Halogen Light source (HL-2000, Ocean Optics).

After the deposition and washing, the MIP coated LPG was tested for phosphate response in DI water, while the washed and unwashed fibre examples were examined by SEM (JEOL JSM 7000F Schottky Emission SEM and Philips XL-30 ESEM).

The LPG response was interpreted by calculating the area above and below the PMTP curve, and taking the ratio of the sum of above and below areas ($S+A$) to the area below the PMTP, **Figure 6-8**, Equation (6-2).

$$R = \frac{S + A}{A} \quad (6-2)$$

Where S is the area above the PMTP limited by a and b wavelength values and the “boundary line” drawn between a and b values of the transmission spectra; A is

the area below the PMTP limited by a and b wavelength values and the x-axes, **Figure 6-8**.

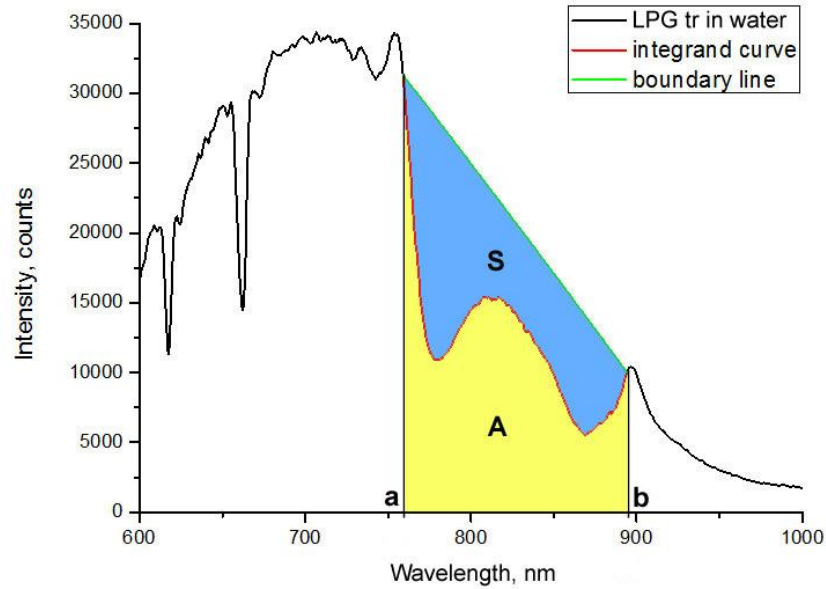


Figure 6-8 Illustration of the PMTP ratio approach for determining the LPG's response to external factors, such as temperature, RI change, strain. The R , ratio yields of the sum of S and A areas to the A area.

The areas were calculated continuously throughout the whole measurement by integration using “trapz” command in MATLAB.

As LPGs are sensitive to temperature change [325], the MIP coated LPG was calibrated for temperature response in DI water by placing the DI water vessel with immersed MIP coated LPG into a temperature controlled water bath (Thermo Scientific Grant SUB Aqua 5). The calibration was done as follows: the temperature of the water bath was set to a certain value (for instance 22.5°C) for 30 minutes, then increased with the step of 0.5°C for 30 minutes and increased again for 0.5°C for 30 more minutes. The temperature was measured continuously using a sealed thermocouple (Pico SE027) via a USB data logger (Pico USB TC-08). Then the R (areas ratio) values were plotted against the temperature values, and a linear fit was performed. Afterwards, the temperature of the tested solution was measured during the phosphate water test continuously, and the slope and intercept values from the calibration linear fit were used to achieve a temperature compensated R ratio, using Equation (6-3).

$$R_{i_comp} = \frac{R_i}{a + b * t_i} \quad (6-3)$$

Where R_{i_comp} is the i-th temperature compensated PMTP areas ratio, R_i is the i-th PMTP areas ratio; a is the intercept of the linear fit; b is the slope of the linear fit; and t_i is the i-th reading of the temperature.

The concentration of the phosphates in water was increased by adding into the tested water a small, previously calculated amount of higher concentration stock solution. For instance, by adding 20.02 μ l of 100 mM KH_2PO_4 stock solution into 20 ml of DI water, would yield a 100 μ M KH_2PO_4 . By adding an additional 80.32 μ l of the 100 mM stock, the resulting concentration will be 500 μ M, and so on. The potassium phosphate monobasic was ordered from Sigma-Aldrich and a stock solution was prepared on the day of the experiment.

6.2.5 Result and discussion for phosphate MIP coated LPG fibre

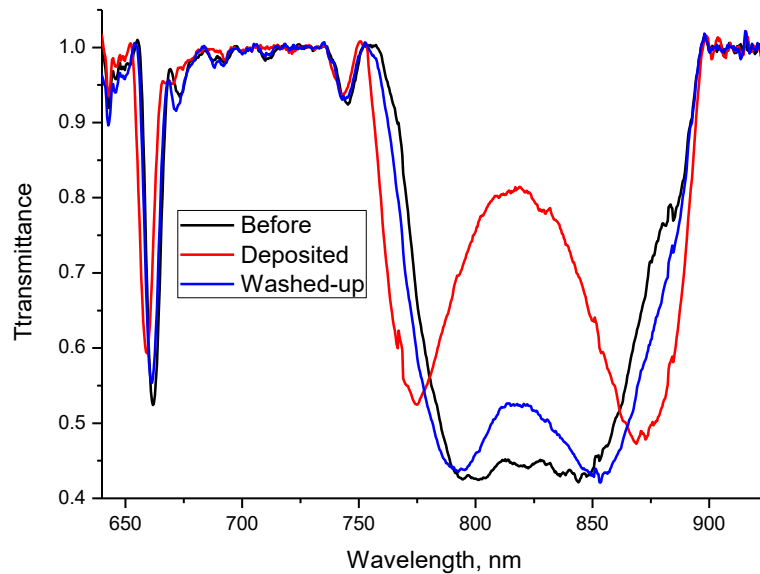


Figure 6-9 Normalized transmission/reflection spectra in air of the 112.0 μ m 1.6 cm long LPG written on SMF750 optical fibre operating in the reflection mode, before deposition - black curve; after deposition - red curve; and after washing in methanol for 2 hours - blue curve.

The deposition of the MIP on a 112.0 μm 1.6 cm long LPG written on SMF750 optical fibre operating in the reflection mode was performed under nitrogen atmosphere by the dip coating technique with withdrawal speed ~ 1.6 mm/sec via the deposition setup described in **Figure 6-6a**. The transmission/reflection spectra of the LPG were recorded continuously during the deposition and during the washing procedure. **Figure 6-9** shows the normalized transmission/reflection spectra in the air before the deposition (black curve), after the deposition (red curve) and after washing in methanol for 2 hours (blue curve). As one can see, after the deposition (red curve) the PM

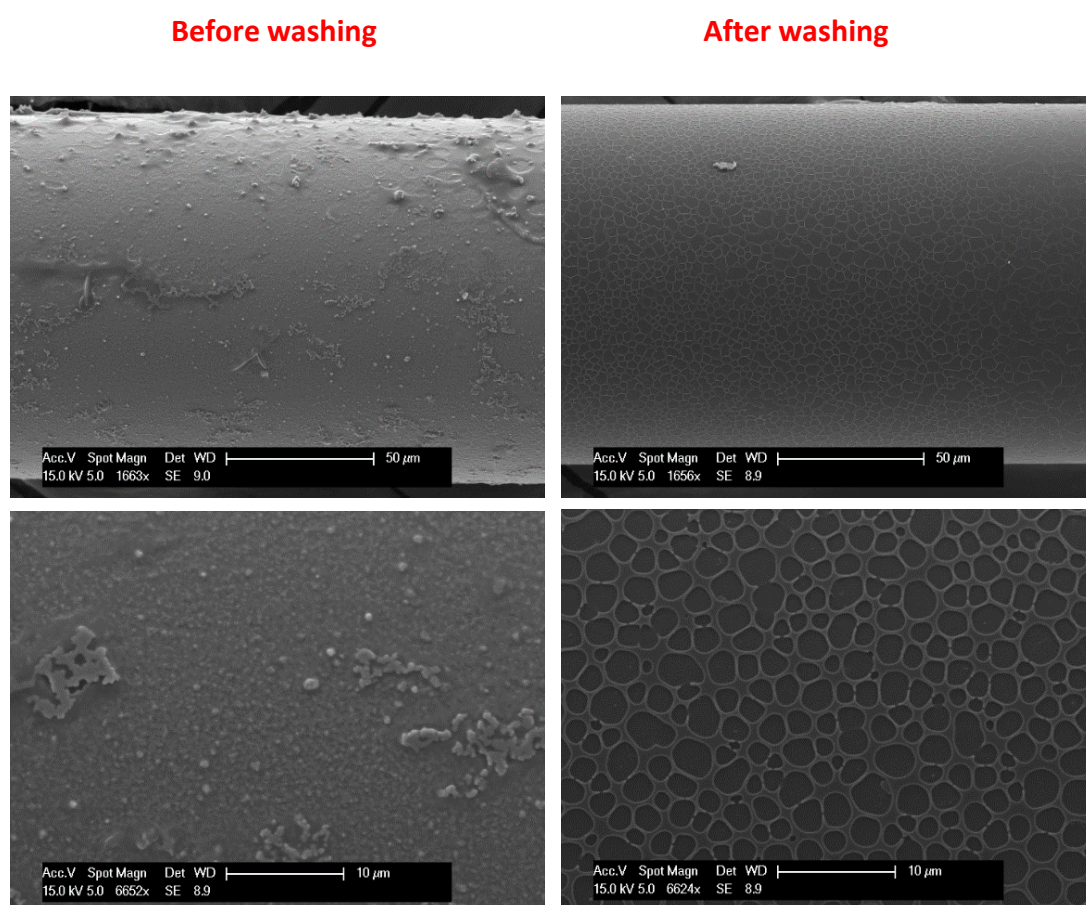


Figure 6-10 SEM images of the ACN MIP deposited SMF750 fibre before and after washing in methanol for ~ 2 hours. Top row scale bar = 50 μm , bottom row scale bar = 10 μm

TP is wider due to the deposited layer thickness, which affects the RI [326], hence the LPG senses it. After washing in methanol for ~ 2 hours, the PMTP narrows (blue curve), however, it remains wider than it was before the deposition (black curve). This suggests a partial removal of the film and the template molecules from

the LPG fibre's surface. **Figure 6-10** shows the SEM images of the ACN MIP deposited fibre before and after washing in methanol for two hours. Before the washing, the fibre can be seen to be covered by a film on top of its surface, after the washing the MIP film is partially removed, and bubble-like holes in the polymer can be seen. However, the MIP film is still present on the surface of the fibre in this porous form.

After the washing procedure, temperature calibration of the MIP coated LPG was performed. The temperature was raised from 21.9°C to 22.5°C where it was held for ~20 minutes after it was raised to 23.0°C where it was held for another 20 minutes and finally the temperature was raised to 23.5°C for another 20 minutes. **Figure 6-11a** shows the temperature (red curve, left y-axis) and the areas ratio (blue curve, right y axis) values during the calibration procedure. As one can see, the areas ratio value follows the opposite trend to the temperature reading. In **Figure 6-11b**, the black curve shows the areas ratio plotted against the temperature, and the red line shows the linear fit of the areas ratio against temperature. Then the slope ($b = -0.05202$) and intercept ($a = 2.418$) values taken from the linear fit were used to calculate the temperature compensated areas ratio value (R_{i_comp}) using **Equation (6-3)**.

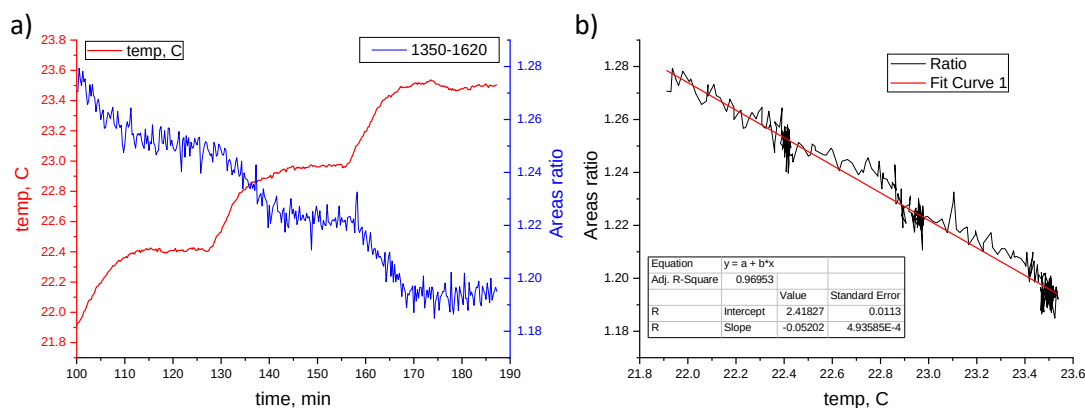


Figure 6-11 Temperature calibration of the ACN MIP coated 112 μm LPG, **a)** Temperature (left y axis) taken by a thermocouple, - red curve and the LPG's area ratio value (right y axis), - blue curve; **b)** Areas ratio plotted against temperature, - black curve, and linear fit of it, - red line

Figure 6-12 shows the ACN MIP coated 112 μm LPG test in water in response to phosphates. In **Figure 6-12a**, one can see the areas ratio R value of the LPG's PMTP (blue curve, right y-axis) and the temperature in $^{\circ}\text{C}$ (red curve, right y-axis) measured by the thermocouple. The temperature was falling steadily from 23.15 $^{\circ}\text{C}$ to 22.56 $^{\circ}\text{C}$.

At 66.5 minutes of the measurement, 20.02 μl of 100 mM potassium phosphate solution was added to the 20 ml DI water in which the LPG was immersed. A spike can be observed in the R value immediately after the phosphate was introduced to the solution. Then the R value continues to increase together with the KH_2PO_4 concentration, and as the temperature falls. **Figure 6-12b** shows the temperature compensated R value, here one can see how the compensated areas ratio remains relatively stable until phosphate enters the vial (65.5 min), where rapid growth can be observed from 0.98 to 0.99 in the first 5 minutes. The R_{comp} value continues to grow with increasing concentrations. However, this growth is not linear against the concentration increase.

Unfortunately, the response of the sensor to phosphates in DI water could not be repeated despite many attempts to replicate it on this or any other sensor fabricated after. Each time the sensors were examined under SEM and similarly tested in DI water. This raises the question, of whether this was a response to something else, or indeed to phosphates, due to some unforeseen reasons during the fabrication of this particular sensor.

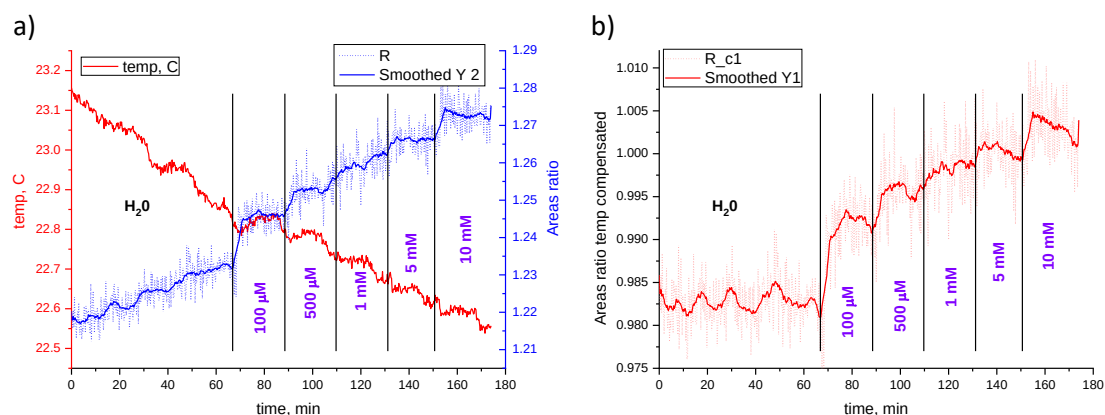


Figure 6-12 phosphate test in water of the ACN MIP coated 112 μm LPG, **a)** Temperature (left y axis) taken by a thermocouple, - red curve and the LPG's area ratio value (right y axis), - blue curve; **b)** Temperature compensated areas ratio value (y axis) over time (x axis) during increasing concentrations of phosphate in water.

6.2.6 Conclusion for phosphate MIP coated LPG fibre sensor

In this subsection, the non-covalent N-allylthiourea phosphate MIP fabrication and evaluation were described. The deposition of the phosphate imprinted MIP on

an LPG surface under a nitrogen atmosphere was explained and analysed. Area of the PMTP of an LPG transmission spectrum was introduced as a response monitoring approach. Phosphate sensing test performed on the phosphate MIP coated LPG, and one time response reported. Unfortunately, the attempts to replicate the response failed.

6.3 MIP nanoparticles

6.3.1 Introduction to MIP nanoparticles

This subsection will describe research into the development of a phosphate sensitive OFS using the MIP nanoparticles (MIP NPs) and MIP materials synthesised by MIP Diagnostics Ltd. under a collaboration agreement with Gill R&D. The materials were given to the author to conduct further research and development, however, the precise chemical nature and structure of the MIP materials remained unknown to the author and will not be disclosed in this thesis because of legal restrictions.

6.3.2 Materials and method for phosphate MIP evaluation

The tested MIP materials were as follows: (i) MIP NPs without functional groups (Normal MIP materials), MIP NPs with Epoxy, Carboxyl, and Amine groups synthesized in ACN (acetonitrile) and DMSO (dimethyl sulfoxide) solvents. According to MIP Diagnostics, their dynamic light scattering (DLS) measurements showed an average size of the MIP nanoparticles (MINPs) to vary from 325 to 415 nm; (ii) MIP “linear polymer”, a MIP has grown on the pre-functionalised surface of a U-shape OFS. The MIP NPs with functional groups were developed in order to make possible the firm attachment to a glass surface via silanization. All the polymers were synthesized by MIP Diagnostics to give a colour change response in presence of phosphates. This should make the OFS response easier because absorption-based OFS can be deployed. Specifically, the U-shape OFS was chosen for this task which is described in Sections 3.2.1 and 3.2.2.

The MIP materials were examined on a JEOL JSM 7000F Schottky Emission Scanning Electron Microscope in both pre and post deposition states. Filtered MIP material was examined on an SEM, with filter pores being significantly smaller than the specified size of the nanoparticles. In addition, the MIP materials were tested in a suspended (or dissolved) state for response to organic and inorganic phosphates.

The MIP materials were examined for response to organic and inorganic phosphates in pure organic solvent (DMF) and with the addition of water. For inorganic phosphate response the potassium phosphate monobasic (KH_2PO_4) was used and for organic phosphates Tetrabutylammonium phosphate monobasic (TBAPh, $(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2)_4\text{N}[\text{OP}(\text{OH})_2\text{O}]$, MW=339.45). All chemicals were ordered from Sigma-Aldrich and used without further purification.

The transition spectra of the diluted MIP materials and added phosphates were recorded using a spectrophotometric setup, **Figure 5-1**. The DMSO COOH (synthesized with carboxyl functional group in DMSO) MIP material was suspended/diluted in DMF solvent in a ratio of 3.1 mg MIP material/1ml of DMF. 1.4 ml of the resulting solution was poured into a quartz cuvette in the spectrophotometric setup described in **Figure 5-1**. Then a freshly prepared 100mM TBAPh solution in DMF was added into the cuvette in a specific quantity to achieve a desired final concentration. For instance, by adding into 14.14 μl of 100 mM TBAPh stock solution into 1.4 ml of MIP materials DMF solution, would yield a 1 mM TBAPh final concentration.

In order to investigate the response to inorganic phosphates, the MIP materials DMF solution was diluted with DI water in a 50/50 ratio. It was a necessary step because the potassium phosphate is not soluble in DMF. In addition, the impact of water was investigated by adding both TBAPh and KH_2PO_4 to a 0.5 mg/ml DMSO COOH MIP materials solution in 50/50 DMF/Water solvent. The desired concentrations were achieved in the same manner as described above.

The received MIP materials were examined with SEM using the following procedure. The MIP materials were diluted in DMF (3 mg/ml), then a 5 μl droplet was placed on a 5x5mm numbered glass substrate. The substrate was left to air-dry, so after the evaporation of the DMF solvent, the MIP materials residue remained on the substrates. After, the substrates were carefully placed and attached to the specimen

holder via conductive SEM double sided tape and coated with an 8-15 nm layer of iridium using Quorum Q150T ES or platinum using Polaron SC7640 sputter coaters. After the specimen holder was placed into the JEOL JSM 7000F Schottky SEM for processing.

To achieve a higher concentration of the nanoparticles a filtering technique was used as follows: first, the MIP materials were suspended/diluted in DMF, after the resulting solution was filtered through a Whatman 3 piece filter funnel 1950-002 fitted with Whatman 7181002 filter paper with 100 μm pores. Therefore, the filter paper should hold the 325-415 nm MIP NPs. The weight of the MIP material stopped by the 100 nm filter was compared to the initial weight of the diluted MIP material. Some of the absorption spectra of the solution were compared before and after the filtering, using the setup shown in **Figure 5-1**. The filter paper was weighed via laboratory scales (XS105 DualRange from Mettler-Toledo) before and after the filtering procedure (when the solvent disappeared). By subtracting the “before” weight from “after” one can obtain the amount of materials detained by the filter. The filtered MIP material was examined via SEM in the same way as described above for the unfiltered MIP material. In addition, the filter paper itself was examined under SEM, by carefully cutting small pieces of the used filter paper, which then was attached to the specimen and coated by iridium.

The raw MIP materials were also examined under SEM. A small amount of each MIP material was spread on a specimen holder with conductive double sided tape, coated by iridium and examined in the electron microscope.

Different deposition techniques were applied to deposit the MIP material to the U-shape fibre’s surface, such as dip coating in sol-gel, covalent bonding to the glass surface via silanization [323]; and polymerization on a U-shape fibre’s surface (this was done at the facilities of MIP Diagnostics). During each deposition two fibres were used, one to test for phosphate response, the other to examine via SEM. The deposited fibres were examined via SEM and tested for phosphate response. The sol-gel matrix was prepared using the same method as described in **section 3.3.1** with the addition of regular MIP material (without any additional functional group). The ratio of the sol-gel mixture is shown in *Table 6-2*.

Table 6-2, Sol-gel ratio fabrication with embedded non-functionalised MIP

TEMS	TEOS	Ethanol	Water	MNIPs	TMAOH (10%)
300 ml	33 ml	1000 ml	330 ml	20 mg	50 ml

After the MIP material embedded sol gel was deposited via dip coating on a U-shape OFS made of FMT-200 multimode optical fibre. The deposition method and setup are described in **Section 4.4.2** and **Figure 4-23**.

For the deposition of the MIP materials with amino functional groups (NH_2), the fibre was silanized using the same APTES-Glutaral protocol as described in Section 6.2.4 adopted from Nicholas et al [264], **Figure 6-13**:

1. Functionalization of the glass surface with hydroxyl groups. The fibre/substrate is dipped into 1% KOH ethanol/water (3/2 respectively) for 20 min. After the fibre/substrate is removed from KOH solution, washed in DI water and air dried;
2. Functionalization of the silica with amino groups. The fibre/substrate is immersed and stirred in 2% APTES solution in methanol for 10 minutes, with proper washing in DI water and 70 min heating in an oven under 110°C afterwards;
3. Binding the silanes' amino group to glutaraldehyde. Immersing and stirring the fibre/substrate in 8% glutaric aldehyde aqueous solution for 30 minutes, with the washing in DI water and air drying after;
4. The fibre then was immersed into a 2 mg/ml Amino MIP material in DMSO for two hours, binding the glutaraldehyde to the MIP materials amino group.

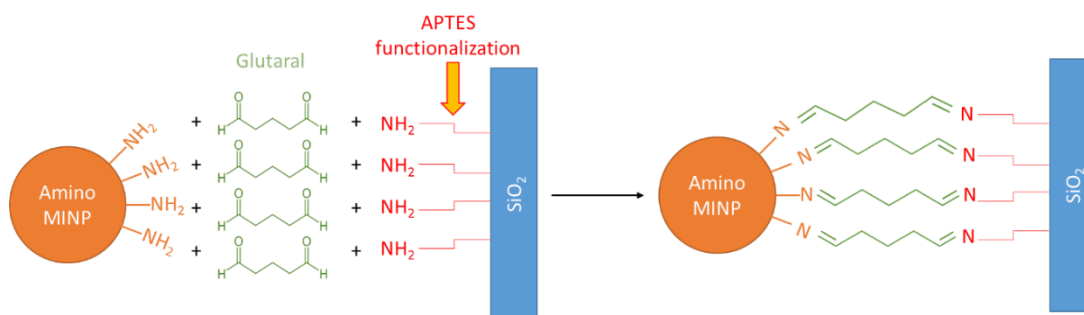


Figure 6-13 Schematic illustration of the Amino MINP binding to glass surface

For the deposition of the MIP materials with epoxy functional groups, the fibre was silanized with APTES only, with the same protocol as above, but without the glutaraldehyde step (No 3), **Figure 6-14**:

1. Functionalization of the glass surface with hydroxyl groups. The fibre/substrate is dipped into 1% KOH ethanol/water (3/2 respectively) for 20 min. After the fibre/substrate is removed from KOH solution, washed in DI water and air dried;
2. Functionalization of the silica with amino groups. The fibre/substrate is immersed and stirred in 2% APTES solution in methanol for 10 minutes, with proper washing in DI water and 70 min heating in an oven under 110°C afterwards;
3. The fibre then was immersed into a 2 mg/ml Epoxy MIP material in DMSO for two hours, binding the surface amino groups to the MIP materials epoxy group.

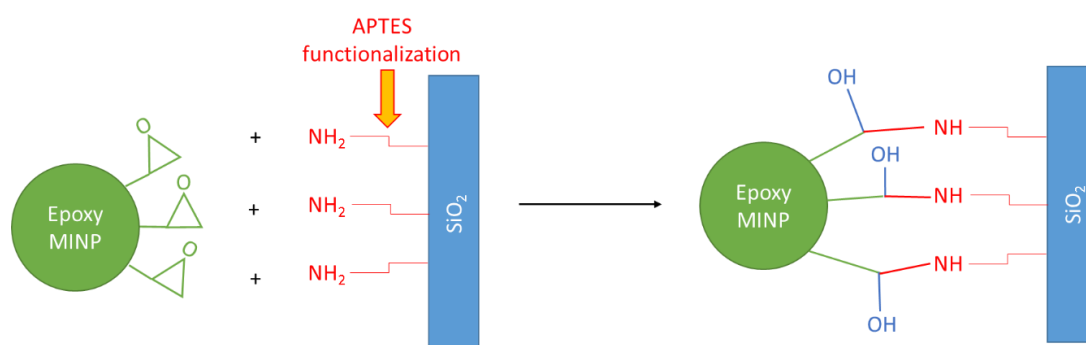


Figure 6-14 Schematic illustration of the Epoxy MINP binding to glass surface

For the deposition of the MIP materials with carboxyl functional groups the fibre was treated as above (APTES functionalization) to achieve the glass' NH_2 functionalisation, **Figure 6-15**:

1. Functionalization of the glass surface with hydroxyl groups. The fibre/substrate is dipped into 1% KOH ethanol/water (3/2 respectively) for 20 min. After the fibre/substrate is removed from KOH solution, washed in DI water and air dried;

2. Functionalization of the silica with amino groups. The fibre/substrate is immersed and stirred in 2% APTES solution in methanol for 10 minutes, with proper washing in DI water and 70 min heating in oven under 110°C afterwards;

However, the carboxyl MIP materials also require additional treatment [327]:

3. 500 μL of the 3 mg/1ml COOH MIP material solution in DMF was added into 400 μL of MES (2-(N-morpholino) ethanesulfonic acid)) buffer (0.1 M, pH = 5.6) containing a 1 mg of NHS (N-hydroxysulfosuccinimide). 0.4 mg of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and 150 μL of DI water was added and stirred 15 min at room temperature (22 °C).
4. 175 μL of carbonate buffer with pH = 11 were added to the solution to increase the pH to ~7;
5. The pre-silanized fibre was immersed into the above solution overnight at room temperature;
6. Afterwards, the LPG was rinsed with DI water to remove loosely bound COOH MIP materials;
7. The fibre was immersed into a carbonate buffer (pH = 9.2) for 2 h to hydrolyse the remaining NHS-esters and recover the original-COOH groups.

For the MIP synthesis on the surface of a fibre, APTES pre-silanized U-shaped fibres were posted to MIP diagnostics where the MIP was “grown” directly on the surface of the U-shape fibre. Then the deposited fibres were posted back to the University of Nottingham for SEM examination and sensitivity tests.

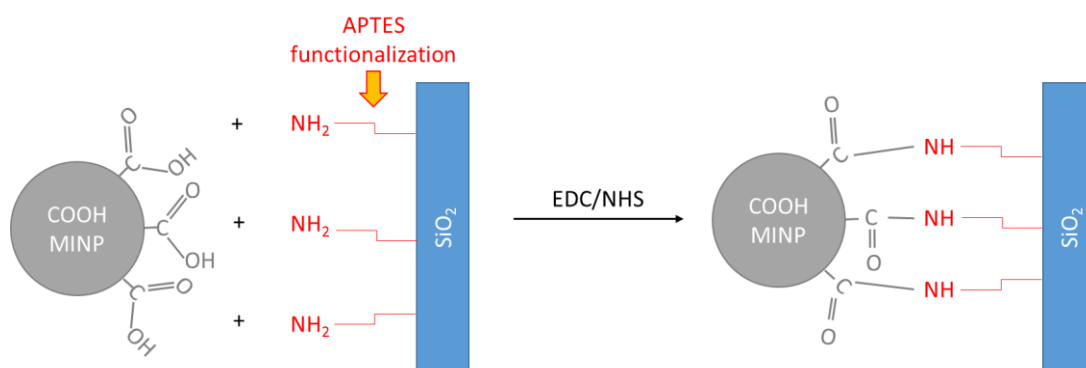


Figure 6-15 Schematic illustration of the carboxyl MINP binding to glass surface

The MIP materials deposited on U-shape OFS were tested for response to phosphate using the setup shown in **Figure 5-17**. The MIP deposited U-shape fibre was immersed into solutions with different concentrations while recording the transmission spectra by a spectrometer (Ocean Optics USB 2000). The light was emitted by a Halogen Light source (HL-2000, Ocean Optics) which gives a wide VIS/IR spectrum.

The concentration of the phosphates in water was raised by adding into the tested water a small, previously calculated amount of higher concentration stock solution. For instance, by adding 20.02 μl of 100 mM KH_2PO_4 stock solution into 20 ml of DI water would yield a 100 μM KH_2PO_4 . By adding than an additional 80.32 μl of the 100 mM stock, the resulting concentration will be 500 μM , and so on. The potassium phosphate monobasic and TBAPh were ordered from Sigma-Aldrich and a stock solution was prepared on the day of the experiment.

6.3.3 Results and discussion of the phosphate MIP materials in a solution

Figure 6-16 a shows the absorbance spectra of 3.1 mg/ml DMSO COOH MIP materials solution in DMF (blue curve), and the same solution's absorption after 10

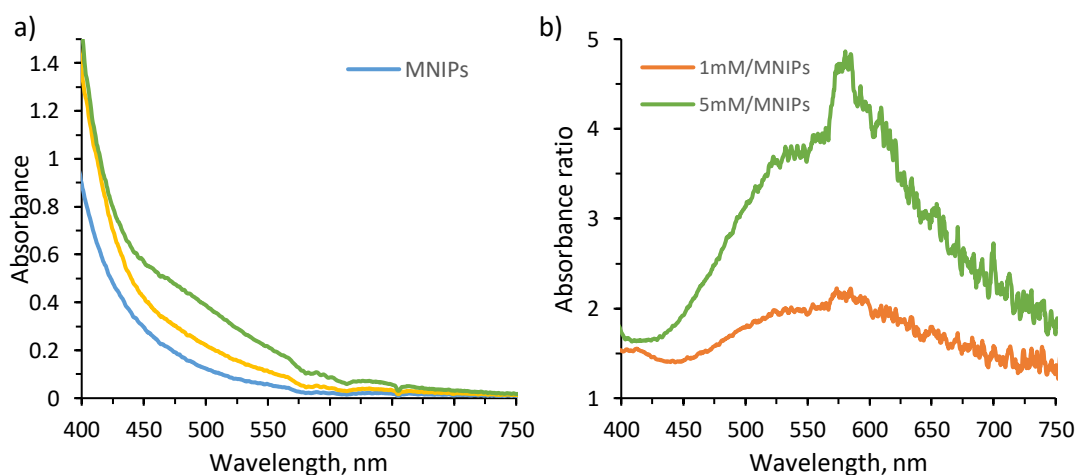


Figure 6-16 a) Absorbance of the 3.1 mg/ml DMSO COOH MIP material in DMF (blue curve), with addition of 1 mM TBAPh after 10 minutes (yellow curve), with addition of 5 mM TBAPh after 10 minutes (green curve); **b)** Ratio of the DMSO COOH MIP material in DMF with and without TBAPh after 10 minutes. Orange curve represents the ratio of 1 mM and green the 5 mM TBAPh DMSO COOH MIP materials solutions in DMF to pure DMSO COOH MIP material in DMF.

minutes of 1 mM TBAPh addition (orange curve). The green curve shows the absorption of the previous solution 10 minutes after the concentration of the TBAPh had risen to 5 mM. One can see that the absorption of the transmission spectra of the DMSO COOH MIP materials solution in DMF grows in presence of organic phosphates (TBAPh) in the region below ~ 700 nm. An absorption shoulder appearance can be observed in the case of a 5 mM concentration of TBAPh in the region between 450 – 650 nm. This means the DMSO COOH MIP materials respond to organic phosphates in a polar aprotic organic solvent (DMF). To find out which part of the absorption spectra differs mostly, the ratio between the absorption of the DMSO COOH MIP materials solution in DMF before the addition, and 10 minutes after addition of 1 mM (orange curve) and 5 mM (green curve) were taken, **Figure 6-16 b**. It can be seen that the absorption ratio peaks between 575 and 600 nm.

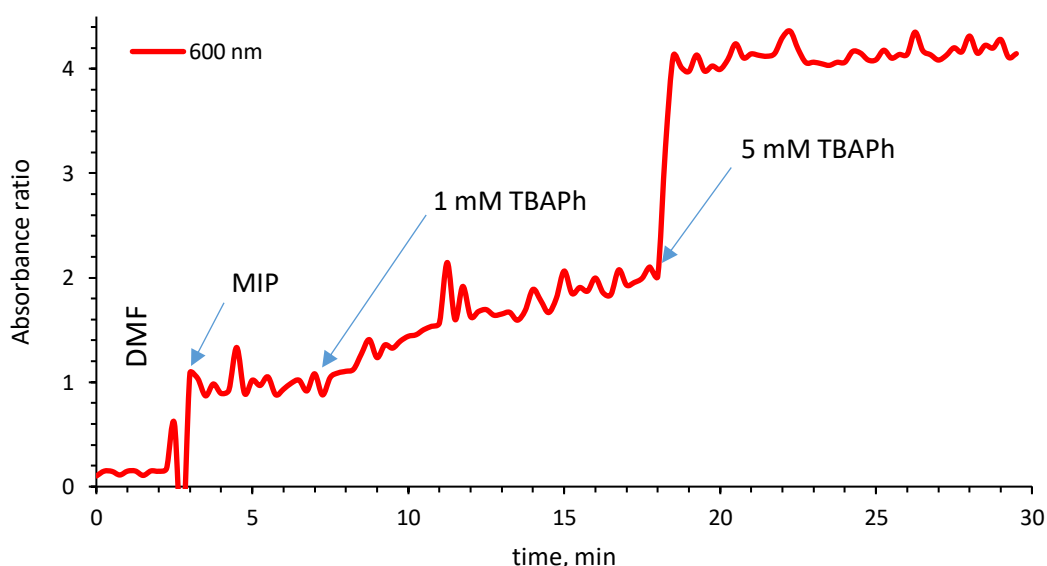


Figure 6-17 Absorbance ratio of the DMSO COOH MIP materials in DMF while 1 and 5 mM of TBAPh was added to the solution. The Ratios denominator is the average absorbance value of the pure 3.1 mg/ml DMSO COOH MIP in DMF.

To observe the dynamic of the response, the 600 nm absorbance wavelength was chosen. The 3.1 mg/ml DMSO COOH MIP DMF's average absorbance value at 600 nm was taken as a baseline (denominator), against which a ratio was taken throughout the whole measurement, **Figure 6-17**. As one can see from the graph, the 1 mM TBAPh was introduced into the solution on the 7th minute, where the absorbance value starts to grow steadily and reaches the ratio value of ~ 2 after 10 minutes approximately. On the 18th minute of the measurement, 5 mM TBAPh was

added to the solution which caused the absorbance ratio to grow rapidly from 2 to 4.1, where it remained in the next 12 minutes despite some fluctuations.

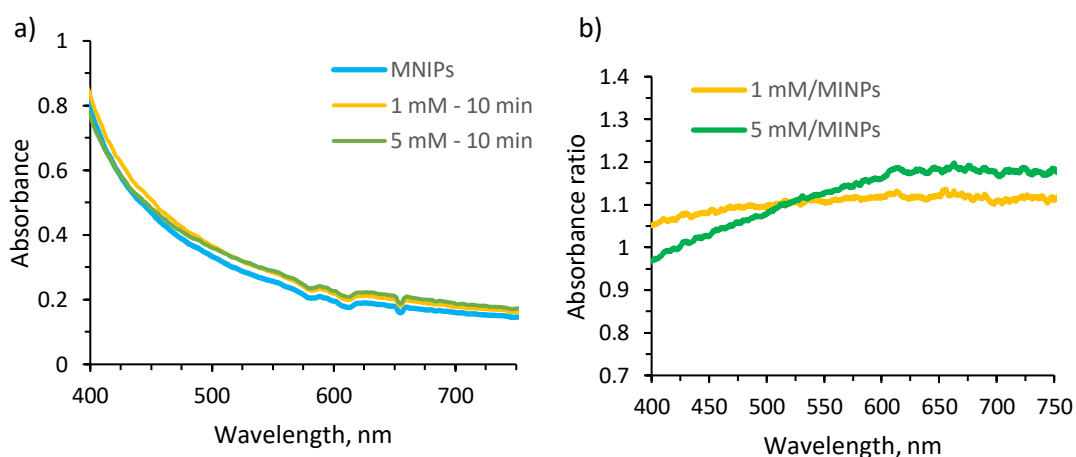


Figure 6-18 a) Absorbance of the 1.55 mg/ml DMSO COOH MIP material in DMF/H₂O (blue curve), with addition of 1 mM KH₂PO₄ after 10 minutes (yellow curve), with addition of 5 mM KH₂PO₄ after 10 minutes (green curve). **b)** Ratio of the 1.55 mg/ml DMSO COOH MIP material in DMF/H₂O with and without KH₂PO₄ after 10 minutes. Orange curve represents the ratio of 1 mM and green the 5 mM KH₂PO₄ DMSO COOH MIP materials solutions in DMF/H₂O to pure DMSO COOH MIP material in DMF/H₂O.

To test the response of the above MIP materials to inorganic phosphate it was necessary to add water, as KH₂PO₄ is not soluble in DMF. Hence, DI water was mixed with DMSO COOH MIP materials in DMF in 50/50 proportion. This resulted in a 1.55 mg/ml DMSO COOH MIP materials DMF/H₂O solution. **Figure 6-18a** shows the absorbance spectra of the 1.55 mg/ml DMSO COOH MIP materials solution in DMF/H₂O (blue curve), and the same solution's absorption after 10 minutes of 1 mM KH₂PO₄ addition (orange curve). The green curve shows the absorption of the previous solution 10 minutes after the concentration of the TBAPh rose to 5 mM. As can be seen from **Figure 6-18a**, no clear absorbance peak or difference can be observed. To find out if any absorption response can be observed, ratios between the absorption of the DMSO COOH MIP materials solution in DMF/H₂O before the addition, and 10 minutes after addition of 1 mM (orange curve) and 5 mM (green curve) were taken, **Figure 6-18b**. As one can see, a small difference can be spotted between the 1 mM and 5 mM KH₂PO₄ as the absorbance of the 5mM curve is slightly lower below 525 nm, and slightly higher, above. However, no significant difference can be noticed. To check if there is a dynamic response to inorganic phosphates the 1.55 mg/ml DMSO COOH MIP DMF/H₂O's average absorbance value at 600 nm was

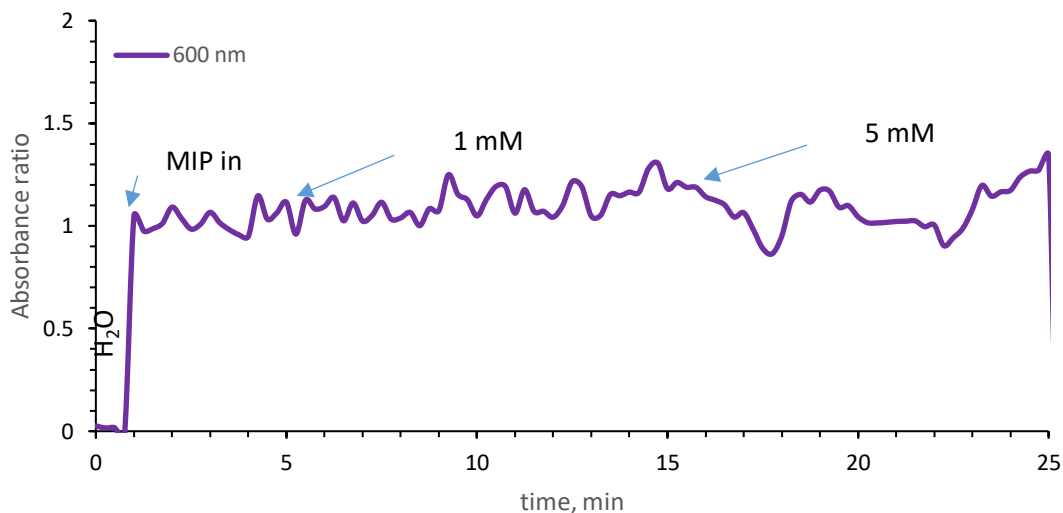


Figure 6-19 Absorbance ratio of the DMSO COOH MIP materials in DMF/H₂O while 1 and 5 mM KH₂PO₄ was added to the solution. The Ratios denominator is the average absorbance value of the pure 1.55 mg/ml DMSO COOH MIP in DMF/H₂O.

taken as a baseline (denominator), against which a ratio was taken throughout the whole measurement, **Figure 6-19**. As can be seen from the graph, despite some fluctuations the ratio value lingers around 1 throughout the whole measurement after the DMSO COOH MIP DMF solution was mixed with water. The addition of, 1 mM nor 5 mM KH₂PO₄ did not increase the value of the absorbance ratio. This shows that the DMSO COOH MIP materials do not respond to inorganic phosphates and may indicate that the presence of water inhibits such a response.

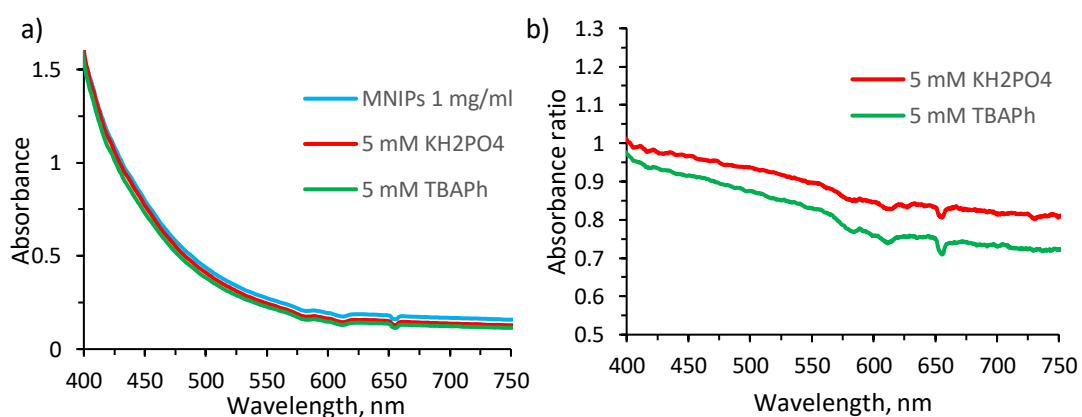


Figure 6-20 a) Absorbance of the 1 mg/ml DMSO COOH MIP material in DMF/H₂O (blue curve), with addition of 5 mM KH₂PO₄ after 10 minutes (red curve), with addition of 5 mM TBAPh after 10 minutes (green curve). **b)** Ratio of the 1 mg/ml DMSO COOH MIP material in DMF/H₂O with and without phosphates after 10 minutes. Red curve represents the ratio of 5 mM KH₂PO₄ and green the 5 mM TBAPh DMSO COOH MIP materials solutions in DMF/H₂O to pure DMSO COOH MIP material in DM/H₂O.

In order to find out if the reason for no response in the inorganic phosphate or water, a DMSO COOH MIP materials solution in DMF/H₂O was tested in the presence of both potassium phosphate and TBAPh, **Figure 6-20**. No clear absorption peak can be seen in **Figure 6-20a** and there is only a small overall decrease of the absorption spectrum by the addition of each 5 mM phosphates into the solution. By taking the ratio between 1 mg/ml DMSO COOH MIP material in DMF/H₂O with and without phosphates after 10 minutes, **Figure 6-20b**, one can see that the absorbance decreases slightly with each phosphate solution which can be explained by precipitation and slight overall dilution of the tested MIP materials solution. To check if there is a dynamic response to both phosphates the 1 mg/ml DMSO COOH MIP DMF/H₂O's average absorbance value at 600 nm was taken as a baseline (denominator), against which a ratio was taken throughout the whole measurement, **Figure 6-21**. As can be observed, despite the addition of 5 mM KH₂PO₄ followed by 5 mM TBAPh the absorbance ratio value remains close to 1, with some fluctuations and a slight steadily decline, probably due to precipitation of the DMSO COOH MIP material in DMF/H₂O and slight dilution of the solution.

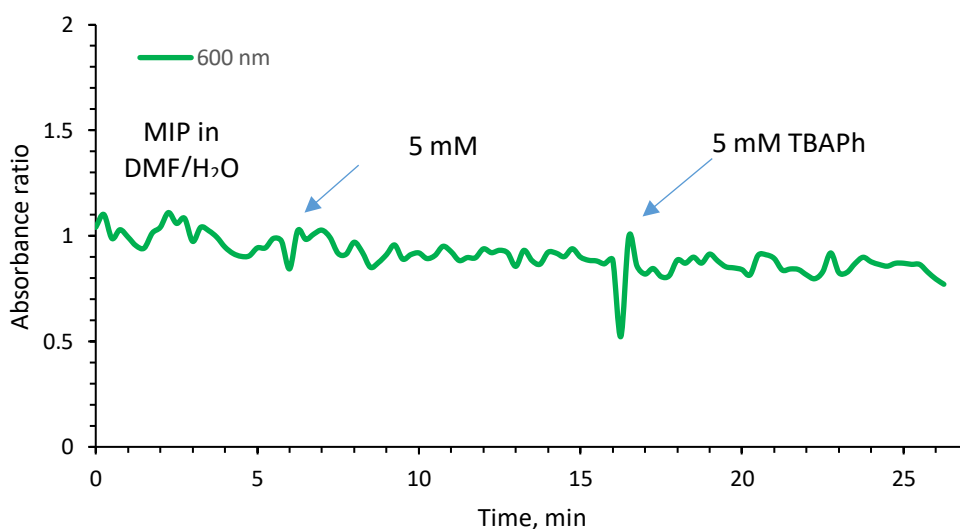


Figure 6-21 Absorbance ratio of the DMSO COOH MIP materials in DMF/H₂O while 5 mM KH₂PO₄ and 5 mM TBAPh was added into the solution. The Ratios denominator is the average absorbance value of the pure 1 mg/ml DMSO COOH MIP in DMF/H₂O.

6.3.4 Conclusion of the phosphate MIP materials in a solution

Taking into account the above results, it can be concluded that the DMSO COOH MIP materials are responsive to organic phosphates in polar aprotic organic solvent (DMF). The presence of water prevents the diluted/suspended MIP materials interaction with phosphates, however, a response of immobilised MINP to phosphates cannot be excluded.

6.3.5 Results and discussion of the SEM examination phosphate MIP materials

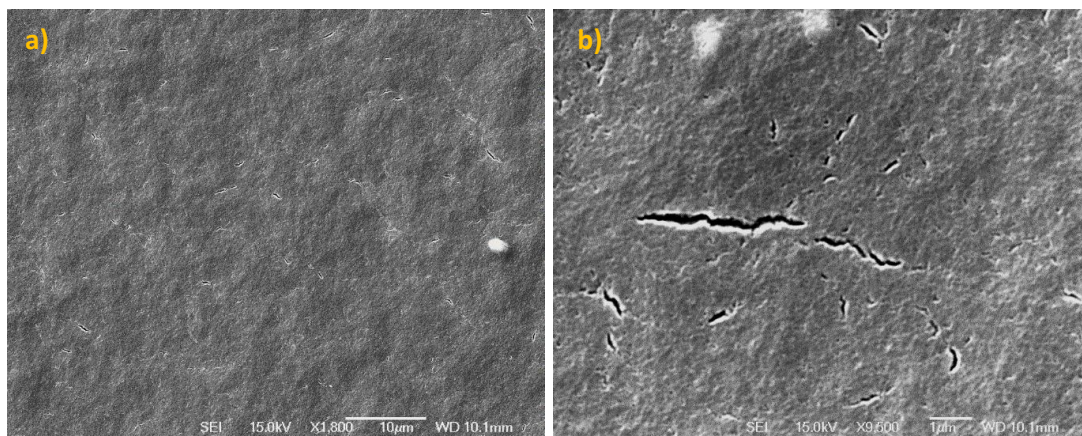


Figure 6-22 SEM of the 5 µl of 1 mg/ml Normal MIP materials solution in DMF placed onto a glass substrate after evaporation of the DMF **a)** magnification x1800, scale shown 10 µm; **b)** magnification x9500, scale shown 1 µm;

Figure 6-22 shows the SEM pictures of a glass substrate with evaporated 5 µl 1 mg/ml normal MIP material solution in DMF. **Figure 6-22a** taken with x 1800 magnification, scale shown on the bottom, - 10 µm. **Figure 6-22b** taken with x 9600 magnification, scale shown on the bottom, - 1 µm. As one can see, a layer of residue material can be observed but there are no clear signs of nanoparticles.

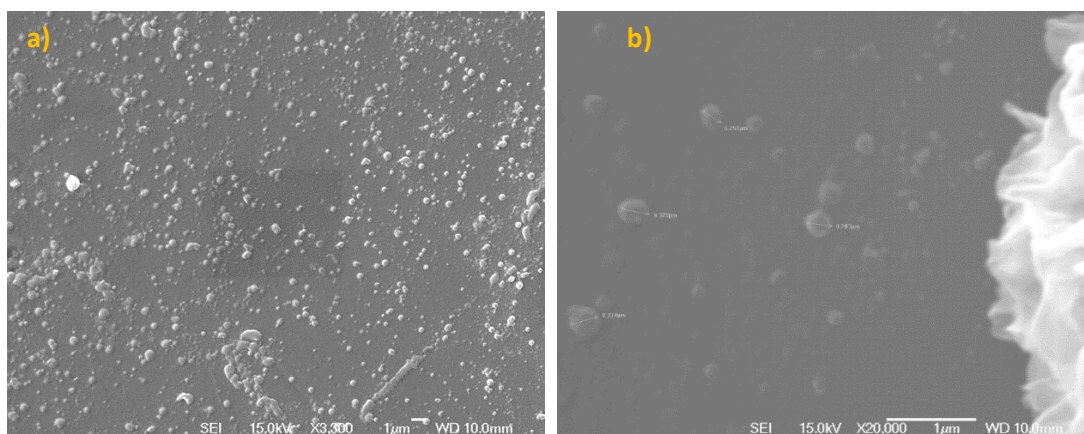


Figure 6-23 SEM of the 5 μ l of 1mg/ml COOH MIP materials solution in DMF placed onto a glass substrate after evaporation of the DMF **a)** magnification x1800, scale shown 10 μ m; **b)** magnification x7500, scale shown 1 μ m. Nanoparticles measured yield (from left to right) 334, 325, 251 and 283nm;

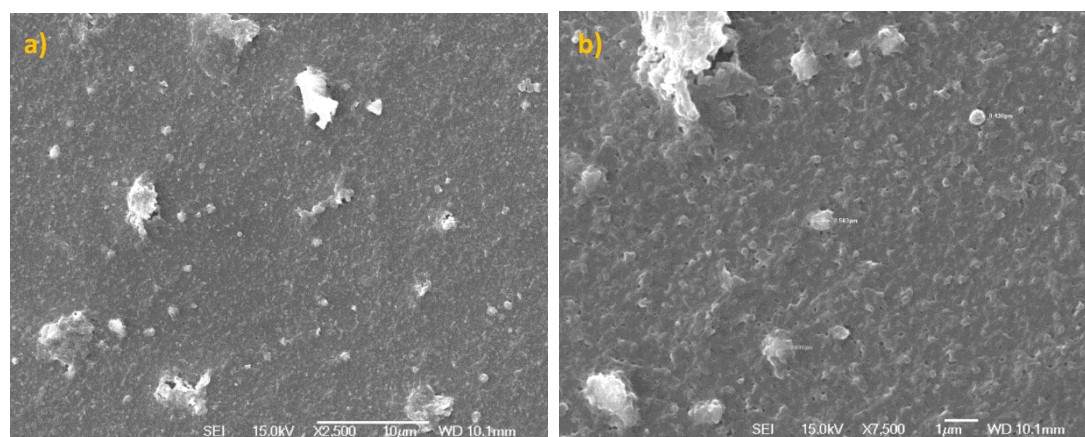


Figure 6-24 SEM of the 5 μ l of 1mg/ml NH₂ MIP materials solution in DMF placed onto a glass substrate after evaporation of the DMF **a)** magnification x2500, scale shown 10 μ m; **b)** magnification x7500, scale shown 1 μ m. Nanoparticles measured yield 490, 563 and 430 nm;

Figure 6-23 shows the SEM pictures of a glass substrate with evaporated 5 μ l 1 mg/ml COOH MIP material solution in DMF. **Figure 6-23a** taken with x 3300 magnification, scale shown on the bottom, - 1 μ m. **Figure 6-23b** taken with x 2000 magnification, scale shown on the bottom, - 1 μ m. Nanoparticles can be observed (the measured on the **Figure 6-23b** yield 334, 325, 251 and 283nm) but they are somewhat covered by a residue material. This could mean that some nanoparticles formed during the synthesis but a large proportion of polymer remained without forming nanoparticles. This polymer presumably can be observed in the form of the residue on the substrate after evaporation of the DMF.

Figure 6-24 shows the SEM pictures of a glass substrate with evaporated 5 μ l 1 mg/ml NH_2 MIP material solution in DMF. **Figure 6-24a** taken with x 2500 magnification, scale shown on the bottom, - 10 μ m. **Figure 6-24b** taken with x 7500 magnification, scale shown on the bottom, - 1 μ m. Here as well, nanoparticles can be observed (the measured ones in **Figure 6-24b** are 490, 563 and 430 nm) but they are

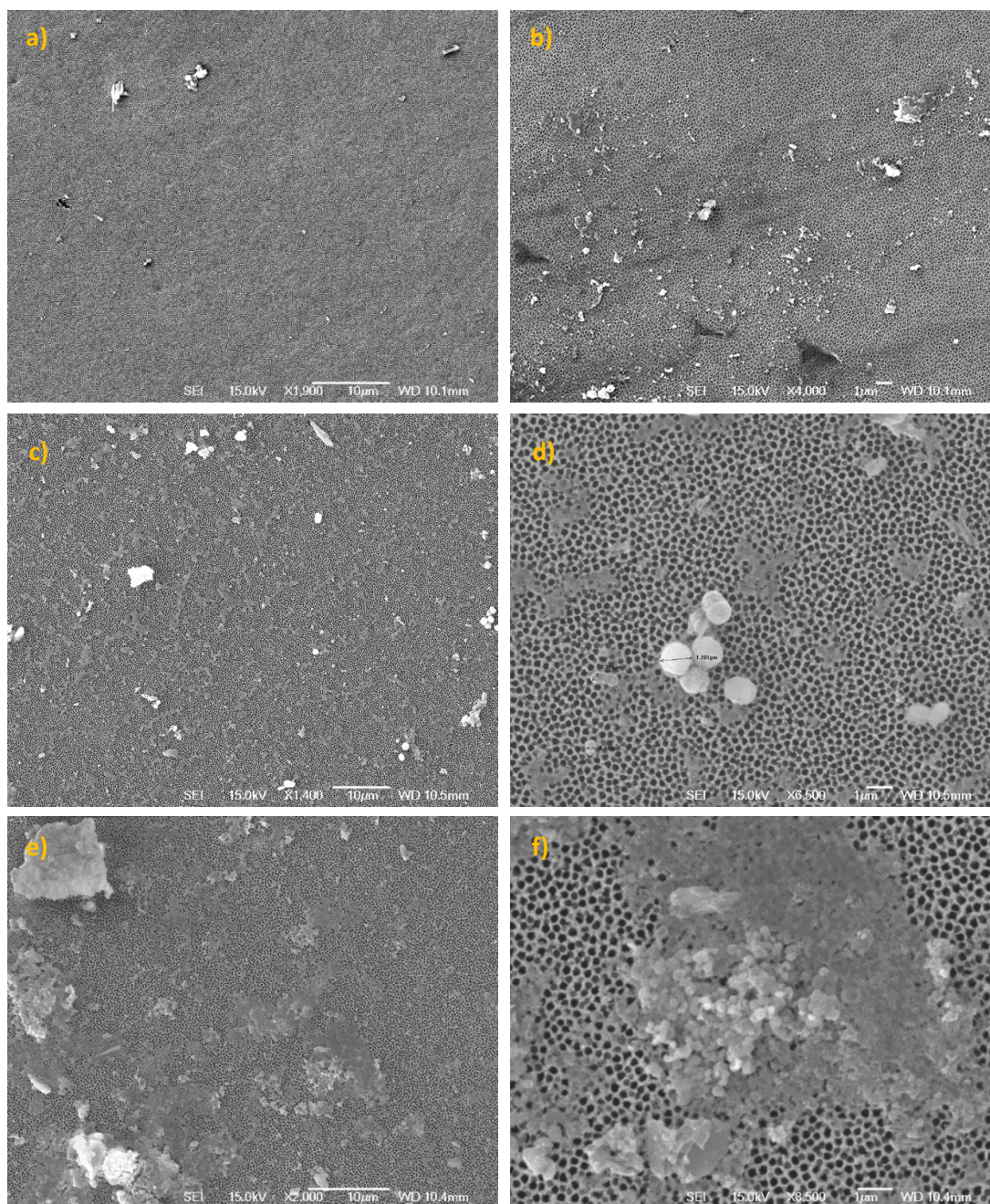


Figure 6-25 Filter paper with detained nanoparticles and residue after filtering of different MIP materials. **a** and **b** Normal MIPs with magnification and scale mark x1900, 10 μ m and x4000, 1 μ m respectively. **c** and **d** COOH MIPs with magnification and scale mark x1400, 10 μ m and x6500, 1 μ m. **e** and **f** Amino MIPs with magnification and scale mark x2000, 10 μ m and x8500, 1 μ m

heavily covered by a residue material that forms a flake-like pattern. This could mean that some nanoparticles formed during the synthesis but a high proportion of polymer remained. This polymer presumably can be observed in a form of the flake-like residue on the substrate after the solvent evaporated.

In order to increase the concentration of the nanoparticles in the MIP materials, each was filtered through a 100 nm pore size Whatman filter paper. Each piece of filter paper was examined under SEM, **Figure 6-25**, the rest was rinsed in DMF to retain the filtered MIP materials.

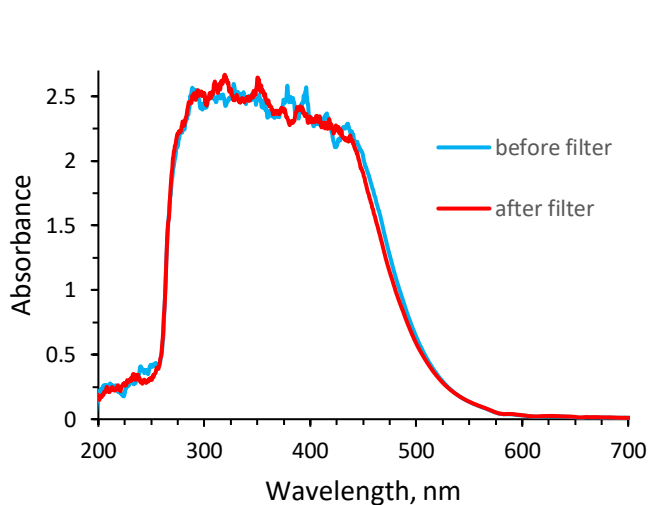


Figure 6-26 Absorption of the 10 mg/ml COOH ACN MIP materials solution in DMF, before and after filtering. Pure DMF spectra was taken as a baseline.

25 mg of COOH ACN MIP materials were dissolved in 2.5 ml of DMF, from which only 2.6 mg were detained by the filter, hence only $\leq 10.4\%$ of the COOH MIP materials are nanoparticles. **Figure 6-26**, shows the absorption spectra of the 10 mg/ml COOH ACN MIP materials solution in DMF, before and after filtering. The difference

in those two absorption spectra is very small. As one can see from the amount of the residue retained by the filter and **Figure 6-26**, the vast majority of the received COOH MIP materials did not form nanoparticles. **Figure 6-25 c and d**, shows that part of the retained materials are not nanoparticles either, however, some nanoparticles could be seen and were measured to vary in size from ~ 348 -1201 nm.

50 mg of NH_2 MIP materials were dissolved in 5 ml of DMF, from which only 3.9 mg were detained by the filter, hence only $\leq 7.8\%$ of the NH_2 MIP materials are nanoparticles. **Figure 6-25 e and f**, shows that part of the retained materials are not nanoparticles either, however, some nanoparticles could be seen.

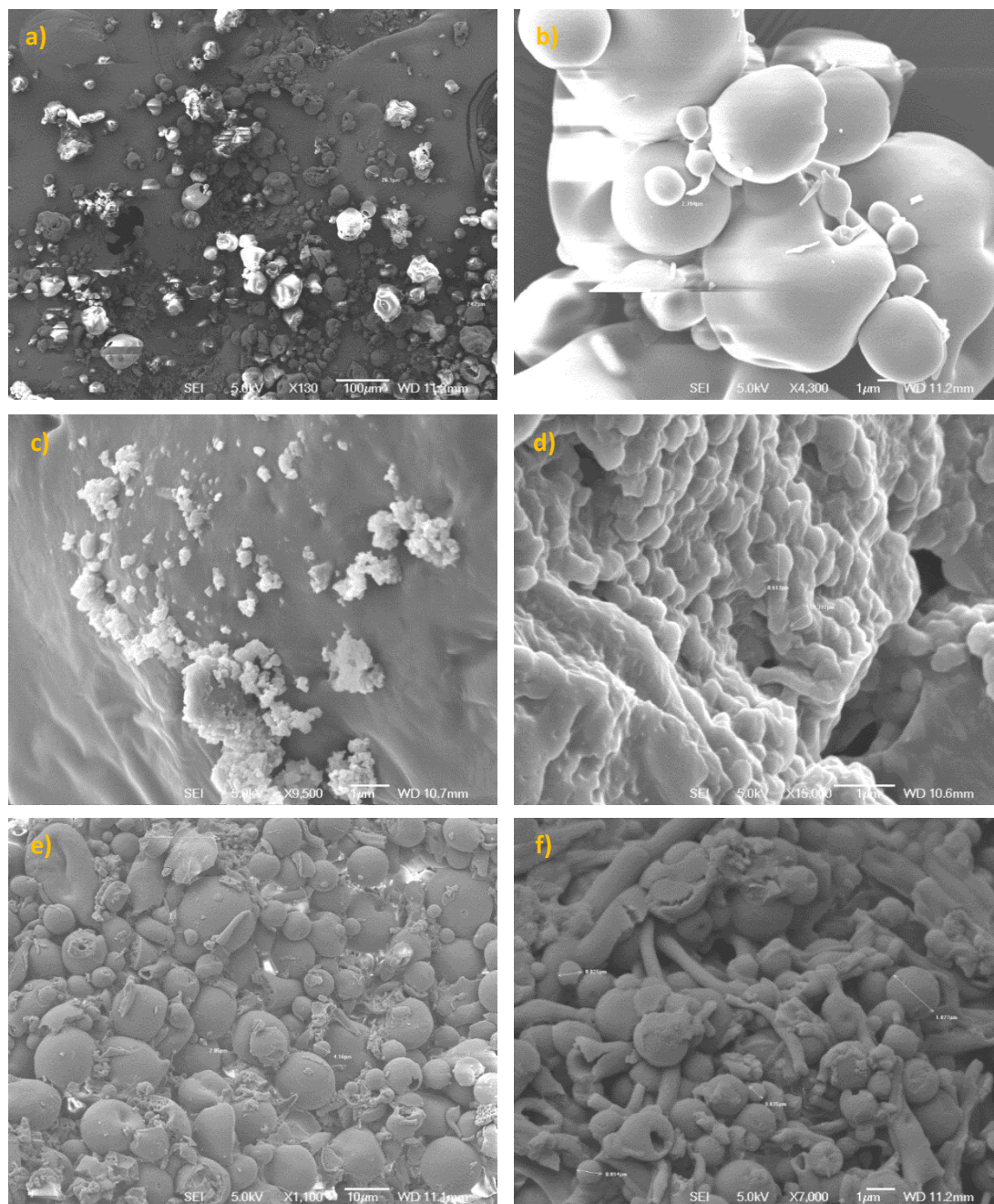


Figure 6-27 SEM pictures of bulk MIP materials spread directly on a specimen holder. Pictures **a** and **b** show the bulk normal MIP materials; **c** and **d** - COOH MIP materials; **e** and **f** - Epoxy MIP materials.

To exclude the highly unlikely damaging effect of the DMF solvent on the MIP materials, they were examined in the bulk solid state directly spread on the specimen holder, **Figure 6-27**. In the case of normal MIP materials (**Figure 6-27 a** and **b**) much bigger particles than specified can be observed (2-16 μm), but no particles can be seen in the majority of the polymer. In the case of COOH MIP materials (**Figure 6-27 c** and **d**) presumably, some nanoparticle like structures covered with the polymer can

be seen with 613 and 392 nm measured cross section. In the case of Epoxy MIP materials (**Figure 6-27 e and f**) alongside nanoparticles (measured 600-800 nm) one can see much bigger particles (exceeding a few micrometres) and debris of the polymer.

6.3.6 Conclusion of the SEM examination phosphate MIP materials

Taking into account the result of the SEM examinations and filtering of the provided phosphate imprinted materials, it can be stated, that the vast majority of the examined MIP materials are not nanoparticles. However, some nanoparticles are present, the best MIP material from this perspective appears to be the COOH ACN MIP, as it showed a grater amount detained by filtering. The filtering procedure can be promising to a certain extent, however the fact that the ratio of the MIP materials detained by filtration is not greater than one tenth, and not only nanoparticles were detained but the residue, makes the filtering approach somewhat questionable.

6.3.7 Result and discussion of deposition of the MIP materials onto U-shape fibre and testing

The sol-gel mixture ratio is shown in *Table 6-2*, the velocity of dip coater is 2mm/sec, 3 layers deposited. **Figure 6-28** shows the SEM picture of the surface of the U-shape OFS after 3 layers sol gel dip coating. As one can see from **Figure 6-28a** very rare nanoparticles can be spotted in the picture. **Figure 6-28b** magnifies further (x14000) on the central part of the previous picture, two nanoparticles size measured to be 462 and 472 nm.

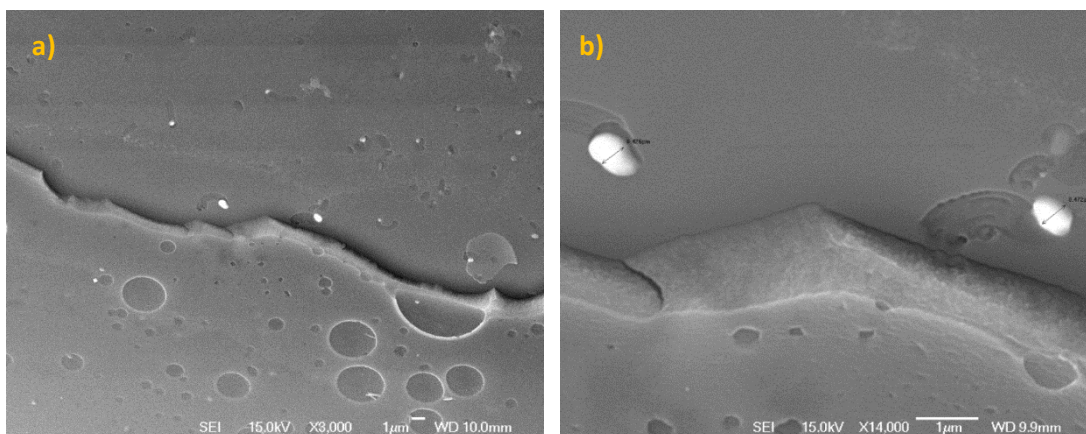


Figure 6-28 3 layer sol-gel deposition of Normal MIP materials on a U-shape OFS; **a)** shows an image of the coated surface with x3000 magnification, and 1 μm scale reference; **b)** magnified up to x14000 and 1 μm scale reference, two nanoparticles observed on the image measured to be 426 and 472 nm large.

In **Figure 6-29a** one can see transmission spectra of U-shape OFS in air after each layer of Normal MIP materials embedded sol-gel deposition. As the sol-gel gets thicker with each layer, the absorption of the U-shape OFS increases, hence the transmitted light decreases. **Figure 6-29b** shows the intensity values ratio at 750 and 600 nm during the test in water for response to high concentration (10 mM) of inorganic phosphate. The ratio is taken instead of pure intensity, in order to minimise other effects affecting the signal such as temperature, RI change and mechanical interaction. As can be seen from **Figure 6-16 a** and **b**, as a response of the MIP materials to phosphates the intensity at 600 nm should decline while at 750 nm the

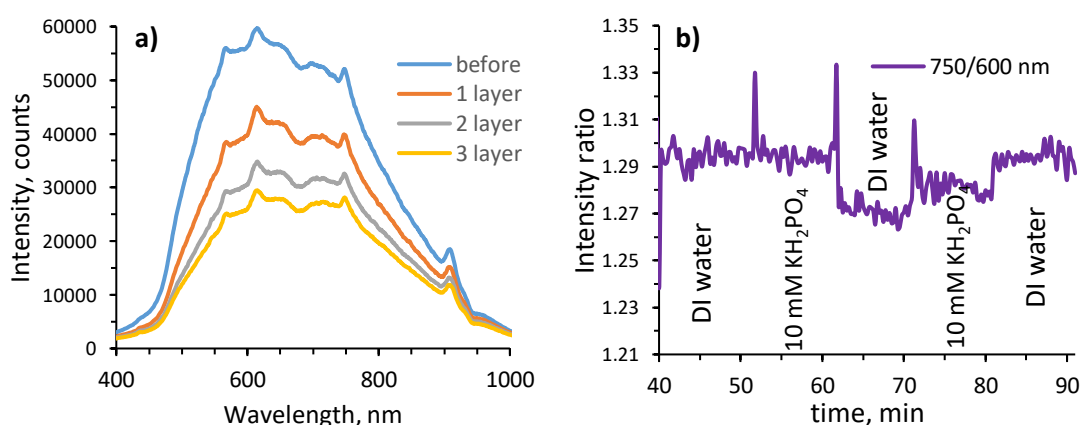


Figure 6-29 a) Shows the transmission spectra of the U-shape OFS in air after each layer of Normal MIP materials embedded sol-gel deposited; **b)** Shows the ratio between intensities at 750 and 600 nm during the inorganic phosphate test in water of the 3-layer Normal MIP materials embedded sol-gel coated U-shape OFS

intensity should remain relatively stable, meaning that the intensity ratio of 750/600 nm should be higher in presence of phosphates. However, in **Figure 6-29b** the violet line representing the 750/600 nm ratio does not show a rise in value in the presence of 10 mM KH_2PO_4 and decline after reinsertion in DI water and looks rather random, which allows to assume other factors influencing the intensity ratio, and no response to inorganic phosphates can be seen.

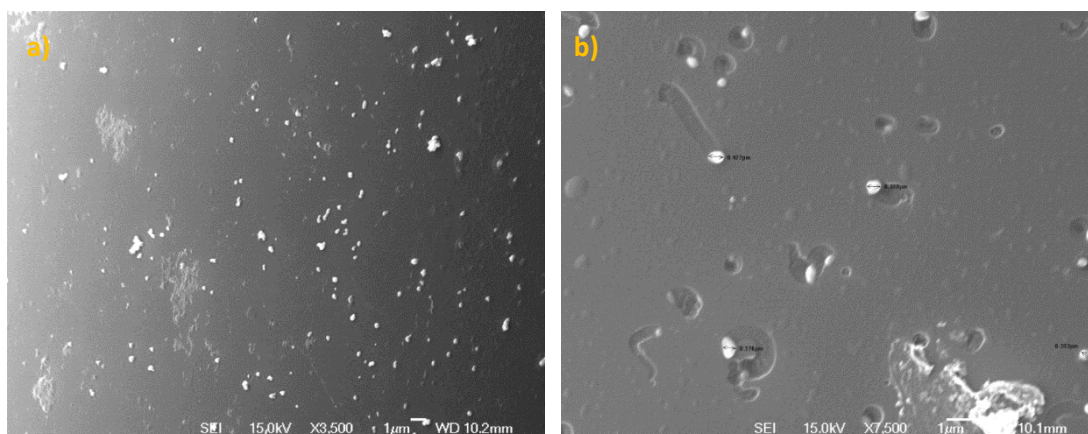


Figure 6-30 a) SEM of the unfiltered COOH MIP materials deposited of a U-shape OFS surface with x3500 magnification, and 1 μm scale reference, rare nanoparticle coverage can be seen. **b)** SEM of the unfiltered COOH MIP materials deposited on a U-shape OFS surface magnified up to x7000 and 1 μm scale reference, few nanoparticles measured on the image (from right to left) to be 427, 376, 380 and 303 nm. The surface appears to be covered with thin film, with movement imprints of some nanoparticles

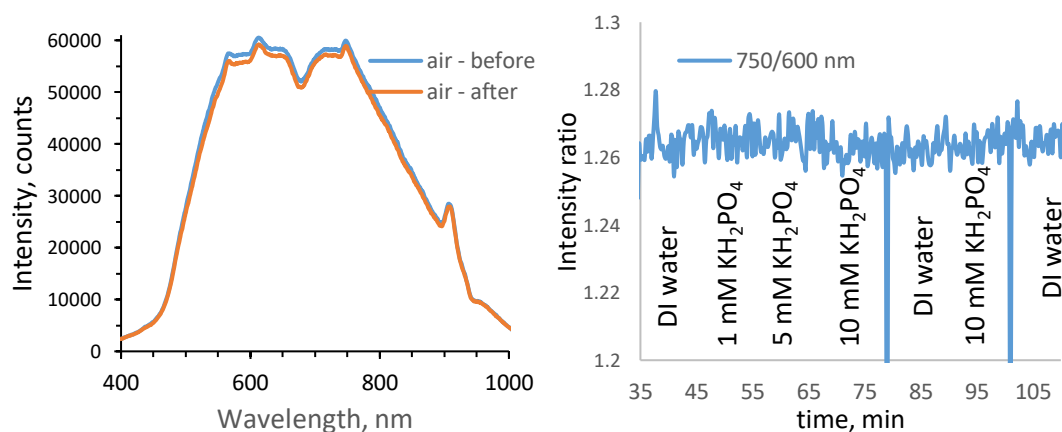


Figure 6-31 a) Transmission spectra of the U-shape OFS in air before (blue curve) and after (orange curve) the deposition of COOH MIP materials; **b)** Shows the ratio between intensities at 750 and 600 nm during the inorganic phosphate test in water of the U-shape OFS coated with COOH MIP materials

Figure 6-30 a and **b** shows the SEM pictures of unfiltered COOH MIP materials deposited onto a pre-functionalised U-shape OFS with magnifications x3500 and x7500 respectively. In **Figure 6-30a** one can clearly see rare nanoparticles deposited on the surface of the U-shape OFS. **Figure 6-30 b** however, reveals a thin film coverage of the glass surface with some trace imprints from nanoparticles. This film can be the polymer residue of the COOH MIP materials, as they constitute the vast majority of the given MIP materials (this was shown in **Section 6.3.5**).

Figure 6-31 a shows the transmission spectra of the U-shape OFS in the air before and after depositions of unfiltered and filtered COOH MIP materials respectively. The blue curve corresponds to the spectra taken before, the orange curve – after the deposition. As one can see, the deposited film and NP result in a slight decline in the transmittance of the U-shape fibre. **Figure 6-31 b** shows the intensity ratio of the transmitted light through COOH MIP material coated U-shape OFS while testing for response to inorganic phosphate. First, the OFS was immersed in DI water, after 45 min 1 mM KH_2PO_4 was added, followed by 5 mM and 10 mM concentration of KH_2PO_4 in the next 20 minutes. Then the solution was substituted by water and in 10 minutes followed by 10 mM KH_2PO_4 . After 10 minutes, the solution was replaced by DI water again. As stated above, the 750/600 intensity ratio should

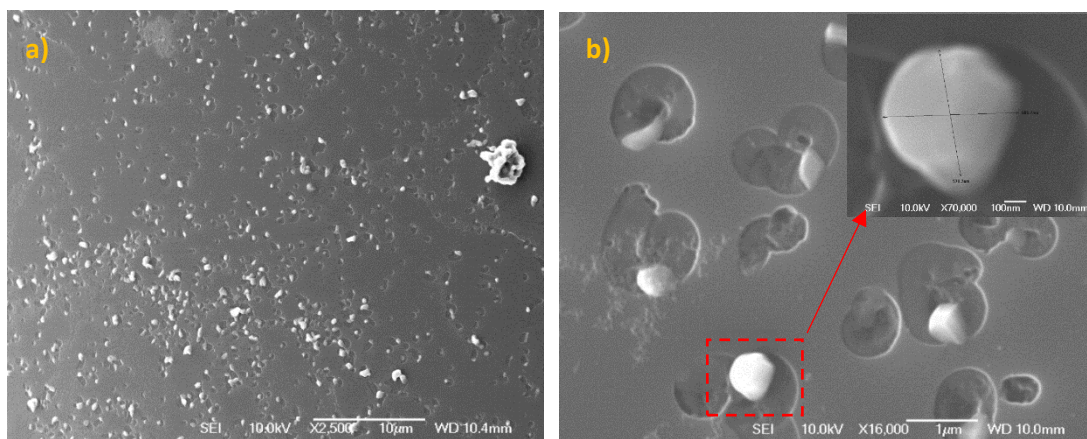


Figure 6-32 a) SEM of the filtered COOH MIP materials deposited on a U-shape OFS surface with x2500 magnification, and 1 μm scale reference, the nanoparticle concentration on the surface seemingly increases after filtration. **b)** SEM of the filtered COOH MIP materials deposited on a U-shape OFS surface magnified up to x16000 and 1 μm scale reference, with a section (red dashed square) magnified up to x70000 and 100 nm reference in the top left, which shows a nanoparticle measured to be 570x505 nm. The surface appears to be covered with thin film, with trace imprints of some nanoparticles

rise in case of response to phosphates, but this is not happening in this case. The ratio

remains relatively stable throughout the experiment with some noise fluctuations. Therefore, no response can be concluded.

In order to increase the concentration of nanoparticles on the surface of the U-shape OFS, the deposition of the COOH MIP materials was repeated after filtration of the COOH MIP materials. **Figure 6-32 a** and **b** show the SEM pictures of filtered COOH MIP materials deposition onto a pre-functionalised U-shape OFS with magnifications x2500 and x1600 respectively. Comparing **Figure 6-30 a** and **Figure 6-32 a**, one can see that the concentration of the nanoparticles is visually larger in the case of the filtered COOH MIP materials. However, **Figure 6-32 b** reveals that the surface of the glass is covered by a film (presumably the polymer residue), similarly to the unfiltered, with trace imprints. **Figure 6-32 b** also shows a zoomed-in part with a nanoparticle (marked by a dashed red square) with x70000 magnification and 100 nm reference. The size of the nanoparticle is measured as 571x606 nm.

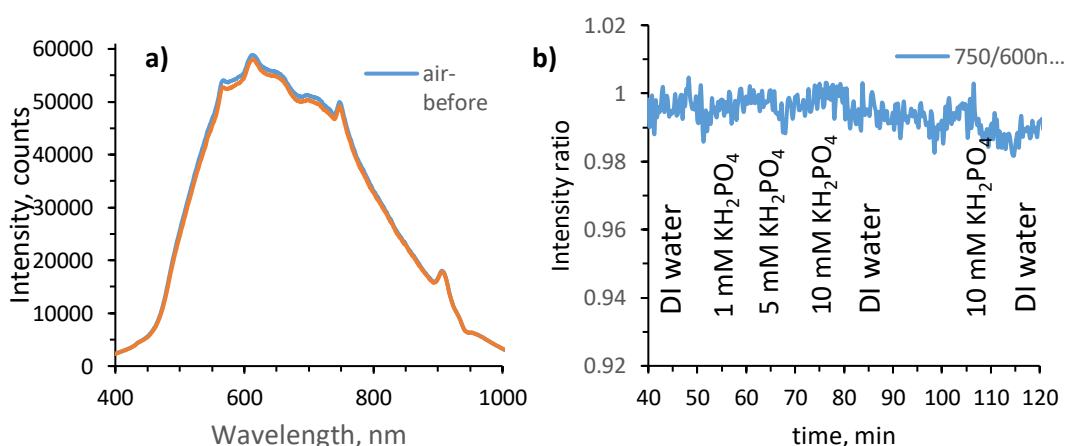


Figure 6-33 a) Transmission spectra of the U-shape OFS in air before (blue curve) and after (orange curve) the deposition of filtered COOH MIP materials; **b)** Ratio between intensities at 750 and 600 nm during the inorganic phosphate test in water of the U-shape OFS coated with filtered COOH MIP materials

All the rest of the MIP materials (Epoxy and Amine) also showed no response to inorganic phosphates, both unfiltered and filtered. Therefore, only the deposition SEM pictures of those will be shown.

Figure 6-34 a and **b** show the SEM pictures of unfiltered Amine MIP materials deposited onto a pre-functionalised U-shape OFS. **Figure 6-34 a** shows an SEM image of the coated surface with x3500 times magnification. Very rare nanoparticles can be seen among a thin layer of residue with holes. **Figure 6-34 b** shows an x18000 times magnified SEM picture where the thin layer of presumable polymer with holes can

be seen, and a few nanoparticles. One of the NPs is measured to be 484x627 nm. **Figure 6-34 c** and **d** show the SEM pictures of the filtered Amine MIP materials deposited on a U-shape OFS. Unfortunately, the magnification and scale information was not recorded with these pictures. However, if one compares pictures **Figure 6-34 a** and **b** with **c** and **d** it can be seen, that the concentration of nanoparticles on the surface of the U-shape increases after filtration of the Amino MIP materials. **Figure 6-34 e** and **f** show the transmission spectra of U-shape OFS in the air before and after depositions of unfiltered and filtered Amino MIP materials respectively. On both figures, the blue curve corresponds to the spectra taken before, the orange curve, – after the deposition. As one can see, the deposited film and NP result in a slight decline in the transmittance of the U-shape fibre.

Figure 6-35 a, b and **c** show the SEM pictures of unfiltered Epoxy MIP materials deposition onto a pre-functionalised U-shape OFS. **Figure 6-35** show the SEM with x6555 magnification and 10 μm scale reference, where on the upper part of the image a rather dense NP coverage can be observed, however, in the lower part the density drops significantly as almost no NP can be seen. **Figure 6-35 b** and **c** show a closer look at the SEM of the Epoxy MIP materials on the surface of the U-shape OFS with x26218 and x52437 magnification respectively. Several nanoparticles can be observed, however, coverage of the glass surface with a film somewhat thicker than in the cases of COOH and Amino MIP materials can be observed. The greater thickness of the U-shape coverage is supported by **Figure 6-35 d**, where the transmission spectra in the air of the U-shape OFS before and after deposition of Epoxy MIP materials can be seen. As one can see, the difference in the transmitted light between the bare and coated U-shape OFS is much bigger than in cases of previous MIP materials.

However, all MIP materials deposited on U-shape OFS showed no response to inorganic phosphate in water.

Figure 6-36 a and **b** show the SEM of the MIP grown directly on the surface of a pre-functionalised U-shape OFS at the facilities of MIP Diagnostics, with x 6000 and x13000 magnification respectively. A rough polymer surface can be seen on the surface of the glass fibre.

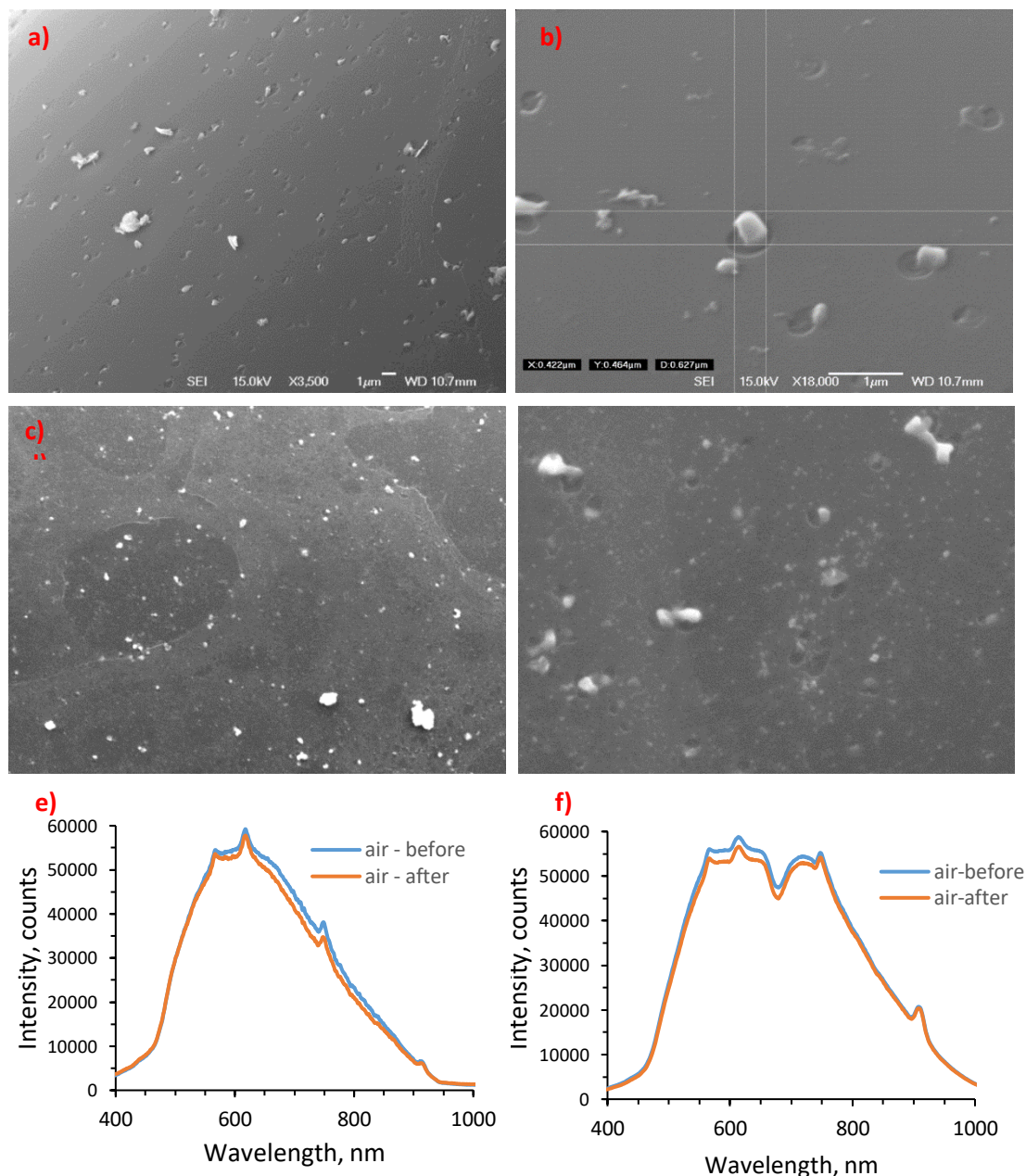


Figure 6-34 **a)** SEM of the unfiltered Amine MIP materials deposited on a U-shape OFS surface with x3500 magnification, and 1 μm scale reference. **b)** SEM of the unfiltered Amine MIP materials deposited of a U-shape OFS surface magnified up to x18000 and 1 μm scale reference, few nanoparticles spotted on the image one of which is measured to be 484x627 nm. **c)** SEM of the filtered Amine MIP materials deposited of a U-shape OFS, where more nanoparticles can be seen (unfortunately the precise magnification was not recorded). **d)** Closer magnification of the U-shape coated with filtered Amino MIP materials (unfortunately, the precise magnification was not recorded). **e)** Transmission spectra of the U-shape OFS before in air (blue curve) and after (orange curve) the deposition of unfiltered Amino MIP materials. **f)** Transmission spectra of the U-shape OFS in air before (blue curve) and after (orange curve) the deposition of filtered Amino MIP materials.

Figure 6-37 **a)** show the intensity values ratio during the test for inorganic phosphates of the U-shape OFS coated with MIP in the facilities of MIP Diagnostics. The MIP

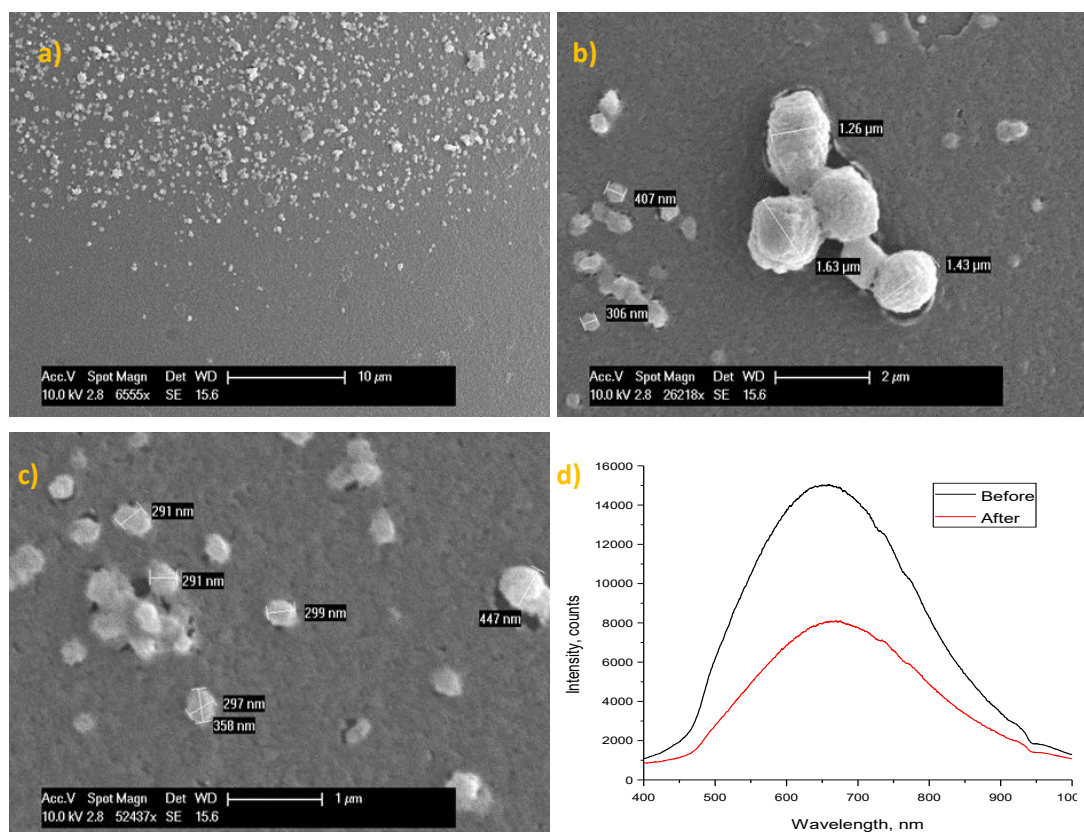


Figure 6-35 **a)** SEM of the unfiltered Epoxy MIP materials deposited on a U-shape OFS surface with x6555 magnification, and 10 μm scale reference. **b)** SEM of the unfiltered Epoxy MIP materials deposited of a U-shape OFS surface magnified up to x26218 and 2 μm scale reference, several nanoparticles measured on the image (from right to left) as 306, 407, 1630, 1260 and 1430 nm. **c)** SEM of the unfiltered Epoxy MIP materials deposited on a U-shape OFS surface magnified up to x52437 and 1 μm scale reference several nanoparticles measured on the image (from right to left) as 291, 291, 358, 297, 299 and 447nm. The surface appears to be covered with a film somewhat thicker than in the case of Amine and COOH MIP materials. **e)** Transmission spectra of the U-shape OFS in air before (black curve) and after (red curve) the deposition of unfiltered Epoxy MIP materials.

coated U-shape OFS was alternately submerged in DI water and 5 mM KH_2PO_4 for 10-15 minutes. A small rise of the ratio value (by ~ 0.02) can be observed during the first immersion into the 5 M KH_2PO_4 followed by a slight decline (by ~ 0.01) in water afterwards. However, the second immersion does not seemingly affect the ratio, and the third immersion into 5 mM KH_2PO_4 solution results in a rise by ~ 0.03 , followed by a decline in DI water. Taking into account that there was no response on the second occasion, and the small magnitude of the changes on the first and third occasion, one can assume, that the reason behind those changes was not related to phosphate response. For comparison, **Figure 6-37 b** shows the intensity values ratio during the test for organic phosphates of the U-shape OFS coated with MIP in the facilities of

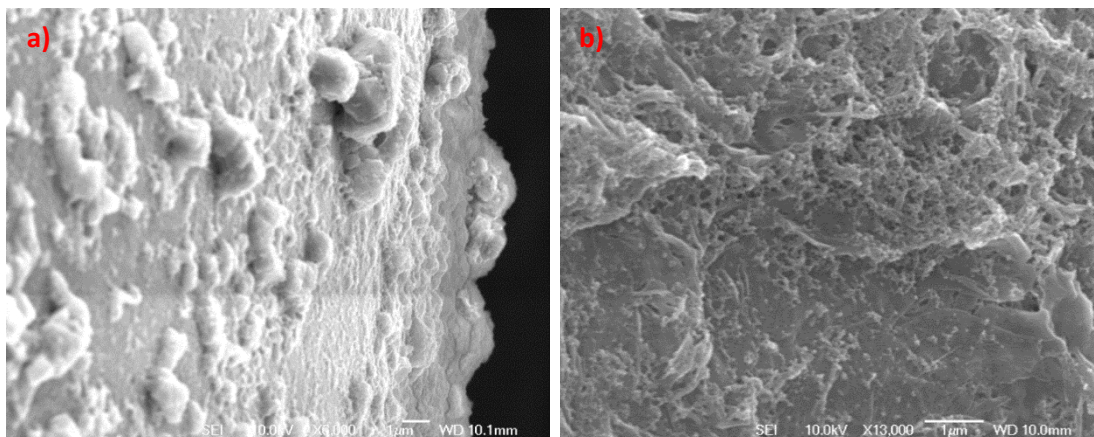


Figure 6-36 SEM picture of MIP directly grown on the surface of the U-shape OFS in the facilities of MIP Diagnostics. **a)** SEM with x6000 magnification and 1 μm reference. **b)** SEM with x13000 magnification and 1 μm reference.

MIP Diagnostics. The MIP coated U-shape OFS was alternately submerged in DMF and 500 μM TBAPh. First, the NIP coated U-shape was immersed into pure DMF for 30 minutes, then 500 μM TBAPh was added for 15 minutes. The addition of the 500 μM TBAPh caused a sharp rise of the ratio value from ~ 2 to 2.4 in the first 1.5 minutes, followed by a slower rise to 2.6 in the next 13 minutes. After changing the solution to pure DMF the ratio declined rapidly to 1.92 followed by a further steady decline in the next 40 minutes to 1.6. After reintroducing the 500 μM TBAPh the ratio has risen, but very slightly, only to 1.62 and stayed there for the following 10 minutes. The next change of the solution to pure DMF caused a further decline of the ratio from 1.59 to 1.51 in 15 minutes, followed by an immediate rise in 500 μM TBAPh to 1.53 with a steady decline down to 1.51 in the next 10 minutes. The reason why the first response was much bigger is that the polymer is removed by and dissolved in DMF solution. This assumption is supported by observing the full transmission spectrum taken at the end of each subsequent immersion into DMF and 500 μM TBAPh, **Figure 6-38**. The first immersion in DMF is shown by the green curve, the followed immersion into 500 μM TBAPh DMF solution by the blue curve. As one can see, the intensity drops significantly in the range between ~ 525 and 650 nm. Then, in subsequent immersion to DMF again (yellow curve) the intensity of the light in the named range rises significantly further to the initial immersion and only slightly decreases after second immersion into 500 μM TBAPh (dark green curve), but it is not even close to the values of the first immersions. The third immersion in DMF (dark blue curve)

increases the sensitivity in the 525-650 nm range slightly further, following a modest decline in the subsequent 500 μM TBAPh addition, however, the intensity in the named region does not return to the values of the second immersions. This shows, that the MIP responds to organic phosphate in the organic solvent, however, the MIP film is not stable in DMF as dissolves in it.

As one can see, the response to 10 times smaller concentration of organic phosphates in DMF caused a significant first response compared to no response to inorganic phosphates in water followed by much smaller, but still observable responses due to dissolution of the MIP coating in DMF.

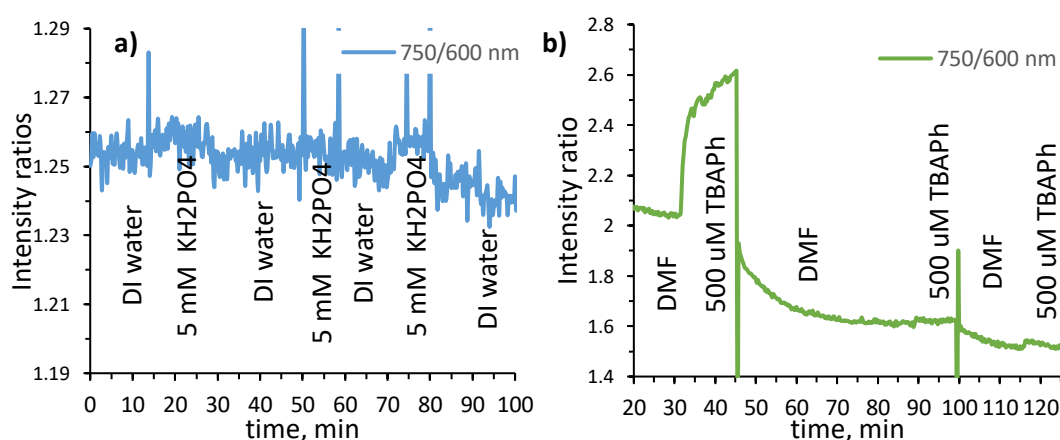


Figure 6-37 *a)* Ratio between intensities at 750 and 600 nm during the inorganic phosphate test in water of the U-shape OFS coated with MIP in the MIP Diagnostics facilities. *b)* Ratio between intensities at 750 and 600 nm during the organic phosphate test in DMF of the U-shape OFS coated with MIP in the MIP Diagnostics facilities.

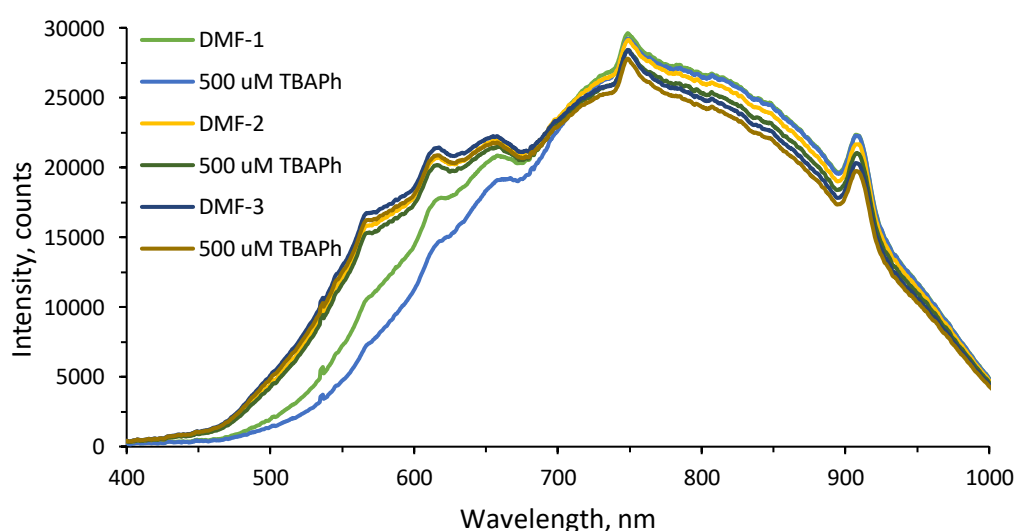


Figure 6-38 *Transmission spectra in the end of each immersion of the MIP coated U-shape OFS into pure DMF and 500 μM TBAPh solution*

6.3.8 Conclusions for the U-shape OFS coated by MIP materials

Unfortunately, none of the above MIP materials and MIP coating responded to phosphates in water. One of the possible explanations could be the hydrophobicity of the polymer, which according to MIP Diagnostics could be potentially overcome in the form of nanoparticles. As one can see from the above, this did not occur and the proposed sensor based on MIP materials, nanoparticles and polymer does not work for phosphate sensing purposes in water.

As the author is not fully aware of the nature of the given polymer (because of legal restrictions), no clear reason can be given why the MIP materials coated U-shape OFS does not work in water.

6.4 Conclusion

In this chapter, a MIP approach for phosphate sensing with OFS was shown. In the first half, an LPG optical fibre sensor in combination with MIP thin polymer film was used. The MIP was synthesised and evaluated. A nitrogen deposition chamber was used to coat the LPG with MIP film. The surface of the coated fibre was examined via SEM, which showed imperfections of the deposited film despite numerous attempts to address the possible issues. In particular, different concentration ratios, nitrogen atmosphere with high purity, different rising velocities and different solvents. The test of the phosphate MIP coated LPG was conducted in parallel with temperature measurement, this allowed for temperature compensation algorithm. The LPG response was quantified via the area of the PMTP. The temperature sensitivity in terms of areas ratio was $\sim 0.05/1^{\circ}\text{C}$. A one-time response to inorganic phosphate in water was achieved (the temperature compensated areas ratio grew by 0.01 in presence of $100\ \mu\text{M}\ \text{KH}_2\text{PO}_4$), however could not be repeated further under similar circumstances.

In the second half of this chapter, MIP nanoparticles and MIP films of unknown to author origin were investigated via U-shape OFS. These MIP materials were a product of a collaboration between Gill R&D and MIP diagnostics with the involvement of the University of Nottingham. The information about the precise MIP nature was restricted because of legal issues. Nevertheless, 5 different types of MIP materials were investigated, evaluated via SEM and tested for both organic and inorganic phosphates with and without the presence of water. It was shown that the mentioned above MIP materials does not respond to both phosphates in presence of water. Three different deposition techniques were used (i) sol-gel matrix dip coating; (ii) direct chemical binding to glass and (iii) polymer synthesis directly on a glass surface (this was done by MIP Diagnostics). These were evaluated via SEM and the response to inorganic phosphates in water was tested. The MIP coated U-shape fibre (by MIP Diagnostics) was also tested in pure DMF for TBAPh response. A response was recorded, however, the coating proved to be unstable in DMF.

Unfortunately, the goal of MIP optical fibre sensing for inorganic phosphate in water was not achieved despite continuous research efforts. The author believes further research is needed to reach this aim. Among the possible solutions, a few approaches can be named such as MINPs of better quality, different deposition techniques and different host materials for phosphate imprint.

Chapter 7 Conclusions and future work

7.1 Summary and conclusions

Optical fibre sensors are small, robust, cheap, reliable, practical and immune to electromagnetic interactions. Optical fibres can be used to create sensors to detect chemical, biological or physical parameters. Generally, there are two different types of OFS, intrinsic, and extrinsic. Intrinsic OFS work on the ability to alter the properties of the propagating or reflecting light inside the fibre as a function of the target entity. This could be a physical parameter such as RI, temperature, strain, or a chemical or even a biochemical target analyte such as ammonia, oxygen, a drug molecule, or antigen. Extrinsic optical fibres use the fibre optic cable just as a waveguide to deliver the light to an external sensor head or medium, analogous to wires in electronics to conduct the electricity to the electronic components.

Nutrients play a key role in our environment and agriculture. Nutrients are responsible for metabolic reactions, eutrophication, play a key role in the Nitrogen cycle and can pose danger to humans and animals at high concentrations. Hence it is very important to monitor the level of nutrients in natural, waste and consumable waters.

There are four main nutrients, ammonia, nitrites, nitrates and phosphates. This PhD thesis is a research attempt to develop optical fibre sensors to monitor three nutrients in an aqueous medium, - nitrites, ammonia and phosphates.

Chapter 2 of this thesis provides a brief overview of the existing nutrient sensors techniques and recent related research papers published. Also, section 2.3 of Chapter 2 gives a short overview of existing optical fibre sensing platforms and their principle of operation, with some recent examples of published OFS.

Chapter 3 focuses on the main experimental techniques and methods used in this PhD research, such as fibre sensing platform fabrications (LPGs and evanescent field fibres), functional coating and their depositions such as sol-gel formation and properties, layer-by-layer, dip coating and silanization techniques.

Chapter 4 describes the research and development on nitrite aqueous OFS. A sensing element on the nitrite OFS the BCB zinc chloride double salt oxazine dye was

chosen based on works published by *Ensafi et al.* [23, 277]. First, the dye itself was examined for interaction with nitrites in an aqueous solution. It was found that in acidic condition (pH ~2) BCB aqueous solution shows a strong spectrophotometric response to nitrites for even 1 μM concentrations, **Figure 4-4b**. However, it was proven to be not fully reversible in the form of aqueous solution **section 4.2.5**. Further, the BCB entrapped sol-gel film deposited on a glass substrate is studied. An optimal base catalysed sol-gel film and dip coating parameters were chosen based on empirical data. The BCB was embedded in the film and the spectral behaviour after the deposition in air, DI water and nitrite aqueous solution was recorded and analysed in **sections 4.3.3 and 4.3.4**. Absorption peaks of ~510 and ~610 nm were highlighted, and their ratio as a detection tool was used to analyse the response dynamic and repeatability, **section 4.3.5**. The pH factor influence on the coating was compared to the sensing response and proven to be opposite but needed to be accounted for. In the discussion (section 4.3.8) a sensing hypothesis was proposed based on exciton excitation distribution change of the BCB [283]. After in **section 4.4.3**, the BCB embedded sol gel was applied onto two types of absorbance-based OFS, U-shape and straight cladding replaced. From those two, the straight cladding replaced absorbance-based platform proved to be more reliable. The dip coating deposition setup and process is described and spectral data analysed. In **section 4.4.6** the sensor's response to nitrite solution in DI water is shown and analysed. The sensor was found to give a repeatable response to nitrites in DI water with 21.81 μM LOD in the case of 0-50 μM DR, and 36 μM in case of 0-500 μM DR. However, the lifetime of the sensor appeared to be limited to few days, with complete insensitivity after 7 days of shelf life. The issue and attempts to solve it are described in detail in Appendix 1. It was found that keeping the sensor under nitrogen atmosphere somewhat prolongs its lifetime, but is not the answer to industrial needs.

Overall the LOD of the proposed sensor is not enough for environmental monitoring which requires LOD ~0.0001-0.1 μM , however, due to ease of fabrication, might be deployed for wastewater treatment where the lowest detection amount for nitrites is around 70 μM and is not that far off the drinking water allowed concentration of ~10 μM see **Table 2-5** and **Table 4-3**.

Chapter 5 describes the research and development of ammonia OFS based on two different platforms, U-shape and LPG OFS. TSPP was chosen as the sensing material based on work published by *Korposh et al.* [300]. As in the previous chapter, first, the TSPP dye itself was examined for response to ammonia. The TSPP dye's response to ammonia was examined in DI, salt and seawater, and it was found to be not responsive in seawater. Moreover, the comparison showed, that the absorption spectra in pure seawater is similar to that of salt water with a high concentration (1mM) of ammonia, which makes the development of seawater sensor somewhat challenging. In the discussion of the TSPP aqueous tests (**section 5.2.4**) a wavelength absorbance or intensity ratio was proposed to monitor the response. **Section 5.3** describes the immobilization and test of the TSPP on a glass substrate via DAR – TSPP layer by layer deposition. In subsection 5.3.3 the deposition process on a glass substrate and its test for gas phase ammonia response is recorded spectrophotometrically and analysed. Covalent binding is assumed between the DAR and TSPP due to the assumed coupling peak appearance around 525 nm [305]. The gas phase response dynamic is analysed. The next subsection describes the DAR-TSPP substrate's test in water where absorbance peaks ratio in water is determined between 504 and 705 nm, as they move in different directions in presence of ammonia in DI water. Next, **section 5.4** describes the deposition and test of DAR-TSPP coating on a U-shape fibre. The deposition process is recorded and analysed, the assumed covalent binding peak is spotted in the case of U-shape OFS as well. The 3 layer U-shape DAR-TSPP OFS' response to ammonia in water on a range for 1 to 200 μM (LOD=3.01 μM at 0-10 μM DR and 25.94 μM at 10-200 μM DR) and repeatable response is shown between DI Water and 50 μM ammonia solution. These sensitivity figures are enough to measure coastal waters where once ammonia concentrations often start with $\sim 5 \mu\text{M}$, drinking water quality monitoring $\sim 15 \mu\text{M}$ and certainly acceptable for wastewater quality control were the concentrations of ammonia begins with $\sim 500 \mu\text{M}$ see **Table 2-5**. Also the results of 3 layer U-shape DAR-TSPP OFS' test in seawater was presented, which shows the irreversible changes in the DAR-TSPP film caused by seawater, which makes such deployment impossible without further development.

Section 5.5 of Chapter 5 describes the research and development of DAR/TSPP aqueous ammonia sensor based on LPG with PMTP in the visible range. The linkage between the complex refractive index and absorption governed by Kramers-Kronig relation gave the idea to use visible LPGs in combination with sensitive dyes. It was assumed, the change in the visible absorbance would yield a change in complex RI thus should be detected by an LPG operating in the visible range. In order to fabricate such LPGs, fibres with visible operating wavelength ranges were chosen (SM450 and SM600 by Thorlabs). However, as those fibres are not photosensitive, therefore not suitable for LPG fabrication via YAG UV-laser, hydrogen loading photosensitive enhancement method was applied, which is described in **section 5.5.2**. The deposition of DAR/TSPP coating was compared between 'visible' and 'NIR' LPG's, which was found to be similar. The testing to aqueous ammonia however, showed a significant difference. While the 'visible' LPG gave a significant response, the NIR showed no response whatsoever. The ratios of transition spectra of both LPGs in DI water and ammonia solution were compared and analysed, **subsection 5.5.3.4**. An interesting spectral picture of the DAR/TSPP 'visible' LPG's response was observed. The response of the PMTP attenuations shifted in one direction (to the blue), while a pure RI response should yield a shift in opposite directions, as happens during the deposition process. These are compared in **Figure 5-41**. The assumed explanation for this phenomenon is in the DAR/TSPP's complex refractive index change behaviour in the visible range, which was calculated based on work published by *Whittaker et al. [318]* explained in **subsection 5.5.3.3**, the calculation's graphical results are presented in **Figure 5-42**. From these results, the change of the refractive index switch directions somewhere in-between the PMTP of the "visible" LPG. Based on the DAR/TSPP visible LPG response, two different ways of ammonia response tracking can be proposed. One is intensity-based, - by taking the intensity ratio between 678.5 and 662 nm and by taking the sum of the PMTP central wavelength shift. The intensity ratio approach yields $\sim 3.24 \mu\text{M}$ and the central wavelength shift sum yields $\sim 4.81 \mu\text{M}$ LOD.

The proposed LODs are not superior to all the existing detection techniques where the LOD sometimes is measured in nM, **Table 2-3**, but it can be enough for drinking water quality monitoring ($\sim 15 \mu\text{M}$) and for wastewater quality control ($\sim$

500 μ M) see **Table 2-5**. Also, the usage of the visible LPGs in combination with sensitive dyes is a novel approach that can be significantly improved.

Chapter 6 describes the research to create a phosphate aqueous OFS based on molecular imprinting approach. The first half of the chapter focuses on a phosphate sensor development based on homemade molecularly imprinted polymer (based on work published by *Warwick et al.* [107]) film using an LPG OFS operating in reflection mode. The second half of **Chapter 6** describes the research attempt to create a phosphate sensor using molecularly imprinted nanoparticles synthesized by MIP Diagnostics Ltd. First, in **sections 6.2.2 and 6.2.3** the homemade MIP using Allylthiourea and the host monomer and PPA as the template molecule, preparation and evaluation is described. The synthesis of MIP was conducted in two different solvents, - ACN and DMF, template retention of those were compared spectrophotometrically and analysed, and in the result of this ACN solvent was chosen. The next section (**6.2.4**) describes the deposition of the MIP on optical fibre with LPG via silanization and dip coating using a homemade nitrogen atmosphere deposition setup. It also describes a novel approach to quantifying an LPG OFS' response, using the areas ratio of the PMTP. The ratio factor compensates for any intensity caused changes. In the result and discussion subsection (**6.2.5**) the deposition and washing (template removal) processes are analysed via a spectrometer and SEM. Temperature calibration of the LPG is shown here as well as the temperature compensated test for phosphates. The test results show the response of LPG to phosphate concentration in water from 100 μ M to 10 mM, however, the response is not linear and wasn't able to be replicated with other similar attempts.

Next, **section 6.3** describes an attempt to use phosphate imprinted nanoparticles (called MIP materials) based on unknown to author polymer provided by MIP Diagnostics Ltd in a collaboration agreement with Gill R&D. The alleged nanoparticles were claimed to be ~350 nm in size synthesised in a way to be responsive colourimetrically in the presence of phosphates, and with different functional groups in order to be attached to glass fibre via silanization as well as without any specific binding group. These were used with sol-gel matrix. First, the MIP materials were examined for response to organic (TBAPh) and inorganic (KH_2PO_4)

phosphates in water and organic solvents (DMSO and DMF) as well as in 50/50 DMSO/H₂O solution. It was established that the MIP materials do respond for at least 1 mM TBAPh in an organic solvent, however, it does not respond to either of the phosphates in presence of water. The MIP materials also were examined using SEM. The SEM images showed very little actual nanoparticles in the MIP materials and filtration through 100 nm pore size filter and weighting showed only ~8-10.5% (depending on the type) of actual nanoparticles in the MIP materials provided. SEM evaluation of each MIP material batch and deposition attempts provided as well as the spectrometric data. Due to a large amount of polymer residue, the deposition of the actual nanoparticles was problematic, which can be seen in the SEM images. The filtration somewhat enhanced the density of the nanoparticles on the glass surface, however, it was still massively covered in residues. A U-shape OFS deposited with MIP grown directly on its surface in the facilities at MIP diagnostics was examined as well via SEM. Unfortunately, none of the U-shapes was responsive to phosphates in water. However, the U-shape with directly grown MIP was found to be responsive to organic phosphate in DMF, but not repeatedly, as the DMF presumably dissolved the MIP film.

Even though the MIP film deposited on LPG's surface showed a one-off sensing result, it can be stated that unfortunately the attempt to create an MIP based aqueous phosphate optical fibre sensor was not successful in this research despite significant efforts applied.

7.2 Future work

The nitrite sensor described in **Chapter 4** showed a LOD which is enough for certain tasks such as wastewater quality measurement, however, it is not enough, but close to drinking water evaluation and far from environmental measurement requirements. However, the LOD possible can be enhanced by improving or changing the deposition matrix. For instance, the usage of hydrogels or polymers instead of sol-gel should be tested. This might also answer the problem of the short shelf life of

the sensor. Real sample measurements besides a calibrated analytical device should be carried out as well.

The ammonia sensor based on DAR/TSPP can be enhanced with an ion-selective or ammonia permeable membrane coating in order to function in seawater, which should keep the other ions out of the coating. This can be done by applying some techniques reported in the literature. For instance, Nafion perfluorosulfonic membrane is more permeable to ammonia than to K^+ [328], or expanded polytetrafluoroethylene, which was proven to be an effective ammonia permeable membrane[329]

The “visible” LPG in combination with sensitive dyes should be investigated further by testing different dyes with different PMTP ranges. Also, the RI calculations should be enhanced to give more precise numerical results as well as mathematical simulation of the “visible” LPGs response to complex refractive index should be carried out. For instance, interesting work was published recently by *Tianqi Sai et al.*[330] where they propose a different approach to calculate the Kramers-Kronig relation and deploy it to calculate aqueous solutions of dyes and compare the calculation results with empirical measurements using a refractometer. Among the dyes tested, BCB is present as well. Development in this direction could lead to a new sensing approach in the area of optical fibre sensors.

The phosphate sensor using thin MIP film should be investigated further by attempting to enhance the deposition techniques and the quality of the film itself. Also. Phosphate sensing should be approached using different sensing bases, such as quantum dots, sensitive dyes or ion-selective films.

Overall the nutrient sensing using optical fibres could and should be investigated by using ionophores in combination with LPGs. For instance nitrite detection could be targeted by deploying Rh(III)-tetra(t-butylphenylporphyrin) [37], or Co(II) tert-butyl-salophen [38] on an LPG surface. Ammonia sensing should be targeted by using nonactin [89] [91], phosphates by applying organotin [127] or molybdenum, ferrocene [131] and tripodal cadmium complexes [109].

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Appendix 1

A1 Sol-gel BCB sensor lifetime challenges

It was observed that the BCB embedded base catalysed sol gel coated optical fibre sensor loses its sensitivity over time. The sensitivity loss could be a result of several processes related to the BCB dye itself, to the base catalysed sol gel matrix, or to the complex they've formed. Such as photo bleaching, sol gel aging related processes: drying, stiffening, matrix shrinkage, or some other processes [287]. In order to understand the nature of sensitivity loss and as an effort to solve it, the next attempts, described below, were deployed to extend the sensor's lifetime.

A1.1 Photo bleaching considerations

To exclude the possibility of photo bleaching the same fibre which was presented in **Figure 4-30a** was placed in darkness for 7 days. Then it was tested with the same sodium nitrite concentration, **Figure 7-1**.

As one can see from the **Figure 7-1**, the sensitivity has dropped dramatically after 7 days in the darkness. In the mentioned figure, the light green curve is the same as was shown in **Figure 4-30a**, and shows the sensor's ratio between 610 nm and 515 nm in water, for 100 and 500 μM sodium nitrite aqueous solution the next day after the coating procedure. The orange curve shows the same ratio but after 7 days. During those 7 days, the fibre was kept in a dark cool place. The orange line with some noise and fluctuation stays constant (especially relative to the green curve) despite being immersed 6 times periodically to water and a 100 μM NaNO_2 solution, with a period of 20-22 minutes for each immersion. Afterwards, the ratio starts to drop steadily when exposed to 500 μM sodium nitrite solution. It started with ~ 1.374 and reached ~ 1.1365 after 20 minutes of immersion. After the solution was replaced by DI water, the ratio starts to grow back slightly, from ~ 1.368 to ~ 1.376 over 20 minutes time.

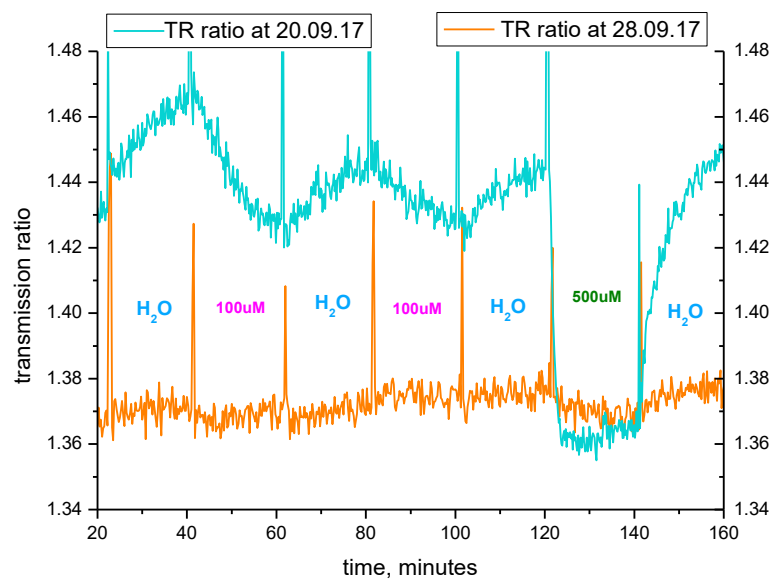


Figure 7-1 Sensitivity comparison of the same optical fibre sensor. The green line shows the response of the fibre to sodium nitrite in the first day after fabrication, while the orange lines shows the response of the same sensor after it was kept in darkness for 5 days.

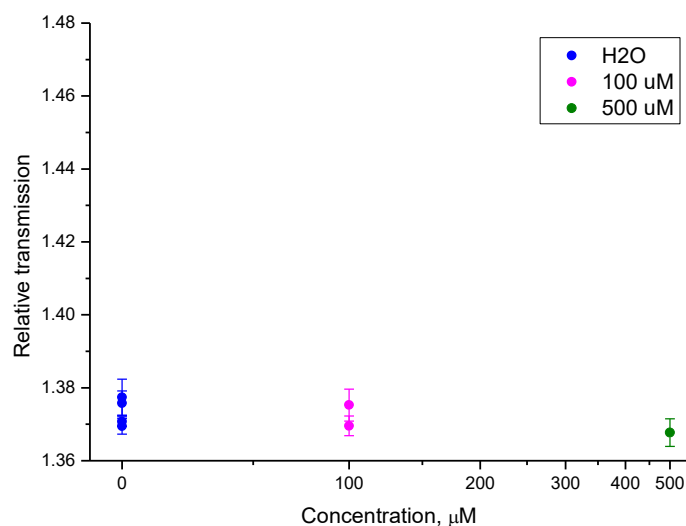


Figure 7-2 Integrated ratio values of the last minute of each measurement and their errors as a function of NaNO_2 concentration. After 7 days in darkness. Blue dots represent the ratio value in DI water, magenta and olive green, - 100 μM and 500 μM respectively.

Figure 7-3 shows the same processes on the same optical fibre sensor and uses the same procedure as described in the case of **Figure 4-31** only with 7-day difference

between the measurements, during which the fibre sensor was kept in a dark cool place.

As can be clearly seen from **Figure 7-1** and from a comparison of **Figure 4-31** and **Figure 4-42** the absence of light itself did not help to increase the sensor's lifetime. This means photo bleaching is not the cause of the sensitivity loss.

A1.2 Nitrogen and dry air

Three similar straight absorbance based fibres were fabricated. The coating was a BCB-NaOH based sol-gel described in Table 4-4, sample No 4 (highlighted by yellow), two layers with a rising velocity of 12 mm/sec. The next day, they went through temperature treatment in the oven with 70°C temperature for one hour and one additional hour of cooling in the oven. One of the sensors was tested immediately with 100 and 500 μM NaNO_2 concentrations in DI water.

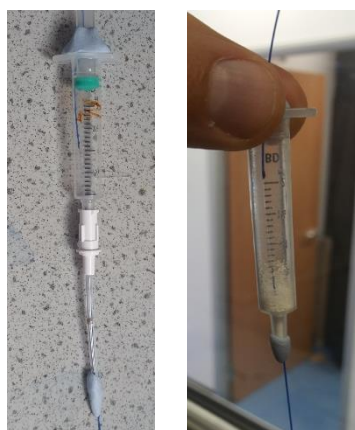


Figure 7-4 photograph of fibres contained in: **a)** N_2 atmosphere; **b)** dehumidified by CaCl_2 atmosphere

Two others were sealed and placed in a dark place for seven days. One was sealed with nitrogen inside, the other with air with added calcium chloride powder for humidity absorption, **Figure 7-4**. After 7 days, they were extracted and tested in order to compare the response. **Figure 7-5** shows the 610/515 nm intensity ratios of those three sensors, freshly fabricated (olive green), confined in Nitrogen for 7 days (blue) and confined in dry air for 7 days (magenta). As one can see the response of green and blue lines are fairly similar. The ratio declines slightly

in 100 μM sodium nitrite solution, with the following restoration in water. The ratio drops more significantly as a response to 500 μM NaNO_2 and restores in water afterwards. However the magenta line shows no response to 100 μM NaNO_2 , and hardly even anything for 500 μM NaNO_2 . Thus dry air does not help to increase the sensor's lifetime, on the other hand, the sensor stored in Nitrogen shows the same

response as a newly fabricated one. This means that the Nitrogen atmosphere does increase the sensor's lifetime.

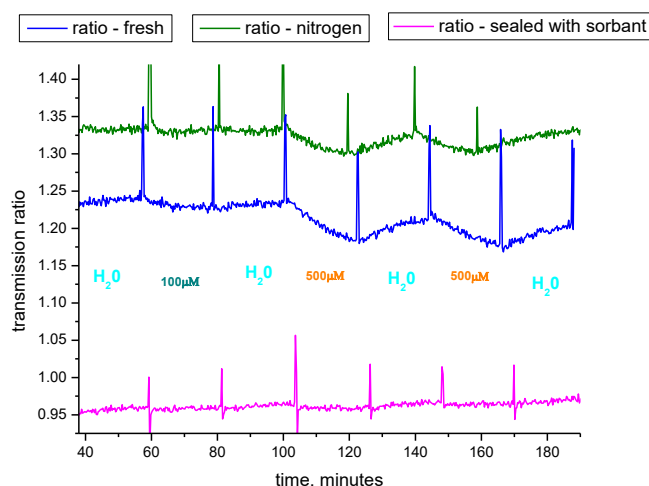


Figure 7-6 Comparison of response to 100 and 500 μM sodium nitrite solution. 610/515 transmission ratio of 2 layered BCB embedded base catalysed sol gel coating fabricated as described in Table 4-4, sample No 4 (highlighted by yellow), with 12 mm/sec elevation speed. The sensors were immersed in water, 100 and 500 μM sodium nitrite solution as denoted in the figure. Olive green line represents the fresh sensor's response, blue represents the sensor confined in Nitrogen for 7 days, the magenta represents the sensor contained in dehumidified (by CaCl_2 powder) air

After the described above test was completed, the sensor kept in Nitrogen was sealed in Nitrogen again for another 13 days. Then it was tested in the same way as above, and the response compared to the previous test, **Figure 7-7**. Blue curve shows

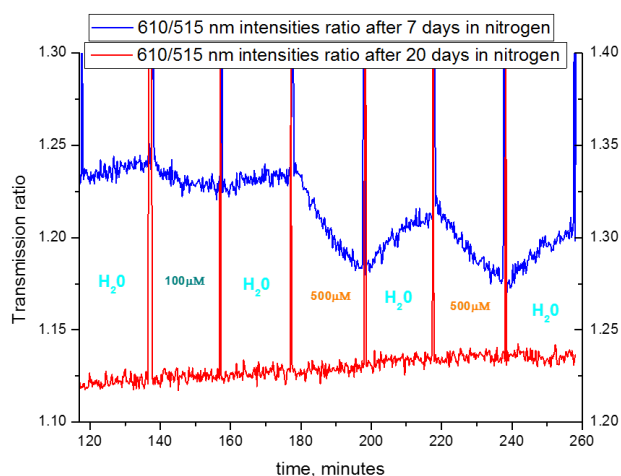


Figure 7-7 Comparison of response to 100 and 500 μM sodium nitrite solution. 610/515 transmission ratio of 2 layered BCB embedded base catalysed sol gel coating fabricated as described in Table 4-4, sample No 4 (highlighted by yellow), with 12 mm/sec elevation speed. The sensors were immersed in water, 100 and 500 μM sodium nitrite solution as denoted in the figure. Blue represents the sensor confined in Nitrogen for 7 days, the red line represents the sensor confined in Nitrogen for 20 days.

the 610/515 nm ratio response of the sensor confined in Nitrogen for 7 days, the red curve shows the same sensor's response after 20 days in N₂. As one can see the red line does not react to 100 and 500 μ M NaNO₂ concentration in DI water. Keeping the fibre in Nitrogen certainly prolongs the sensor's lifetime but does not answer to industrial needs made by Gill R&D of continuous using of the sensor for at least 3 months.

It is reasonable to suggest that the sensitivity loss could lay in the sol gel's ageing behaviour [287].