Optical microfiber sensor for heavy metal ion detection

Yap, Stephanie Hui Kit

2016

Yap, S. H. K. (2016). Optical microfiber sensor for heavy metal ion detection. Master's thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/68936>

<https://doi.org/10.32657/10356/68936>

NANYANG
TECHNOLOGICAL
UNIVERSITY

**OPTICAL MICROFIBER SENSOR
FOR HEAVY METAL ION DETECTION**

YAP HUI KIT STEPHANIE

SCHOOL OF ELECTRICAL & ELECTRONIC ENGINEERING

2016

**OPTICAL MICROFIBER SENSOR
FOR HEAVY METAL ION DETECTION**

YAP HUI KIT STEPHANIE

School of Electrical & Electronic Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Master of Engineering

2016

ABSTRACT

Heavy metal pollution in water environment poses a serious threat and of great environmental concern because they cannot be degraded or destroyed completely. In fact, heavy metal tends to accumulate over time within living organism and the rate of accumulation can be faster than they are excreted. Therefore, continuous monitoring of the level of heavy metal contaminants in water resources is crucial to keep them below the standard maximum permissible limit of the particular geographical area.

Addressing this matter, optical tapered fiber that is well known for excellent sensing capabilities due to strong evanescent field in the tapered region was adapted in this study to measure different concentrations of metal ion in aqueous environment. Tapered fiber with waist diameter of 7.1 μm was used and the surface of the tapered region was functionalized with amine group in order to form strong covalent bond between amine and the carboxylic group of a compound known as chelating agents. Chelating agent is capable of binding metal ions to form stable metal chelates, thus, when metal ions are bound on the surface of the tapered region, there will be some changes in refractive index around the tapered region. These changes can result in the alteration of characteristic of light passing through the tapered region.

A series of data sets were collected using non-functionalized tapered fiber for detection of low lead (II), zinc (II) and copper (II) ion concentration (1000ppm and below) and was compared with chelating agent-functionalized tapered fiber of the same taper profile. Findings from this study showed functionalized tapered fiber enhances detection limit of regular non-functionalized tapered fiber from 250 ppm (parts per million) to 30 ppm. On top of this, findings from the in-depth study of taper profile

characterization and properties of chelating agents made another highlight for the whole research project. It was found that the choice of taper profile and chelating agents is the primary key to fabricate a functionalized tapered fiber sensor that is capable of detecting extremely low concentration of heavy metal ions in metal solutions. In this study, we report the lowest detection limit achievable using D-Penicillamine (DPA)-functionalized tapered fiber of taper waist 3.9 μm is 10 ppb (parts per billion). These encouraging results uncovered the potential of chelating agent-functionalized tapered fiber to be developed into a good heavy metal ion sensor.

Further work was done with a proposed design of a reader unit to eliminate the use of an optical spectrum analyzer to observe the output wavelength spectra of the tapered fiber sensor. While the preliminary data from the study showed a standard deviation of ± 0.4 nm, the proposed design also showed a significant weight and cost reduction, which suggests the sensing system is likely to be made portable in order to facilitate on-site detection.

ACKNOWLEDGEMENT

First and foremost, my sincere thanks and appreciation to my supervisor, Professor Tjin Swee Chuan for his constant guidance, understanding and support. Special thanks to Assoc. Prof Yong Ken Tye for his unfailing support and his patience in sharing valuable insights of relevance information related to the study of metal chelation. I am thankful to my fellow lab mates and friends for the help and inspirations they extended. Last but not the least, I would like to extend my heartfelt gratitude to my family for their vital encouragement and much needed motivation.

TABLE OF CONTENTS

	PAGE
PREFACE	i - x
1. GENERAL INTRODUCTION	1
2. LITERATURE REVIEW	5
2.1 Optical Fibre	5
2.1.1 Mode Theory for Circular Waveguide	6
2.2 Optical Microfiber	9
2.2.1 Principle of Evanescent Field Sensing in Non-Adiabatic Tapered Microfiber	11
2.3 Optical Microfiber as Chemical Sensor	14
2.3.1 Absorption based Optical Chemical Sensor	16
2.3.2 Fluorescence based Optical Chemical Sensor	17
2.3.3 Refractive Index based Optical Chemical Sensor	18
2.4 Adhesion of Chemical Compound on Inorganic Substrate for use in Chemical Sensors	19
2.4.1 Surface Functionalization of Inorganic Substrates	21
2.4.1.1 Silane Coupling Agent	22
2.4.1.2 Modification of Silica-based Surface	24
2.4.2 Crosslinker	26
2.4.2.1 Zero-Length Crosslinker	29
2.5 Chelation	31
2.5.1 Principle of Metal Chelation	32
2.5.2 Types of Metal Chelators	34
2.5.2.1 Poly-amino carboxylic Acids	34
2.5.2.2 Derivatives of alpha-amino acids	35
2.5.2.3 Chelators with Vicinal Sulfhydryl	36
2.6 Heavy Metals	38
2.6.1 Lead (Pb)	38
2.6.2 Copper (Cu)	39
2.6.3 Zinc (Zn)	40
2.7 Standards for Safe Drinking Water	40
2.8 Conclusion	41
3. RESEARCH METHODOLOGY AND SURFACE VERIFICATION OF FUNCTIONALIZED OPTICAL MICROFIBER	43
3.1 Introduction	43
3.2 Fabrication of Microfiber	44
3.3 Microfiber Surface Modification	45
3.3.1 Materials	45
3.3.2 Preparation of Surface Functionalized Microfiber	46
3.4 Experimental Procedure	48
3.4.1 Experimental Arrangement	48

3.5 Surface Verification of Functionalized Optical Microfiber	49
3.5.1 Materials and Methods	49
3.6 Results and Discussions	50
3.6.1 Scanning Electron Microscope (SEM) Imaging	50
3.6.2 Energy Dispersive X-Ray Spectroscopy (EDX) Analysis	50
3.7 Conclusion	53
4. STRAIN AND TEMPERATURE CHARACTERIZATION OF OPTICAL MICROFIBER SENSOR	54
4.1 Introduction	54
4.2 Materials and Methods	55
4.3 Results and Discussions	55
4.3.1 Axial Strain Characterization	55
4.3.2 Temperature Characterization	57
4.4 Conclusion	62
5. HEAVY METAL DETECTION USING FUNCTIONALIZED OPTICAL MICROFIBER SENSOR	63
5.1 Introduction	63
5.2 Materials and Methods	64
5.2.1 Packaging of Microfiber Sensor	64
5.2.2 Experimental Procedure	65
5.3 Results and Discussions	66
5.3.1 Chelating Agent Presence Verification	66
5.3.2 Heavy Metal Detection Using Non-Functionalized Optical Microfiber with Taper Waist Diameter 7.1 μ m	68
5.3.3 Heavy Metal Detection Using Functionalized Optical Microfiber with Taper Waist Diameter 7.1 μ m	70
5.3.4 Comparison between Ethylenediaminetetraacetic acid (EDTA) and D-Penicillamine (DPA) for Copper (II) Ions Detection	74
5.3.5 Effect of Taper Waist Diameter on Sensing Potential of Functionalized Microfiber Sensor	75
5.3.6 Proposed Reader Unit	77
5.3.6.1 Identifying Direction Shift	78
5.3.6.2 Identifying Amount of Wavelength Shift	81
5.4 Conclusion	83
6. CONCLUSION	85
6.1 Conclusion	85
6.2 Recommendation for Future Work	87
AUTHOR'S PUBLICATION	88
REFERENCES	88

LIST OF FIGURES

Figure		Page
2.1	Electric field distribution in several guided modes in ascending order from left to right	8
2.2	Plots of normalized propagation constant versus normalized frequency for a few of the lowest order fiber modes	8
2.3	Flame brushing technique setup. Optical fiber is clamped onto two translation stages which are moving apart when heat is supplied and ultimately decreases the local diameter (tapered region). Arrows indicate movement performed by each component which is controlled by computer or controller	10
2.4	Schematic diagram of a biconical microfiber	12
2.5	Light source power, P_s transmittance measurements required to use absorption to measure concentration of an analyte in a solvent. Light power, P_o is power measured after light passing through pure solvent sample, while P_s is power measured after passing through solvent and analyte	16
2.6	General process of covalent bonding of chemical compound onto inorganic substrate for use in chemical sensors	20
2.7	Common functional groups involve in crosslinking and chemical modification process (a) Primary Amines ($-\text{NH}_2$), (b) Sulfhydryls ($-\text{SH}$), (c) Carboxyls ($-\text{COOH}$), (d) Carbonyls ($-\text{CHO}$)	21
2.8	General chemical structure of silane coupling agent	22
2.9	Reaction scheme of silane coupling agent and inorganic surface containing available $-\text{OH}$ groups. (a) Hydrolysis of reactive groups to form silanols. (b) Polymer matrix was formed after condensation process (c) Hydrogen bonding between silane coupling agent and inorganic surface containing hydroxyl group (d) Stable siloxane linkages through dehydration	23
2.10	Conjugation process using homobifunctional crosslinking reagent	27
2.11	Conjugation process using heterobifunctional crosslinking reagent	28
2.12	Chemical structure of EDC	29
2.13	Reaction scheme of carboxyl to amine crosslinking using carbodiimide EDC and EDC/sulfo-NHS	31
2.14	Formation of metal ligand complexes using polydentate ligand	32
2.15	Chemical structure of EDTA	33
2.16	Chemical structure of DPA	36
2.17	Chemical structure of DMSA	37

3.1	Microscopic view of typical microfiber. (a) Geometry of fabricated microfiber with taper waist diameter of $7.1\mu\text{m}$ (b) Image of tapered region obtained using scanning electron microscope	45
3.2	Surface functionalization of microfiber by silanization and the reaction scheme of EDC/NHS coupling for bonding of chelating agent onto the surface	47
3.3	Experimental Arrangement	48
3.4	Figure 3.4 (a)-(f): SEM image of microfiber with taper waist diameter of $7.1\mu\text{m}$ with magnification $\times 15000$ of (a) silanized microfiber using APTES (b) EDTA-functionalized microfiber and (c) EDTA-functionalized microfiber with lead (II) ion trapped on the surface by EDTA. SEM image of un-tapered fiber with magnification $\times 5000$ of (d) silanized fiber using APTES (e) functionalized fiber with EDTA attached on the surface and (f) functionalized fiber with lead (II) ion trapped on the surface by EDTA	51
3.5	The possible silanization process of microfiber surface	51
3.6	The spectrum of microfiber sensor obtained from SEM-EDX analysis. Green colour frame in the inset diagram represents the scanning area	52
4.1	Wavelength response of a microfiber and its shift upon applying strain on it	56
4.2	Resonant wavelength shift versus applied strain of tapered fiber with fix taper length of 5mm but different taper waist diameter	57
4.3	Variation of wavelength shift with increasing in temperature for microfiber with taper waist diameter of $4.7\mu\text{m}$ and taper length of 5mm	59
5.1	Three-dimensional view of an optical microfiber heavy metal ion sensor packaged in an acrylonitrile butadiene styrene (ABS) made cartridge	64
5.2	Side view of an optical microfiber heavy metal ion sensor packaged in an acrylonitrile butadiene styrene (ABS) made cartridge	65
5.3	Top view of an optical microfiber heavy metal ion sensor packaged in an acrylonitrile butadiene styrene (ABS) made cartridge	65
5.4	Actual experimental arrangement	66
5.5	Wavelength response (a) non-functionalize microfiber sensor (b) functionalized microfiber	67
5.6	Wavelength response of non-functionalized microfiber sensor surrounded by different concentrations of lead (II) chloride solutions	69
5.7	Wavelength response of (a) non-functionalized and (b) functionalized microfiber sensor surrounded by 250ppm lead chloride solution	71
5.8	Wavelength shift versus lead (II) chloride concentration for non-functionalized and functionalized microfiber	72

5.9	Wavelength shift versus zinc (II) chloride concentration for non-functionalized and functionalized microfiber	72
5.10	Wavelength shift versus copper (II) chloride concentration for non-functionalized and functionalized microfiber	73
5.11	Comparison between EDTA and DPA	75
5.12	Wavelength response of (a) EDTA functionalized microfiber of taper waist diameter 4.7 μ m (b) EDTA functionalized microfiber of taper waist diameter 3.9 μ m in lead (II) ion solution.	76
5.13	Wavelength responses for deionized water and 10 ppb copper (II) ion solution using a DPA functionalized microfiber sensor functionalized with taper waist diameter of 3.9 μ m	77
5.14	Optical connection setup for overall sensing system, incorporating the reader unit	78
5.15	Changes in peak intensity of two FBGs serve as markers to measure amount of dip wavelength shift. (Output spectra (dotted line) remains in each figure to serve as reference spectra. Each diagram shows reference spectra experiencing left shift in chronological order)	79
5.16	Examples showing the role of FBG 2 as a marker when (a) output spectra was blue shifted causing reflection peak power intensity of FBG 2 to increase (positive sign (+)) (b) output spectra was red shifted causing reflection peak power intensity of FBG 2 to decrease (negative sign (-))	80
5.17	Comparison between actual and calculated wavelength shift based on reflection peak's intensity changes	81
5.18	Least square regression analysis	82
5.19	Comparison between actual and revised calculated wavelength shift	83

LIST OF TABLES

Table		Page
2.1	Silane effectiveness on inorganic substrates	25
2.2	Drinking water quality guidelines (unit in ppm) prepared by organizations and committees throughout the world	41
3.1	Microscopic composition of microfiber at the scanning area	52
4.1	Summary of strain sensitivity for different types of taper profile	58
4.2	Microfiber temperature sensing sensitivity of varying taper waist diameter	61
5.1	Refractive index for different types of solution	69
5.2	Summary of measured power intensity of FBGs reflection peaks, corresponds to figure 5.15	79

LIST OF SYMBOLS AND ABBREVIATIONS

ABS	Acrylonitrile Butadiene Styrene
APTES	3-Aminopropyltriethoxysilane
BAL	British Anti-Lewisite
Cu	Copper
DI	Deionized
DMPS	sodium 2,3-dimercapto-1-propanesulfonate
DMSA	meso-2,3-dimercaptosuccinic acid
DPA	D-Penicillamine
DTPA	Diethylenetriaminepentaacetic acid
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy dispersive X-ray spectroscopy
EM	Electromagnetic
EPA	United States Environmental Protection Agency
FBG	Fiber Bragg grating
FOCS	Fiber optic chemical sensors
FSR	Free spectral range
IR	Infrared
MCL	Maximum Contaminant Level
MZI	Mach-Zehnder interferometers
n_1	Core refractive index
n_2	Cladding refractive index
NHS	N-hydroxysuccinimide
NTA	Nitrilotriacetic acid
OSA	Optical spectrum analyzer
Pb	Lead
PDMS	Polydimethylsiloxane
ppb	Parts per billion
ppm	Parts per million
RI	Refractive index
RIU	Refractive index unit
SEM	Scanning electron microscope
TE	Transverse electric
TIR	Total internal reflection
TM	Transverse magnetic
WHO	World Health Organization
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION

Heavy metal pollution in natural water environment is an area of concern in both human and ecosystem health. Many heavy metals are lethal not only at high concentrations but can be deleterious even at very low concentrations, because they may be accumulated in human organs, causing long term negative health effects [1]. High levels of heavy metal concentration have been found in the source of drinking water in Asian countries like China, Bangladesh, Thailand, Nepal and India [2]. For instance, high arsenic compound ($107\text{ }\mu\text{g/L}$) found in drinking water consumed by India's population is approximately 11 times higher than maximum permissible limit set by World Health Organization (WHO) [3]. Despite the need of having good heavy metal water filtration system, continuous monitoring in the level of heavy metals contamination in natural water environment is important to ensure safe drinking water for the sake of human health.

Conventional ways of quantitatively detecting levels of heavy metal contamination include atomic absorption/emission spectroscopy, inductively coupled plasma mass spectrometry and cold vapor atomic fluorescence spectrometry [4]. Even though these methods give good measurement sensitivities, they involve complicated chemical processes for extracting metal ions from sampled water collected from reservoir of interest. In addition, sophisticated instruments were used in these techniques which do not encourage on-site detection to be carried out and the costs of these instruments are usually very high. As a result, there is a demand of a rapid on-site heavy metal

detection system that is low in cost and does not involve complicated operation procedures. One plausible solution for this is to use optical sensors. In recent years, fiber optic sensors are finding increasing applications in industry, environmental monitoring, medicine and chemical analysis. In fact, many existing electrical sensors are slowly being replaced with optical sensors due to the extremely high sensitivity offered by the optical sensors. Other fascinating characteristics of optical sensors include [5]:

- i. Compact and light weight
- ii. Suitable to be used in harsh environment
- iii. Remote sensing capability
- iv. Ease of implementation in any structure due to their small size and cylindrical geometry

Various kinds of optical fiber structures, each with unique design and optical properties have been explored to fit the needs of a heavy metal sensing system. Moreover, each fiber design usually involves chemical surface treatment. For instance, M. Benounis et. Al. demonstrated successful preparation of polymeric cladding by depositing calixarene molecule onto the plastic cladding silica fiber using dip-coating method [6], meanwhile, B. Gu et. Al. also reported self-assembled nano coating on a thin core fiber [7]. Both works involve chemical modifications of the fiber sensing region prior to attachment of specific chemical compound which serves as the key element in heavy metal detection. These compounds are usually capable of holding onto metal ions to form metal complexes, which in turn, influences the output light transmission along the fiber sensor. Hence, these output information (e.g., intensity, wavelength, or phase) can be used to correlate with the concentration of heavy metal

ions presence in water samples.

Despite the increasing interest in optical fiber based heavy metal sensor, all reported absorbance based fiber sensor devices [6-9] mostly operates within visible (400 – 700nm) and near-infrared (IR) (750 – 1400nm) wavelength range, while short wavelength IR (1400 – 3000nm) remained less explored. This is because short wavelength IR is not typically absorbed by chemical compounds. These designs are also restricted to the use of in-laboratory bench top setup. On top of that, study on portable and smaller in size of fiber based heavy metal ion sensor device is also lacking.

This study aims to bridge the gap in existing research literature by designing a compact fiber-based heavy metal sensing device and fabricating a surface functionalized tapered microfiber, operating at short IR wavelength. The reason for this is that tapered microfiber occupies a prominent place due to its outstanding achievable sensitivity, ease of fabrication and low cost. As mentioned above, most of the fiber-based heavy metal sensor choose operating wavelength that is typically absorbed by target chemical compound, however, this is not necessary for tapered microfiber because it is capable to detect based on refractive index (RI) changes alone, provided that these changes occur close to the surface of sensing region. Moreover, fiber sensor operating at short IR wavelength has sufficiently low attenuation, which promotes the feasibility of remote sensing applications [10].

In this study, the sensing region of tapered microfiber sensor is functionalized and treated with a chemical compound known as chelating agent, which is capable to bind with common heavy metal contaminants in drinking water such as lead (Pb), zinc (Zn) and copper (Cu). Chelating agent is an organic compound, often used in metal

detoxification due to their ability to form a stable metal chelates (i.e. complex compound composed of metal ion and chelating agent) that are easily excreted from target site. Therefore, attachment of chelating agent onto the sensing region serves to trap heavy metal ions onto the surface and this will alter the effective RI of the microfiber which in turn results in a spectral shift. This information is then useful to correlate with the concentration of heavy metal ions in water samples. In addition, each type of chelating agents have their own ligand structure characteristics, and for this reason, these chelating agents are unique in a sense that each of them has distinct affinities toward specific metal type. As a result, the study to compare the detection capability of tapered microfiber heavy metal sensors that have been treated with different types of chelating agents is worth exploring. Another highlight of this study includes solution to prevent cross sensitivities that may occur during heavy metal detection with tapered microfiber. The term cross sensitivities in this context refers to ability of microfiber sensor to detect not only effective RI changes caused by trapping of metal ion on the sensing region, but also other unfavorable parameters such as temperature and axial strain. A well designed packaging of tapered microfiber sensor is important to keep changes in light propagation due to these unfavorable parameters as low as possible.

In summary, the core objectives of this study are:

- i. To investigate metal binding properties of various types of chelating agent
- ii. To develop a surface treated microfiber through binding of chelating agent for heavy metal detection in water environment
- iii. To improve the sensitivity of microfiber in heavy metal detection through microfiber surface functionalization, in short wavelength IR range
- iv. To design a packaging for surface treated microfiber sensor

CHAPTER 2

LITERATURE REVIEW

2.1 Optical fiber

Optical silica fiber is a cylindrical waveguide made up of a silica core and a silica cladding. The core is usually doped with special dopants such as germanium dioxide, GeO_2 , causing the core refractive index, n_1 to be slightly higher than the cladding refractive index, n_2 . Such characteristic allows light to propagate through the core by total internal reflection (TIR) when the light incident angle is larger than the critical angle. According to work done by Maxwell in 1864, light wave is electromagnetic in nature. Electromagnetic energy in the form of light is confined and guided within the waveguide itself, in a direction parallel to its axis. Common configuration structure of an optical waveguide consist of a single solid dielectric cylinder (i.e. core) surrounded by a solid dielectric cladding. Optical fiber can be divided into two categories: (i) *step index fiber*, where the refractive index of the core, n_1 is constant throughout and undergoes an abrupt change at the core-cladding interface; (ii) *graded index fiber*, where the refractive index of the core decreases parabolically from the center of the fiber. On top of that, both step and graded index fibers can be further categorized into single and multimode classes. Single mode fiber allows only one mode of propagation, whereas multimode fiber is capable of sustaining hundreds or thousands of modes. The total number of guided modes, M , depend upon a parameter called the V number, given as follows [11]:

$$M = \frac{V^2}{2} \quad (1)$$

Meanwhile, the V number is given by:

$$V = \frac{2\pi a}{\lambda} (n_1^2 - n_2^2)^{1/2} \quad (2)$$

where,

a = core radius

λ = operating wavelength of light

n_1 = refractive index of the core

n_2 = refractive index of the cladding

As seen in equation (2), V number summarizes all the important physical characteristics of an optical fiber. V number is important to determine whether an optical fiber will be single-mode or not at a particular wavelength. For instance, optical fiber designed with V number ≤ 2.405 , allows only one mode to propagate, hence, such fiber is known as single mode fiber.

2.1.1 Mode Theory for Circular Waveguide

Light propagation along an optical circular waveguide can be described as a set of guided electromagnetic (EM) waves, known as modes of the waveguide. Each guided mode is a combination of electric and magnetic field distributions that are perpendicular to each other and repeat itself at equal intervals [11]. As seen in equation (1), optical fiber carries finite number of guided modes along its axis. However, there

is also infinite number of radiated modes that exists due to refraction of incoming light that is outside the range of fiber acceptance angle. These radiated modes are not bound within the fiber core but are trapped in the cladding instead, known as cladding modes. Thus, core and cladding modes occur simultaneously as light wave propagates down the waveguide. Small portions of the guided modes' electric field may spread into the cladding and the same goes for the cladding modes. Consequently, mode coupling between cladding and higher order core modes is likely to eventuate. Besides guided and radiated mode, there is another type of mode called leaky mode. This mode is partially confined within the core region, and continuously gets attenuated as they propagate along the fiber.

Transverse electric (TE) and transverse magnetic (TM) fields are guided modes found within the cylindrical waveguide. Contrary to TM modes, TE modes have zero electric field and non-zero magnetic field in the direction of light propagation. Unlike one-dimensional planar waveguides, it is common that cylindrical waveguide modes are labelled with integers m and n since the waveguide is bounded in two dimensions. Besides TE_{mn} and TM_{mn} modes, presence of core-cladding boundary conditions of an optical fiber stimulate coupling between electric and magnetic field components. As a result, non-zero electric and magnetic fields in the direction of light propagation give rise to hybrid modes. In this case, the amount of electric field can be larger than magnetic field or vice versa, and therefore hybrid modes, HE_{mn} or EH_{mn} are designated depending on which field contributes the most in the direction of propagation. The order of a mode is defined as the number of field zeros across the waveguide. Figure 2.1 depicts different order of TE modes in a planar dielectric slab waveguide [11].

Figure 2.1: Electric field distribution in several guided modes in ascending order from left to right. [11]

As aforementioned, V number plays an important role in determining the cutoff condition for all guided modes (Figure 2.2). The lowest order mode of an optical fiber is the HE_{11} mode. It is also known as the fundamental mode since it does not have a cutoff and is always present in the optical fiber unless the core diameter reduces to zero. For instance, single mode fiber designed with V number of ≤ 2.405 tends to cut off all higher order modes, leaving only the fundamental mode.

Figure 2.2: Plots of normalized propagation constant versus normalized frequency for a few of the lowest order fiber modes [13]

In order to remain guided within the waveguide, propagation factor, β is required to satisfy the following condition [12]:

$$n_2k < \beta < n_1k \quad (3)$$

where, k is the propagation constant in free space and is given as:

$$k = \frac{2\pi}{\lambda} \quad (4)$$

From equation (3), when β happens to be equal or less than n_2k , optical power flowing through the optical waveguide is said to be in leaky mode, where optical power leaks from the core into cladding as it travels for few centimeters to kilometers.

2.2 Optical Microfiber

Tapered optical fiber or microfiber is a type of optical fiber having cladding diameter (along with the core) of hundreds of nanometers to several micrometers. Microfiber is also commonly known as evanescent wave based optical fiber since it operates based on the principle of fluctuations in the evanescent field in the tapered region. Common approach to fabricate a microfiber is usually done by heating and stretching a regular sized optical fiber (Figure 2.3). There are five types of tapering techniques that have been successfully reported so far: (i) self-modulated taper drawing [14] (ii) flame elongation [15-16] (iii) flame brushing technique [17] (iv) modified flame brushing technique [18] and (v) direct drawing from the bulk [19].

Figure 2.3: Flame brushing technique setup. Optical fiber is clamped onto two translation stages which are moving apart when heat is supplied and ultimately decreases the local diameter (tapered region). Arrows indicate movement performed by each component which is controlled by computer or controller [17].

Apart from fabrication methods, various kinds of microfiber structures have also been explored. Typical structures include biconical microfibers, microfiber gratings, microfiber circular cavities and microfiber Mach-Zehnder interferometers (MZI) [20]. Among these structures, biconical microfiber has the simplest configuration. In 2006, Kieu and Mansuripur reported the potential of modifying standard single mode fiber into biconical microfiber, to be used as a simple displacement, temperature and refractive index sensors [21]. In the report, they do not only demonstrated the simplicity of biconical microfiber fabrication using flame brushing technique, but also showed fine tuning of stretching rate, length of heated zone and flame temperature is capable of constructing different shapes and optical properties of biconical microfibers, indicating a wide range of design flexibility of microfibers.

In recent years, biconical microfiber attracted huge amount of interest in optical sensing applications because it possesses several unique characteristics including large fraction of evanescent fields, strong optical confinement, mechanical flexibilities, low power consumption and exceptionally fast response toward surrounding changes [20, 22-23]. Owing to its outstanding optical and mechanical properties, microfiber is

widely investigated for numerous sensing applications such as measurement of chemical concentration and physical properties like refractive index, temperature, humidity and strain [22-26].

2.2.1 Principle of Evanescent Field Sensing in Non-Adiabatic Tapered Microfiber

Biconical microfiber can be classified into adiabatic and non-adiabatic microfibers. Since the primary aim of an adiabatic or gradual taper is to reduce the insertion loss, it is fabricated with small taper angles, causing only a small change in radius along the taper length. As a result, most of the power remain in the fundamental mode and do not couple with other higher order cladding modes as it travels along the taper. In contrast to adiabatic, non-adiabatic or abrupt taper has much larger taper angle and thus, coupling of fundamental mode to higher order cladding modes is inevitable [27].

Electric field of guided modes are not completely confined to the central core, instead, small portions will decay exponentially into the cladding, known as evanescent field. In low-order modes fields are mostly concentrated close to the center of the core with minimal penetration into the cladding region. On the other hand, higher order modes have evanescent fields which extend further beyond the cladding region because fields are distributed more toward the edges of the core (Figure 2.1) [11]. Even though single mode fiber support only one mode, nonetheless, it is possible that higher order modes can be supported in the tapered region due to the large refractive index difference between the core and surrounding (Figure 2.4). As a result, microfiber makes a very good sensor since large portions of light entering the tapered waist region is guided by

cladding/air, thereby increasing amount of evanescent wave, causing it to be extremely sensitive to small changes in the surrounding medium [21,28].

Figure 2.4: Schematic diagram of a biconical microfiber

The operation principle of tapered optical fiber begins when light wave is launched into the core of un-tapered fiber, propagating as fundamental mode, HE_{11} of the single mode fiber. As the light entered the tapered region, significant portions of the power will be coupled into the higher order mode, usually HE_{12} , having the closest propagation constant to HE_{11} mode [16, 29-30]. Light continues to travel down the taper and is again coupled back into fundamental mode at the end of the up-tapered region. At this point, apparent phase difference between HE_{11} and HE_{12} mode is seen through modal interference that is represented as follows [31-32]:

$$I = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos \phi \quad (5)$$

where,

I = power coupling between fundamental mode and HE_{1m} mode

ϕ = phase difference between the modes

For a uniform beating region, the phase difference is given as:

$$\phi = \Delta\beta L \quad (6)$$

where,

L = length of beating region

Difference between propagation difference of the modes, $\Delta\beta$ can be represented as:

$$\Delta\beta = \frac{\lambda (U_2^{\infty 2} - U_1^{\infty 2})}{4\pi n_2 \rho^2} \exp\left(-\frac{2}{V}\right) \quad (7)$$

where,

U_m^{∞} = asymptotic values of the U parameters of the modes. (U_1^{∞} corresponds to HE_{11} = 2.405 and U_2^{∞} corresponds to HE_{12} = 5.520 [29].

ρ = radius of taper waist

V = normalized frequency, slightly difference than equation (2), expressed as:

$$V = \frac{2\pi\rho}{\lambda} (n_2^2 - n_{surrounding}^2)^{1/2} \quad (8)$$

where $n_{surrounding}$ is the refractive index of surrounding medium.

Sensitivity of non-adiabatic microfiber is mainly determined by the interaction of evanescent wave with surrounding medium in tapered region. In other words, the stronger the evanescent wave penetrating into the surrounding medium, the output spectrum detected at the end of the microfiber will be more responsive to small changes in the surrounding medium [31]. Evanescent light penetration is described by its penetration depth, D_p , which is the distance away from the core-cladding interface,

at which the magnitude of the electric field at the surface begin to decays to its 1/e value, and is given by [33]:

$$D_p = \frac{\lambda}{2\pi\sqrt{n_1^2 \sin^2 \theta - n_2^2}} \quad (9)$$

where,

λ = operating wavelength of light

θ = angle of incidence at the core-cladding interface

Cladding thickness of a bare fiber is several times than that of the core layer. Therefore, evanescent field will not be able to interact with fiber surrounding environment since it decays to negligible value as it reaches beyond the cladding. This explains the reason why an evanescent wave based fiber sensor is done by removing the cladding or tapering the fiber down to a diameter less than initial core diameter. In addition, variation in radius along the tapered region also contributes to the coupling of light among the modes in the tapered region, which in turn causes the fiber transmission properties to change. For instance, continuous reduction in the waist diameter will results in a perturbation leading to coupling of existing modes, and thus, supporting oscillatory spectra to be seen in the output [34].

2.3 Optical Microfiber as Chemical Sensor

Chemical sensors have been defined as an analytical device capable to deliver real-time information in the presence of specific compounds in complex samples [35]. Ability of remote sensing, immunity to electromagnetic interference and the potential of distributed sensing along the fiber length are some of the capabilities offered by

fiber optic chemical sensors (FOCS) that motivates extensive research in optical sensing. FOCS became available in the late 1970s when Hesse [36] patented the first FOCS for oxygen. In his work, Hesse applied oxygen sensitive chemistry to the optical waveguide which was injected with fluorescence light at one end of the fiber, followed by oxygen detection at another end through measurement of fluorescence intensity change which is affected by the presence of oxygen.

FOCS can be divided into two main categories, extrinsic and intrinsic. Extrinsic FOCS require the use of indicators, probes, or color-forming reactions for analyte sensing. In this scheme, optical fiber does not play the role for sensing functions. In 2010, Yoo and Song conducted a study of heavy metal ion sensing using FOCS. The sensor described in their work was prepared by embedding conjugate polymer, known as polydiacetylene (PDA) to microfiber using 3-D hydrodynamic focusing technique. PDA changes its color from visible blue to fluorescent red when it comes into contact with certain metal ions such as Al^{3+} and Zn^{2+} . In addition, when the PDA sensor microfiber is exposed to metal ion solution for ten minutes, rapid increase in fluorescence intensity was observed in the first minute and reaches equilibrium after nine minutes [37].

In contrast to extrinsic FOCS, intrinsic FOCS, or direct sensing, involves measuring the effect of analyte on optical properties of optical fiber such as refractive index, absorption, emission and polarization [38]. Due to its potential of providing real-time analyte detection, intrinsic FOCS with different detection principles such as absorption, fluorescence, reflectance and others have been widely investigated and developed using various kinds of fiber structures, including optical microfiber.

2.3.1 Absorption Based Optical Chemical Sensor

The fundamental principle of chemical analyte detection based on optical absorption can be explained through the expression of Beer-Lambert law [39]:

$$A = a_{\lambda} L c \quad (10)$$

where,

A = measured absorbance

a_{λ} = wavelength dependent absorptivity coefficient

L = path length

c = analyte concentration

Majority of the chemical molecules are capable of absorbing 200-400nm (ultraviolet) and 400-800nm (visible) light. Moreover, exact detection of certain molecule is probable since different molecules absorb radiation of different wavelengths [39]. The process of measuring absorption usually involves two transmittance measurements, as depicted in Figure 2.5. In this case, light source power, P_o and P_a are compared in order to determine the concentration of analyte in a chemical solvent.

Figure 2.5: Light source power, P_s transmittance measurements required to use absorption to measure concentration of an analyte in a solvent. Light power, P_o is power measured after light passing through pure solvent sample, while P_s is power measured after passing through solvent and analyte [39].

An optical microfiber sensor based on evanescent absorption spectroscopy in visible wavelength range using tapered multimode fiber was designed for the detection of ethanol with concentration ranging from 5% to 40% [40]. Linear relationship was found between light absorption and ethanol concentration in this work. Another study using absorption based evanescent wave sensor for mercury sensing was done using an organic compound known as dithizone. This compound forms metal-dithiozonates complex and produces distinct absorption spectra in the presence of metal ions in aqueous solution [9].

2.3.2 Fluorescence Based Optical Chemical Sensor

Fluorescence based chemical sensor involves excitation of light on the fluorescent molecule deposited on FOCS. This light of particular wavelength will be absorbed by the molecules, followed by emission of light at higher wavelength [38]. It is the changes in optical properties of the emission such as intensity, color and polarization that can be correlated to the presence of chemical analyte. Linear relationship between fluorophore concentration and the intensity of its emission is given as [41]:

$$I_F = K\epsilon I c \quad (11)$$

where,

K = quantum efficiency

ϵ = fluorophore absorption coefficient at the excitation wavelength

I = intensity of the excitation light

c = analyte concentration

Zhujun and Seitz [42] have reported immobilization of fluorimetric reagent, quinolin-8-ol-5-sulfonate (QS) to FOCS for the determination of heavy metal ions. Presence of metal ions triggered the formation of strong fluorescent QS-complex and was measured as fluorescence intensity. Recent advancement in fluorescence based FOCS for heavy metal detection can be seen from work done by Aknuser [43]. In his work, the fluorescence optical heavy metal sensor was prepared by using polyvinyl chloride films with triazolo-thiadiazin derivative embedded on it to form strong double bond with lead (II) ion. This sensor does not only show good selectivity toward lead (II) ion with respect to other coexisting interference metal ions but also capable of providing detection limit of 2.2×10^{-8} M.

2.3.3 Refractive Index Based Optical Chemical Sensor

Refractive index (RI) of a medium is defined as the ratio of the velocity of light in a vacuum to the velocity of light in the medium. Light propagates slower in optically dense medium than in one that is less dense [12]. The essence of RI based FOCS lies in the change of optical properties of light passing through different mediums with different RI. In the case of microfiber, difference in fiber core and the surrounding medium's RI occur due to the presence of chemical analyte of certain concentration [38]. Fundamental and higher order modes travelling side by side in the core and surrounding medium of tapered region experience phase shift upon recombining at the up-tapered region. This is reflected as total resonant wavelength shift when the output spectra of the two samples with different RI are compared [31]. Such sensors are of great interest since they offer broad measuring ranges and are extremely sensitive.

Cohoon et al. [44] used a non-adiabatic biconical fiber with taper waist diameter about 15 – 20 μm to monitor effects of the change in RI of aqueous samples. The tapered fiber was first immersed in deionized solution followed by incrementally adding in isopropyl alcohol to alter the RI of aqueous solution. Transmission spectrum observed at the output end was found to shift to the longer wavelength (red shift) as the concentration/RI of aqueous sample was increased, and showed detection limit of 0.6×10^{-5} RI units (RIU). Another study using adiabatic tapered microfiber long period grating of taper waist diameter of 55 – 60 μm was demonstrated to investigate its potential for use as refractive index sensor. Interesting findings from this study include noticeable contradiction of transmission spectrum behavior compared with the conventional long period grating. Red shift in resonant wavelength of tapered long period grating occurred when RI of surrounding medium increases. This phenomenon contradicts the conventional long period grating, where the resonant wavelength shifts to the shorter wavelength (left shift). The tapered long period grating RI sensor offered detection limit of 1.67×10^{-5} RIU around aqueous sample with RI range about 1.38 [45].

2.4 Adhesion of Chemical Compound on Inorganic Substrate for use in Chemical Sensors

The key figure of all chemical sensors is the known chemical compound used to identify and quantify the target analyte. These compounds are wisely chosen for their specificity to the target analyte. Moreover, they should also be able to convert successful detection of analyte into a measurable physical change for analytical purposes. These chemical compounds, which are usually organic (i.e. compounds

whose molecules contain carbon) in nature, are adhered onto the sensing surface, and the means used to attach them to the surface plays a critical role in sensor development and optimization. Although a number of adhesion methods had been reported [46], these approaches share a common concept, and are generally composed of three main steps, as shown in Figure 2.6.

Figure 2.6: General process of covalent bonding of chemical compound onto inorganic substrate for use in chemical sensors

Many developed FOCS found in the literature often involved chemical modification of the optical fiber surface [7, 47-48]. This is because common optical fiber is made of inorganic material and there is a need to functionalize the surface prior to attachment of organic substrate/compound on it. Once the fiber surface is ready to bond with organic substrate/compound, a crosslinker, which acts as a bridge, is required to create a strong bond between them. The substrate/compound layer developed on the fiber surface will react with target analyte, causing variation in the intensity of the light travelling down the fiber. Quantitative analysis can be done since the intensity change is related to the analyte concentration [47].

2.4.1 Surface Functionalization of Inorganic Substrates

Modification of inorganic surface through functionalization of chemical groups is often done to alter the substrate's physical and chemical properties. Chemical or functional group is defined as part of a molecule which consists of groups of atoms that determines the chemical behavior of the molecule. It is attached to the backbone (carbon atom) of the organic molecules. Despite the vast number of recognized functional groups in organic chemistry, functional groups that are commonly targeted for majority of chemical modification and conjugation techniques are shown in Figure 2.7.

Figure 2.7: Common functional groups involve in crosslinking and chemical modification process (a) Primary Amines ($-\text{NH}_2$), (b) Sulfhydryls ($-\text{SH}$), (c) Carboxyls ($-\text{COOH}$), (d) Carbonyls ($-\text{CHO}$)

Functionalization is a process of adhering molecules with specific functional groups to the substrate's surface through chemical grafting, or simply through adsorption without forming a covalent or ionic bond. Adsorption approach is done by means of

mutual attractive interactions between chemical groups and the surface. As a result, the total amount of adsorbed functional groups may vary at different spots. On top of that, bounded molecules may be easily released to the solution when the substrate is immerse in the analyte solution. For this reason, signal loss and cross contamination of the spots is most likely to happen. On the other hand, covalent grafted molecules are attached to the surface by means of chemical bond. These grafted molecules tend to grasp strongly to the surface which in turn, possess no detachment problem as in adsorption. However, thorough cleaning of the surface (repeated rinsing) with deionized water is possible to remove contaminants and weakly bounded molecules [49].

2.4.1.1 Silane Coupling Agent

Silane coupling agent is a compound whose molecules contain functional groups that act as an intermediary to form durable bond between inorganic and organic materials. The process of functionalizing inorganic surface using silane coupling agent is known as silanization [50]. General molecular structure of a silane coupling agent is illustrated in the figure below [51]:

Figure 2.8: General chemical structure of silane coupling agent [51]

Silane coupling agents are composed of a central silicon atom with three reactive groups, R, attached to it. These reactive groups (e.g. methoxy, ethoxy) are hydrolysable and are responsible for forming chemical bonds with inorganic materials.

On the other side, core silica atom is linked to non-hydrolysable functional groups, X, usually amine, epoxy, vinyl etcetera, via an alkyl chain(CH₂)_n. It is this characteristic which enables covalent linkages to another organic molecule [52]. There is plenty of silane coupling agents available with different chemical structure to meet different needs of inorganic-to-organic bonding, but the basic mechanism behind all of these agents are naturally the same (Figure 2.9).

Figure 2.9: Reaction scheme of silane coupling agent and inorganic surface containing available -OH groups. (a) Hydrolysis of reactive groups to form silanols. (b) Polymer matrix was formed after condensation process (c) Hydrogen bonding between silane coupling agent and inorganic surface containing hydroxyl group (d) Stable siloxane linkages through dehydration

Silanization begins with hydrolysis of the three reactive groups to form silanols. Water is needed for hydrolysis and may be either added intentionally, appear freely at surrounding atmosphere, or originally present on the inorganic substrate surface [53]. Next, silane coupling agents experienced first round of condensation to form a

polymer matrix linked together by -Si-O-Si- bonds. While silane network begin to develop, part of the network form hydrogen bonding with the hydroxyl (-OH) groups on the surface of inorganic substrate. Successively, the second round of condensation reaction will take place. Removal of water that is present on the inorganic surface is required to form robust siloxane linkages [48,54].

2.4.1.2 Modification of Silica-based Surface

Concentration of -OH groups on inorganic surface predominantly decides the effectiveness of silane coupling. Therefore, not all substrates exhibit suitable physical and chemical properties for effective silanization. According to Rapra Technology (Table 2.1), good substrates for silanization are silica, quartz, glass and aluminium. Meanwhile, graphite and carbon black are labelled as poor substrates [55].

Among the substrates listed in Table 2.1, silica is the most commonly used inorganic material in silane studies [53, 56-58]. Aside from this, there are also a large number of research which involve silica surface modification using silanization process, particularly for use in optical sensing [59-61]. For example, Yeo et al. fabricated a polymer coated fiber Bragg grating (FBG) to measure moisture absorption in concrete for structural monitoring. In order to improve adhesion between silica and polymer layer, Yeo first treated the FBG using 3-Aminopropyltriethoxysilane (APTES) prior to applying polymer coating onto it [62]. APTES is an amino-silane coupling agent that is widely used to modify silica surface. It contains reactive groups for inorganic surface attachment at one end and functional amine group at another end. These primary amine groups are then available for subsequent crosslinking with other chemical compounds.

For this reason, silane agents are often regarded as primary ingredient for initial optical fiber surface modification.

Silane Effectiveness	Substrates
Excellent	Silica
Quartz
	Glass
	Aluminium
	Alumino-Silicates
Good	Mica
Talc
	Inorganic Oxides (Fe_2O_3 , TiO_2 etc.)
	Steel, Iron
	Asbestos
Moderate	Nickel
Zinc
	Lead
	Chalk
	Gypsum
	Barytes
	Graphite
Poor	Carbon Black

Table 2.1: Silane effectiveness on inorganic substrates [55]

2.4.2 Crosslinker

The process of chemically joining two or more molecules through covalent bond is known as crosslinking. Subject to types of chemical groups available on target molecules, it is crucial to select an appropriate crosslinker with compatible reactive functionalities at both ends of its spacer arm to achieve successful conjugation process. Therefore, careful considerations are needed when choosing a suitable crosslinker are:

- i. *Crosslinking target* refers to reactive groups at either end of the crosslinker. These reactive ends are reactive toward functional groups commonly found in proteins and can be the same or different to each other. Suitable crosslinker type will be chosen according to the desired conjugation needs.
- ii. *Spacer arm attributes* refer to the characteristics of the chemical chain between the reactive ends. For instance, length of the spacer arm determines the flexibility of conjugation. Longer spacer arms provide greater flexibility but concurrently introduce more sites for nonspecific binding. Furthermore, availability of cleavage site in the spacer arm that allows reverse conjugation also affects the choice of the crosslinker used in certain applications.
- iii. *Reaction time* of a crosslinker when exposed to target molecule can be either instantaneous or delayed by some time to get activated through UV light exposure. Generally, crosslinkers can be classified as homobifunctional and heterobifunctional. Crosslinking reagents with identical reactive groups at both ends of its spacer arm was first used by Hartman and Wold [63] to modify bovine pancreatic ribonuclease A, a type of RNase, with diimido ester. Homobifunctional crosslinkers have identical reactive groups at both ends of its spacer arm that link the same common groups on both target molecules (Figure 2.10).

Figure 2.10: Conjugation process using homobifunctional crosslinking reagent

The primary drawback of homobifunctional linkers is that they are likely to create poorly defined conjugates [64]. Homobifunctional conjugation process begins when the reagent first reacts with molecule A, to form an active intermediate. This intermediate may then form crosslinks with molecule B or unexpectedly react intramolecularly with other functional groups on part of its own chain instead. This shortcoming of homobifunctional linker gets worse when it is used in single-step reaction procedures, where reagent and reaction mixtures are added together at the same time. This method gives the least control over crosslinking process, causing only a small percentage of the desired conjugate constituting the final conjugate products. Addressing this matter, a two-step procedure has been developed to alleviate the problem. In the two-step protocols, molecule A is first activated with homobifunctional linker followed by removing the excess reagent and by-products. Subsequently, the activated intermediate is mixed with molecule B, creating the final desired conjugate products. Even though the two-step procedure gives better control of the conjugation process, they do have a problem with hydrolysis, where the activated intermediate may get hydrolyzed (or degrade) before the addition of molecule B. Regardless of the limitations possess by homobifunctional linkers, they remain as a

primary choice for many applications since they are sufficiently good enough to form effective desired conjugates.

Unlike homobifunctional crosslinkers, heterobifunctional reagents comprise of two different reactive ends that can tie to two different functional groups on the target molecules (Figure 2.11).

Figure 2.11: Conjugation process using heterobifunctional crosslinking reagent

Hence, direct crosslinking reaction to designated parts of target molecules is possible thus giving better control of the conjugation process. Practically, all heterobifunctional linkers are often used in the two-step process that limits the possibility of self-conjugation, which happen frequently in single-step homobifunctional crosslinking. It is probable that molecule B does not have groups needed to couple with second end of the crosslinker. In this case, an additional step to modify molecule B is needed prior to completion of the second stage of conjugation process. As a result, direct conjugation is attainable, provided the activated molecule A does not have functional groups similar to other end of the heterobifunctional crosslinker.

2.4.2.1 Zero-Length Crosslinker

Zero-length crosslinker is a unique type of crosslinking reagents. As its name implies, zero-length crosslinkers bring two molecules together through a bond having no additional atoms. In other words, functional groups of the two molecules are covalently attached together with no intervening linker or spacer [65]. One of the most popular types of zero-length crosslinker that form linkage between amines and carboxylates or phosphates is known as carbodiimides [66].

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)

Figure 2.12: Chemical structure of EDC

EDC is a zero-length, water soluble carbodiimide heterobifunctional crosslinking reagent (Figure 2.12). Crosslinking reaction using EDC in the presence of N-hydroxysuccinimide (NHS) or N-hydroxysulfosuccinimide (sulfo-NHS), a sulfonated analogue to NHS, is a notable example of two-step zero-length crosslinking protocol. EDC enable direct conjugation between carboxyls (-COOH) and primary amines (-NH₂) without becoming part of the linkage (amide bond). The mechanism of crosslinking action is initiated by activation of carboxyl group, creating an o-acylisourea intermediate. During the subsequent reaction, when the activated intermediate is exposed to amine group from molecule B, they will be cross-linked through an amide bond without inclusion of any atoms from EDC [67]. The o-

acylisourea intermediate is highly unstable in aqueous solutions and will most likely get hydrolyzed if they fail to react with the amine groups, causing carboxyl groups to be regenerated [68].

In 1982, Staros [69] has successfully demonstrated high crosslinking yields of two new crosslinking reagents, namely 3,3'-dithiobis(sulfosuccinimidyl propionate) and bis(sulfosuccinimidyl)suberate due to incorporation of sulfo-NHS into these reagents. This is because sulfo-NHS is a hydrophilic organic compound that hydrolyzes slower compared to their rates of reaction with primary amine groups [70]. Further, Staros and his group reported significant increase of glycine to keyhole limpet hemocyanin conjugation products, approximately 15-times in EDC/sulfo-NHS compared to that obtainable solely with EDC [69]. Nakajima and Ikada's (1995) work further support the claim when they figured out carboxylate containing molecules that form an anhydride from the o-acylisourea intermediate can result in an increase of amide bond formation [66]. Similarly, formation of a sulfo-NHS intermediate from the reaction of hydroxyl group on sulfo-NHS with EDC active complex will give rise to the resultant amide bond formation [52].

Overall, addition of sulfo-NHS in the EDC coupling protocol does not only increase the solubility but also assist in creating a more stable active intermediate, which efficiently reacts with amine groups at physiological Ph. Final conjugation product of this two-step crosslinking procedure is the same even in the absence of sulfo-NHS, that is, a stable amide linkage. A summary of EDC coupling reaction is shown in Figure 2.13.

Figure 2.13: Reaction scheme of carboxyl to amine crosslinking using carbodiimide EDC and EDC/sulfo-NHS (Note: A and B are from distinct proteins) [71].

2.5 Chelation

Chelating agents (or chelators) are defined as multidentate ligand whose structure is capable of binding the donor atoms (or sites) to metal ions, producing complex ring-like structure called *chelates*. The word ‘chelation’ originated from the Greek word, *chele*, which means claw of a lobster, describing the concept of strong grasp of the metal-ligand bonding (Figure 2.14) [72-73]. Chelating agents are widely used in industrial, domestic, agriculture and clinical applications. It is often related to metal detoxification due to their ability to form a very stable metal chelates (i.e. compound composed of metal ion and chelating agent) that are easily isolated from the target site [74].

Figure 2.14: Formation of metal ligand complexes using polydentate ligand [75]

2.5.1 Principle of Metal Chelation

The concept of chelation is based on simple coordination chemistry. In general, ligand is an ion or molecule that binds to central metal atom to form coordination complexes. One important characteristic of chelating agent that helps to perform metal binding is the chelating ligand that consists of binding atoms in the form of chemical groups like $-\text{SH}$, $-\text{S-S}$, $-\text{NH}_2$, $=\text{NH}$, $-\text{OH}$, $-\text{OPO}_3\text{H}$ or $>\text{C}=\text{O}$ [75]. Although not absolute, chelating agents can be divided into six categories based on their ligand structure type: (i) polyaminocarboxylic acids; (ii) chelators with vicinal sulfhydryl ($-\text{SH}$) groups, (iii) β -mercapto- α -aminoacids; (iv) hydroxamic acids; (v) orthohydroxycarboxylic acids and (vi) miscellaneous agents [76].

Early discovery of chelation by Werner (1983) was through his work on metal complexes structure. Fundamental to all Werner's work was the concept of coordination number in which he defined the term as the number of ligands attached directly to the central metal ion. In his work, Werner utilized chelating properties of ethylenediamine, $\text{C}_2\text{H}_4(\text{NH}_2)_2$ to react with tetraammineplatinum(II) chloride, $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$ and found four molecules of ammonia have been replaced by two

molecules of ethylenediamine in the final compound, $[\text{Pt}(\text{C}_2\text{H}_4(\text{NH}_2)_2)_2]\text{Cl}_2$. Werner concluded that each molecule of ethylenediamine takes up two of the four coordination positions, creating a five-membered heterocyclic ring [77]. Bonding between chelating ligands and central metal atom through coordinate bonds is the result of metal atom (acceptor atom) accepting an electron pair from each coordinating atom of chelating agent (donor atom).

Overall, the process of metal chelation can be described using a simple balanced equation given as [78]:

where,

HY = chelating agent

MX = metal salt

M = targeted metal atom

MY = metal chelate

In summary, a molecule ought to meet two basic requirements in order to perform as a metal chelator. First, the substance needs to own at least two suitable functional group (i.e. multidentate ligand), in which the donor atoms are capable of donating a pair of electrons in order to combine with targeted metal atom. Second, the donor atoms must be located in the molecule itself to form a heterocyclic ring with metal atom as a closing participant [77].

2.5.2 Types of Metal Chelators

To date, there are varieties of chelating agents of differing affinities toward specific metal types available for distinct metal chelation applications. As mentioned in Section 2.4.1 earlier, chelating agents can be distinguished based upon their ligand structure characteristics.

2.5.2.1 Poly-amino carboxylic Acids

Poly-amino carboxylic acid is a substance made up of one or more nitrogen atoms that are bonded to two or more carboxyl groups via carbon atoms. Some chelating agents containing poly-amino carboxylic ligands are ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), and diethylenetriaminepentaacetic acid (DTPA). These chelators are often used in metal extraction from soil and natural waters for analytical purposes [79].

Ethylenediaminetetraacetic acid (EDTA)

EDTA was first prepared using ethylenediamine and chloroacetic acid by Ferdinand Munz (1935), but are now mainly prepared from ethylenediamine, formaldehyde and sodium cyanide [80]. It is a poly-amino carboxylic acid and physically, a water-soluble and colorless solid, usually marketed as sodium salt. It binds to metal ions through its hexadentate ligand (i.e. six potential binding sites) which consists of four carboxylate and two amine groups (Figure 2.15) [81].

Figure 2.15: Chemical structure of EDTA [82]

EDTA is capable to chelate a wide range of metal ions, thus making them very useful in many important industrial works. Abundant experimental works have been reported on the use of EDTA for metal excretion such as lead, nickel, zinc, iron and others. Furthermore, EDTA is also found in many commercialized household products and industrial use such as detergents, cosmetics, pulp and paper industry, textile industry and concrete admixtures [83-84]. In addition to the extensive investigations on possible use of EDTA, there has also been a growing emphasis on its ecotoxicology effect. EDTA is found to be quite persistent in the environment due to its low biodegradability [85-86]. However, there is no valid indication of harmful effects found to date regarding long-term exposure of low concentrations EDTA in the environment [87-88].

2.5.2.2 Derivatives of alpha-amino acids

Alpha amino-acids are amino acids whereby amine and carboxylic acid groups are attached to the first carbon atom. D-Penicillamine (DPA), N-acetylcysteine (NAC) and 2-Mercaptopropionyl glycine (Tiopronin) are some of the known examples of amino acids derivatives, reported to be effective in metal detoxification treatment [89].

D-Penicillamine (DPA)

Among all other chelating agents, DPA was extensively investigated and many of them showed increase renal copper excretion. The use of DPA for copper excretion in Wilson's disease is originally discovered by Walshe [90]. DPA is sulfhydryl containing amino-acid (Figure 2.16) and effectively chelate copper through reductive chelation process [73]. In addition to copper, DPA is also used as the chelating agent for lead, mercury, and arsenic intoxication.

Figure 2.16: Chemical structure of DPA [82]

2.5.2.3 Chelators with Vicinal Sulfhydryl (-SH) groups

2,3-Dimercaptopropanol, or more commonly known as British Anti-Lewisite (BAL), meso-2,3-dimercaptosuccinic acid (DMSA) and sodium 2,3-dimercapto-1-propanesulfonate (DMPS) are some of the prominent metal chelators which comprise of two or more sulfhydryl (or thiol) groups. BAL was first introduced as a remedy to lewisite, arsenic containing gas use in chemical warfare during World War II. Even though BAL is meant for clinical treatment of arsenic poisoning since 1950s, Aposhian et al. highlighted BAL medication could result in possible redistribution of arsenic into patients' brain [84]. Consequently, BAL was later on replaced by DMSA and DMPS which features less toxicity and higher therapeutic index than BAL itself [90].

Meso-2,3-dimercaptosuccinic acid (DMSA)

DMSA is akin in chemical structure to BAL, both comprised of dithiol groups in their ligands (Figure 2.17). Moreover, coordination of both sulfur atoms with selected metal atom varies according to the metal types to form metal chelates. Physically, DMSA appear as water soluble and available in the form of white crystalline powders. It has been proposed as an effective antidote to lead, mercury, arsenic and cadmium poisoning since 1957 by Liang et al. [91-92]. Substantial number of animal studies showed that DMSA is favored over BAL in clinical use for treatment of metal detoxification because it is less toxic and available in capsule form, hence suitable for oral administration. [89, 82].

Figure 2.17: Chemical structure of DMSA [82]

2.6 Heavy Metals

Heavy metals are metallic chemical compounds with density of at least 5 g/cm³, that appear to be toxic or poisonous even at low concentrations in water environment [94]. They are naturally ubiquitous and non-biodegradable. Most of the metal ions are highly soluble in water environments and easily absorbed by living organism, which in turn becomes incorporated into the food chain [95]. Consequently, exposure to elevated levels of hazardous heavy metals from chemical-intensive industries such as cadmium, chromium, arsenic, lead, mercury, copper and zinc can cause various adverse health effects to mankind. In the following section, common occurrence of several types of these heavy metals and its corresponding effect on human's health are discussed briefly.

2.6.1 Lead (Pb)

Lead is soft metal commonly used in the production of solder, cable sheathing and antiknock compounds in petrol. Although primary source of lead exposure usually comes from air and food, lead is nominated in the list of top five contaminants in drinking water. Lead from water pipes particularly in old buildings may partially dissolve in the water flow, thus, reaching human via the food chain. The amount of lead that may dissolve in water depends on temperature, water hardness and acidity (Ph) of the water [96].

Health Effect

The symptoms of severe lead poisoning include abdominal pain, fatigue, headaches and other symptoms related to the nervous system. Infants and young children are at

greater risk since their actively growing bodies are especially vulnerable and susceptible to absorbing and retaining lead [97]. A number of studies have been conducted to investigate neurological effects of lead exposure toward infants and children. Findings drawn from these studies demonstrated children with high dentine lead levels suffer behavioral and cognitive deficits [98-99].

2.6.2 Copper (Cu)

Copper's origin in the water is very diverse. It appears in the form of complexes and is commonly found in ground water, seawater and drinking water. Similar to lead, copper contamination usually occur due to erosions of natural deposits and corrosion of interior copper pipelines [100].

Health Effect

Copper is an essential nutrient needed by human body in small amount. However, this type of metal does possess acute health effect when the concentration is high. One will likely experience gastrointestinal disturbance such as nausea, diarrhea and vomiting. In some cases, people with genetic disorder, such as Wilson disease are more sensitive toward the effect of copper contamination. Patients with Wilson disease are not capable to excrete excess amount of copper through bile, but accumulate in the liver instead, possibly to a life threatening level, leading to liver damage [101].

2.6.3 Zinc (Zn)

Zinc is one of the most common elements that occurs naturally in the environment and spreads through air, soil and groundwater. Industrial groundwater pollution of zinc is mostly induced by untreated wastewater released from battery production industries and galvanic industries. On top of that, leaching of zinc elements from piping and fittings of a plumbing system often causes zinc concentrations to exceed its permissible rate in tap water supply [102].

Health Effect

Excessive intake of zinc, either accidentally or intentionally as dietary supplement may lead to stomach and abdominal cramps, fever, nausea, and diarrhea [103]. Even though chances of intoxication by excessive exposure rarely happen [104], chronic low-dose exposure of zinc over an extended period of time may lead to impairment of pancreatic function as a result of prolonged accumulation of zinc [102].

2.7 Standard for Safe Drinking Water

The signs of contaminated water are not always apparent, hence it is crucial to have a regulatory criteria for all types of heavy metal presence in aquatic environment. For instance, United States Environmental Protection Agency (EPA) regulates Maximum Contaminant Level (MCL) standard, defined as the maximum concentrations of chemical compounds allowable in public drinking water system [105]. Table 2.2 illustrates several drinking water quality guidelines enforced by organizations and committees in different countries.

Heavy Metal	Guideline for Maximum Allowable Contaminant Level (parts per million)			
	Australian Drinking Water Guidelines [105]	World Health Organization [106]	National Environment Agency of Singapore [107]	United States Environmental Protection Agency [108]
Antimony	0.003	0.020	0.020	0.006
Arsenic	0.010	0.010	0.010	0.010
Cadmium	0.002	0.003	0.003	0.005
Copper	2.000	2.000	2.000	1.300
Iron	0.300	--	--	0.300
Lead	0.010	0.010	0.010	0.015
Mercury	0.001	0.006	0.006	0.002
Zinc	3.000	--	--	5.000

Table 2.2: Drinking water quality guidelines (unit in ppm) prepared by organizations and committees throughout the world.

2.8 Conclusion

Overall, this chapter provides an insight to the understanding of optical fiber and one of its structure variations, namely microfiber. Microfiber makes a good RI sensor since they have strong evanescent field and high nonlinearity [109]. On top of that, RI based microfiber sensor has drawn much attention in recent years, particularly in the area of water quality monitoring application. Since most of the target compound of interest within water resources are usually organic based, microfiber need to undergo a series of surface treatment processes, which include functionalization of microfiber surface with amine functional group (silanization) and attachment of metal chelators (i.e. chelating agents) onto the sensing region to bind with central metal atom. Chelating

agents differ on their ligand structure characteristics, and for this reason, these chelating agents are unique in a sense that each of them has distinct affinities toward specific metal types. Henceforth, choosing an appropriate chelating agent to specifically target a particular heavy metal ion type enable the possibility of low concentration detection limit of an RI based heavy metal microfiber sensor. In the next chapter, we shall discuss in details about the creation of a RI based heavy metal microfiber sensor for this study, which involves the fabrication and functionalization of the sensor, as well as the actual in-laboratory experiment setup.

CHAPTER 3

RESEARCH METHODOLOGY AND SURFACE VERIFICATION OF FUNCTIONALIZED OPTICAL MICROFIBER

3.1 Introduction

In previous chapter, it was understood that microfiber is a good choice for refractive index based chemical sensor. In addition, an overview of chelating agent usage in heavy metal intoxication therapy has also been introduced. In this project, the preparation of microfiber heavy metal sensor was done by functionalizing the sensing region (tapered region) via chemical modification, followed by attachment of chelating agent compound onto the surface. This chapter will describe the main materials and common methods used across multiple parts of the study. For other specific materials and methods, they are described in respective chapters. In the latter part of this chapter, verification of surface functionalized microfiber was done to validate the effectiveness of functionalization protocol as explained in Section 3.2.2. Visual verification as well as quantitative analysis was performed for each stage of functionalization process using scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDX).

3.2 Fabrication of Microfiber

Generally, tapering a single mode optical fiber is normally done by reducing the cladding diameter by chemical etching or by heating and pulling both ends of the fiber. Chemical etched taper can be done using hydrofluoric acid while heat-pulled taper uses heat source from oxygen-butane torch, CO₂ laser, or an electric arc formed between a pair of electrodes in a fusion splicer, together with mechanical pulling. In this project, standard single mode fiber of attenuation 0.2 dB/km was purchased from Yangtze Optical Fiber & Cable Co Ltd. Commercial microfiber fabrication machine (Vytran, GPX-3000) equipped with motorized fiber holding stage is utilized. This machine uses graphite filament as the source to provide heat when the fiber ends are pulled in opposite directions. Ability of this machine to provide precise control of taper transition length, waist length and waist diameter allow convenient manipulation of the adiabaticity, free spectral range and desirable sensitivity of the microfiber. In this work, tension monitor of the microfiber fabrication machine plays an important role to calibrate the normalized power (50W) of the filament and its respective power degradation rate (-15%) during the fabrication. The power level was ensured to stay close to the normalized value during the fabrication, while maintaining the fiber tension at a certain positive level with slight periodic fluctuations. The fabricated microfiber has a total length of 9mm with left transition length of 2.4 ± 0.5 mm, waist length of 5 ± 0.5 mm and right transition length of 1.6 ± 0.5 mm. Figure 3.1 illustrates microscopic view of typical fabricated microfiber.

(a)

(b)

Figure 3.1: Microscopic view of typical microfiber. (a) Geometry of fabricated microfiber with taper waist diameter of $7.1\mu\text{m}$ (b) Image of tapered region obtained using scanning electron microscope

3.3 Microfiber Surface Functionalization

3.3.1 Materials

Hydrochloric acid (HCl, Ar), 3-Aminopropyltriethoxysilane (APTES, 99%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS, 98%), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, > 99%), zinc chloride (ZnCl_2 , > 98%), lead chloride (PbCl_2 , >98%), and copper chloride (CuCl_2 , 97%) were all purchased from Sigma Aldrich. Sodium Hydroxide (NaOH, Ar) was purchased from Schedelco and sulfuric acid (H_2SO_4 , Ar) was purchased from JT Baker. Hydrogen peroxide (H_2O_2) was purchased from HCS Scientific and Chemical.

All chemicals were used as received without further purification. Deionized water used was purified by Mili-Q water purification system.

3.3.2 Preparation of Surface Functionalized Microfiber

Surface treatment of fabricated microfiber is accomplished by soaking the U-shape bended microfiber into engineered chemical solvents. By doing so, the entire taper length will get to be functionalized. The treatment process begins by washing the microfiber in acetone solution for ten minutes in order to remove dust particles, contaminants or other impurities on the fiber surface. Prior to silanization, the microfiber was cleaned with 1M H_2SO_4 for 30 minutes, conducted at 90°C, followed by Piranha solution (mixture of sulfuric acid and hydrogen peroxide in a volume ratio of 3:1) for 10 minutes inside the fume hood and 1M NaOH for 10 minutes at 120°C. Between each of these cleaning steps, the microfiber is rinsed thoroughly a few times with deionized water. This step is important to form high density of hydroxyl functionalities necessary for silane modification. Next, two percent of silane coupling agent, APTES was mixed into deionized water at pH 4.5-5.5 (adjusted by acetic acid). The microfiber is dipped into the solution for two hours at 75°C under low humidity environment. Silanized microfiber was then dried overnight in an oven at 60°C. Following this, each ml of carboxyl groups containing chelating agent solution such as EDTA were activated using 0.4mg of EDC and stabilized with 1.1mg of NHS and left undisturbed for 15 minutes at room temperature. The activated chelating agent was then added to the silanized microfiber and was left to stand for 2 hours to allow covalent bonds to be formed. At the end of the bonding reaction, hydroxylamine in a

final concentration of 10Mm was then added to stop the reaction. An illustration of this surface functionalization protocol is shown in Figure 3.2.

Figure 3.2: Surface functionalization of microfiber by silanization and the reaction scheme of EDC/NHS coupling for bonding of chelating agent onto the surface

It is worth noting that apart from EDTA, there was another type of chelating agent used to functionalized microfiber sensor in this study, namely DPA. Both of these chelating agents are composed of carboxyl functional group, hence, the same protocol was applied. This is because the crosslinker (i.e. EDC) used in the above protocol is a carboxyl-to-amine crosslinker, which targets amine group on silanized microfiber and carboxyl group of EDTA or DPA. Therefore, in case where non-carboxyl containing chelating agents need to be used, similar protocol can still be applied, except that EDC need to be replaced with other suitable crosslinker which is capable of linking targeted functional groups together.

3.4 Experimental Procedure

3.4.1 Experimental Arrangement

Figure 3.3 shows the experiment setup used in this project. Light coupled from the laser source will propagate down the microfiber and get reflected by the end facet of the patch cord. This reflected light will be guided out to the optical spectrum analyzer (OSA) via the circulator after passing through the microfiber a second time. A circulator is used to increase the extinction ratio of received power in order to yield sharper resonant wavelength dips [30]. This configuration resembles a Mach-Zehnder interferometer where a double pass measurement scheme is used [44].

Figure 3.3: Experimental Arrangement

As seen in the figure, oscillatory spectra can be observed in the OSA as a result of light coupling between HE_{11} and HE_{12} mode. Microfiber which experiences RI change in the surrounding medium of the tapered region will induce a wavelength shift (red or blue shift) in the oscillatory spectra and this can be clearly observed in real-time via OSA. Thus, experiment is conducted in such a way where sufficient amount of DI water was first drop cast onto the tapered region, covering along the taper length, followed by

taking note of the spectra observed in OSA. Next, DI water was removed and replaced with the same amount of heavy metal solution. Due to RI difference between DI water and heavy metal solution, the output spectra will be seen to have red or blue-shifted. The amount of wavelength shifts differ depending on the amount of RI difference between heavy metal solution and DI water. Thus, a series of calibration work was done in order to correlate amount of wavelength shifts with heavy metal solution of different concentration. This information will then be useful to estimate presence of unknown heavy metal concentration in the water samples.

However, since the microfiber is as well very sensitive to both strain and surrounding temperature, the design of the fiber holder plays a critical role to avoid cross talk of external factors and sensing measurements. The design of fiber holder will be discussed in detail in Chapter 6.

3.5 Surface Verification of Functionalized Optical Microfiber

3.5.1 Materials and Methods

Scanning of successful functionalized microfiber was done using SEM. Effectiveness of metal chelation by chelating agent molecule bonded on the microfiber surface was verified quantitatively through EDX. Elemental quantification analysis using SEM-EDX works by incident of electron beam onto the sample causing shell transition and resulting in the emission of X-rays. These X-rays are utilized to identify the sample's elemental composition by analyzing the energy and wavelength characteristics of these rays.

3.6 Results and Discussions

3.6.1 Scanning Electron Microscope (SEM) Imaging

Figure 3.4(a)-(c) illustrates the surface of microfiber samples (7.1 μm) at different stages of functionalizing process. Although not clear, apparent bright metallic nano dots that could be the metal ions were seen in Figure 3.4(c). This showed that metal ions were indeed got trapped on the microfiber's surface by chelation.

In order to have a clearer view of the functionalized surface, Figure 3.4(d)-(f) illustrates standard fiber (un-tapered) samples at different stages of functionalizing process, arranged in sequence of functionalizing stages, corresponds to Figure 3.4(a)-(c). Under large magnification, a uniform layer of APTES was seen on the silanized sample, and this is critical to promote evenness and sufficient sites for the attachment of chelating agent. On the other hand, Figure 3.4(f) depicts fiber sample that has been tested with lead (II) ion solution. From these figures, apparent increase in surface roughness was seen as the fiber underwent more stages of chemical functionalization.

3.6.2 Energy Dispersive X-Ray Spectroscopy (EDX) Analysis

In Table 3.1, all element composition found on the non-functionalized and functionalized microfibers are listed. Silanized microfiber showed a dramatic increase in carbon element compared to non-functionalized microfiber. Silanization of microfiber with APTES was carried out to form silanol and amino sites, as seen in Figure 3.5. Thus, results obtained demonstrated that APTES had reacted with the hydroxyl group on the microfiber surface, and the ratio of silicon and oxygen atom is approximately 1:2.5. This showed that APTES is bonded to two or three active hydroxyl sites.

Figure 3.4 (a)-(f): SEM image of microfiber with taper waist diameter of $7.1\ \mu\text{m}$ with magnification $\times 15000$ of (a) silanized microfiber using APTES (b) EDTA-functionalized microfiber and (c) EDTA-functionalized microfiber with lead (II) ion trapped on the surface by EDTA. SEM image of un-tapered fiber with magnification $\times 5000$ of (d) silanized fiber using APTES (e) functionalized fiber with EDTA attached on the surface and (f) functionalized fiber with lead (II) ion trapped on the surface by EDTA

Figure 3.5: The possible silanization process of microfiber surface

Element Weight (%)	Non-Functionalized Microfiber (Figure 3.6a)	Non-Functionalized Microfiber Tested with Pb ²⁺ solution	Silanized Microfiber (Figure 3.6b)	Functionalized Microfiber Tested with Pb ²⁺ solution (Figure 3.6c)
O	69.55 ± 1.25	68.90 ± 1.23	53.71 ± 1.45	64.47 ± 1.22
Si	24.63 ± 0.59	21.52 ± 0.52	21.66 ± 0.65	24.87 ± 0.55
C	5.82 ± 1.55	9.58 ± 1.48	26.64 ± 1.76	9.39 ± 1.54
Pb	nd	nd	nd	0.78 ± 0.28

*nd means not detected

Table 3.1: Microscopic composition of microfiber at the scanning area

Figure 3.6(a)-(c): The spectrum of microfiber sensor obtained from SEM-EDX analysis. Green colour frame in the inset diagram represents the scanning area

In order to verify the capability of functionalized microfiber to chelate heavy metal ion, non-functionalized and functionalized microfiber was dipped separately into lead (II) solution and rinsed thoroughly with deionized water, followed by EDX analysis. Results obtained indicate that there was no trace of lead element found on the non-functionalized microfiber. Conversely, about $0.78 \pm 0.28\%$ was detected on the surface of EDTA functionalized microfiber. For this reason, the ability of functionalized microfiber sensor to trap heavy metal ion onto the sensing region was proven.

3.7 Conclusion

Surface scanning of functionalized microfiber sensor has successfully verified the attachment of chelating agent compound onto the sensing region. Under large magnification, changes made to the microfiber surface were clearly noticeable. On top of that, encouraging results were obtained from surface analysis using EDX, demonstrating the capability of functionalized microfiber to trap heavy metal ion through chelating agent compounds bonded on the sensing region. Henceforth, we shall investigate the sensor's characteristics and their sensing performance.

CHAPTER 4

AXIAL STRAIN AND TEMPERATURE CHARACTERIZATION OF OPTICAL MICROFIBER SENSOR

4.1 Introduction

In general, the use of evanescent field from a microfiber makes the sensor extremely sensitive to refractive index changes of the surrounding medium. On the flip side, it is also sensitive to at least two or more parameters simultaneously making it difficult to differentiate between the parameters of interest. Multi-parameter measurement is beneficial in a sense, where it could minimize design complexity, structure size and cost of the sensing system. However, cross-sensitivity issue often arise if unfavourable parameters are not well controlled or multi-parameter discrimination is not performed. In this work, we identified refractive index, strain and temperature variation in the environment as the major parameters that will influence the propagation of light signal within the microfiber. The ultimate intention of our work is to only monitor changes in the effective refractive index caused by the presence of metal ions within the sensing region of the microfiber. Therefore, it is important to examine the influence of strain and temperature on microfiber sensor for different tapered geometry with the objective of minimising or eliminating cross-talk issues due to these factors.

4.2 Materials and Methods

Tapered fibers of different waist diameter were fabricated using the method described in section 3.1. Strain characterization of tapered fiber was done by clamping both fiber ends on the x-axis translational stage. One end remained in a fixed position while the other end was pulled at 10 μ m steps. The tapered region was immersed in deionized water as strain is applied on it. Spectrum of each step was captured and recorded. Meanwhile, temperature characterization experiment was done by immersing the tapered fiber in a water bath containing deionized water. The temperature was slowly increased from room temperature to 100°C in steps of 10°C and amount of wavelength shifts due to increment of temperature were noted.

4.3 Results and Discussions

4.3.1 Axial Strain Characterization

Through observations, we noticed when the tapered region of microfiber was intentionally placed under a relaxed condition (bent), pulling one end of the microfiber will generate red shift in its wavelength spectrum. As the tapered region was further stretched (tapered region approaching straight condition), blue shift was observed. At this point, the tapered region undergoes real strain applied on it and data was collected until the microfiber breaks. Figure 4.1 depicts the typical wavelength response for different axial strains.

Figure 4.1: Wavelength response of a microfiber and its shift upon applying strain on it

The amount of applied strain in this experiment is equal to the ratio of the change in length to the initial length of the fiber. From Figure 4.1, the resonant wavelength experienced a shift to shorter wavelengths with increasing applied strain. According to [110], attenuation peak wavelength, λ_m can be expressed as,

$$\lambda_m = \frac{2 \Delta n_{eff} L}{(2m + 1)} \quad (4.1)$$

where

Δn_{eff} is the effective refractive index difference between the core and cladding modes,

L is the effective length of the interferometer, and

m is the order of Mach-Zehnder interference.

When axial strain is applied, the length of the microfiber elongates along with changes in the Δn_{eff} . Therefore, blue shift in Figure 4.1 indicate Δn_{eff} decreasing at a much faster rate than the elongation of interferometer length, L . Figure 4.2 shows the

microfiber (of different taper waist diameter) resonant wavelength shift as a function of applied strain. Decreasing the taper waist diameter lead to increase of sensitivity toward strain. However, it is worth noting that smaller diameter in the range of less than 1mε exhibit lower strain sensitivity compared to the larger diameter ones.

Figure 4.2: Resonant wavelength shift versus applied strain of tapered fiber with fix taper length of 5mm but different taper waist diameter

As seen in Table 4.1, microfibers with fixed taper length of 5 mm and waist diameter of 4.7 and 7.1μm demonstrate less sensitivity to strain in the range of 0 to 1 mε, but increases gradually and crosses 12.6 μm's slope at 3mε (Figure 4.2).

Besides taper waist diameter, the length of the tapered region also affects the sensitivity of the microfiber toward strain. Table 4.1 summarizes strain sensitivities of microfiber with different taper profiles.

Taper length = 5 mm		Strain Sensitivity (nm/mε)		
Taper waist diameter (μm)	Strain, S (mε)			
	0 < S < 1	2 < S < 3	4 < S < 5	
4.7	-5.689 ± 1.647	-13.772 ± 1.048	-15.868 ± 0.225	
7.1	-9.281 ± 0.847	-13.772 ± 1.572	-13.772 ± 0.393	
12.6	-11.677 ± 0.899	-11.677 ± 0.450	-11.677 ± 0.150	
Taper length = 6 mm		Strain Sensitivity (nm/mε)		
Taper waist diameter (μm)	Strain, S (mε)			
	0 < S < 1	2 < S < 3	4 < S < 5	
4.7	-8.084 ± 0.463	-13.514 ± 0.619	n.a	
7.1	-5.988 ± 0.742	-11.712 ± 0.873	-11.676 ± 0.433	
12.6	-7.485 ± 0.449	-9.581 ± 0.136	-9.880 ± 0.449	

Table 4.1: Summary of strain sensitivity for different types of taper profile

* n.a means not available (fiber broke)

In the table above, the total amount of strain applied was divided into three distinct ranges. As mentioned previously, microfiber with larger waist diameter, regardless of taper length, is more capable of generating consistent amount of shift per each strain step applied. On the other hand, longer taper length microfiber showed lower sensitivity towards strain in the range of 0 to 5 mε compared to shorter taper length microfiber. This finding was as expected since longer tapered length is known to have lower sensitivity [111]. This is most likely due to microfiber with longer tapered length having much higher tension limit that it can sustain, and thus its sensitivity could be much larger than shorter tapered length at total strain applied above 5 mε.

4.3.2 Temperature Characterization

Temperature sensitivity is a measure of the interference wavelength shift divided by the corresponding temperature change. Figure 4.3 illustrates variation of wavelength

shifts with increasing ambient temperature of microfiber with taper waist diameter of $4.7\mu\text{m}$.

Figure 4.3: Variation of wavelength shift with increasing in temperature for microfiber with taper waist diameter of $4.7\mu\text{m}$ and taper length of 5mm

As mentioned in Section 2.2.1, there is a phase difference between the coupling of higher order and fundamental mode at the up tapered region. This phase difference will change with surrounding temperature due to thermo-optic effect and thermal expansion of the fiber, which in turn, induces wavelength shifts as observed in Figure 4.3. Thermo-optic effect and thermal expansion is defined as the thermal modulation of refractive index and length of optical fiber [112]. Effect of these temperature-related factors to the change in phase difference can be seen in a quantitative way by expressing equation (2.6) as given below:

Phase difference between fundamental and higher order modes is given as,

$$\phi = \frac{2\pi}{\lambda} \Delta n L \quad (4.2)$$

If $\Delta n (T)$ and $L (T)$ represent changes of refractive index and taper waist length with temperature respectively, equation (4.2) can be rewritten as,

$$\phi = \frac{2\pi}{\lambda} \Delta n(T) L(T) \quad (4.3)$$

and,

$$\Delta n (T) = \Delta n (T_o)[1 + \eta . \Delta T] \quad (4.4)$$

$$L (T) = L (T_o)[1 + \alpha . \Delta T] \quad (4.5)$$

where,

$\Delta n (T_o)$ and $L (T_o)$ is the refractive index and waist length at room temperature, T_o ,

$\eta = (\partial \Delta n / \partial \Delta T)_{T=T_o}$ is thermo-optic coefficient of silica glass, and

$\alpha = 5.5 \times 10^{-7} \text{ K}^{-1}$ is the thermal expansion coefficient of silica glass.

Using equation (4.3) to (4.5), the relationship between relative wavelength shift due to temperature change, ΔT can be expressed as,

$$\frac{\Delta \lambda}{\lambda} = - \frac{1}{\Delta n (T_o) L (T_o)} \left(\frac{\partial \Delta n}{\partial T} \right)_{T=T_o} . L(T_o) \Delta T \quad (4.6)$$

From Figure 4.3, it is clear that in the temperature range of 20°C to 100°C, blue shift increases linearly with increasing temperature. A linear curve fitting the experimental data showed the sensitivity of microfiber as temperature sensor. As seen in equation (4.6), thermo-optic effect dominates over thermal expansion of the microfiber sensor in temperature sensing. Moreover, negative thermo-optic coefficient of the fiber core also explains the negative wavelength shifts.

In order to investigate temperature-sensing response of varying taper lengths and waist diameters, several microfibers were fabricated with varying taper waist diameters while keeping down tapered, up tapered and taper waist lengths constant, at 1, 2, and 5 (or 6) mm respectively. Table 4.2 show the comparative results of temperature sensitivities obtained.

Taper length = 5 mm	
Taper waist diameter (μm)	Temperature Sensitivity ($\text{nm}/^{\circ}\text{C}$)
4.7	-0.748 ± 0.172
7.1	-0.359 ± 0.032
12.6	-0.163 ± 0.003
Taper length = 6 mm	
Taper waist diameter (μm)	Temperature Sensitivity ($\text{nm}/^{\circ}\text{C}$)
4.7	-0.769 ± 0.274
7.1	-0.387 ± 0.030
12.6	-0.204 ± 0.003

Table 4.2: Microfiber temperature sensing sensitivity of varying taper waist diameter

From the results, the sensitivity of microfiber sensor increases with decreasing taper waist diameter. However, the large standard deviations suggest that microfiber sensor with smaller taper waist diameters experience instability more than larger taper waist diameters. Meanwhile, microfiber with longer taper length showed higher temperature sensitivity than microfiber with shorter taper length. Overall, the temperature sensitivity of the sensor is contributed by the difference in thermo-optic dependence of the fiber core and the aqueous cladding. Moreover, the effective refractive index of the core and cladding vary with temperature, causing linear phase shifts of the interference wavelength peaks.

4.4 Conclusion

In summary, output wavelength response from the microfiber was found to react not only to refractive index changes of the surrounding medium, but also to other external factors such as strain and temperature. Characterizations for each of these parameters were conducted in attempt to keep cross-talk influence as low as possible. We investigated microfibers with taper waist diameters of 4.7, 7.1 and 12.6 μm , and discovered that microfibers with smaller tapered waist diameter gave higher sensitivity to change in axial strain and ambient temperature. Maximum sensitivity of -15.868nm/m ϵ and -0.7694/ $^{\circ}\text{C}$ were recorded with microfiber of taper waist diameter 4.7 μm due to changes in the effective refractive index of the microfiber sensor caused by axial strain and temperature.

Results obtained from all experiments conducted in this chapter showed that the performance of microfiber sensor toward these external factors is clearly determined by the taper profile parameters, such as taper length and taper waist diameter. For instance, microfiber with longer taper length and smaller taper waist diameter has the highest sensitivity toward strain and temperature changes, and shows minor instability and fragility issue. Overall, our experimental observations indicate that the geometry of tapered region does affect microfiber sensor's sensitivity toward external factors and suggest that there are evidences for a trade-off between sensitivity and performance stability. Thus, careful selection of optimum taper profile is important for this trade off to be well balanced as well as better control of cross-sensitivity. Following this chapter, we shall look into a more in depth quantitative analysis of heavy metal ion detection using functionalized microfiber sensor with optimize taper geometry to gather more convincing data of its metal ion detection capability.

CHAPTER 5

HEAVY METAL DETECTION USING FUNCTIONALIZED OPTICAL MICROFIBER SENSOR

5.1 Introduction

In the previous chapter, we have noted that our microfiber sensor does not only respond to refractive index changes alone, but also to strain and temperature changes. In this chapter, we will explain in detail the method used to package the microfiber sensor in such a way that strain and temperature influences can be kept negligible. The highlights of this chapter also include discussions of detection limit achieved from using bare microfiber sensor and surface functionalized microfiber sensor, both with taper diameter of $7.1\mu\text{m}$ and taper length of 5mm. Furthermore, a comparison was made between two distinct functionalized microfiber sensors, one attached with EDTA while another with DPA, for the purpose of investigating the chelation properties of both chelating agents toward different metal ion types. In addition to indications obtained from surface verification in Chapter 3, a subsection of this chapter will describe the additional step taken to validate the presence of chelating agents on the surface functionalize sensing region. Following this, we will also highlight the limit of sensing potential of our microfiber sensor through manipulation of taper waist diameter.

Finally, a compact and cost effective reader system will be presented as the final part the whole study. This reader system was specially designed to serve as an economical alternative to high end laboratory equipment and further reducing the size of overall sensing system.

5.2 Materials and Methods

5.2.1 Packaging of Microfiber Sensor

As mentioned in Section 4.3, microfiber of smallest taper waist diameter exhibits maximum strain sensitivity of approximately $-15 \text{ nm/m}\epsilon$ while maximum sensitivity for temperature could reach up to $-750 \text{ pm}/^\circ\text{C}$. Addressing this cross-sensitivity issue, a cartridge was designed to minimize both the strain and temperature effects. The sensing region of microfiber sensor was sealed in a cartridge fabricated by a 3D printer using a plastic material called Acrylonitrile Butadiene Styrene (ABS). The slot in the center is a fluidic channel that is designed by padding polydimethylsiloxane (PDMS) sheets at top and bottom of the cartridge. The water samples are injected by a syringe penetrating into the top PDMS sheet and can be discharged through a channel embedded at the bottom PDMS sheet. The outflow in the channel is controlled by a clip and directed into a beaker underneath the cartridge. Two pairs of magnetic buttons are deployed inside the edges of the cartridge in order to offer a primary strain control. The fiber is then fixed by a pair of magnetic clamps mounted on the translation stages that are screwed on magnetic holders (Figure 5.1 and 5.2).

Figure 5.1: Three-dimensional view of an optical microfiber heavy metal ion sensor packaged in an acrylonitrile butadiene styrene (ABS) made cartridge

Figure 5.2: Side view of an optical microfiber heavy metal ion sensor packaged in an acrylonitrile butadiene styrene (ABS) made cartridge

Figure 5.3: Top view of an optical microfiber heavy metal ion sensor packaged in an acrylonitrile butadiene styrene (ABS) made cartridge

5.2.2 Experimental procedure

Note that all experiments were conducted at room temperature. Experimental arrangement is shown in Figure 5.4. All samples of solutions, which include deionized water and heavy metal solutions of different concentrations (30, 75, 100, 250, 500, 750 and 1000 parts per million (ppm)), were pumped into the sample chamber until it covers the whole sensing region. For reference purposes, deionized water was first pumped into the sample chamber. The output spectrum observed in the OSA was

saved once the spectrum is deemed stable (spectrum remain the same after a certain period of time). The same procedure was repeated with different concentrations of heavy metal solutions such as lead (II) chloride, zinc (II) chloride and copper (II) chloride. This time, heavy metal solution was left to stay within the sample chamber for about 30 minutes. Any changes in the output spectrum within this period of time were closely observed.

Figure 5.4: Actual experimental arrangement

5.3 Results and Discussion

5.3.1 Chelating Agent Presence Verification

Apart from the positive outcome showing the presence of chelating agent in the sensing region via surface imaging as demonstrated in Chapter 3, this section aims to provide an additional proof to show that chelation process has occurred.

One way of doing this is by comparing the output spectrum with the sensing region surrounded with DI water, before and after contact with heavy metal solution. The reason for this is to ensure that the change in the spectrum is solely due to the presence of heavy metals on the surface of the microfiber and not due to the refractive index of

the sample solution. Observable changes in dip wavelength shift between these two spectra indicate that successful chelation has occurred on the surface of the fiber. Figure 5.5 (a) and (b) illustrate the comparison of wavelength responses obtained from non-functionalized and functionalized microfiber, both with waist diameters of $7.1\mu\text{m}$.

Figure 5.5: Wavelength response (a) non-functionalize microfiber sensor (b) functionalized microfiber

In Figure 5.5(a), the non-functionalized microfiber sensor experienced a 0.3 nm wavelength shift when immersed in 250 ppm of Pb (II) solution, with reference to deionized water. Next, the sensor was flushed thoroughly with deionized water to rinse off all residues before immersing it into clean deionized water for a second time. As can be seen in the figure, the difference in wavelength shift with the microfiber sensor immersed into deionized water for the first time (dash line) and second time (dotted line) was found to be very small and assumed negligible. This suggests that the non-functionalized microfiber sensor did not retain any metal ion on its surface after thorough flushing with deionized water.

On the other hand, functionalized microfiber sensor in Figure 5.5(b) clearly showed a wavelength response (dotted line) which did not return to the deionized water reference spectrum. Thus, it can be deduced that, the functionalized microfiber sensor experienced a change in its effective refractive index after the sensor was in contact with sample solution. The reason behind this is due to formation of stable chelates on the sensing region after chelation process took place. These findings further validate that the wavelength shifts were caused by the chelation process, which in turn, validate the presence of heavy metal being trapped in the sensing region.

5.3.2 Heavy Metal Detection Using Non-Functionalized Optical Microfiber with Taper Waist Diameter 7.1 μ m

Several lead (II) ion solutions of concentrations 30, 75, 100, 250, 500, 750 and 1000ppm were prepared for the experiments. The refractive index (RI) values of the solutions were measured using a commercial portable refractometer (Kyoto Electronic, RA-130) with resolution of 0.0001. Table 5.1 shows the measured refractive indices of deionized water and three heavy metal solutions at different concentrations to be tested in our experiments. For the experiments using the microfiber, non-functionalized microfiber was first used to determine the spectra shifts for different concentrations of lead. Figure 5.6 shows the output spectra obtained from the OSA.

Types of solution Concentration (ppm)	Deionized Water	Lead (II) Chloride	Zinc (II) Chloride	Copper (II) Chloride
0	1.3329	1.3329	1.3328	1.3330
30	n.a	1.3330	1.3328	1.3330
75	n.a	1.3330	1.3328	1.3330
100	n.a	1.3330	1.3328	1.3330
250	n.a	1.3330	1.3331	1.3330
500	n.a	1.3330	1.3332	1.3331
750	n.a	1.3331	1.3334	1.3331
1000	n.a	1.3331	1.3336	1.3332

*n.a means not available

Table 5.1: Refractive index for different types of solution

Figure 5.6: Wavelength response of non-functionalized microfiber sensor surrounded by different concentrations of lead (II) chloride solutions

Experiments were conducted following the procedure mentioned in Section 5.2.2. In Figure 5.6, the leftmost spectrum (dark blue plot) was obtained when the sensing region was surrounded with deionized water. The other spectra were obtained when the sensing region was surrounded with different concentrations of lead (II) chloride

solution. As seen in the figure, it is apparent that the wavelength shifted to the right as the concentrations of lead (II) chloride solution increases. However, at a concentration of 100ppm, the wavelength response obtained from the OSA was nearly the same as that of the deionized water. One plausible reason that may explain this finding is that the RI value of this particular solution is nearly the same as the RI of the deionized water (Table 5.1). Although non-functionalized microfiber is a refractive index sensor itself, it may not be sensitive enough to detect the presence of very small quantity of lead (II) ion in the aqueous solution since there is insignificant change in the RI of the solution at low concentration of lead chloride. On the other hand, for concentrations of lead chloride above 250ppm, the dip wavelength shifts were measured and were found to increase linearly with concentration and hence the RI. Through observations, non-functionalized microfiber surrounded with deionized water and heavy metal solutions yield a stable (fix) wavelength response within a minute and did not show any changes for the next 30 minutes.

5.3.3 Heavy Metal Detection Using Functionalized Optical Microfiber with Taper Waist Diameter 7.1 μ m

Experiments were repeated using surface functionalized microfiber. It is important to note that EDTA was bonded onto the surface of tapered region and all microfiber were made for one-time use, which means that we do not reuse the same microfiber for subsequent metal detection in this study. Figure 5.7 illustrates a comparison of non-functionalized and functionalized microfiber to detect the presence of lead (II) ion for the 250ppm lead (II) chloride solution.

Figure 5.7: Wavelength response of (a) non-functionalized and (b) functionalized microfiber sensor surrounded by 250ppm lead chloride solution

As can be seen in the figure, the dip wavelength was found to be red shifted, similar to the case of non-functionalized microfiber. For the functionalized microfiber, a 0.7 ± 0.1 nm wavelength shift was observed (Figure 5.7(b)), in contrast to the 0.3 ± 0.1 nm wavelength shift as compared for the non-functionalized microfiber (Figure 5.7(a)).

All measured wavelength shifts of different lead (II) ion solutions are summarized in Figure 5.8.

Unlike the non-functionalized microfiber, surface functionalized microfibers required response time of at least 15 minutes (when immersed in heavy metal solutions) before the wavelength response became stable. Interaction of chelating agent and metal ion could be the reason for this long stabilization time. On top of this, metal ion was trapped on the microfiber surface due to presence of EDTA. This will generate a change in Δn_{eff} of the microfiber, which in turn causes resonant wavelength to be red shifted. From these experiments, we have verified that functionalized tapered fiber does enhance detection sensitivity of the microfiber, (Figure 5.8), from detection limit

of 250 ppm to 75 ppm. Similar experiments were repeated for zinc (II) chloride solution and Figure 5.9 shows the summary of wavelength shifts obtained from different concentrations of zinc (II) chloride solutions.

Figure 5.8: Wavelength shift versus lead (II) chloride concentration for non-functionalized and functionalized microfiber

Figure 5.9: Wavelength shift versus zinc (II) chloride concentration for non-functionalized and functionalized microfiber

Note that functionalized tapered region of the microfiber surrounded by 75 ppm and 100 ppm of zinc (II) chloride solutions caused a $0.4 \pm 0.1\text{nm}$ and $0.6 \pm 0.1\text{nm}$ wavelength shift respectively with respect to wavelength response of deionized water. This is comparable to previous results obtained with lead (II) chloride, where both indicate detection limits of 75 ppm, but differ in the amount of wavelength shifts. At both 100 and 250 ppm concentrations, the wavelength shifts were found to be the same. This might be due to the slight difference in the actual tapering profile of the microfibers. Even though all microfibers used in these experiments were done using motorized fabrication machine, it is very difficult to produce identical tapered fibers all the time. Therefore, experiments need to be repeated at least a few times to determine the actual value of wavelength shift for each concentration. Other than lead (II) and zinc (II) ion, EDTA was reported to be able to chelate a wide range of other heavy metal ions. A validation of this was done by repeating the experiment procedure with copper (II) chloride solutions, as shown in Figure 5.10. In contrast to lead and zinc, detection limit of copper (II) ion was recorded at 100 ppm.

Figure 5.10: Wavelength shift versus copper (II) chloride concentration for non-functionalized and functionalized microfiber

5.3.4 Comparison between Ethylenediaminetetraacetic acid (EDTA) and D-Penicillamine (DPA) for Copper (II) Ions Detection

Aside from EDTA, various types of chelating agents that contains carboxyl functional groups are also suitable to bond the amine group onto the silanized microfiber surface. This gives us a wide flexibility of choosing appropriate chelating agents of greater affinity toward specific metal types. For instance, DPA is a well-known chelating agent to copper [113], thus its capability to chelate copper (II) ions was also explored in this study and all the data were plotted in Figure 5.11. The results in Figure 5.11 shows that microfiber sensor with DPA bonded on its surface greatly enhances the detection limit of copper (II) ions. The plot indicates improvement of copper ion detection limit from 100 ppm to 30 ppm. Abundant literatures are available that describe the effectiveness of DPA for chelating copper, whether in the excretion of copper in animals or environmental study [114-115]. The mechanism of the formation of DPA-copper (II) complex began when electrons of Cu-S transfer reaction take place, causing the metal ion to reduce to copper (I). This reduction process causes weaker bound of copper (I) (since total charge become less and thus, less ionic contribution to the bond) and appear more accessible for the attachment by other penicillamine molecule. This way, DPA possesses a higher chelative capacity for copper in comparison to EDTA, and is deemed more effective in extracting copper ion from a compound [114].

Figure 5.11: Comparison between EDTA and DPA

5.3.5 Effect of Taper Waist Diameter on Sensing Potential of Functionalized Microfiber Sensor

It is of interest to investigate lowest detectable concentration of heavy metal solutions using functionalized microfiber of different taper waist diameters. As mentioned in Section 2.2.1, microfiber with very small taper waist diameter is extremely sensitive to the surrounding changes. Thus, an EDTA functionalized microfiber with taper waist diameter of 4.7 and 3.9 μm were fabricated to investigate possible improvement in their detection limit, and the results obtained are shown in Figure 5.12.

Figure 5.12: Wavelength response of (a) EDTA functionalized microfiber of taper waist diameter 4.7μm (b) EDTA functionalized microfiber of taper waist diameter 3.9μm in lead (II) ion solution

From Figure 5.12, dip wavelength shifts are apparent, where a total wavelength shift of $0.4 \pm 0.1\text{nm}$ and $1.2 \pm 0.1\text{nm}$ were measured for both Figure 5.12(a) and (b) respectively. This shows that detection limit can be improved significantly to parts per billion (ppb) level using functionalized microfiber sensor with taper waist diameter of less than 5μm. These results are encouraging since safe drinking water sometimes contains extremely toxic heavy metal compounds (such as lead, cadmium, etcetera) of not more than 10ppb. Figure 5.13 shows the output wavelength spectra obtained from sensing of 10 ppb copper (II) ion solution using DPA functionalized microfiber sensor with taper waist diameter of 3.9μm. Referring to Table 2.2 in Section 2.7, WHO and EPA stipulates that the maximum allowable concentration of copper (II) ion in public drinking water is 2 ppm and 1.3 ppm respectively. Thus the limit of detection for the present functionalized microfiber sensor evidently meets the needs as defined by these standards.

Figure 5.13: Wavelength responses for deionized water and 10 ppb copper (II) ion solution using a DPA functionalized microfiber sensor functionalized with taper waist diameter of $3.9\mu\text{m}$

5.3.6 Proposed Reader Unit

The conventional OSA used in present study has several limitations such as large in size and expensive. Therefore, a solution which addresses these limitations is crucial and would reduce the total cost and weight of sensing system. For these purposes, a concept of a compact and cost effective reader unit was proposed in this study. This reader incorporates two FBGs which serve as markers to measure amount of dip wavelength shifts and to determine the shift direction as well. The reason for choosing FBG as a marker is because the intensity of its reflection peak reflects the intensity of the microfiber's output spectra in real time. In other words, when the output wavelength spectra of the microfiber experienced a shift due to refractive index change, the peak power intensity of the grating will also change linearly. While intensity detection can be easily influenced by the energy fluctuation of the light source, a stable superluminescent diode (SLED) was employed to replace the tunable laser source used in previous experiments. Figure 5.14 illustrates the optical connection setup for the sensing system, incorporating the proposed reader.

Figure 5.14: Optical connection setup for overall sensing system, incorporating the reader unit

5.3.6.1 Identifying Direction of Shift

In order to implement the use of reader unit for the purpose of identifying the direction of wavelength shift, two FBGs were employed. The Bragg wavelength, λ_b , of these FBGs were chosen to be separated by a wavelength difference of approximately three quarter of the free spectral range (FSR) of the waveform observed by the OSA. The first Bragg wavelength, λ_{b1} (1551 nm), was positioned at the peak/dip of the output interference spectrum each time when the sensor was first immersed in the deionized water. (Figure 5.15(a)). On the other hand, second Bragg wavelength, λ_{b2} (1562.9 nm) will be positioned at the rising/falling edge of the spectrum when λ_{b1} is positioned at peak/dip of the spectrum correspondingly. This is done by introducing secondary axial strain using the translational stage as mentioned in Section 5.3.1 earlier, to cause the output spectrum to shift to a desired position. By doing so, we will also be able to screen across one cycle of the wavelength response to identify the approximate maximum and minimum power intensity value. For illustration purposes, Figure 5.15(a)-(f) show a series of output spectrum experiencing a blue shift, in chronological order. Table 5.2 shows the collected data, corresponding to Figure 5.15. It should be noted that the FSR for this sample set is 14.9 nm.

Figure 5.15(a)-(f): Changes in peak intensity of two FBGs serve as markers to measure amount of dip wavelength shift. (Output spectra (dotted line) remains in each figure to serve as reference spectra. Each diagram shows reference spectra experiencing left shift in chronological order)

<i>i-th</i>	Peak Power (dBm)		Intensity Change (Db)	
	FBG 1 at 1551 nm ($P_{\lambda_{b1_i}}$)	FBG 2 at 1562.9 nm ($P_{\lambda_{b2_i}}$)	$P_{\lambda_{b1_i}} - P_{\lambda_{b1_{i-1}}}$	$P_{\lambda_{b2_i}} - P_{\lambda_{b2_{i-1}}}$
0	-36.353	-39.296	0	0
1 (Figure 5.15(a))	-36.793	-37.656	-0.440	1.640
2 (Figure 5.15(b))	-39.895	-37.590	-3.102	0.066
3 (Figure 5.15(c))	-47.267	-40.392	-7.372	-2.802
4 (Figure 5.15(d))	-56.416	-47.180	-9.149	-6.788
5 (Figure 5.15(e))	-44.290	-51.363	12.126	-4.183
6 (Figure 5.15(f))	-38.712	-44.660	5.578	6.703

Table 5.2: Summary of measured power intensity of FBGs reflection peaks, corresponds to figure 5.15

Figure 5.16: Examples showing the role of FBG 2 as a marker when (a) output spectra was blue shifted causing reflection peak power intensity of FBG 2 to increase (positive sign (+)) (b) output spectra was red shifted causing reflection peak power intensity of FBG 2 to decrease (negative sign (-))

The direction of shift can be determined through the sign (positive (+) or negative (-)) of the amount of intensity change (Figure 5.16). For instance, peak power for FBG 2 first experience an increase in power intensity because the position of the reflection peak (FBG 2) move from the rising edge to the peak when shifted to the left (Figure 5.16 (a)). As the left shift increases, power intensity will slowly decrease as the reflection peak slowly shifted to the dip position (see Table 5.2). Conversely, the reflection peak will first experience a decrease in power intensity if the wavelength was shifted to the right (Figure 5.16 (b)). Rapid sampling of the power intensity readout is critical in this design to make close prediction of the shift direction and eliminating the need of OSA.

5.3.6.2 Identifying Amount of Wavelength Shift

Calibration was done to identify relationship between the change in dip wavelength and the corresponding reflection peak intensity of FBG 1, at which FBG 1 was initially positioned either at the peak or dip of wavelength spectrum. As aforementioned, we will be using some of the information from FBG 1 to make close approximation of the amount of wavelength shift. For instance, minimum (P_{min}) and maximum (P_{max}) intensity of FBG 1's reflection peak, and the intensity changes that were induced when wavelength spectrum was shifted are both required to perform the proposed mathematical algorithm. The intensity change per 1 nm wavelength shift was given as;

$$\frac{|P_{max} - P_{min}|}{0.5 \text{ FSR}} \quad (5.1)$$

From equation (5.1), amount of wavelength shift can be calculated based on the reflection peak's intensity change measured. Figure 5.17 illustrates a set of data which shows both the actual and calculated wavelength shifts.

Figure 5.17: Comparison between actual and calculated wavelength shift based on reflection peak's intensity changes

Referring to Figure 5.17, the calculated wavelength shifts were found not to match with the actual value. This is expected because equation (5.1) assumed that the wavelength shift varied linearly (straight line) with reflection peak's intensity change, however, this is in contrast to the reality whereby, the wavelength shift varied in a polynomial manner. However, it is of interest to know that both graphs shown in Figure 5.17 were strongly correlated by 96.89%, which means that both the actual and calculated shifts are correlated by a positive correlation factor. Figure 5.18 shows the least square correlational regression equation. Log function was applied to fit all data sets into the linear least square regression analysis.

Figure 5.18: Least square regression analysis

Using the regression equation, the subsequent step is to make a closer estimation to actual wavelength shift using the calculated wavelength shift mentioned previously. For this purpose, the regression equation was tested with several microfiber sensors which possess similar FSR (i.e. 16 nm in this example). Figure 5.19 shows the

comparison between actual and revised calculated wavelength shift using the regression equation in Figure 5.18.

Figure 5.19: Comparison between actual and revised calculated wavelength shift

As seen in Figure 5.19, the standard deviation for the estimated value lies within the range of ± 0.4 nm. The accuracy of this proposed reader unit (based on all data sets acquired in this study) slightly deviates from the desired performance since a wavelength shift of 0.4nm could possibly affect the estimation of the actual heavy metal concentration. However, this proposed design does provide a valuable insight for further investigation because the standard deviation can be improved to ± 0.2 nm by increasing number of samples and improving the system performance.

5.4 Conclusion

Thus far, all data presented in previous sections showed surface functionalized microfiber sensors yield better metal ion detection sensitivity due to chelation by chelating agent attached on the sensing region. Moreover, the findings showed that

different types of chelating agents exhibit different chelation properties towards different types of metal ions. For instance, DPA-functionalized microfiber sensor yields better copper (II) ion detection as compared to EDTA-functionalized microfiber sensor, because DPA has higher chelative capacity for copper compared to EDTA. Besides choosing the right chelating agent to target desired metal ion types, another way to improve the sensor's detection limit is to reduce the taper waist diameter. Results showed the reduction of waist diameters from $7.1\mu\text{m}$ to $3.9\mu\text{m}$ significantly increases the detection limit by 3000 times (30 ppm to 10 ppb), introducing the potential use of functionalized microfiber sensor in detecting maximum permissible level of heavy metal contaminant in our daily drinking water. Although small taper waist diameter requires careful handling due to its fragility, the printed Acrylonitrile Butadiene Styrene (ABS) cartridge which was previously described in Section 5.2.1 overcomes the problem by protecting and securing the sensor in place, thus improving the robustness of the sensor. On top of this, the working principle and the concept of a designed reader unit was explained and tests were conducted to demonstrate its functionality to identify the direction of wavelength shifts, as well as providing close approximation of amount of wavelength shifts, with standard estimated error of 0.4375, based on all the acquired data sets. With this reader unit, OSA can be eliminated in the microfiber based heavy metal sensing system design, further reducing the cost while improving the portability of the whole sensing system. However, more tests and calibrations are needed to make proper adjustment of the least square regression analysis to improve the system.

CHAPTER 6

CONCLUSION

6.1 Conclusion

Heavy metal contamination in water environment raises public concern about its impact on human health and the ecosystem. Due to the alarming rate of the accumulation of heavy metal over time, heavy metal ion detection is important to allow the necessary remedial actions to be taken to reduce concentration of metal ions before these toxic metals get into our food chain. In this study, an optical tapered fiber, also known as microfiber, with chelating agent functionalized on the tapered region for heavy metal ion detection was demonstrated. The functionalized microfiber sensor is capable to trap heavy metal ions present in the sample solutions which can be quantitatively measured through the dip wavelength shift in the output spectra, due to changes of the effective refractive index of the microfiber sensor. Addressing this claim, concrete evidences were shown throughout this study to support the ability of the functionalized microfiber to detect heavy metal ions. For instance, surface scanning of the functionalized microfiber using SEM has confirmed the successful attachment of chelating agent on the microfiber surface. Moreover, composition analysis through EDX also revealed the presence of lead (II) ions immobilized onto the EDTA-functionalized microfiber sensor's surface after the sensor was used to detect lead (II) ion in lead (II) chloride solution. While quantitative data is equally important for analysis purposes, major highlight of this study also includes the correlation between amount of spectra shift and the concentration of metal ions in sample solutions.

Moreover, external factors such as strain and temperature characterizations for microfiber of different tapering profiles are equally important to investigate the influence of these parameters toward the microfiber sensor and to identify appropriate taper profile to achieve optimum sensing performance. Based on the results obtained, the maximum sensitivity achievable were recorded at $-15.868\text{nm}/\text{M}\epsilon$ and $-0.7694/^{\circ}\text{C}$ using microfiber sensor with taper waist diameter of $4.7\text{ }\mu\text{m}$. In addition, the degree of strain and temperature influences on the performance of microfiber sensor was found to vary depending on the taper profile, whereby, microfiber with longer taper length and smaller taper waist diameter possesses the highest sensitivity and vice versa. In order to overcome multiple parameter sensing of the microfiber sensor, all single used microfiber sensor in this experiments were packaged in a 3D- printed ABS-made cartridge (Section 5.2.1). The cartridge was economically designed and also exhibit several interesting features to keep strain and temperature influences to a minimum. These include constant inflow and outflow rate to bring sample solution in/out of the sample chamber, good grip for microfiber sensor, consistent sample volume and placement of microfiber. Under such controlled environment, the trapping of the metal ion by the chelating agent compounds on the surface of microfiber causes an observable shift in the output spectra when compared to the spectra from the deionized water. All data sets obtained in this study showed a red shift when heavy metal ion concentration increases. DPA-functionalized microfiber showed lowest detection limit of 30 ppm for copper (II), while EDTA-functionalized microfiber showed the best detection limit of 75 ppm for lead (II) and zinc (II) ion, but 100 ppm for copper (II) ion. The detection limit of the functionalized microfiber sensor was further improved by using smaller taper waist diameter which yield stronger evanescent wave. For instance, a reduction of taper waist diameter from $7.1\text{ }\mu\text{m}$ to $3.9\text{ }\mu\text{m}$ enables the system

to detect down to ppb concentration range. Overall, a heavy metal ion sensor can be developed using evanescent wave based optical fiber with extra advantages of lower cost, smaller size and high flexibility in choosing appropriate types of chelating agent for targeting specific metal ion detection.

6.2 Recommendation for Future Work

Although the results presented in this thesis have demonstrated the effectiveness of a microfiber based heavy metal ion sensor, it can be further developed in a number of ways:

1) Extending the study with wider choice of chelating agents

In this study, we have verified that each chelating agent has its unique chelative properties and affinity towards particular types of heavy metal ion. A complete series of heavy metal ion detection database using wider choice of chelating agent-functionalized microfiber for specific metal ion detection will be very useful, not only that this will broaden the sensor's detection capabilities, but also to develop deeper understanding of metal binding properties for various kinds of chelating agents for future use.

2) Extending the development of a digitized reader unit

The proposed reader unit explained in previous chapter unveils the feasibility of assembling the whole system into a compact and portable system to facilitate on-site detection. In the near future, it is practical to digitize the system with a microcontroller or field programmable gate array to perform all mathematical algorithm, while providing a simple user interface for easy handling.

AUTHOR'S PUBLICATION

1. W. B. Ji, S. H. K. Yap, N. Panwar, L. L. Zhang, B. Lin, K. T. Yong, *et al.*, "Detection of low-concentration heavy metal ions using optical microfiber sensor," *Sensors and Actuators B: Chemical*, vol. 237, pp. 142-149, 2016.

REFERENCES

- [1] R. Singh, N. Gautam, A. Mishra, and R. Gupta, *Heavy metals and living systems: An overview* vol. 43, pp. 246-253, 2011.
- [2] H.-W. Chen, "Gallium, Indium, and Arsenic Pollution of Groundwater from a Semiconductor Manufacturing Area of Taiwan," *Bulletin of Environmental Contamination and Toxicology*, vol. 77, pp. 289-296, 2006.
- [3] T. Roychowdhury, H. Tokunaga, and M. Ando, "Survey of arsenic and other heavy metals in food composites and drinking water and estimation of dietary intake by the villagers from an arsenic-affected area of West Bengal, India," *Science of The Total Environment*, vol. 308, pp. 15-35, 2003.
- [4] C. Xing, L. Liu, X. Zhang, H. Kuang, and C. Xu, "Colorimetric detection of mercury based on a strip sensor," *Analytical Methods*, vol. 6, pp. 6247-6253, 2014.
- [5] S. Ghetia, R. Gajjar, and P. Trivedi, "Classification of fiber optical sensors," *International Journal of Electronics Communication and Computer Technology (IJECCCT)*, vol. 3, pp. 442-445, 2013.
- [6] M. Benounis, N. Jaffrezic-Renault, H. Halouani, R. Lamartine, and I. Dumazet-Bonnamour, "Detection of heavy metals by an optical fiber sensor with a sensitive cladding including a new chromogenic calix[4]arene molecule," *Materials Science and Engineering: C*, vol. 26, pp. 364-368, 2006.
- [7] B. Gu, M.-J. Yin, A. P. Zhang, J.-W. Qian, and S. He, "Fiber-optic metal ion sensor based on thin-core fiber modal interferometer with nanocoating self-assembled via hydrogen bonding," *Sensors and Actuators B: Chemical*, vol. 160, pp. 1174-1179, 2011.
- [8] H. Tan Chun, J. Mohd. Zubir Mat, S. Lim Hwee, and C. Moo Yow, "Detection of heavy metal ions in aqueous solution using fiber optic sensor," in *Computer and Communication Engineering (ICCCE), 2012 International Conference on*, pp. 267-270, 2012.
- [9] K. Bhavsar, R. Prabhu, and P. Pollard, "Development of dithizone based fibre optic evanescent wave sensor for heavy metal ion detection in aqueous environments," in *Journal of Physics: Conference Series*, vol. 450, pp. 1-5, 2013.
- [10] K.T.V. Grattan and B. T. Meggitt, Eds., *Optical Fiber Sensor Technology: Chemical and Environmental Sensing* (Optoelectronics, Imaging and Sensing 4). Springer Netherlands, p15, 1999.
- [11] G. Keiser, *Optical Fiber Communications*, 4 ed.: McGraw Hill, 2008.
- [12] J. M. Senior, *Optical fiber communications: principles and practice*, 3 ed.: Pearson 2009.
- [13] G. P. Agrawal, *Fiber-Optic Communication Systems*, 4 ed., 2011.
- [14] T. Limin, L. Jingyi, Y. Zhizhen, T. S. Geoff, and M. Eric, "Self-modulated taper drawing of silica nanowires," *Nanotechnology*, vol. 16, pp. 1445, 2005.
- [15] T. A. Birks, W. J. Wadsworth, and P. S. J. Russell, "Supercontinuum generation in tapered fibers," *Optics Letters*, vol. 25, pp. 1415-1417, 2000.
- [16] H. S. Lin, Y. N. Phua, M. R. Mokhtar, H. A. Abdul-Rashid, M. Othman, and Z. Yusoff, "Strain effect on spectral response of biconic tapered fiber interferometer," in *2012 IEEE 3rd International Conference on Photonics*, pp. 107-109, 2012.

- [17] G. Brambilla, F. Xu, and X. Feng, "Fabrication of optical fibre nanowires and their optical and mechanical characterisation," *Electronics Letters*, vol. 42, pp. 517-519, 2006.
- [18] G. Brambilla, F. Koizumi, X. Feng, and D. J. Richardson, "Compound-glass optical nanowires," *Electronics Letters*, vol. 41, pp. 400-402, 2005.
- [19] L. Tong, L. Hu, J. Zhang, J. Qiu, Q. Yang, J. Lou, *et al.*, "Photonic nanowires directly drawn from bulk glasses," *Optics Express*, vol. 14, pp. 82-87, 2006.
- [20] J. Lou, Y. Wang, and L. Tong, "Microfiber Optical Sensors: A Review," *Sensors*, vol. 14, pp. 5823, 2014.
- [21] K. Q. Kieu and M. Mansuripur, "Biconical Fiber Taper Sensors," *IEEE Photonics Technology Letters*, vol. 18, pp. 2239-2241, 2006.
- [22] G. Brambilla, "Optical fibre nanowires and microwires: a review," *Journal of Optics*, vol. 12, pp. 043001, 2010.
- [23] G. Y. Chen, M. Ding, T. Newson, and G. Brambilla, "A review of microfiber and nanofiber based optical sensors," *The Open Optics Journal*, vol. 7, pp. 32-57, 2013.
- [24] L.-P. Sun, J. Li, L. Jin, Y. Ran, and B.O.Guan, "High-sensitivity humidity sensor based on microfiber Sagnac interferometer," in *Asia Communications and Photonics Conference 2015*, Hong Kong, 2015.
- [25] J. Wo, G. Wang, Y. Cui, Q. Sun, R. Liang, P. P. Shum, *et al.*, "Refractive index sensor using microfiber-based Mach-Zehnder interferometer," *Optics Letters*, vol. 37, pp. 67-69, 2012.
- [26] M. Z. Muhammad, A. A. Jasim, H. Ahmad, H. Arof, and S. W. Harun, "Non-adiabatic silica microfiber for strain and temperature sensors," *Sensors and Actuators A: Physical*, vol. 192, pp. 130-132, 2013.
- [27] J. Shi, S. Xiao, L. Yi, and M. Bi, "A Sensitivity-Enhanced Refractive Index Sensor Using a Single-Mode Thin-Core Fiber Incorporating an Abrupt Taper," *Sensors*, vol. 12, pp. 4697, 2012.
- [28] E. Baude, "Numerical study of tapered fiber optics as evanescent field sensors," in *Microwave & Optoelectronics Conference (IMOC), 2013 SBMO/IEEE MTT-S International*, pp. 1-5, 2013.
- [29] S. Lacroix, F. Gonthier, R. J. Black, and J. Bures, "Tapered-fiber interferometric wavelength response: the achromatic fringe," *Optics Letters*, vol. 13, pp. 395-397, 1988.
- [30] W. B. Ji, Y. C. Tan, B. Lin, S. C. Tjin, and K. K. Chow, "Nonadiabatically Tapered Microfiber Sensor With Ultrashort Waist," *IEEE Photonics Technology Letters*, vol. 26, pp. 2303-2306, 2014.
- [31] W. B. Ji, H. H. Liu, S. C. Tjin, K. K. Chow, and A. Lim, "Ultrahigh Sensitivity Refractive Index Sensor Based on Optical Microfiber," *IEEE Photonics Technology Letters*, vol. 24, pp. 1872-1874, 2012.
- [32] S. W. Harun, K. S. Lim, C. K. Tio, K. Dimyati, and H. Ahmad, "Theoretical analysis and fabrication of tapered fiber," *Optik - International Journal for Light and Electron Optics*, vol. 124, pp. 538-543, 2013.
- [33] L. w. Li and X. h. Sun, "Investigation on the Tapered Fiber Evanescent-Field Sensor Based on the Comsol Software," in *Photonics and Optoelectronics (SOPO), 2012 Symposium on*, pp. 1-3, 2012.
- [34] L. C. Bobb, P. M. Shankar, and H. D. Krumboltz, "Bending effects in biconically tapered single-mode fibers," *Journal of Lightwave Technology*, vol. 8, pp. 1084-1090, 1990.
- [35] A. Hulanicki, S. Glab, and F. Ingman, "Chemical sensors: definitions and classification," *Pure and Applied Chemistry*, vol. 63, pp. 1247-1250, 1991.
- [36] H. H. Hesse, East German Patent, 1974.
- [37] I. Yoo and S. Song, "Metal Ion Detection of Novel Conjugated-Polymer Sensor Fibers Fabricated With 3-D Hydrodynamic Focusing," in *14th International Conference on Miniaturized Systems for Chemistry and Life Sciences*, Groningen, The Netherlands, Oct. 2010.

- [38] H. Qazi, A. Mohammad, and M. Akram, "Recent Progress in Optical Chemical Sensors," *Sensors*, vol. 12, pp. 16522-16556, 2012.
- [39] F. Baldini, A. Chester, J. Homola, and S. Martellucci, "Optical chemical sensors" Springer Science & Business Media, vol. 224, 2006.
- [40] S. H. Girei, A. A. Shabaneh, P. T. Arasu, S. Painam, and M. H. Yaacob, "Tapered multimode fiber sensor for ethanol sensing application," in *Photonics (ICP), 2013 IEEE 4th International Conference*, pp. 275-277, 2013.
- [41] S. S. Chong, A. Aziz, and S. W. Harun, "Fibre optic sensors for selected wastewater characteristics," *Sensors*, vol. 13, pp. 8640-8668, 2013.
- [42] Z. Zhujun and W. R. Seitz, "A fluorescent sensor for aluminum(III), magnesium(II), zinc(II) and cadmium(II) based on electrostatically immobilized quinolin-8-ol sulfonate," *Analytica Chimica Acta*, vol. 171, pp. 251-258, 1985.
- [43] N. Aksuner, E. Henden, I. Yilmaz, and A. Cukurovali, "A novel optical chemical sensor for the determination of nickel(II) based on fluorescence quenching of newly synthesized thiazolo-triazol derivative and application to real samples," *Sensors and Actuators B: Chemical*, vol. 166-167, pp. 269-274, 2012.
- [44] G. Cohoon, C. Boyter, M. Errico, K. Vandervoort, and E. Salik, "Enhancing sensitivity of biconical tapered fiber sensors with multiple passes through the taper," *Optical engineering*, vol. 49, pp. 034401-034401-5, 2010.
- [45] W. B. Ji, S. C. Tjin, B. Lin, and C. L. Ng, "Highly Sensitive Refractive Index Sensor Based on Adiabatically Tapered Microfiber Long Period Gratings," *Sensors (Basel, Switzerland)*, vol. 13, pp. 14055-14063, 2013.
- [46] L. Shriver-Lake, P. Charles, and C. Taitt, "Immobilization of Biomolecules onto Silica and Silica-Based Surfaces for Use in Planar Array Biosensors," in *Biosensors and Biodetection*. vol. 504, A. Rasooly and K. Herold, Eds., ed: Humana Press, pp. 419-440, 2009.
- [47] A. Guthrie, R. Narayanaswamy, and D. Russell, "Optical fibres in chemical sensing-a review," *Transactions of the Institute of Measurement and Control*, vol. 9, pp. 71-80, 1987.
- [48] P. K. Jal, S. Patel, and B. K. Mishra, "Chemical modification of silica surface by immobilization of functional groups for extractive concentration of metal ions," *Talanta*, vol. 62, pp. 1005-1028, 2004.
- [49] V. Dugas, A. Elaissari, and Y. Chevalier, "Surface Sensitization Techniques and Recognition Receptors Immobilization on Biosensors and Microarrays," in *Recognition Receptors in Biosensors*, M. Zourob, Ed., ed: Springer New York, pp. 47-134, 2010.
- [50] J. Wiegmann, "The chemistry of silica. Solubility, polymerization, colloid and surface properties, and biochemistry. Von RALPH K. ILLER. New York/Chichester/Brisbane/Toronto: John Wiley & Sons 1979. XXIV, 866 S., Lwd., £ 39.50," *Acta Polymerica*, vol. 31, pp. 406-406, 1980.
- [51] Arkles, B., "Silane Coupling Agents: Connecting Across Boundaries 3rd Ed.", from <http://www.gelest.com/goods/PDF/brochures/couplingagents.pdf>, June 11, 2006.
- [52] Hermanson, G. T., *Bioconjugate techniques*. Academic press, 2013.
- [53] J. A. Howarter and J. P. Youngblood, "Optimization of Silica Silanization by 3-Aminopropyltriethoxysilane," *Langmuir*, vol. 22, pp. 11142-11147, 2006.
- [54] E. Plueddemann, "Nature of Adhesion Through Silane Coupling Agents," in *Silane Coupling Agents*, ed: Springer US, pp. 115-152, 1991.
- [55] R. T. Limited, "Polymer Bonding 2004: 1st International Conference Focussing on the Bonding of Rubber and Plastics to Various Substrates : Munich, Germany, 2004.
- [56] A. Simon, T. Cohen-Bouhacina, M. C. Porté, J. P. Aimé, and C. Baquey, "Study of Two Grafting Methods for Obtaining a 3-Aminopropyltriethoxysilane Monolayer on Silica Surface," *Journal of Colloid and Interface Science*, vol. 251, pp. 278-283, 2002.
- [57] Y. An, M. Chen, Q. Xue, and W. Liu, "Preparation and self-assembly of carboxylic acid-functionalized silica," *Journal of Colloid and Interface Science*, vol. 311, pp. 507-513, 2007.

- [58] C. J. Stanford, M. Dagenais, J.-H. Park, and P. DeShong, "Real-time monitoring of siloxane monolayer film formation on silica using a fiber Bragg grating," *Current Analytical Chemistry*, vol. 4, pp. 356-361, 2008.
- [59] A. Yalcin, K. C. Popat, J. C. Aldridge, T. A. Desai, J. Hryniewicz, N. Chbouki, *et al.*, "Optical sensing of biomolecules using microring resonators," *Selected Topics in Quantum Electronics, IEEE Journal of*, vol. 12, pp. 148-155, 2006.
- [60] S. Akita, H. Sasaki, K. Watanabe, and A. Seki, "A humidity sensor based on a hetero-core optical fiber," *Sensors and Actuators B: Chemical*, vol. 147, pp. 385-391, 2010.
- [61] Y. Yuan, C. Xue, H. Wang, and Q. Zhang, "Research on optical fiber sensor based on nanoparticles-modified," in *Electronics and Optoelectronics (ICEOE), 2011 International Conference on*, pp. V2-314-V2-316, 2011.
- [62] T. L. Yeo, D. Eckstein, B. McKinley, L. F. Boswell, T. Sun, and K. T. V. Grattan, "Demonstration of a fibre-optic sensing technique for the measurement of moisture absorption in concrete," *Smart Materials and Structures*, vol. 15, p. N40, 2006.
- [63] F. C. Hartman and F. Wold, "Bifunctional Reagents. Cross-Linking of Pancreatic Ribonuclease with a Diimido Ester1," *Journal of the American Chemical Society*, vol. 88, pp. 3890-3891, 1966.
- [64] S. Avrameas, "Coupling of enzymes to proteins with glutaraldehyde: Use of the conjugates for the detection of antigens and antibodies," *Immunochemistry*, vol. 6, pp. 43-52, 1969.
- [65] Z. Grabarek and J. Gergely, "Zero-length crosslinking procedure with the use of active esters," *Analytical Biochemistry*, vol. 185, pp. 131-135, 1990.
- [66] N. Nakajima and Y. Ikada, "Mechanism of Amide Formation by Carbodiimide for Bioconjugation in Aqueous Media," *Bioconjugate Chemistry*, vol. 6, pp. 123-130, 1995.
- [67] A. Williams and I. T. Ibrahim, "A new mechanism involving cyclic tautomers for the reaction with nucleophiles of the water-soluble peptide coupling reagent 1-ethyl-3-(3'-(dimethylamino)propyl)carbodiimide (EDC)," *Journal of the American Chemical Society*, vol. 103, pp. 7090-7095, 1981.
- [68] M. A. Gilles, A. Q. Hudson, and C. L. Borders Jr, "Stability of water-soluble carbodiimides in aqueous solution," *Analytical Biochemistry*, vol. 184, pp. 244-248, 1990.
- [69] J. V. Staros, R. W. Wright, and D. M. Swingle, "Enhancement by N-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions," *Analytical Biochemistry*, vol. 156, pp. 220-222, 1986.
- [70] P. S. Anjaneyulu and J. V. Staros, "Reactions of N-hydroxysulfosuccinimide active esters," *International Journal of Peptide and Protein Research*, vol. 30, pp. 117-124, 1987.
- [71] Scientific, T. (2011). "NHS and Sulfo-NHS", https://tools.lifetechnologies.com/content/sfs/manuals/MAN0011309_NHS_SulfoNHS_UG.pdf, June 11, 2015.
- [72] G. T. Morgan and H. D. K. Drew, "CLXII.-Researches on residual affinity and co-ordination. Part II. Acetylacetones of selenium and tellurium," *Journal of the Chemical Society, Transactions*, vol. 117, pp. 1456-1465, 1920.
- [73] S. J. S. Flora, "3.35 - Chelation Therapy," in *Comprehensive Inorganic Chemistry II (Second Edition)*, J. R. Poeppelmeier, Ed., Amsterdam: Elsevier, pp. 987-1013, 2013.
- [74] I. S. Pinto, I. F. Neto, and H. V. M. Soares, "Biodegradable chelating agents for industrial, domestic, and agricultural applications—a review," *Environmental Science and Pollution Research*, vol. 21, pp. 11893-11906, 2014.
- [75] S. J. S. Flora and V. Pachauri, "Chelation in Metal Intoxication," *International Journal of Environmental Research and Public Health*, vol. 7, pp. 2745-2788, 2010.
- [76] M. M. Jones and M. A. Basinger, "A hypothesis for the selection of chelate antidotes for toxic metals," *Medical Hypotheses*, vol. 9, pp. 445-453, 1982.

- [77] D. P. Mellor, "CHAPTER 1 - Historical Background and Fundamental Concepts," in *Chelating Agents and Metal Chelates*, F. P. D. P. Mellor, Ed., ed: Academic Press, pp. 1-50, 1964.
- [78] M. M. Jones, "New Developments in Therapeutic Chelating Agents as Antidotes for Metal Poisoning," *Critical Reviews in Toxicology*, vol. 21, pp. 209-233, 1991.
- [79] F. Crea, C. De Stefano, A. Gianguzza, D. Piazzese, and S. Sammartano, "Speciation of poly-amino carboxylic compounds in seawater," *Chemical Speciation & Bioavailability*, vol. 15, pp. 75-86, 2003.
- [80] Z. Yuan and J. M. VanBriesen, "The Formation of Intermediates in EDTA and NTA Biodegradation," *Environmental Engineering Science*, vol. 23, pp. 533-544, 2006.
- [81] Z. Mohammadi, S. Shalavi, and H. Jafarzadeh, "Ethylenediaminetetraacetic acid in endodontics," *European Journal of Dentistry*, vol. 7, pp. S135-S142, 2013.
- [82] O. Andersen, "Chemical and Biological Considerations in the Treatment of Metal Intoxications by Chelating Agents," *Mini Reviews in Medicinal Chemistry*, vol. 4, pp. 11-21, 2004.
- [83] C. Oviedo and J. Rodríguez, "EDTA: the chelating agent under environmental scrutiny," *Química Nova*, vol. 26, pp. 901-905, 2003.
- [84] J. R. Hart, "Ethylenediaminetetraacetic Acid and Related Chelating Agents," in *Ullmann's Encyclopedia of Industrial Chemistry*, ed: Wiley-VCH Verlag GmbH & Co. KGaA, 2000.
- [85] B. Nowack, "Environmental Chemistry of Aminopolycarboxylate Chelating Agents," *Environmental Science & Technology*, vol. 36, pp. 4009-4016, 2002.
- [86] S. Tandy, K. Bossart, R. Mueller, J. Ritschel, L. Hauser, R. Schulin, *et al.*, "Extraction of Heavy Metals from Soils Using Biodegradable Chelating Agents," *Environmental Science & Technology*, vol. 38, pp. 937-944, 2004.
- [87] C. K. Schmidt and H.-J. Brauch, "Impact of aminopolycarboxylates on aquatic organisms and eutrophication: Overview of available data," *Environmental Toxicology*, vol. 19, pp. 620-637, 2004.
- [88] Lanigan, R. S., & Yamarik, T. A. "Final Report on the Safety Assessment of EDTA, Calcium Disodium EDTA, Diammonium EDTA, Dipotassium EDTA, Disodium EDTA, TEA-EDTA, Tetrasodium EDTA, Tripotassium EDTA, Trisodium EDTA, HEDTA, and Trisodium HEDTA," *International Journal of Toxicology*, vol. 21, pp. 95-142, 2002.
- [89] H. V. Aposhian, R. M. Maiorino, D. Gonzalez-Ramirez, M. Zuniga-Charles, Z. Xu, K. M. Hurlbut, *et al.*, "Mobilization of heavy metals by newer, therapeutically useful chelating agents," *Toxicology*, vol. 97, pp. 23-38, 1995.
- [90] H. Mückter, B. Lieb, F.-X. Reich, G. Hunder, U. Walther, and B. Fichtl, "Are we ready to replace dimercaprol (BAL) as an arsenic antidote?," *Human & Experimental Toxicology*, vol. 16, pp. 460-465, 1997.
- [91] H. V. Aposhian and M. M. Aposhian, "MESO-2,3-Dimercaptosuccinic Acid: Chemical, Pharmacological and Toxicological Properties of an Orally Effective Metal Chelating Agent," *Annual Review of Pharmacology and Toxicology*, vol. 30, pp. 279-306, 1990.
- [92] P. Asiedu, T. Moulton, C. B. Blum, E. Roldan, N. J. Lolacono, and J. H. Graziano, "Metabolism of meso-2,3-dimercaptosuccinic acid in lead-poisoned children and normal adults," *Environmental Health Perspectives*, vol. 103, pp. 734-739, 1995.
- [93] O. Andersen, "Chemical and Biological Considerations in the Treatment of Metal Intoxications by Chelating Agents," *Mini Reviews in Medicinal Chemistry*, vol. 4, pp. 11-21, 2004.
- [94] M. A. Barakat, "New trends in removing heavy metals from industrial wastewater," *Arabian Journal of Chemistry*, vol. 4, pp. 361-377, 2011.
- [95] P. Khanna, "Assessment of heavy metal contamination in different vegetables grown in and around urban areas," *Research Journal of Environmental Toxicology*, vol. 5, p. 162, 2011.

- [96] P. J. Udeh, A Guide to Healthy Drinking Water: All You need to know about the Water You Drink: iUniverse, 2004.
- [97] L. Järup, "Hazards of heavy metal contamination," *British Medical Bulletin*, vol. 68, pp. 167-182, 2003.
- [98] H. L. Needleman, C. Gunnoe, A. Leviton, R. Reed, H. Peresie, C. Maher, *et al.*, "Deficits in Psychologic and Classroom Performance of Children with Elevated Dentine Lead Levels," *New England Journal of Medicine*, vol. 300, pp. 689-695, 1979.
- [99] M. Fulton, G. Thomson, R. Hunter, G. Raab, D. Laxen, and W. Hepburn, "Influence Of Blood Lead on The Ability And Attainment Of Children In Edinburgh," *The Lancet*, vol. 329, pp. 1221-1226, 1987.
- [100] Minesota Department of Health, "Copper in Drinking Water," <http://www.health.state.mn.us/divs/eh/water/factsheet/com/copper.pdf>, June 11, 2015
- [101] WHO, World Health Organization (2004). Copper in Drinking Water. Retrieved from http://www.who.int/water_sanitation_health/dwq/chemicals/copper.pdf, June 11, 2015.
- [102] J. O. Nriagu, *Zinc in the environment Pt. 1, Pt. 1*. New York; Chichester [etc.]: Wiley, 1980.
- [103] H. H. Sandstead and W. Au, "Chapter 47 - Zinc," in *Handbook on the Toxicology of Metals (Third Edition)*, G. F. Nordberg, B. A. Fowler, M. Nordberg, and L. T. Friberg, Eds., ed Burlington: Academic Press, 2007, pp. 925-947.
- [104] L. M. Plum, L. Rink, and H. Haase, "The Essential Toxin: Impact of Zinc on Human Health," *International Journal of Environmental Research and Public Health*, vol. 7, pp. 1342-1365, 2010.
- [105] "Australian Drinking Water Guidelines 6" https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/eh52_australian_drinking_water_guidelines_150527.pdf, June 11, 2015.
- [106] WHO, World Health Organization, "Guidelines for drinking-water quality, fourth edition" http://whqlibdoc.who.int/publications/2011/9789241548151_eng.pdf, June 11, 2015.
- [107] NEA, National Environment Agency, "Quality of Piped Drinking Water Regulations 2008" <http://statutes.agc.gov.sg/aol/search/display/view.w3p?page=0;query=DocId%3A%22c6503669-7d36-4a1a-836a-459a3ad88cdc%22%20Status%3Ainforce%20Depth%3A0;rec=0>, June 11, 2015.
- [108] USEPA, United States Environmental Protection Agency, "National Primary Drinking Water Regulations, <http://water.epa.gov/drink/contaminants/index.cfm#List>, June 11, 2015.
- [109] L. Tong, R. R. Gattass, J. B. Ashcom, S. He, J. Lou, M. Shen, *et al.*, "Subwavelength-diameter silica wires for low-loss optical wave guiding," *Nature*, vol. 426, pp. 816-819, 2003.
- [110] P. Lu, L. Men, K. Sooley, and Q. Chen, "Tapered fiber Mach-Zehnder interferometer for simultaneous measurement of refractive index and temperature," *Applied Physics Letters*, vol. 94, p. 131110, 2009.
- [111] B. Musa, Y. M. Kamil, M. H. A. Bakar, A. S. M. Noor, A. Ismail, and M. A. Mahdi, "INVESTIGATING THE EFFECT OF TAPER LENGTH ON SENSITIVITY OF THE TAPERED-FIBER BASED TEMPERATURE SENSOR," *Jurnal Teknologi*, vol. 78, 2016.
- [112] T. K. Yadav, M. A. Mustapa, M. H. Abu Bakar, and M. A. Mahdi, *Study of single mode tapered fiber-optic interferometer of different waist diameters and its application as a temperature sensor* vol. 9, 2014.
- [113] S. R. Gooneratne and D. A. Christensen, "Effect of chelating agents on the excretion of copper, zinc and iron in the bile and urine of sheep," *The Veterinary Journal*, vol. 153, pp. 171-178, 1997.
- [114] J. H. Park, E. J. Hill, T.-H. Chou, V. LeQuire, R. Roelofs, and C. R. Park, "MECHANISM OF ACTION OF PENICILLAMINE IN THE TREATMENT OF

- AVIAN MUSCULAR DYSTROPHY *," *Annals of the New York Academy of Sciences*, vol. 317, pp. 356-369, 1979.
- [115] M. Tegoni, D. Valensin, L. Toso, and M. Remelli, "Copper chelators: chemical properties and bio-medical applications," *Current medicinal chemistry*, vol. 21, pp. 3785-3818, 2014.