Development of chitosan based optical fiber chemical sensors

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NANYANG
TECHNOLOGICAL
UNIVERSITY

**DEVELOPMENT OF CHITOSAN BASED OPTICAL
FIBER CHEMICAL SENSORS**

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**SCHOOL OF CHEMICAL AND BIOMEDICAL
ENGINEERING**

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Raghunandhan Ravikumar

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LIST OF ABBREVIATIONS

APTES	(3-aminopropyl) triethoxysilane
ASE	Amplified Spontaneous Emission
CS	Chitosan
DD	Degree of Deacetylation
ECH	Epichlorohydrin
EDX	Energy Dispersive X-Ray Spectroscopy
EMI	Electromagnetic Interference
FBG	Fiber Bragg Grating
FOS	Fiber Optic Sensors
FPI	Fabry Perot Interferometer
LBL	Layer-By-Layer
LPG	Long Period Grating
MES	2-(N-morpholino)ethanesulfonic acid (MES)
MI	Michelson Interferometer
MZI	Mach-Zehnder Interferometer

NCF	No Core Fiber
OSA	Optical Spectrum Analyzer
PAA	Poly (acrylic acid)
PBOS	Polymer Based Optical Sensors
PCF	Photonic Crystal Fiber
RI	Refractive Index
SEM	Scanning Electron Microscopy
SI	Sagnac Interferometer
SMF	Single Mode Fiber
SPR	Surface Plasmon Resonance

Abstract

In recent years there has been a tremendous progress in the development of polymer based fiber optic sensors. Particular emphasis on research has been focused on in-line fiber optic interferometric sensors owing to their many advantages like compact size, high sensitivity, immunity to electromagnetic interference etc. Among the polymers used in sensing applications, chitosan a natural biopolymer, has attracted a significant amount of interest. This thesis explores the possibilities of integrating chitosan with inline interferometric sensors for the sensing of chemical and biological species.

The first work presented in the thesis involves an interferometric sensor with a no-core fiber (NCF) spliced between single mode fibers (SMF) and functionalized with chitosan (CS)/poly acrylic acid (PAA) self-assembled polyelectrolyte layers for the detection of heavy metal ion Nickel (II) (Ni^{2+}). The sensing scheme is based on multi mode interference (MMI) i.e. interference of fundamental modes and excited higher order modes in the NCF, to detect changes in refractive index induced by Ni^{2+} adsorption on the functionalized sensor. Wavelength shifts were measured real-time for the continuous monitoring of adsorption of Ni^{2+} at different concentrations. The proposed sensor exhibits a linear response in the concentration range upto 500 μM , with a Ni^{2+} detection sensitivity of 0.0554 nm/ μM and concentration detection limit of 0.1671 μM .

The second work presents an interferometric fiber sensor for detection of hexa-

histidine tagged microcin (His-MccS). This intermodal fiber sensor is implemented by a no-core fiber (NCF) functionalized with chitosan (CS)-nickel (Ni) film for direct detection of small peptide: microcin. The fiber intermodal sensor relies on the refractive index modulations due to selective adsorption event at the chitosan (CS)-nickel (Ni) film. Owing to the strong affinity between Ni^{2+} ions and histidine, the immobilized Ni^{2+} ions in the chitosan film were utilized as binding agents for the direct detection of hexa-histidine tagged microcin. A comparative study in relation to different target size was conducted: full proteins trypsin, bovine serum albumin (BSA) and human serum albumin (HSA), with high histidine content on their surface and His-MccS (peptide, 11.6 KDa), have been employed for sensor evaluation. Results have shown selectivity for His-MccS relative to trypsin, BSA and HSA. The most telling contribution of this study is the fast detection of small biomolecule His-MccS compared to standard detection procedures like SDS-PAGE and western blot. The proposed sensor exhibits His-MccS detection sensitivity of 0.0308 nm/(ng/ml) in the range of (0-78) ng/ml with concentration detection limit of 0.8368 ng/ml.

The third work involves an inline reflection mode intermodal sensor based on direct ligand immobilization for detection of nickel ions (Ni^{2+}). Covalently immobilized nickel specific ligand, Meso-Tetra(4-carboxyphenyl)porphine, on the NCF surface serves as adsorption site for Ni^{2+} and can induce ambient refractive index (RI) change around the NCF on occurrence of binding events. The change in RI results in spectrum wavelength shifts, which was measured for continuous

monitoring of Ni^{2+} concentration. The proposed sensor was observed to exhibit a sensitivity of 0.1210 nm/ μM towards Ni^{2+} ions.

The final work demonstrates the use of molecularly imprinted chitosan functionalized PCF based MZI interferometric sensor for the detection of Ni^{2+} ions. The chitosan was crosslinked with epichlorohydrin (ECH) to increase its mechanical strength and thereby to improve the sensor stability. The sensor exhibits a Ni^{2+} detection sensitivity of 0.0604 nm/ μM in the linear range and a limit of detection of 0.2008 μM . The sensor also shows a good specificity to Ni^{2+} ions compared to Cu^{2+} , Ca^{2+} , and Na^+ ions. Investigation was carried out to examine the effect of crosslinking on the sensor performance by varying the ECH to chitosan molar ratio (5:1, 10:1, and 15:1). It is observed that the sensor achieved its best performance when the molar ratio was 10:1.

Chapter 1

Introduction

1.1 Motivation & Objectives

Research on polymers for sensing applications has been gaining tremendous amount of interest in the recent years. Polymers possess desirable properties like their ability to be coated easily and effectively on solid substrates and the facile methods of chemical modifications that can be performed on them to tailor certain desirable properties.

Polymers can be broadly classified as synthetic and natural polymers. Natural polymers occur in nature, are biodegradable and biocompatible, and can be imparted desirable sensing characteristics through chemical modifications. Some of the natural polymers are proteins, DNA and polysaccharides like cellulose, agarose and chitosan. On the other hand synthetic polymers are man-made polymers. Synthetic polymers have been extensively used in the development of sensors as different monomers can be chemically incorporated to synthesis polymers with a broad range of chemical, physical and biological properties.

However with the emphasis shifting towards the development of non-polluting and eco-friendly smart polymers, natural polymers have been gaining a considerable amount of interest in sensing applications. Among natural polymers, chitosan has attracted significant attention owing to its properties like film and diaphragm

formation, low optical loss, chelation of heavy metal ions, response to physical stimuli like pH, temperature and humidity, biocompatibility and biodegradability. These attributes make chitosan a viable alternative to synthetic polymer based chemical sensors for sensing of chemical and biological species.

There are different types of sensors like electrochemical sensors, resonant sensors, semiconductor sensors and optical sensors. Among optical sensors, inline interferometric sensors have gained considerable amount of interest owing to their favourable attributes like compact size, high dynamic range, good sensitivity and stability. Hence development of chitosan integrated inline fiber optic interferometric sensors for detection of heavy metal ions and proteins would be the focus of this thesis.

1.2 Thesis Organization

In Chapter 2, literature survey leading to the research is presented. The properties of the natural polymer chitosan are introduced and different types of optical sensors are reviewed, followed by a brief discussion on the sensing principles of various types of in-line fiber optic interferometric sensors.

In Chapter 3, a chitosan/PAA (polyacrylic acid) bilayer based intermodal sensor is introduced for detection of Ni^{2+} ions. Sensor fabrication and sensing principles are discussed in details, and experimental data are presented in terms of sensitivity, specificity, and stability and response times.

Chapter 4 focuses on the development of chitosan-nickel film based interferometric sensor for detection of hexa-histidine tagged proteins. This chapter presents the characterization of the thin film, functionalization process, the sensitivity and specificity of the sensor to histidine tagged proteins, response time of the sensor and reusability of the sensor.

Chapter 5 introduces a new polymer free fiber optic interferometric sensor for the detection of Ni^{2+} ions. Even though chitosan possess many desirable characteristics that enable it to be used for heavy metal detection, chitosan/polymer based sensors suffer from instability issues as detailed in chapter 2. An alternative to the polymer based heavy metal ion sensors is discussed and a facile method for the immobilization of a metal specific ligand on the sensor substrate through silane coupling is presented and the performance of the sensor is discussed.

Chapter 6 discusses the design and implementation of a photonic crystal fiber (PCF) Mach Zehnder interferometer (MZI) functionalized with metal ion imprinted chitosan and crosslinked with epichlorohydrin (ECH) for the detection of heavy metal ions. Crosslinking of chitosan could be used to counter the instability problem associated with chitosan based sensors but this could lead to a loss in the performance of the sensor due to the loss of metal chelating amine groups due to crosslinking. Hence a method is presented for the surface functionalization of a metal ion imprinted chitosan crosslinked with ECH on the PCF sensor and the sensor's performance in terms of sensitivity, limit of detection and specificity and regeneration are presented and discussed.

Chapter 2

Literature Survey

Polymers are macromolecules made up of connecting identical structural units (monomer) together through a process known as polymerization. The structure of polymer chains depends on the polymerization method and the structure of the monomer.

There are two main categories of polymer: synthetic and natural. Synthetic polymers are man-made polymers synthesized by polymerization process using chemical substances. Nylon, Teflon, poly propylene and poly vinyl chloride (PVC) are some of the common synthetic polymers. On the contrary natural polymers are widely found in nature (plants and animals) and are formed by condensation reactions of the monomers, with water being the by-product. Nucleic acids and proteins are natural polymers found in humans whereas cellulose is another natural polymer, which is the main structural component of plants.

Synthetic polymers have dominated a large part in sensing industry. The particular reasons that make them attractive for sensing applications are relatively low cost and able to gain a specific sensing behavior on its surface region or into bulk material through side chain modification. Moreover, synthetic polymers can be easily deposited as a sheet or thin/thick film into inorganic solid state devices. The deposition methods include: casting, photolithography, spin coating,

electrochemical, vacuum deposition (sputtering, plasma), Langmuir-Blodgett method, etc.

2.1 Chitosan

Chitosan is generally found in nature as a macromolecular compound called chitin. Chitin was discovered by a French professor Henri Braconnot in 1811, by repeated chemical treatment of mushroom with a dilute alkali solution and he named it as fungine. The name chitin was coined by Odier. J. L. Lassaigne proved that chitin is not cellulose by confirming the existence of nitrogen and later presence of glucosamine and acetic acid was identified by G. Ledderhose through hydrolysis. Further research confirmed that chitin was made of repeating units of N-acetylglucosamine. F. Hoppe-Seiler confirmed that chitin could be deacetylated into a new product by boiling chitin in an alkali solution and named this product chitosan [1].

The chemical structures of chitin and chitosan are presented in Figure 2-1. Chitin consists of repeating units of N-acetyl-D-glucosamine and deacetylation of chitin leads to formation of chitosan. The removal of acetyl groups from chitin results in amine groups. The chemical structures of chitosan and chitin are similar to cellulose except that C-2 position hydroxyl group of cellulose is replaced by acetyl group in chitin and amino group in chitosan. Comparing to chitin, chitosan is more efficient in adsorption capacity due to the present of amino groups on polymer chain.

Figure 2-1: Chemical Structures of (a) chitin and (b) chitosan

Generally if the degree of deacetylation (DD) of chitin is more than 60% and it can dissolve in weak acid, it can be called chitosan [1]. Physical and chemical properties of chitosan largely depend on its molecular weight and degree of deacetylation [2]. Chitosan ($[\text{C}_6\text{H}_{11}\text{O}_4\text{N}]_n$) is receiving a great deal of interest in

biomedical applications such as immobilization matrix or scaffold, artificial vessels/skin as well as a drug carrier due to its biocompatibility and non-toxicity properties.

2.1.1 Cationic polyelectrolyte

Many properties of chitosan can be explained by the presence of primary amine and hydroxyl groups. Chitosan has a pK_a value of around 6.5 and thus at lower pH (<6.5), the amines will be protonated and acquire positive charge, rendering polycationic behavior to chitosan. Degree of deacetylation and pH value dictates the charge density of chitosan. At high pH (>6.5), de-protonation of amine groups occurs causing chitosan to lose charge and become insoluble.

This cationic property of chitosan enables it to be applied as an agent that can be used with other chemical species like metal anions, dyes, etc. to improve coagulation and as a filtration membrane for the removal of contaminants from water [3, 4].

2.1.2 Film forming ability

One of the most fascinating attribute of chitosan is its ability to form films and membranes easily. Chitosan solutions are highly viscous and hence can be cast into films and membranes possessing good mechanical properties and are permeable. The chitosan films/membranes have been utilized in various fields such as membrane separation, food packaging, and biomedical applications.

The amine and hydroxyl functional groups form inter and intra molecular bonds between them, thereby contributing to the formation of chitosan films. The mechanical properties of the film can be altered by manipulation of degree of deacetylation (DD), molecular weight, pH of solvent, and type of solvent. Based on these parameters it is found that tensile strength of chitosan films can be altered between 7 to 150 MPa [2, 5]. Higher molecular weight chitosan has relatively better tensile strength compared to lower molecular weight chitosan. This can be attributed to the presence of greater amount of amine and hydroxyl functional groups, resulting in stronger inter and intramolecular hydrogen bonds [6]. Chitosan films from higher DD possess greater positive charge density compared to films prepared from lower DD chitosan. This results in chain flexibility reduction and renders the film crystalline, resulting in greater tensile strength [5].

Chitosan is insoluble in water and dissolves in weak acids. The acid type also influences the mechanical properties of films. Chitosan dissolved in acetic acid solution forms dimers, which indicates relatively strong intermolecular interaction. Thus chitosan films cast from solutions with acetic acid as solvent had greater tensile strength than those from other acid solutions [6]. The Young's modulus of chitosan is in the range between 2 to 6.3 GPa [7], which implies that chitosan has relatively better elasticity compared to polyurethane (Young modulus value around 70 GPa) which is a commonly used sensing diaphragm [8]. These mechanical properties of chitosan make it a suitable candidate for acoustic and pressure sensing.

2.1.3 Swelling effect

Chitosan is classified as a hydrogel due to the presence of a large number of hydrophilic functional groups resulting in high affinity for water and at the same time it doesn't dissolve in water due to inter and intra molecular bonding between the functional groups. Hence water molecules can penetrate through the polymer matrix causing chitosan to swell. Chitosan hydrogel can be made into four generic forms (1) Three dimensional gel, (2) small particles or micro gel, (3) brush layer and (4) thin hydrogel film on a solid substrate. Among these, rate of swelling is higher in thin hydrogel film due to smaller dimensions and sponge-like nanostructure [9].

As seen earlier, charge density of amine function groups of chitosan depends on the pH of the solvent. At lower pH (< 6.5), the amine groups in the film are protonated and hence repel each other resulting in the swelling of chitosan film. This environment pH-dependent swelling effect has been exploited to facilitate a range of applications such as target drug release and hybrid hydrogel [10, 11]. In all, both humidity and pH sensing can be realized by using the swelling effect of chitosan.

2.1.4 Optical properties

Polymeric materials have relatively low optical loss at communication wavelengths of 830 nm, 1300 nm and 1550 nm and thus aid using them with optical devices. Among all the polymeric materials, research using chitosan has drawn great attention and is not restricted to areas such as waste water treatment and medical

applications, but also in optoelectronics [12] and as a on-chip waveguide/sensing material [13].

The optical loss value of chitosan was reported to be less than 0.5 dB/cm and it also possessed good thermal stability, thereby making chitosan film a suitable material for optoelectronic applications [12]. Nosal et al. (2005) studied the optical properties of chitosan film over a wide range of wavelengths and values of adsorption and refractive index were verified [14, 15].

Koralewski et al. (2006) studied optical behavior of chitosan in aqueous solution in terms of refraction, optical rotatory dispersion (ORD) and Faraday effects and found degree of deacetylation weakly influenced refractive index and the specific rotation $[\alpha]$ varied with concentration of chitosan solution and induces levorotatory effect in the visible range [16].

Chitosan's low adsorption in optical windows and the tailoring of refractive index of chitosan film by introducing metal ions into the film [17] or optically active nanocrystals [18], make it an attractive alternative as optical material for a variety of applications.

Advances in chitosan deposition technique utilizing photolithography and etching have resulted in integration of chitosan film on lab-on-a-chip devices. Most of these devices are based upon detection techniques involving the adsorption of light upon binding of analyte onto chitosan film, which results in alteration of the light intensity. A micro fluidic optical sensing system with an electrodeposited chitosan film has been reported and is based on the change of output spectrum by binding of

analyte onto the chitosan film [19]. These sensors based on laboratory optical analyzing tools are relatively sensitive and reliable in comparison to other types of polymer based sensors. However, they are usually associated with expensive equipment, high fabrication cost and time-consuming preparation steps. Therefore, there is a growing need for the development of a robust, real time and low cost optical detection technique, which is the main objective of this thesis.

2.2 Sensing devices based on responsive polymers

Design and fabrication of a highly sensitive physical transducer is paramount for effective transformation of the polymer's chemical or physical changes into measurable signals. Table1 illustrates the typical transducers that can be used to detect the physical/chemical changes in a polymer due to external stimuli.

Sensor Type	Sensing Device	Sensing Principle	References
Resonant sensors	Quartz Crystal Microbalance (QCM)	Small mass changes of the quartz crystal due to chemical/biological induced changes in the responsive polymer modulates the resonant frequency of the crystal	[20-22]
	Micro Electro Mechanical Systems (MEMS)	Transduction of mechanical energy in the form of movement or deformation of micro-machined	[23-25]

		parts to an electronic or optical readout reporting a change in the physical, chemical or biological environment.	
Semiconductor based sensors	Ion-Sensitive Field Effect Transducer (ISFET)	Gate material replaced by ion selective polymer. Binding of a charged target analyte modulates the gate voltage thereby inducing a change in the current flowing between source and drain.	[26-28]
Electrochemical cell based sensors	Potentiometric Sensors based on ISE (ion selective electrodes)	Target analyte causes a change in the potential difference between the ISE and reference electrode.	[29-31]
	Amperometric sensors	The change in concentration of target analyte causes a change in current between the sensing and counter electrode.	[32-34]
	Conductometric sensors	Change in analyte concentration induces a change in the electrolyte conductivity of the measuring cell.	[35, 36]

Table 2-1: Sensing devices based on responsive polymers

2.3 Optical sensors

Traditional optical sensors are based on spectroscopy techniques such as Fourier Transform Infra-Red (FTIR) spectroscopy, Ultra-Violet-Visible (UV-Vis) spectroscopy, Raman spectroscopy and fluorescent measurement. In these devices a guided light from a light source interacts with the analyte and is then captured by a receiver either as absorbance, transmittance, fluorescence and Raman scattering and is further processed to provide required information.

Chitosan film based optical devices have been reported and experimentally demonstrated. Chitosan films are used as an immobilization matrix or as a selective coating in optical sensors for chemical and biosensing. Binding of target analyte (proteins, metal ion etc.) to chitosan film alters optical/physical properties of chitosan film. This alteration can be measured as optical absorbance, change of mass or electrical conductivity which can be detected by the sensors.

Among the optical sensors, Surface Plasmon Resonance (SPR) sensors have been used frequently and have been successfully commercialized. SPR method is based on the measurement of reflectance from the metal-coated surface. At a critical angle, excitation of surface plasmon occurs and the excited plasmon propagates along the metal surface, which results in a dip in the reflectance. The target analyte detection depends on change of dielectric profile of metal surface and can be inferred from measured parameters (minimum value of reflectivity, critical angle and resonance curve's width). SPR sensors for detection of heavy metal ions has

been reported. Fabrication of the sensor involves depositing crosslinked chitosan thin film onto gold layer on the SPR substrate. The sensitivity of this sensor can be attributed to chelating property of chitosan film and it binds metal ions from the sample. This leads to a change in the resonance angle, which is the measured parameter [37-40].

2.3.1 Fiber optic sensors

Fiber optic sensors (FOS) represent a new generation of optical sensors. Low cost of the optical fiber, advanced laser technology and spectroscopy principles serve as the basis for the development of these sensors. In FOS sensing system, the optical fiber is commonly functionalized with a polymer layer [41] sensitive to external stimuli, on its surface or tip to enhance sensitivity and selectivity. External stimuli can result in variation in refractive index, adsorption or reflection coefficient in the sensitive layer. This in turn will modulate amplitude, phase velocity, wavelength and polarization characteristics of the transmitted or reflected light propagating in the fiber. The external stimuli can be determined and quantified by using a suitable demodulation technique.

In this work, the FOS is chosen as a physical transducer to implement an environmental sensor for detection of heavy metals (Nickel in the current work). The reasons for this are, FOS are immune to electromagnetic interference as no electrical current flows at the sensing point and they are able to withstand in harsh environment such as high temperature and high doses of radiation. Furthermore, the sensors are compact, lightweight, and able to guide light of different

wavelength at the same time. This last attribute allows multiplexing of many sensors on a single fiber network thereby enhancing throughput and resulting in a highly parallelized sensing system [42].

2.3.2 Fundamental of an optical fiber

The basic element of fiber-optic sensors is optical fiber and this section is dedicated to the understanding its transmission characteristics.

Figure 2-2: Basic structure of an optical fiber.

As shown in Figure 2-2, an optical fiber has a cylindrical central core, a cladding and an outer coating (Jacket) to protect the fiber from physical damage and moisture. For a standard single-mode fiber (SMF), the diameter of the core is around $8\text{ }\mu\text{m}$ and the core refractive index is around 1.465 and cladding refractive index is 1.45. The fiber cladding is usually made up of pure silica and the core is silica doped with germanium. Doping is done to make refractive index of the core

n_0 higher than the cladding n_1 . If incident angle θ_i at the internal core-cladding interface is greater than the critical angle θ_c light is confined in the core and propagated along the fiber by total internal reflection. Light rays which satisfy Eq. (2.1) are guided rays, and the remainder is leaky rays which leak to the surrounding and are lost.

$$\theta_i > \theta_c = \sin^{-1}\left(\frac{n_1}{n_0}\right) \quad (2.1)$$

Transmission of light in optical fibers can be described by the mode theory which considers electromagnetic wave behavior to describe the propagation of light along a fiber. The mode theory proposes that a light wave can be represented as a plane wave whose surfaces constant (electric field distribution) does not change and normal to the direction of propagation. The parallel planes propagate with same phase are defined as wavefronts as shown in Figure 2-3.

Figure 2-3: Light Propagation in optical fiber

As the wavefront propagates along the fiber, it undergoes a gradual phase change due to reflections at the core/cladding interface. If interfering wavefronts are out of phase relative to each other, they will eventually vanish due to destructive

interference. Thus interference is the reason for a finite number of modes that can propagate along the fiber. Therefore, in order to propagate along fiber axis, the incident angle of wavefront must be greater than the critical angle and the guided rays must remain in phase after multiple reflections from the interface.

2.3.3 In-line fiber-optic interferometric sensors

The field of FOS is very vast with a number of configurations and sensing techniques that can be employed to detect a wide variety of measurands. Grating-based FOS such as fiber Bragg gratings (FBG) or long-period gratings (LPG) is the most widely used configuration [43]. The principle of detection is based on resonant wavelength shifts due effective refractive indices change of cladding modes with the change in measurand. Extensive improvements of these grating-based FOS have led to them being commercialized. Another important configuration is Surface Plasmon Resonance (SPR) FOS and Liedberg *et al.* [44] first demonstrated its application for biosensing in 1983. Since then it has been extensively explored and has become a reliable label-free detection methodology for biosensing.

Another sensing scheme that exhibits a remarkable performance is the fiber-optic interferometer. In addition to general advantages of FOS such as EMI immunity and high stability, it also offers a large dynamic range and high sensitivity [45, 46]. However, owing to the high cost of optical components or complex sensing element, they are not so far commercialized.

In recent years, “In-line” fiber optic interferometer also known as all-fiber interferometer has attracted intensive attention for their potential sensing applications. This type of sensor allows compact structure, low cost, easy fabrication and spatial coherence as it is constructed mainly by single mode fiber. On top of that, since in-line interferometric sensing systems are mostly made up of optical fiber, so the traditional bulky optic component such as lenses and beam splitters/combiners can be replaced by miniature fiber devices which allow the interferometer to be employed for micro-scale applications and increase its chance in commercialization.

The detailed description and working principles of different types of in-line fiber-optic interferometers will be presented in the following sections.

2.3.3.1 Interferometry principles

Interferometric FOS detection is based on the change in the optical path length or polarization properties of the optical fiber induced by measurand change. Assuming a well-defined wide band spectrum for the optical source, the resultant wavelength- depended electric field $E^l(\lambda)$ after passing through a interferometer can be expressed as [47]:

$$E^l(\lambda) = P(X, \lambda)E(\lambda) \quad (2.2)$$

where $E(\lambda)$ is the electric field from the light source, $P(X, \lambda)$ is the propagation matrix describing the sensing element of the interferometer in which X represents the external stimuli such as stress, pressure, strain or refractive index.

In-line interferometers have been used for a wide range of measurands like pressure, temperature, strain, magnetic field and biological events due to its simple configuration, fast response, wide dynamic measuring range and immunity to electromagnetic interference. Based on their configuration in-line interferometers can be categorized in to four types namely: Fabry-Perot (FP), Mach-Zehnder (MZ), Michelson (MI) and Sagnac. In the following sections, each of the above mentioned sensors is analyzed by means of configuration and working mechanism.

2.3.3.2 Fabry-Perot interferometer

Fabry-Perot interferometer (FPI) usually consists of a cavity separating two parallel reflecting surfaces. Interference signal is generated due to superposition of multiple reflected/transmitted beams at these reflecting surfaces. FPI based on fiber can be divided into two major categories: Extrinsic Fabry-Perot interferometers (EFPI) Figure 2-4 (a) and Intrinsic Fabry-Perot interferometers (IFPI) Figure 2-4(b). For EFPI, the air cavity is usually constructed by a supporting structure and compare to the IFPI, the fabrication is relatively simple and low cost in comparison to the IFPI, but it suffers from the low coupling efficiency and alignment problem [48, 49]. On the other hand, the IFPI has a high coupling efficiency as reflecting components are formed within the fiber itself. Most of the in-line Fabry-Perot interferometers are considered as IFPI because the cavity is formed within the fiber. The fiber cavity can be fabricated by any means such as Fiber Bragg Gratings (FBG) [50], micro machine [51], chemical etching [52] or thin film deposition [53].

Figure 2-4: (a) Extrinsic Fabry Perot Interferometer & (b) Intrinsic Fabry Perot Interferometer

The Fabry-Perot interferometer is considered as a multiple beam interferometer as the light beams go through multiple transmission and reflection within cavity by two reflecting surfaces. Assuming incoming light is linear polarized and air cavity is a non-birefringence material. When all the beams are interfered, the total accumulated phase ϕ in Eq. (2.3) will only be influenced by the physical path difference, so ϕ_I will be a dominant effect and can be expressed as:

$$\phi = \phi_I = \frac{2\pi}{\lambda} 2nd \cos \theta \quad (2.3)$$

where d is the length of the cavity, n is the refractive index of cavity material, λ is the operating wavelength and θ is the incidence angle of light at the reflecting surfaces. Assuming the reflectivity of the two mirrors are the same, the transmittance function can be given as [54]:

$$T = \frac{1}{1 + F \sin^2 \frac{\phi_I}{2}} \quad (2.4)$$

Where, F is the coefficient of fineness which characterizes phase resolution and is determined by the reflectivity of the reflecting surface R :

$$F = \frac{4R}{(1 - R)^2} \quad (2.5)$$

Therefore, T is a function of ϕ_I and R . When perturbation is introduced to the Fabry-Perot interferometer, it will induce physical path difference and the phase will be modulated. Therefore, by measuring the wavelength spectrum shift, the applied perturbation can be derived quantitatively. The free spectral range (FSR) is the space between two adjacent interference peaks and is influenced by the path difference; a shorter path difference allows larger FSR which will result in a wide dynamic measuring range to the interferometer [55]. On top of that, the higher the reflectivity of the reflecting surfaces, the greater the number of circulations within the cavity can lead to the enhancement of the visibility (contrast) of the spectrum as well as the phase sensitivity.

2.3.3.3 Mach-Zehnder interferometer

Similar to FPI, the fiber type Mach-Zehnder interferometer is also considered as a two beam-interferometer with a simple example shown in Figure 2-5. It consists of two optical couplers to function as beam splitter/combiner. The light is coupled

into a single mode fiber and is amplitude divided into two fiber arms: reference arm and sensing arm by the first optical coupler. After travelling through two separated arms, the divided two beams are recombined by the second coupler and the interference signal is captured by the detector. The measurand modifies the path of the signal beam, whereas the reference light beam experiences a constant environment. Since measurand directly influences physical path in sensing arm, so the phase modulation of ϕ_I is a dominant effect, which in turn, modulates the intensity of interference spectrum expressed by Eq.(2.6) [54].

Figure 2-5: Mach-Zehnder Interferometer

$$I(\lambda) = I_S + I_R + 2\sqrt{(I_S I_R)} \cos(\Delta\phi_I) \quad (2.6)$$

where I_S and I_R are the intensities of the sensing beam and reference beam, $\Delta\phi_I$ is the phase variation between two beams due to change in physical path in sensing arm. Thus, the physical parameter can be measured easily by detecting the phase shift of the output spectrum.

Figure 2-6: Different configuration of In-line MZI: (a) LPG, (b) core mismatch, (c) collapsing, (d,e) core mismatch, (f) tapering.

The two arm MZI have been replaced by in-line MZI, in which beam splitter/combiner are integrated in one fiber, so the two optical couplers are no longer required increasing robustness of the interferometer for micro-scale sensing. The in-line beam splitter/combiner can be formed by various methods as shown in Figure 2-6 (a-f). The first one illustrated in Figure 2-6(a), employs Long-Period Grating (LPG) pairs as the beam splitter/combiner. The LPG is a periodic modulation of refractive index in fiber core induced by CO₂ laser or mechanical

stress which is well known for its core/cladding coupling ability. The first LPG couples incident light from the core modes into cladding modes and the coupled cladding modes propagate in the cladding are then recoupled back to the core by the second LPG and interfered with the core modes. In this type of in-line MZI, the reference arm and sensing arm have equal physical lengths but different optical path difference (OPD) due to difference in refractive index between core and cladding.

Another technique of splitting a light beam into core and cladding modes is by fiber core mismatch Figure 2-6(b). In this approach, two fiber are fusion spliced with an intentional offset along the x-axis, so that fraction of the light propagating in the lead-in fiber leak to the cladding of the second fiber through the misalignment. The propagating cladding modes will be collected by another offset-spliced fiber and interfered with the core modes. The MZI make from core mismatch technique is cost-effective comparing to the LPG technique and the number of cladding mode can be controlled by amount of offset.

In-line MZI can also be fabricated by splicing fiber with a section of photonic crystal fiber (PCF) Figure 2-6(c). PCF is made of pure silica with air-hole cladding material running along the fiber core. By collapsing the air-hole structure of the PCF, the core mode beam in a PCF is expanded into cladding modes. In-line MZI make from this way is rather easy as it does not involve high cost equipment or troublesome aligning process and exhibits low temperature cross sensitivity owing to its pure silica structure. However, the insertion loss is relatively high in comparison to the core mismatch technique.

Another method to fabricate in-line MZI is splicing the lead-in fiber with a fiber having different core size as shown in Figure 2-6(d, e). Figure 2-6(d) involves a two short sections of multimode fiber (MMF) spliced with a single-mode fiber (SMF) while Figure 2-6(e) involves a small core fiber sandwiches between two SMFs. By both ways, the core modes are dispersed at the splicing region due to mode-field diameter mismatch and spread out into the core and cladding which then couple back at the following splicing region.

The coupling of the light from core into cladding can also be achieved by using fiber tapers as shown in Figure 2-6(f). The fiber taper is fabricated by heating to fiber's softening temperature follow by pulling. If taper angle is large enough, the coupling effect takes place. The fiber taper method offers easy construction but it suffers from weak mechanical strength and usually more than one cladding mode are excited, hence, the interference will be distorted due to contributions from multiple waves.

2.3.3.4 Michelson interferometer

The Michelson interferometer (MI) is also a two-beam interferometer and closely related to MZI in term of structure, but the operation principle is based on the reflection mode. Figure 2-7 (a-b) shows the examples of a conventional fiber type MI and in-line MI. The MI is much like a half of the MZI in configuration but the light beams at the reference and sensing arm are terminated at the reflector(s), so that they are folded back on themselves to recombine at the same optical couple or in-line splitter/combiner (eg. LPG). Since the in-line MI is very similar to the in-

line MZI and the main differences are existence of the reflector(s) and with only one in-line splitter/combiner.

Figure 2-7: (a) Conventional Fiber type Michelson Interferometer (MI) & (b) Inline MI

As in-line MI operates in reflection mode, they are more compact than the in-line MZI and exhibits relatively high sensitivity as the light beam double passes the sensing arm. However, the reflected light beams feedback into the light source will cause instability, so it is usually necessary to incorporate an optical isolator between the light source and optical coupler [81]. Further, the light beams reflected from the reflector (mirror) might not have the same polarization state when they combine at the in-line splitter/connector. This polarization mismatch may cause

significant reduction of the visibility of interference signal and hence, degrades the performance [56].

2.3.3.5 Sagnac interferometer

Figure 2-8: Sagnac Interferometer

Another two-beam in-line interferometer is Sagnac interferometer (SI) which consists of an optical fiber loop as shown in Figure 2-8. A light beam is split by the 3-dB optical coupler and propagates in counter direction along the fiber loop. After that, they recombine again at the same splitter and interfere.

Unlike other two-beam interferometers, the beams travel in counter direction experience the same physical and optical path, so the phase difference between the beams would always be zero. Therefore, in order to provide OPD in the fiber loop, birefringent fiber is usually integrated into the Sagnac loop. The birefringent fibers are anisotropic fibers that display two distinct refractive indices in two orthogonal principle axes which are described as the fast (lower refractive index) and slow axes (higher refractive index), referring to the phase velocity of the light travelling within them. As a result, the linearly-polarized light beams are polarized with

different velocity along the slow axis and the fast axis, yielding the OPD and the modulation of the phase term ϕ_2 . The phase ϕ_2 modulation induced by this type of in-line interferometer is given in Eq.(2.7):

$$\phi = \phi_2 = \frac{2\pi}{\lambda} BL, B = |n_f - n_s| \quad (2.7)$$

where B is the birefringence which is a function of n_f effective index of the fast axis and n_s effective index of the slow axis, L is the length of birefringent fiber. In general, the integrated birefringent fiber is utilized as a sensing fiber as any external perturbation will easily induce the variation in the B of the birefringent fiber, so the external perturbation can be quantified by measuring the phase shift of the wavelength spectrum.

2.4 Chapter conclusion

In this chapter, various properties (mechanical, optical and biological) of chitosan have been reviewed, which suggests a high potential for chitosan to replace synthetic polymer as a sensing element in mechanical, chemical, environmental and biological sensing applications. To develop chitosan based sensor, sensing device structures that can be employed to detect the changes in the properties of polymer film are studied and evaluated. The in-line fiber-optic interferometric sensors have attracted much attention due to its high performances in term of flexibility, sensitivity and stability. Chitosan based interferometric sensors have been previously reported for acoustic sensing [57], humidity sensing [58] and immunosensing [59, 60]. The acoustic sensor combines the diaphragm forming

ability of chitosan to a FPI sensor to sense changes in acoustic pressure and also for ultrasound measurement. The humidity sensor is based on chitosan's ability to absorb water and swell in an aqueous environment. Immunosensors were based on antibody (immunoglobulin, IgG) immobilization to a sensor functionalized with chitosan/polysodium styrene sulfonate. The immobilization was achieved through electrostatic attraction and hydrophobic interactions and the sensor was subsequently used for detection of anti-immunoglobulin G (anti-IgG). All these sensors are based on the physical property of Chitosan like diaphragm forming ability and polycationic property of chitosan to bind proteins through electrostatic interactions. Chitosan possesses another important property namely chelation of heavy metal ions and the focus of this thesis would be on developing chitosan based optical fiber interferometric chemical sensors for detection of heavy metal ions and extending this metal binding property of chitosan for developing optical fiber interferometric sensors for the detection of histidine tagged proteins.

Chapter 3

Chitosan/PAA based fiber-optic interferometric sensor for heavy metal ions detection^{*}

3.1 Introduction

Contamination by heavy metals is very relevant from health point of view due to their toxicity and carcinogenicity [61]. These effects include central nervous system disorders (Hg^{2+} , Pb^{2+} , As^{3+}), kidneys or liver disorders (Cu^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+}), disorders in skin or bones (Ni^{2+} , Cu^{2+}). Hence, monitoring the concentration of these species in water bodies is imperative and new sensing strategies are consistently being developed for heavy metals detection [62-66].

Miniature fiber optic sensors are being developed as viable alternatives for these applications. Use of fiber optic sensors is intensively researched due to their unique characteristics such as multiplexing, remote sensing, high flexibility, high sensitivity, low cost, and immunity to electromagnetic interference etc. [45, 67, 68]. Surface plasmon resonance based fiber optic sensors have been reported for detection of manganese [69] and cadmium ions [39]. Fiber optic heavy metal ion sensors based on surface enhanced raman scattering (SERS) (Cd^{2+}) [70] and

^{*}© 2016 Elsevier. Adapted, with permission, from Raghunandhan, R., et al., *Chitosan/PAA based fiber-optic interferometric sensor for heavy metal ions detection*. Sensors and Actuators B: Chemical, 2016. **233**: p. 31-38.

fluorescence (Cu^{2+}) [71] have also been reported. Among the different types of fiber optic sensors, intermodal interference based sensor has gained a great interest, owing to its high stability, compact structure and large dynamic range [45]. Interferometers in general rely on the interference of light beams in the sensing and reference arm for sensing changes in the external stimuli. In the case of inline fiber optic interferometric sensors since the optical fiber acts as both sensing and reference arms and interference spectrum is due to the interference of fundamental and higher order modes, these type of sensors are also called as intermodal sensors. The fiber optic intermodal interferometer can be categorized into four groups namely: Fabry-Perot (FPI), Sagnac (SI), Mach Zehnder (MZI) and Michelson interferometers (MI). Among all of these configurations, Fabry-Perot, Michelson and Sagnac interferometers are usually associated with extensive fabrications and require additional components such as an optical circulator or coupler to guide the reflected light [58, 59]. On the contrary, the intermodal Mach Zehnder interferometer involves a rather simple and robust fabrication process as no coupler/circulator is required.

In this paper, a new intermodal Mach Zehnder interferometer for Ni^{2+} sensing is proposed. The sensor was fabricated by splicing a NCF in between standard single mode fibers and functionalized with chitosan (CS) / poly acrylic acid (PAA) multilayer film to facilitate Ni^{2+} ions adsorption on the sensor for detection. The functionalization was carried out by Layer-By-Layer (LBL) assembly (also known as electrostatic self assembly), which is one of the effective methods for

functionalization of fiber surface [60, 72, 73]. The simplicity of the process and controllable nanoscale coating make this a preferred process [60]. Protonation of free amino groups makes chitosan cationic when dissolved in organic acids, while PAA is anionic when dissolved in deionized water due to the presence of carboxyl groups. Therefore, multilayer film [CS/PAA]_n (where n is the number of bilayers) can be formed on the fiber surface through electrostatic interaction. This pair of polyelectrolytes provides ample chelating sites for direct Ni²⁺ adsorption [74] on the fiber surface. For sensor evaluation, the spectrum shifts due to the binding event of various Ni²⁺ concentrations on the sensor was monitored in real time and the Langmuir isotherm model was used to analyze the binding kinetics.

3.1 Materials and methods

3.2.1 Materials

Optical fibers including single mode fiber (8.2/125 μm) (SMF-28) and no core fiber (125 μm) (NCF) are purchased from Corning and Prime Optical Fiber Corporation respectively.

Chitosan (medium molecular weight), polyacrylic acid (PAA), 2-(N-morpholino) ethanesulfonic acid (MES) and anhydrous nickel chloride salt are purchased from Sigma Aldrich. Sulfuric acid (95%) was purchased from Merck and hydrogen peroxide (30%) was purchased from VWR. The reagents used were of analytical grade. Ultrapure water (18.2 MΩ cm resistivity) from Mill-Q system was used in preparing the solutions.

3.2.2 Methods

3.2.2.1 Sensor fabrication

To fabricate the sensor, a 20 mm segment of NCF (Prime Optical Fiber Corporation) was spliced in between SMF (8.2/125 μm SMF-28) by using an electric arc splicer (Type 39, Sumitomo) to form an intermodal Mach Zehnder interferometer.

3.2.2.2 Sensor functionalization

To functionalize the sensor, 2% chitosan solution was prepared by dissolving 2g of chitosan in 4% acetic acid solution and 8% PAA solution was prepared by diluting 35% PAA solution in Milli-Q water (Millipore). The solutions were continuously stirred for 24 h at room temperature. Prior to coating, NCF segment was treated with piranha solution (sulphuric acid (concentrated, 98%) and hydrogen peroxide (30%) in 3:1 ratio) for 60 min and sonicated with distilled water and dried under a stream of purified nitrogen gas. This procedure removed organic residues and hydroxylated the fiber surface. To carry out LBL self-assembly, the treated fiber was first dipped into chitosan solution for 5 minutes and withdrawn at a rate of 70 mm/minute by using a dip coater (KSV NIMA). The fiber was allowed to dry for 60 seconds, followed by washing with Milli-Q water. The procedure was repeated for PAA and this constitutes a full bilayer deposition cycle (Figure 3-1). Once desired number of bilayers was achieved, the functionalized fiber was then allowed to dry overnight in a vacuum oven at 60⁰C.

Figure 3-1: Bilayer functionalization procedure schematic

3.2.2.3 Experimental setup

The schematic diagram of the sensing system is presented in Figure 3-2, which consists of an Amplified Spontaneous Emission (ASE) broadband light source (center wavelength: 1570 nm), optical spectrum analyzer (OSA) (YOKOGAWA AQ6370) and our interferometric sensor. The light emitted from the ASE light source is coupled into the lead-in SMF and the lead-out SMF is coupled to the OSA.

Figure 3-2: Schematic diagram of the sensing system.

3.3 Experimental results and discussion

3.3.1 Sensing principle

NCF is a high-precision pure silica fiber with an undoped core and no cladding and has been widely utilized in the fabrication of heterocore structures for sensing of refractive index [75] and humidity [76]. The schematic diagram of the sensor is shown in Figure 3-3. As light propagates, the fundamental mode in the lead-in SMF will be excited to higher order modes in the NCF region and the interference between fundamental and higher order modes is then coupled back by the lead-out SMF.

Figure 3-3: Schematic diagram of sensor.

Figure 3-4 shows the simulation performed by using BeamPROP toolbox of RSoft, which illustrates that as the fundamental mode in the lead-in SMF enters NCF, subsequent excitation of higher order modes occurs and interference between the modes is formed at the lead-out SMF. Based on the simulation, the NCF can be considered as a multimode waveguide (number of propagating modes >3).

Figure 3-4: Mode propagation in the SMF-NCF-SMF configuration.

Excitation of the higher order modes in the NCF is achieved by launching a field using the lead-in SMF and interference of propagating modes leads to the formation of either single or multiple self-images of the input field along the NCF [77]. The effective length of NCF can be calculated using (3.1):

$$L = p \left(\frac{3L_{\pi}}{4} \right) \quad p=0,1,2,3,\dots \quad (3.1)$$

where p is the self-image number and L_{π} is the beat length which can be expressed as:

$$L_{\pi} = 4 \left(\frac{n_{ncf} d_{ncf}^2}{3\lambda_d} \right) \quad (3.2)$$

where n_{ncf} is the effective refractive index, d_{ncf} is the core diameter of NCF and λ_d is spectral dip wavelength. Combining (1) and (2) the equation for spectral dip wavelength λ_d can be obtained as follows,

$$\lambda_d = p \left(\frac{n_{ncf} d_{ncf}^2}{L} \right) \quad p=0,1,2,3,\dots \quad (3.3)$$

Based on Eq.(3.3), the spectral dip wavelength λ_d is a function of effective refractive index n_{ncf} , diameter of NCF d_{ncf} and length of NCF L . Since the NCF has no cladding, evanescent waves account for a significant portion of the transmitted optical power and hence the effective refractive index n_{ncf} of the guided modes is influenced by the external refractive index [75, 78]. Hence any changes in the refractive index of the ambient environment results in a shift in the interference spectrum of the sensor as determined by (3.3).

Analysis of the sensor spectrum after functionalization with [CS/PAA]₁₀ bilayers is carried out using the equations of mode propagation. Assuming ideal alignment of the SMF and the NCF and considering the circular symmetry of input field, as light propagates from SMF to NCF only LP_{0m} modes will be excited in the NCF region. Denoting the field profile of LP_{0m} mode as $\phi_m(r)$, the NCF input field can be described by [79],

$$E(r, 0) = \sum_{m=1}^M b_m \phi_m(r) \quad (3.4)$$

where M is the number of modes excited in the NCF; b_m is the coefficient of excitation of the LP_{0m} mode and can be given by [80],

$$b_m = \frac{\int_0^\infty E(r,0) \phi_m(r) r dr}{\int_0^\infty \phi_m(r) \phi_m(r) r dr} \quad (3.5)$$

The total light field distribution at a propagation distance z in the NCF can be expressed as [81]

$$E(r, z) = \sum_{m=1}^M b_m \phi_m(r) \exp(-\gamma_m z) \exp(j\beta_m z) \quad (3.6)$$

where β_m is the propagation constant of LP_{0m} mode; γ_m is the coefficient of evanescent absorption of the LP_{0m} mode and can be calculated by [82],

$$\gamma_m = \frac{a_\lambda \lambda n_{cs} \cos \theta_m \cot \theta_m}{4\pi a n_{ncf}^2 \cos^2 \theta_c \sqrt{\sin^2 \theta_m - \sin^2 \theta_c}} \quad (3.7)$$

where λ is the free space wavelength, a_λ is coefficient of attenuation of the chitosan/PAA film at λ , n_{cs} and n_{ncf} are the refractive indices of the $[CS/PAA]_{10}$ bilayers and NCF respectively, a is the NCF radius, θ_c is the critical angle, and

$$\theta_m = \sin^{-1} \frac{\beta_m}{k} \quad (3.8)$$

where $k = 2\pi n_{ncf}/\lambda$ is the wave number.

The transmission of the proposed NCF sensor can be calculated by the overlap integral between $E(r,z)$ and the fundamental mode of the lead-out SMF $E_S(r)$ and can be expressed as [80],

$$T = 10 \log_{10} \left[\frac{\left| \int_0^\infty E_S(r) E(r,z) r dr \right|^2}{\int_0^\infty |E_S(r)|^2 r dr \int_0^\infty |E(r,z)|^2 r dr} \right] \quad (3.9)$$

As the Ni^{2+} ions bind to the chitosan/PAA functionalized sensor both the refractive index n_{cs} and the coefficient of attenuation a_λ of $[\text{CS/PAA}]_{10}$ bilayers changes, which in turn modulates the propagation constant β_m and coefficient of evanescent absorption γ_m and ultimately translates to wavelength shifts of the interference spectrum, as predicted by the transmission equation (3.9).

3.3.2 Sensor calibration

The sensing principle of the proposed sensors is based on refractive index (RI) change of a chelating multilayer $[\text{CS/PAA}]_{10}$ film coated on the fiber surface and thus the sensor was calibrated to measure interference dip shifts in response to external refractive index prior to functionalization.

The sensor was immersed in sucrose solutions of different refractive index values and the wavelength of the interference dip ($\lambda_d = 1551.86$ nm when external RI = 1) in response to external refractive index changes was monitored and recorded. As observed from Figure 3-5, with increase in refractive index the interference dip shifts towards longer wavelengths as predicted by Eq.(3.3).

Figure 3-5: Spectral response of sensor to varying external refractive index.

Figure 3-6 shows the wavelength shift for different refractive index levels, and it is observed that the sensor exhibits a non linear response which can be fitted to a 2nd degree polynomial ($R^2=0.9812$). For determination of the RI sensitivity, the response of the sensor can be fitted into 3 quasi linear regions (Figure 3-7); first region from 1.3329 RIU to 1.3864 RIU with a sensitivity of 135.31 nm/RIU, second region from 1.3864 RIU to 1.4141 RIU with a sensitivity of 289.17 nm/RIU and a final region from 1.4141 RIU to 1.4355 RIU, with a sensitivity of 711.18 nm/RIU. Refractive index sensitivity of our sensor is relatively higher than other reported interferometric sensors [83-85] and long period grating sensors [86, 87].

Figure 3-6: Calibration curve for wavelength shifts with external RI.

Figure 3-7: Sensitivity of the sensor to external RI.

3.3.3 Sensor functionalization

The schematic of the sensor after 10 bilayer deposition is shown in Figure 3-8. The spectral response of the sensor before and after the $[\text{CS/PAA}]_{10}$ functionalization process, with the sensor immersed in MES buffer is shown in Figure 3-9. After the functionalization process, it is observed that there is a shift in the spectrum dip towards longer wavelength, indicating an alteration in the effective refractive index of the NCF. This is attributed to the functionalization of CS/PAA bilayers on the sensor surface.

Fig. 6(a)

Figure 3-8: Sensor scheme after functionalization of $[\text{CS/PAA}]_{10}$.

Fig. 6(b)

Figure 3-9: Sensor spectral response in MES buffer, before and after functionalization of [CS/PAA]₁₀.

3.3.4 Binding of Ni²⁺ to CS/PAA multilayer

After calibration, the sensor was functionalized with [CS/PAA]₁₀ and examined with different concentrations (10-10000 μ M) of NiCl₂ in pH 6, 2-(N-morpholino) ethanesulfonic (MES) acid buffer. The spectral response of the sensor is shown in Figure 3-10 (inset) and shift of the interference fringes to longer wavelengths is observed when Ni²⁺ concentration is increased. The wavelength modulation is contributed by amino groups in chitosan which help in adsorption of Ni²⁺ by chelation [88]. Ni²⁺ being a metal cation, the binding takes place predominantly through chelation, wherein the metal cation is bound to the amine groups of the same or different chitosan polymer chains through inter or intramolecular binding

[89, 90]. Similarly, Ni^{2+} also forms complexes with the carboxyl groups in different PAA chains [91] and further increases crosslinking of the polymeric network. As a result, with the increase in concentration of Ni^{2+} , more coordination bonds can be formed, either by amino groups of chitosan or carboxyl groups of PAA, thereby increasing degree of crosslinking in the CS/PAA network, leading to an increase in refractive index and thus shifting the dip to longer wavelengths. Figure 3-10 shows the plot of dip shift vs concentration and it is observed that at higher concentrations of Ni^{2+} the shift in the dip wavelength is significantly lower. This can be attributed to non availability of binding sites on the sensor surface due to a high concentration of Ni^{2+} , thereby leading to a saturation in the sensor response.

Figure 3-10: Response of $[\text{CS/PAA}]_{10}$ functionalized sensor for changes in Ni^{2+} concentration (Inset) and calibration curve for the sensor.

The sensitivity curve of the proposed sensor towards Ni^{2+} concentration in the linear range is presented in Figure 3-11. The sensor exhibits a sensitivity of 0.05537 nm/ μM in the concentration range upto 500 μM ($R^2 = 0.99669$).

Figure 3-11: Sensitivity curve of $[\text{CS/PAA}]_{10}$ functionalized sensor.

3.3.5 Effect of number of bilayers

To investigate the effects of number of bilayers in Ni^{2+} sensing, the sensor was functionalized with 20 bilayers using the dip coating procedure described in Section 2 and tested with different concentrations of Ni^{2+} . The sensitivity curve of this sensor is presented in Figure 3-12 and sensitivity is determined to be 0.046 nm/ μM ($R^2=0.96774$). Based on the results, the increase in number of bilayers does not reflect in an increase in the sensitivity, rather the sensitivity decreased. The

drop in sensitivity and linearity may be attributed to the instability of the coated films on account of increased thickness of the films [92].

Figure 3-12: Sensitivity curve of [CS/PAA]₂₀ functionalized sensor.

3.3.6 Concentration detection limit

Langmuir isotherm model is used for the analysis of wavelength shifts as a function of Ni²⁺ concentration and subsequent determination of the concentration detection limit of the sensor. The Langmuir isotherm equation is expressed as follows:

$$\Delta\lambda = \Delta\lambda_{max} \left(\frac{KC}{1 + KC} \right) \quad (3.10)$$

where $\Delta\lambda$ is the wavelength shift due to adsorption of the Ni^{2+} onto the $[\text{CS/PAA}]_{10}$ functionalized NCF surface, $\Delta\lambda_{\max}$ is the maximum wavelength shift due to complete monolayer formation, C is the concentration of Ni^{2+} in solution and K is the equilibrium binding constant under steady state conditions. Langmuir isotherm based non linear regression model is used for determining values of K and $\Delta\lambda_{\max}$ [93, 94]. Figure 3-13 shows the Langmuir model based nonlinear regression fit of the wavelength shifts vs Ni^{2+} concentration plot. Based on the curve fitting results, the Langmuir isotherm parameter values are determined to be $K = 0.00322 (\mu\text{M})^{-1}$ and $\Delta\lambda_{\max} = 37.2 \text{ nm}$. The experimental data shows good fit with the Langmuir model ($R^2 = 0.94998$) thus suggesting the formation of mono layer on the self assembled layers.

Without consideration of environmental noise and inherent thermal noise of OSA, resolution of the OSA mainly limits the detection limit of this sensing system [95]. Therefore, by making $\Delta\lambda$ value equal to OSA resolution (0.02 nm) , the lowest nickel concentration detection limit C_{LOD} can be expressed by the modified Langmuir isotherm as [93]:

$$C_{\text{LOD}} = \frac{\Delta\lambda}{K(\Delta\lambda_{\max} - \Delta\lambda)} \quad (3.11)$$

Figure 3-13: Change in wavelength as a function of Ni^{2+} concentration and associated Langmuir isotherm of $[\text{CS/PAA}]_{10}$ functionalized sensor.

Using K and $\Delta\lambda_{\max}$ values obtained from the curve fitting using Langmuir isotherm model, concentration detection limit of the sensor is calculated to be $0.1671 \mu\text{M}$. This is lower than the recommended limit of $1.13 \mu\text{M}$ for drinking water set by WHO [96].

3.3.7 Sensor regeneration

The sensor was immersed in pH 3.5 MES buffer and the dip wavelength was measured, followed by the immersion of sensor in a 100 μM NiCl_2 solution and measurement of dip wavelength. The sensor was again immersed in the pH 3.5 MES buffer. From Fig.11, it can be observed that immersion in the 100 μM NiCl_2 solution causes a wavelength shift of about 4 nm and subsequent immersion in the MES buffer causes the dip wavelength to shift to the original value. This can be attributed to the reversible binding of Ni^{2+} on CS/PAA multilayers. At $\text{pH} < 4$, amine groups in chitosan are protonated which results in the unbinding and release of Ni^{2+} ions thereby regenerating the sensor. The process mentioned above is for one regeneration cycle and the sensor was regenerated upto 5 times without any significant loss in performance as shown in Figure 3-14.

Figure 3-14: Regeneration Cycle: Shift in dip wavelength of the sensor in pH 3.5 MES buffer and 100 μM Ni^{2+} solutions sequentially.

3.3.8 Stability

The sensor was immersed in pH 6 MES buffer and the interference spectrum of the sensor was recorded over a period of 2 weeks. It is observed that the dip wavelength drifts from 1565.28 nm to 1565.24 nm after 14 days as illustrated in Figure 3-15. Thus the drift in dip wavelength during this period is not very significant (variation of $\sim 0.002\%$), indicating the stability of the sensor.

Figure 3-15: Sensor stability: Variation in dip wavelength with sensor immersed in pH 6 MES buffer for 2 weeks.

3.3.9 Specificity analysis

The response of the functionalized sensor was examined for Na^+ and Ca^{2+} ions of different concentrations. It was observed that the sensor is not sensitive to either Na^+ or Ca^{2+} ions upto a concentration of 100 μM . The wavelength shifts of the sensor for different concentration of Ca^{2+} and Na^+ ions (in the range of 500 μM) were compared with that of Ni^{2+} ions and this is illustrated in Figure 3-16. It can be observed that the response of sensor towards Na^+ ions is very poor since chitosan does not chelate alkali metals. The sensor shows a low sensitivity towards Ca^{2+} ions, but it is insignificant when compared to that of Ni^{2+} ion, as evident from the bar chart. Chitosan does not chelate Ca^{2+} and hence the sensitivity has to be attributed to affinity of PAA to Ca^{2+} ions. The sensor is therefore specific towards Ni^{2+} ions compared to alkali and alkaline earth metals. However chitosan is sensitive to other transition metals like Hg^{2+} , Cu^{2+} and hence the presence of these metal ions would create cross-sensitivity issues. Chelating agents with enhanced capability to bind specific metals is an intense area of research and chitosan can be used as an immobilization matrix for these ligands [97].

Figure 3-16: Specificity Analysis: Response of [CS/PAA]₁₀ sensor to Na⁺, Ca²⁺ and Ni²⁺ ions.

3.3.10 Response time

The response time of the CS/PAA multilayer functionalized sensor is influenced by (1) diffusion of Ni²⁺ ions to the multilayer-solution interface and (2) rate of complexation of the metal by chitosan and PAA [98]. The response time of the sensor was examined for 200 μM and 500 μM Ni²⁺ concentrations. As observed from Figure 3-17, the sensor was found to reach 95% of the final dip shift at 20–40 s, depending on Ni²⁺ concentration, with a faster response time for higher concentration (about 23s for 500 μM and 32s for 200 μM).

Figure 3-17: Response time curves with sensor immersed in 200 μM and 500 μM Ni^{2+} solution.

3.3.11 Temperature cross-sensitivity

Examination of sensor cross sensitivity to temperature was carried out before and after functionalization of $[\text{CS/PAA}]_{10}$ multilayer film. Figure 3-18 shows the temperature calibration curve of the unfunctionalized sensor. It can be observed that as temperature increases the spectral dip shifts to longer wavelengths. This temperature induced dip shift depends on three factors - change in length, change in diameter and change in effective refractive index of the NCF. Change in length and diameter are a result of thermal expansion, whereas change in effective refractive index is due to thermo-optic effect. It can also be observed that the

sensor exhibits a linear response to temperature changes between 25⁰C and 55⁰C. The sensitivity of unfunctionalized sensor to temperature changes was found to be 11.43 pm/⁰C which is less than Long Period Gratings [99].

Figure 3-18: Temperature calibration curve of unfunctionalized sensor.

Figure 3-19 shows the temperature calibration curve of sensor functionalized with [CS/PAA]₁₀ multilayer. It can be observed that as temperature increases the spectral dip shifts to shorter wavelengths. This is due to thermally induced expansion of the multilayer, resulting in a decrease in RI due to increased swelling. It can also be observed that the shift is significantly reduced when the temperature is increased from 50 to 55⁰C and can be attributed to the maximum swelling of the multilayer film. The temperature sensitivity of the functionalized sensor is significantly higher at -38.97 pm/⁰C compared to the unfunctionalized sensor.

Temperature compensation by incorporating an in-line Fiber Bragg Grating (FBG) for simultaneous temperature measurement can eliminate this temperature cross-sensitivity [100].

Figure 3-19: Temperature calibration curve of [CS/PAA]₁₀ functionalized sensor.

3.4 Chapter conclusion

A new type of fiber-optic interferometric sensor for Ni²⁺ detection has been developed and experimentally demonstrated. The proposed sensor has many advantages such as ease of fabrication, compact structure, fast response, low cost and a relatively wide measuring range. The fiber was functionalized with electrostatically self-assembled multilayers [CS/PAA]₁₀ for effective Ni²⁺ adsorption and the detection sensitivity was determined to be 0.0554 nm/μM. The kinetic profile of Ni²⁺ adsorption onto the sensor was further analyzed by the

Langmuir isotherm parameters and a lower detection limit of 0.1671 μM was achieved. The specificity and the lower detection limit of these heavy metal ion sensors could be further improved by immobilizing a suitable cation receptor (ligand) onto the polymer matrix [101], which would be the focus of future work. The promising results of this sensor encourage it to be employed in many potential applications such as monitoring of heavy metal ions in drinking water.

Chapter 4

Chitosan-nickel film based interferometric optical fiber sensor for label-free detection of histidine tagged proteins^{*}

4.1 Introduction

In the field of molecular biology recombinant expression of proteins by cloning is state of the art and has been in practice for the last few years [102, 103]. Detection and purification of these proteins are done efficiently by genetically incorporating an epitope tag. This process involves addition of amino acid sequences to the terminal ends of the protein, which act as specific sites for binding partners like antibodies [104]. One of the most used methods for protein tagging involves genetically adding poly-histidine tags to the terminal ends of the protein. The strong affinity between the histidine tag and a divalent metal ion such as nickel (Ni^{2+}) enables the purification of proteins with a high degree of specificity using metal affinity chromatography [105-107]. Subsequently, detection and quantification of His-tagged proteins after purification will be carried out so that the yield can be determined. SDS_PAGE is a standard technique used for identification of the His-tagged protein after purification. Western blot technique is

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also used in some cases when there is availability of anti-His-tag antibodies [108, 109]. However, the characterization of His-tagged proteins using these standard procedures is time consuming and tedious. A fast detection method for specific detection of His-tagged proteins could therefore simplify the procedure dramatically. Hence, alternative methods have been developed for detection of His-tagged proteins such as surface plasmon resonance (SPR) sensors and nano-material (eg: silicon nanowires) based field effect transistors [110, 111]. Even though the responses of these sensors are faster than the standard procedures, they suffer from issues like complex fabrications and high costs. On top of that, functionalizing of Ni^{2+} -NTA (nitrilotriacetic acid) film onto solid surfaces requires complex chemical strategies [112, 113]. This necessitates the design of a new sensor which is robust, relatively easier to fabricate and cost-effective without compromising on the performance.

In this work, we propose a core fiber (NCF) based MZI sensor functionalized with CS-Ni film for the detection of hexa-histidine tagged protein. The CS-Ni film not only provides specific binding sites for direct immobilization of histidine tagged protein but also offers 'soft' surface to prevent protein from denaturation and preserves their bio specific activity. Fiber optic sensors have been used extensively in the field of sensing due to their unique characteristics such as low cost, immunity to electromagnetic interference, high sensitivity, ease of multiplexing and remote sensing capability[45]. Among fiber optic sensors, those based on intermodal interference have gained considerable interest on account of their compact structure, large dynamic range and high stability [45] and have been

reported extensively [114-117]. NCF is a pure silica fiber with an undoped core and no cladding. The absence of cladding makes it possible for the higher order modes to interact more effectively with the external environment and thus provides enhanced sensitivity to changes in external refractive index. Additionally no time consuming and dangerous process like chemical etching using hydrofluoric acid for removal of cladding is involved which aids in the ease of fabrication. This enables fabrication of a highly sensitive MZI by using NCF as the sensing fiber. Therefore, owing to all these advantages the proposed intermodal sensor is able to provide fast detection and enable both quantitative and kinetic recordings of the specific analyte-probe binding events. In our effort, the sensor is designed for the detection of histidine tagged microcin (His-MccS). Microcins are low molecular weight antibacterial peptides produced by enterobacteria [118] and the synthesis is activated under conditions of stress such as nutrient depletion [119, 120]. Microcins belong to the class of bacteriocins and exhibit antibacterial mechanisms, especially against infections with gram-negative bacteria [121], and are believed to assist intestinal microbiota against possible takeover by competing enterobacteria[118]. Thus, they offer promise in the development of novel and efficient antibiotics [122, 123]. Amongst the gut flora, probiotic *E. coli* G3/10 produces a novel microcin that is named microcin S (MccS) [124]. The gene clusters for microcin S synthesis involves four genes grouped in a single operon. The clustered genes for microcin S is composed of (i) microcin gene (*mcsS*) (ii) self-immunity gene (*mcsI*) that encodes the protein that nullifies the microcin

activity thereby protecting the producing strain, (iii) microcin export and external secretion genes (*mcsA* and *mcsB*).

In order to selectively detect His-MccS, the NCF is functionalized with a suitable bioreceptor. The immobilization of the bioreceptor has to be done with utmost care, since the sensor's performance is largely governed by the stability of the bioreceptor immobilization. Chitosan based electrochemical biosensors have been reported previously and extensively involve electrochemical methods for detection and quantification of the target species [125-127]. Singh et al. have demonstrated an electrochemical based chitosan-nickel oxide film biosensor for the detection of cholesterol [128]. Even though these chitosan composite based electrochemical biosensors show good sensitivity and selectivity to target molecules like glucose, cholesterol, catechol etc., their ability to be used for protein detection is hindered by the need to use mediators since many proteins analytes are not intrinsically capable of being redox partners and this complicates the sensor fabrication process [129]. In this work we have utilized a CS-Ni film based optical intermodal sensor for detection of hexa-histidine tagged proteins. Intermodal sensors in addition to the advantages mentioned above, monitor the binding of protein to the CS-Ni functionalized sensor surface by tracking the ambient refractive index changes and hence can detect any histidine tagged protein which binds on to the CS-Ni film. The CS-Ni film acts as a bioreceptor since hexa-histidine tag in the protein selectively binds to divalent metal ions like Ni^{2+} [130, 131]. Chitosan is selected as the chelating agent for Ni^{2+} ion owing to its film forming ability, biocompatibility and ability to bind divalent metal ions like Ni^{2+} [132]. In our previous work, we

have reported a fiber optic interferometric sensor functionalized with self-assembled polyelectrolyte multilayers of chitosan/poly acrylic acid for detection of Ni^{2+} ions [132]. Amine groups present in same or different chitosan polymer chains can bind to Ni^{2+} through inter or intramolecular bonds [89, 90]. Upon capturing His-MccS by the CS-Ni film, the sensor will effectively transduce refractive index change of the CS-Ni film into a quantifiable interferometric fringes signal. The sensor's response to a full protein: trypsin, which inherently contains histidine residues on the surface, was verified to evaluate the specificity of the sensor towards His-MccS. Our work features the first experimental evidence about reliable and fast detection of His tagged proteins with interferometric fiber optic biosensors.

4.2 Materials and methods

4.2.1 Materials

Optical fibers including single mode fiber (8.2/125 μm) (SMF-28) and no core fiber (125 μm) (NCF) are purchased from Corning and Prime Optical Fiber Corporation respectively.

Chitosan (medium molecular weight), phosphate buffered saline (PBS) and anhydrous nickel chloride salt are purchased from Sigma Aldrich. Sulfuric acid (95%) was purchased from Merck and hydrogen peroxide (30%) was purchased from VWR. The reagents used were of analytical grade. Ultrapure water (18.2 $\text{M}\Omega$ cm resistivity) from Mill-Q system was used in preparing the solutions.

4.2.2 Methods

4.2.2.1 Sensor fabrication

The fiber optic interferometric sensor was fabricated by splicing 20 mm of no-core fiber (NCF) (Prime Optical Fiber Corporation) between single mode fibers (SMFs) (8.2/125 μm SMF-28) using a Sumitomo Type 39 electric arc splicer.

4.2.2.2 Sensor functionalization

2g of chitosan was dissolved in 4% acetic acid solution to prepare a 2% chitosan solution and the pH was adjusted to 6. To 20ml of this chitosan solution 0.1g of NiCl_2 was added and the solution was stirred continuously at room temperature for 24h and subsequently filtered to remove the excess NiCl_2 . Based on our previous work [132], 10mM NiCl_2 would completely occupy the binding sites of chitosan and a higher number of nickel ions translate to a wider sensing range. Hence to ensure the complete coverage of the binding sites of chitosan, a NiCl_2 concentration slightly higher than 10mM (approx. 30 mM) was chosen. Prior to the functionalization process, NCF sensor was first immersed for 60 min in piranha solution (30% Hydrogen peroxide and concentrated sulphuric acid (98%) in 1:3 ratio) and subsequently immersed in distilled water and finally dried under a stream of nitrogen gas. Piranha treatment cleaned the NCF surface of organic impurities and hydroxylated the fiber surface. Next, the treated fiber was placed in a custom-made polydimethylsiloxane (PDMS) micro-channel and chitosan-nickel blend was injected into the channel and dried overnight in a vacuum oven at 60⁰C.

4.2.2.3 Microcin S expression and purification

The recombinant expression of microcin S was performed in 250 ml of LB broth where exponentially grown *E. coli* Top10 cells with the recombinant plasmid pBbE8k-pBAD-McsS-His were grown to an optical density of 0.7 - 1, and their expression was induced fully by 0.2% L-arabinose ($C_5H_{10}O_5$, Sigma-Aldrich, item no. A3256) at 37 °C and 225 rpm for 4-5 h. Subsequent centrifugation at 4000 x g for 20 min yielded cell pellets and homogenization was carried out using Emulsiflex-C3 homogenizer (Avestin, Inc.). Ni-NTA (Ni^{2+} -nitrilotriacetate) column (Qiagen) was used for the purification of microcin S (His-MccS) under native conditions. Washing was carried out with 50 mM Na_3PO_4 , 300 mM NaCl, and 10 mM imidazole (pH 7.4). Elution was performed with 50 mM Na_3PO_4 , 300 mM NaCl, and 500 mM imidazole (pH 7.4). Since the estimated molecular weight of microcin S is approximately 12 kDa, the eluate was first added to Amicon Ultra-15 Centrifugal Filter Unit, Millipore and concentrated using a 30-kDa molecular mass cut-off membrane. The concentrated fractions containing the microcin S was further concentrated using a 3-kDa cut-off membrane. After this, the resulting retentate was collected and the microcin S concentration was determined using Quant-iTTM protein (ThermoFisher Scientific, Cat. no.: Q33210) for subsequent use.

4.2.2.4 Sensing system setup

The sensing system consists of an Amplified Spontaneous Emission (ASE) broadband light source (center wavelength: 1570nm), the NCF sensor and an

optical spectrum analyzer (OSA) (YOKOGAWA AQ6370) and is presented in Figure 4-1. The proposed sensor is an inline fiber optic intermodal sensor based on transmission mode. To operate, light emitted from the ASE light source is coupled to the lead-in SMF and after propagating through the NCF region, the transmitted light is guided by the lead-out SMF to OSA for analysis.

Figure 4-1: Experimental setup

4.3 Experimental results and discussion

4.3.1 Sensing principle

The histidine tagged protein sensor is implemented with a CS-Ni film functionalized NCF surface as the sensing element. The NCF is a cladding-less pure silica fiber with an undoped core and has previously been utilized for development of refractive index [75] and humidity sensors [76]. The sensing and reference arms are self contained within the NCF and have the same physical length but different optical path lengths due to modal dispersion [133]. The image of the spliced region between lead-in SMF and NCF is shown in Figure 4-2(b).

Within the NCF region excitation of the fundamental mode of the incoming light to higher order modes occurs and subsequent interference between the higher order modes and fundamental mode leads to the formation of self-images of the input field along the length of NCF [77].

Assuming ideal alignment of the SMF and the NCF and considering the circular symmetry of input field, as light propagates from SMF to NCF only LP_{0m} modes will be excited in the NCF region. Denoting the field profile of LP_{0m} mode as $\phi_m(r)$, the NCF input field can be described by [79],

$$E(r, 0) = \sum_{m=1}^M b_m \phi_m(r) \quad (4.1)$$

where M is the number of modes excited in the NCF; b_m is the coefficient of excitation of the LP_{0m} mode and can be given by [80],

$$b_m = \frac{\int_0^\infty E(r,0) \phi_m(r) r dr}{\int_0^\infty \phi_m(r) \phi_m(r) r dr} \quad (4.2)$$

The total light field distribution at a propagation distance z in the NCF can be expressed as [81]

$$E(r, z) = \sum_{m=1}^M b_m \phi_m(r) \exp(-\gamma_m z) \exp(j\beta_m z) \quad (4.3)$$

where β_m is the propagation constant of LP_{0m} mode; γ_m is the coefficient of evanescent absorption of the LP_{0m} mode and can be calculated by [82],

$$\gamma_m = \frac{a_\lambda \lambda n_{cs} \cos \theta_m \cot \theta_m}{4\pi a n_{ncf}^2 \cos^2 \theta_c \sqrt{\sin^2 \theta_m - \sin^2 \theta_c}} \quad (4.4)$$

where λ is the free space wavelength, a_λ is coefficient of attenuation of the chitosan nickel film at λ , n_{cs} and n_{ncf} are the refractive indices of the chitosan nickel film and NCF respectively, a is the NCF radius, θ_c is the critical angle, and

$$\theta_m = \sin^{-1} \frac{\beta_m}{k} \quad (4.5)$$

where $k = 2\pi n_{ncf}/\lambda$ is the wave number.

The transmission of the proposed NCF sensor can be calculated by the overlap integral between $E(r,z)$ and the fundamental mode of the lead-out SMF $E_S(r)$ and can be expressed as [80],

$$T = 10 \log_{10} \left[\frac{\left| \int_0^\infty E_S(r) E(r,z) r dr \right|^2}{\int_0^\infty |E_S(r)|^2 r dr \int_0^\infty |E(r,z)|^2 r dr} \right] \quad (4.6)$$

As the histidine tagged proteins bind to the sensor both the refractive index n_{cs} and the coefficient of attenuation a_λ of CS-Ni film changes, which in turn modulates the propagation constant β_m and coefficient of evanescent absorption γ_m and ultimately translates to wavelength shifts of the interference spectrum, as predicted by the transmission equation Eq.(4.6). As a result, by monitoring the shift in the position of the interference fringes, the analyte can be identified and quantified. The spectrum of the fabricated sensor is shown in Figure 4-2(c) and the observed minimum insertion loss of the proposed sensor in air is ~ 3.5 dB (at 1569.80 nm).

Figure 4-2: (a) Schematic of sensing system & (inset) sensor configuration, (b) after splicing image of Lead-in SMF with NCF and c) spectral response of bare sensor.

4.3.2 Sensor calibration

Prior to surface functionalization with CS-Ni film, the sensor was calibrated for refractive index changes by immersing into sucrose solutions with refractive index values ranging between 1.33 and 1.44. The shift in spectral dip wavelength ($\lambda_d = 1569.80$ nm when sensor in air) with change in external refractive index was monitored and recorded. From Figure 4-3 (inset) it is observed that increase in external refractive index causes the shift of spectral dip towards longer wavelengths. The refractive index calibration curve of the sensor is obtained by

plotting wavelength shifts and the corresponding refractive index values and is shown in Figure 4-3. The NCF sensor is observed to exhibit a non-linear response described by an exponential function ($R^2=0.9916$).

Figure 4-3: Calibration curve for wavelength shifts with external refractive index ($n=3$) & (inset) sensor response for different external refractive index.

4.3.3 CS-Ni film characterization by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX)

Figure 4-4 (a) presents the SEM image of chitosan film and Figure 4-3(b) presents the SEM image of CS-Ni film. It is observed that the surface of the chitosan film is relatively smoother compared to that of the CS-Ni film. The CS-Ni film is also observed to have a lot more folds presumably due to the binding of Ni^{2+} with various chitosan polymer chains through co-ordination bonds. Figure 4-4(c) and 4-4(d) presents the EDX micrographs of chitosan and CS-Ni respectively. It is observed that nickel does not give a recognizable spike in the chitosan spectra

whereas in the case of CS-Ni film a distinct peak for nickel is seen in the XRD spectra with a normalized atomic concentration of about 16.5 %.

4.3.4 Binding of His-MccS to CS-Ni functionalized sensor

Figure 4-4(e) and Figure 4-4(f) presents the microscopic images of the fiber surface before and after functionalization with CS-Ni film respectively. It is observed that a very thin uniform layer of CS-Ni film is formed over the sensor surface and the thickness of the film was determined to be around 450 nm. Increasing the thickness of the CS-Ni film does not guarantee better sensitivity; the evanescent field of the sensor can penetrate only a few hundred nanometers on the exterior and decays exponentially. The response time of the sensor is directly proportional to the square of its thickness [134]. Hence a sensor with thicker film coating would have no improvement on the sensitivity but can increase the sensor response time, which is not desirable.

Figure 4-4 (g) presents the spectral response of the sensor before and after CS-Ni film functionalization. It is observed that the dip wavelength shifts after functionalization with CS-Ni film. The wavelength shift can be attributed to the change in RI at the NCF-ambient interface which confirms the film has been functionalized onto the sensor.

Figure 4-4: (a) SEM image of chitosan film, (b) SEM image of CS-Ni film, (c) EDX micrograph of chitosan film, (d) EDX micrograph of CS-Ni film, (e) microscopic image of fiber before functionalization and (f) after functionalization of CS-Ni film, and (g) sensor spectrum.

The CS-Ni functionalized sensor was examined with different concentrations (0-40000 ng/ml) of His-MccS in PBS buffer solution. As shown in Figure 4-5, shifts of the interference fringes are observed with an increase in His-MccS concentration. This wavelength modulation is contributed by the binding of His-

MccS to Ni^{2+} ions in the CS-Ni film. The imidazole functional group of histidine is responsible for such binding with nickel metal ion as the nitrogen atoms present in imidazole functional group act as electron donors and form coordinate bonds with metal ion [135].

Hexa-histidine tag was inserted into microcin, since the binding affinity of hexa histidine tag to Ni^{2+} is relatively stronger and stable than a mono or di-histidine tag [136]. As shown in Figure 4-6, Ni^{2+} ion can bind to multiple imidazole groups in a hexa-histidine tag through coordinate bonds. This leads to stronger binding affinity between the metal ion and histidine tagged protein [137] and also helps in the selective binding of histidine tagged protein over other proteins [138].

Figure 4-5: Response of CS-Ni film functionalized sensor to changes in His-MccS concentration.

Figure 4-6: Ni^{2+} and His-MccS binding scheme.

Due to the binding of His-MccS, the refractive index at the sensor-ambient interface changes, causing a shift in the dip wavelength and the extent of the shift is proportional to the concentration of microcin. The calibration curve of the proposed sensor towards His-MccS is presented in Figure 4-7 and sensor response in the linear range is presented in Figure 4-7 (inset). The sensor exhibits a sensitivity of 0.0308 nm/(ng/ml) in the range of 78(ng/ml).

Figure 4-7: Calibration curve for the CS-Ni film functionalized sensor & (inset) linear range of the sensor response to changes in His-MccS (n=3).

To prove the significance of Ni^{2+} in the binding of His-MccS, negative control experiment was conducted. The sensor with only CS film was exposed to different concentrations of His-MccS and the response is shown in Figure 4-8. It is observed that there is no significant wavelength shift nor a significant data trend with increasing concentration of His-MccS, indicating that His-MccS exhibits a low affinity towards the CS film in the absence of Ni^{2+} ions. Small shifts observed are possibly due to the non-specific adsorption of His-MccS onto the chitosan film.

Figure 4-8: Performance of Chitosan film functionalized sensor to changes in His-MccS concentration (n=3).

4.3.5 Concentration detection limit

The wavelength shift as a function of His-MccS concentration is analysed by Langmuir isotherm model and subsequently the sensor's concentration detection limit is determined. The Langmuir isotherm is described by the following equation:

$$\Delta\lambda = \Delta\lambda_{max} \left(\frac{KC}{1+KC} \right) \quad 4.7$$

where $\Delta\lambda$ is the shift in wavelength induced by the adsorption of the His-MccS onto the CS-Ni functionalized sensor, $\Delta\lambda_{max}$ is the maximum shift in wavelength

induced by the formation of a complete monolayer, C is His-MccS concentration in solution and K is the steady state equilibrium binding constant. The values of K and $\Delta\lambda_{max}$ are determined using a non-linear regression fit based on Langmuir isotherm [93, 94]. Figure 4-9 shows the Langmuir model based fit of the sensor wavelength shifts and the corresponding His-MccS concentration and it is observed that the experimental data displays a good fit with Langmuir isotherm model ($R^2=0.9852$). The Langmuir isotherm parameter values are determined to be $K = 0.00312 \text{ (ng/ml)}^{-1}$ and $\Delta\lambda_{max} = 7.68 \text{ nm}$.

Figure 4-9: Performance of CS-Ni film functionalized sensor to changes in His-MccS concentration and associated Langmuir isotherm fit (n=3).

Neglecting the effects of inherent thermal noise and environmental noise of OSA, the limit of detection of the sensing system is majorly limited by the resolution of OSA [95]. By rearranging the Langmuir isotherm equation, the limit of detection of His-MccS, C_{LOD} can be expressed as [93]:

$$C_{LOD} = \frac{\Delta\lambda}{K(\Delta\lambda_{max}-\Delta\lambda)} \quad 4.8$$

By making the value of $\Delta\lambda$ equal to the resolution of OSA (0.02nm) and using the K and $\Delta\lambda_{max}$ values determined from Langmuir isotherm based curve fitting, C_{LOD} of the sensor is calculated to be 0.8368 ng/ml.

4.3.6 Specificity analysis

For evaluating the specificity of the CS-Ni functionalized sensor, the sensor was examined with various concentrations of a full protein; trypsin. The spectral response of the sensor to trypsin is presented in Figure 4-10 (inset), and in Figure 4-10, it can be observed that the sensor responds to trypsin in the concentration range of 0-200 $\mu\text{g/ml}$. This range is different from the measuring range for His-MccS (0-40000 ng/ml) which demonstrates a specificity of the sensor to His-MccS in this concentration range.

Figure 4-10: Spectral response of Chitosan-Nickel film functionalized sensor to changes in trypsin concentration (Inset) and associated plot of Dip shift vs. Trypsin concentration (n=3).

To further evaluate the specificity of the sensor towards His-MccS, the sensor was first immersed in a 78 ng/ml concentration of His-MccS and followed by immersing the sensor in a 78 ng/ml concentration of trypsin. The stepwise variation of dip shift for this procedure is presented in Figure 4-11 and it can be seen that dip shift for His-MccS is significantly higher than trypsin. The dip shift for trypsin is lower owing to the binding sites being occupied by the His-MccS. To verify this point, another cycle of experiment was conducted with reversed sequence; the sensor was dipped into the trypsin solution first, followed by immersion in the His-MccS solution as shown in Figure 4-12. It is still observed that the dip shift for His-MccS is significantly higher than that of trypsin. On dipping the sensor into the trypsin solution nickel ions bound to chitosan can bind to some histidine groups present on the surface of trypsin. Subsequent immersion of the sensor in His-MccS

solution leads to most of the trypsin bound to the sensor surface replaced by His-MccS since binding of Ni^{2+} to poly-histidine tags is much stronger than binding to mono-histidine group [139, 140]. This causes the sensor to shift significantly more for His-MccS than trypsin. The specificity of the sensor was further evaluated by immersing the sensor in 78 ng/ml solutions (in PBS buffer) of bovine serum albumin (BSA) and human serum albumin (HSA) respectively and is shown in Figure 4-13. The sensor exhibits a significantly greater dip shift to His-MccS compared to trypsin, BSA and HSA. The specificity of the sensor was further improved by adding a low concentration of imidazole (20mM) to the PBS buffer and using this buffer to prepare 78 ng/ml solutions of His-MccS, trypsin, BSA and HSA. At low concentrations imidazole can displace the non specific proteins binding to the sensor surface but does not displace the hexa-histidine tagged protein [141] [142]. After the addition of 20 mM imidazole, the sensor's response to trypsin, BSA and HSA decreases considerably whereas the response to microcin did not change significantly, as observed from Figure 4-13. These experimental results conclusively prove the specificity of the sensor towards His-MccS.

Figure 4-11: Sensor response to 78 ng/ml microcin followed by 78 ng/ml trypsin (n=3).

Figure 4-12: Sensor response to 78 ng/ml trypsin adsorption followed by 78 ng/ml microcin (n=3).

Figure 4-13: Specificity analysis: sensor response to 78 ng/ml of His-MccS, trypsin, BSA and HSA in PBS and (PBS+20mM imidazole) (n=3).

4.3.7 Response time

The CS-Ni functionalized sensor's response time is influenced by: (1) His-MccS diffusion from solution to the CS-Ni film and (2) rate of binding of the His-MccS to Ni^{2+} [143]. The response time of the sensor was examined with 39 ng/ml and 78 ng/ml His-MccS concentrations and presented in Figure 4-14. The sensor is observed to attain 95% of the final dip shift around 150 s for both the concentrations. The response time of the proposed sensor is relatively faster in comparison to other reported histidine tagged protein sensors [144, 145].

Figure 4-14: Response time plot of the Chitosan-Nickel film functionalized sensor to His-MccS concentrations of 39 ng/ml and 78 ng/ml.

4.3.8 Sensor regeneration

Prior to regeneration, the sensor was first treated with 10 mM ethylenediaminetetraacetic acid solution for 10 minutes. EDTA is a very good chelator of Ni^{2+} ions and hence strips the His-MccS attached Ni^{2+} ions from the sensor surface. Subsequently the sensor was dipped in a 30 mM NiCl_2 solution for 10 minutes to allow the chitosan film to reabsorb Ni^{2+} ions. At 30 mM concentration of Ni^{2+} ions most of the binding sites of the chitosan film are occupied leading to the saturation of chitosan film [132]. The sensor was then immersed in a 78 ng/ml solution of His-MccS and the dip shift as observed. This

completes one cycle of regeneration of the sensor. The dip shift of the sensor evaluated with 78 ng/ml of His-MccS for 5 cycles of regeneration is shown in Figure 4-15. It is observed that for the first 3 cycles of regeneration there was no significant loss in the response of sensor to His-MccS. A significant drop is observed in the sensor performance after the 4th regeneration cycle and a very drastic drop is observed after the 5th cycle.

Figure 4-15: Sensor regeneration: Sensor response to 78 ng/ml of His-MccS after each regeneration cycle.

4.4 Chapter conclusion

A new type of CS-Ni film based fiber-optic interferometric sensor for label-free detection of polyhistidine tagged proteins has been proposed and experimentally demonstrated. By monitoring wavelength shift of the sensor spectrum, the proposed biosensor was able to detect His-MccS effectively with sensitivity of

0.0308 nm/(ng/ml). The kinetics of His-MccS adsorption onto the CS-Ni functionalized NCF sensor was analyzed by fitting the sensor response to Langmuir isotherm model and the sensor's limit of detection was determined to be 0.8368 ng/ml. The selectivity of the proposed sensor towards histidine tagged proteins was comparatively evaluated with a full protein–trypsin and it was concluded that specificity mainly depends on the affinity of His-Tag to CS-Ni film. The proposed biosensor combines the advantages of small size and high sensitivity of NCF sensors and exhibited rapid response compared to existing techniques such as SDS-PAGE and western blot. The positive result of this sensor makes it a viable candidate to be used for high throughput screening in bioprocesses.

Chapter 5

Fiber optic nickel ion sensor based on direct ligand immobilization^{*}

5.1 Introduction

Detection of metal ions in aqueous solution is carried out using conventional methods like anodic stripping voltammetry (ASV), inductively coupled plasma mass spectrometry (ICPMS) and atomic absorption spectroscopy (AAS). These methods are complex and involve tedious procedures for sample preparation, expensive, require extensive training to use and are destructive. This has led researchers to focus on polymer based optical sensors (PBOS) such as surface plasma resonance (SPR) sensors or optical fiber sensors as an alternative to the conventional methods. These sensors possess fast response times, don't require extensive sample preparation, and are non-destructive and cost-effective. In the last few years many fiber-optic based SPR sensors and fiber-optic interferometric sensors for ion detection have been demonstrated. The significant aspect of these sensors is the presence of an active chelating polymeric layer on the silica surface for specific detection of a target ion (Figure 5-1).

^{*}© 2017 IEEE, Adapted with permission from Raghunandhan, R., L.H. Chen, and C.C. Chan. *Fiber optic nickel ion sensor based on direct ligand immobilization*. in *2017 25th Optical Fiber Sensors Conference (OFS)*. 2017.

Figure 5-1: Surface modification of silica substrate for heavy metal ion detection.

The sensitivity of PBOS depends on the thickness of the polymeric matrix: best sensitivity is achieved when polymeric matrix's thickness equals the penetration depth of the evanescent light wave (several tens of nanometers). Hence, if the polymeric matrix is very thick, it causes a dip in sensitivity since the evanescent wave decays exponentially with penetration distance. For prism based SPR sensors, traditional techniques such as spin coating and dip coating are employed to coat a thin polymer film on the sensor surface. In the case of fiber optic SPR sensors grafting techniques are employed to form a thin film on the surface, but the downside to this technique is it involves immobilization of polymerization initiators to enable surface polymerization which increases the complexity of the process [146]. Even though it is desirable to have the polymeric matrix sensitive to a specific ion, it is usually not the case. The chelating groups in the polymeric matrix usually suffer from interference from other undesirable ions which share

similar chemical or physical properties as the target ions. Many works on fiber-optic ion sensors involves investigation performed with only one type of target ion at a time [147-151]. This seriously limits the use of these sensors for real world applications. Additionally these sensors suffer from stability issues due to the swelling effect of hydrogels in aqueous environment. These sensors operate based on the change in refractive index of the polymeric medium triggered by binding of the target ions. The swelling effect results in refractive index change of the polymeric film causing cross sensitivity to undesirable ions. In addition, the chelating ability of hydrogel is pH dependent: many polymers lose their binding ability at acidic pH environments. In a low pH environment, the chelating groups in the polymeric matrix like amines or carboxyl groups are positively charged and thus unable to donate electrons to the target cation [152]. As a result, binding of target ion at lower pH is poor and unstable.

The major problem encountered with polymeric based optical sensors is its lack of specificity to the target molecule, as the chelating groups in the polymer film can bind to other molecules/ions with a weaker force introducing false positive reading. In addition many polymers are ineffective at acidic pH values due to the protonation of functional groups such as amino or carboxylic acids. As a result, in low pH environment, they attain positive charge and are unable to donate electrons to the target molecules/ions to retain them in the polymeric network. Instability is attributed to the swelling effect of the hydrogel in the aqueous environment. Moreover, optical sensors generally operate based on the change of refractive index of the polymeric medium when target molecules/ions binding takes place.

This swelling effect due to water adsorption or temperature will result in thickness/refraction index change of the polymeric film causing cross sensitivity error. To overcome these limitations an alternative sensing system for heavy metal ion detection has to be developed that doesn't require the use of a polymer matrix.

5.2 Materials and methods

5.2.1 Materials

Optical fibers including single mode fiber (8.2/125 μm) (SMF-28) and no core fiber (125 μm) (NCF) are purchased from Corning and Prime Optical Fiber Corporation respectively.

(3-aminopropyl) triethoxysilane (APTES) and Meso-Tetra (4-carboxyphenyl) porphine ($\text{C}_{48}\text{H}_{30}\text{N}_4\text{O}_8$) are purchased from Sigma Aldrich. Sulfuric acid (95%) was purchased from Merck and hydrogen peroxide (30%) was purchased from VWR. The reagents used were of analytical grade. Ultrapure water (18.2 $\text{M}\Omega\text{ cm}$ resistivity) from Mill-Q system was used in preparing the solutions.

5.2.2 Methods

5.2.2.1 Sensor fabrication

NCF is a heterocore silica fiber with no cladding and an undoped core and has been previously reported for sensing applications [75, 132]. The intermodal sensor is fabricated by splicing a 35mm length of nocore fiber (NCF) with a standard SMF fiber.

5.2.2.2 Sensor functionalization

The first step in functionalizing the sensor involves activating its surface. This was achieved via a piranha treatment. A piranha solution consisting of a 3:1 ratio of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) was prepared. The sensing (NCF) end of the fabricated sensor was immersed into the piranha solution for duration of 30 minutes during which time the spectrum of the sensor formed on the OSA was constantly monitored and recorded. After 30 minutes, the sensing end was removed from the piranha solution and left to air dry for 10 minutes before being rinsed with DI water to hydroxylate the sensor surface.

The second step in functionalizing the sensor involves performing a silane treatment. After the sensor was hydroxylated via piranha treatment, it was treated with the silane coupling agent (3-aminopropyl) triethoxysilane (APTES). To carry out the silane treatment, the activated sensor surface was immersed in a solution of APTES for a period of 10 minutes. After that, the sensing end was removed from the solution and the corresponding spectrum formed on the OSA was observed and recorded. The NCF end was then immersed back into the APTES solution. This procedure was repeated for a total duration of two hours so as to achieve a uniform silane layer on the surface of the sensor. The sensing region was then left to air dry for an hour.

The third and final step of the functionalization process involved the immobilization of the ligand Meso-Tetra (4-carboxyphenyl) porphine ($\text{C}_{48}\text{H}_{30}\text{N}_4\text{O}_8$) onto the surface of the silanized sensor. Figure 5-2 illustrates the chemical structure of Meso-Tetra (4-carboxyphenyl)porphine.

Figure 5-2: Chemical structure of Meso-Tetra(4-carboxyphenyl)porphine.

5.2.2.3 Sensing system setup

The sensing setup schematic is shown in Figure 5-3. The fabricated sensor was interrogated using the broadband light source connected via a three-port circulator and its reflectance spectrum was measured and recorded using the Optical Spectrum Analyzer (OSA). Light from the light source is coupled into port 1 of the circulator and the reflected light from the sensor head is coupled to port 3 of the circulator which is connected to the OSA.

Figure 5-3: Sensing Schematic and (inset) Sensor configuration.

5.3 Experimental results and discussion

5.3.1 Sensor working principle and calibration

The sensor operates on the basis of the principle of Multi Mode Interference (MMI), which can be explained as follows: As light propagates along the SMF and enters the multi mode region of the NCF, the higher order modes get excited due to the mode field mismatch and an interference occurs between the different modes as the beam travels through the NCF [153]. The coupling efficiency between the back reflected field, as a result of Fresnel reflection in the NCF and the fundamental mode in the input SMF is dependent on parameters such as the length of the NCF section, wavelength and refractive index of the surrounding medium [116].

The formation self-images as a result of the interference pattern produced in the NCF section is governed by the following equation [154]:

$$\lambda_{dip} = p \left(\frac{n_{mmf} W_{mmf}^2}{L} \right) \quad p=0,1,2,3\dots \quad 5.1$$

where, λ_{dip} denotes the wavelength of spectral dip, p is the self image number, n_{mmf} is the effective refractive index of the NCF, W_{mmf} is the effective diameter of the NCF and L denotes the length of the NCF. From the eq. (5.1) it is apparent that λ_{dip} shifts to longer wavelengths when the effective refractive index increases.

The sensor was immersed into different RI solutions and the spectrum was monitored by using OSA. The spectral response of the sensor is presented in Figure

5-4. It is observed that with increasing RI, λ_{dip} shows a red shift which is in accordance with eq. (5.1).

Figure 5-4: Sensor RI spectral responses.

The dip shift vs RI plot is shown in Figure 5-5. The plot is divided into two quasi-linear regions and the RI sensitivity of each region is calculated. The first linear region ranges from 1.33 to 1.3861 RIU with a sensitivity of 103.88 nm/RIU and the second region ranges from 1.3861 to 1.4135 RIU with a sensitivity of 245.27 nm/RIU.

Figure 5-5: Relationships between Dip Shift and RI.

5.3.2 Sensor functionalization

The ligand Meso-Tetra (4-carboxyphenyl) porphine ($C_{48}H_{30}N_4O_8$) is an aza, cyclic molecule with electron rich interior cavities within which the heavy metal ion is encapsulated via ion - dipole interactions. This can be explained on the basis that the two-pyrroline nitrogens have the ability to accept two protons while the two $-NH$ groups are capable of losing an equal number of protons [155].

The ligand is immobilized on the sensor surface via a condensation reaction between amine groups on the silanized surface and carboxyl groups of the ligand, resulting in the formation of an amide bond. The reflectance spectrum of the sensor was monitored throughout the silane treatment and ligand functionalization process and is illustrated in Figure 5-6 and the relative dip shift after each step in the functionalization procedure is presented in Figure 5-7. It can be observed that there is a shift in the interference dips of the reflectance spectrum towards longer wavelengths after the silane treatment and ligand functionalization. This is due to the increase in RI around the sensing (NCF) interface induced by the functionalization of the ligand.

Figure 5-6: Sensor responses after silanization and ligand functionalization.

Figure 5-7: Shift in interference dips after silane treatment and ligand functionalization.

5.3.3 Ni²⁺ ions sensing

In order to test the functionalized sensor's response to nickel ions, the functionalized sensor was exposed to Ni²⁺ solutions in the concentration range of 0.001mM to 1mM prepared by dissolving Nickel Chloride (NiCl₂) in DI water and the spectrum was recorded in the OSA. From Figure 5-8, it is observed that the interference dips of the reflectance spectrum shifts towards longer wavelengths with increasing nickel ion concentration.

Figure 5-8: Sensor spectral responses to different Nickel ion concentrations.

The ligand Meso-Tetra(4-carboxyphenyl)porphine (C₄₈H₃₀N₄O₈) is an aza, cyclic molecule with electron rich interior cavities within which the heavy metal ion is encapsulated via ion - dipole interactions. This can be explained on the basis that

the two-pyrroline nitrogens have the ability to accept two protons while the two –NH groups are capable of losing an equal number of protons [155]. As a result of the binding of nickel ions present in the solution to the electron rich interior cavity of the ligand there is a change in the ambient RI around the NCF interface, which causes the interference dips to shift. The plot of sensor dip shift with change in Ni^{2+} concentration is presented in Figure 5-9. It is observed that the sensor exhibits a linear response ($R^2 = 0.8411$) in the range up to 0.02mM with a Ni^{2+} detection sensitivity of 0.1210 nm/ μM .

Figure 5-9: Relationship between the dip shift and Ni^{2+} concentration.

Langmuir isotherm model is used for the analysis of wavelength shifts as a function of Ni^{2+} concentration and subsequent determination of the concentration detection limit of the sensor. The Langmuir isotherm equation is expressed as follows:

$$\Delta\lambda = \Delta\lambda_{max} \left(\frac{KC}{1+KC} \right) \quad 5.2$$

where $\Delta\lambda$ is the wavelength shift due to adsorption of the Ni^{2+} onto the ligand functionalized NCF surface, $\Delta\lambda_{max}$ is the maximum wavelength shift due to complete monolayer formation, C is the concentration of Ni^{2+} in solution and K is the equilibrium binding constant under steady state conditions. Langmuir isotherm based non linear regression model is used for determining values of K and $\Delta\lambda_{max}$. Figure 5-10 shows the Langmuir model based nonlinear regression fit of the wavelength shifts vs Ni^{2+} concentration plot. Based on the curve fitting results, the Langmuir isotherm parameter values are determined to be $K= 70.625 \text{ (mM)}^{-1}$ and $\Delta\lambda_{max} = 3.75 \text{ nm}$.

Figure 5-10: Change in wavelength as a function of Ni^{2+} concentration and associated Langmuir isotherm.

Without consideration of environmental noise and inherent thermal noise of OSA, resolution of the OSA mainly limits the detection limit of this sensing system [95]. Therefore, by making $\Delta\lambda$ value equal to OSA resolution (0.02 nm), the lowest nickel concentration detection limit C_{LOD} can be expressed by the modified Langmuir isotherm as [93]:

$$C_{LOD} = \frac{\Delta\lambda}{K(\Delta\lambda_{max} - \Delta\lambda)} \quad 5.3$$

Using K and $\Delta\lambda_{max}$ values obtained from the curve fitting using Langmuir isotherm model, concentration detection limit of the sensor is calculated to be 0.076 μM .

This is lower than the recommended limit of 1.13 μM for drinking water set by WHO [96].

5.4 Chapter conclusion

A novel fiber optic intermodal sensor for detection of Ni^{2+} ions has been demonstrated. A very specific ligand to Ni^{2+} , Meso-Tetra(4-carboxyphenyl)porphine ($\text{C}_{48}\text{H}_{30}\text{N}_4\text{O}_8$) is directly immobilized onto the NCF sensor through covalent bonding and acts as binding sites for Ni^{2+} ions. Sensitivity of the sensor to Ni^{2+} ions was evaluated to be 0.1210 nm/ μM and the sensor is a viable alternative to polymer based fiber optic heavy metal ion sensors.

The positive results prove the feasibility of covalent immobilization strategy of ligands directly on to the fiber optic sensor surface. In this study we had used a commercially available ligand which is specific for Ni^{2+} ions. The proposed sensor offers several advantages over polymer based sensors. The sensitivity of the [CS/PAA] bilayer heavy metal ion sensor discussed in Chapter 3 is limited by the thickness of the bilayer coating and hence care should be taken to build up the bilayers to optimal thickness. The direct ligand coating has no such limitations since the ligand is directly functionalized onto the sensor surface enabling effective evanescent wave interaction. Furthermore the [CS/PAA] functionalized sensor does not bind specifically to Ni^{2+} ions since the amine groups in chitosan can bind to other bivalent transition metal ions like Cu^{2+} , Pb^{2+} , and Hg^{2+} etc. The direct ligand functionalized sensor on the other hand is very specific to the binding of Ni^{2+} ions since the ligand used is a Ni^{2+} specific ligand. Lastly the [CS/PAA]

sensor is pH dependent owing to the polycationic nature of chitosan. At low pH values the amine groups in the chitosan are protonated leading to loss in chelation of Ni^{2+} ions. This limits the operating conditions of the polymer based sensors. We propose to utilize a similar strategy to immobilize other novel ligands directly onto the intermodal sensor surface and use it subsequently for the detection of target molecules. The advantages of using this type of sensors are it is compact, easy to fabricate and can be used for testing a wide range of ligand-target species interaction. The proposed sensing system also facilitates the study of binding events using adsorption isotherm models as shown in the preliminary study. Further the sensing system can be utilized to evaluate the selectivity of the synthesized ligands to target species in the presence of other competing species.

Chapter 6

Molecularly imprinted chitosan based photonic crystal fiber sensor for detection of heavy metal ions

6.1 Introduction

Heavy metals are a group of metals possessing high atomic weights and relatively high densities and are toxic or poisonous even at low concentrations [156]. The increase in industrial activities has led to the entry of heavy metals into the environment through air, water and soil. Heavy metals like Pb, Hg, Ni, Cd, and As poses a very serious threat to humans and environment [157]. Nickel in particular is prevalent in the environment and in very low concentrations is vital for some cellular functions. But above a critical concentration nickel can cause allergic skin reactions [158], cardiovascular disorders [159], respiratory disorders [160] and is carcinogenic [161]. Hence monitoring of these heavy metal ions in the environment becomes essential. Current methods of heavy metal detection include ultraviolet—visible spectroscopy, inductively coupled plasma-mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), etc [162-164]. Even though these techniques can detect trace quantities of heavy metal ions and are highly sensitive and selective, they suffer from expensive apparatus, complex procedure, tedious complex preparation and highly trained personnel [157]. Additionally these

methods cannot be employed for the continuous and remote monitoring in situ. Therefore a simple, inexpensive and direct sensing system for the remote and continuous monitoring of heavy metal ions is necessary.

Alternative methods of detection of heavy metal ions utilize different signal transduction techniques including optical [165], electrochemical [166], and field effect transistors [167]. Many different types of optical sensors including fluorescence sensors [168], colorimetric sensors [169], surface enhanced raman spectroscopy (SERS) sensors [170] have been reported. A new class of optic sensors based on optic fibers has gained significant research interest since the last few years. Fiber optic sensors offer several advantages like miniature size, low cost, immunity to electromagnetic interference and multiplexing capability. Among the different types of fiber optic sensors, interferometric sensors have attracted a great deal of interest due to their large dynamic range, high sensitivity and accuracy [45]. The Mach Zehnder interferometer (MZI) configuration offers significant advantages like simple fabrication, direct readout and relatively high sensitivity over the other configurations. In this work, the MZI is fabricated by fusion splicing a segment of photonic crystal fiber (PCF) between two normal single mode fibers (SMFs). A very thin layer of chitosan-nickel film is deposited on the surface of PCF by dip coating and modified chemically through molecular imprinting to be sensitive to Ni^{2+} ions.

Chitosan is a biopolymer derived from the deacetylation of an abundant natural polysaccharide chitin and possesses many desirable properties like excellent chelating ability, biocompatibility and anti bacterial capability. These endear

chitosan to be used in wastewater treatment and in food and pharmaceutical industry. The amine groups present in the chitosan helps in chelation of many heavy metal ions like Ni^{2+} , Cu^{2+} , Pb^{2+} , Zn^{2+} , etc [171, 172]. However there are serious shortcomings of chitosan like poor physical properties and instability due to swelling in aqueous systems, which hinders its practical application [173]. The specific adsorption of metal ions is also an issue as chitosan can chelate a variety of metal ions [174]. Crosslinking is the most often utilized strategy for improving the mechanical properties of chitosan [175]. The downside to crosslinking is the reduction in the adsorption capacity of chitosan towards metal ions. This is due to the crosslinking of chelating NH_2 groups in the chitosan. Inoue et al proposed to solve this problem by crosslinking the chitosan after the formation of Chitosan-metal complex [176]. This method resulted in a higher adsorption capacity of metal ions by the crosslinked chitosan but suffered from a very complicated preparation methodology [177]. To overcome the aforementioned problems molecular imprinting technology has been used to prepare metal ion imprinted chitosan using glutaraldehyde and epichlorohydrin (ECH) as cross-linkers [178-180].

Molecular imprinting technology was first introduced by Ekberg and Mosbach for synthesizing materials for specific molecular separation [181]. Molecular imprinting process involves the creation of a specific space for the imprinting molecule in the adsorbent and thereby results in enhanced sensitivity of the adsorbent for the imprinting molecule [182-184]. During the early days the molecular imprinting process was used majorly for synthesis of molecularly-imprinted polymers (MIPs) as specific chromatographic separation columns for

enantiomer separation [185, 186]. Currently research on MIPs has gained considerable momentum owing to the various advantages offered such as high selective capability, simple and low cost synthesis, and high stability and can be used to target a number of molecules [187, 188]. Recent research mainly focuses on developing MIPs for a range of biomimetic functions including recognition of specific target molecules [189, 190], development and screening of drugs [191, 192], sensors [193-195], chromatographic separation [196, 197], and catalysis [198, 199].

The ease of fabrication of the PCF based MZI sensor and the facile method of coating the sensor surface with chitosan film and the ease with which the chitosan film can be modified by molecular imprinting process to be selective for different metal ions offers a promising alternative to the conventional methods of heavy metal detection.

6.2 Materials & Methods

6.2.1 Materials

Optical fibers including single mode fiber (8.2/125 μm) (SMF-28) and photonic crystal fiber (12/125 μm) (PCF) are purchased from Corning and Thor Labs respectively.

Chitosan (Medium molecular weight), anhydrous nickel chloride salt (>98% pure), and epichlorohydrin (ECH) were purchased from Sigma Aldrich. Sulfuric acid (95%) was purchased from Merck and hydrogen peroxide (30%) was purchased

from VWR. The reagents used were of analytical grade. Ultrapure water (18.2 M Ω cm resistivity) from Mill-Q system was used in preparing the solutions.

6.2.2 Methods

6.2.2.1 Sensor Fabrication

The SMFs (SMF-28, 8.2/125 μ m) and endlessly single mode fiber (ESM-12B, 12/125 μ m) were purchased from Corning and Thorlabs respectively. To fabricate the MZI, a PCF segment of 20 mm length was fusion spliced between the standard SMFs.

6.2.2.2 Sensor Functionalization

2g of chitosan was dissolved in 100ml of 4% acetic acid solution and continuously stirred for 24 hours. 0.1g of NiCl₂ was added to this chitosan solution and stirred continuously until the mixture is homogenous. Subsequently the mixture was filtered to remove the undissolved NiCl₂ particles. The sensor was treated with piranha solution (70% sulphuric acid and 30% hydrogen peroxide) for 60 minutes, followed by immersion in Millipore water and subsequent drying under a stream of nitrogen gas. The piranha treatment activates the sensor surface by hydroxylation and prepares the sensor for functionalization. For carrying out functionalization the surface activated sensor was immersed in the (CS)-nickel (Ni) mixture and was drawn out at a rate of 70 mm/min by using KSV Nima dip coater. A thin layer of CS-Ni film is functionalized on the fiber and is air dried for 5 minutes followed by drying overnight in a vacuum oven at 60 ⁰C. The fibers were then treated with epichlorohydrin (ECH) for 120 minutes to facilitate cross linking. The crosslinked

CS-Ni film functionalized sensor as then immersed in 1mM hydrochloric acid solution for 120 minutes to ensure the release of Ni^{2+} ions. At the end of this process the sensor was functionalized with Ni^{2+} imprinted ECH crosslinked chitosan (Ni-ECH-CS) film. The spectrum of the sensor was monitored after each stage of the functionalization process and recorded.

6.2.2.3 Preparation of nickel chloride test solutions

A 1mM stock solution of nickel chloride was prepared by dissolving 0.013g of NiCl_2 in 100 ml of 10mM, pH 6, 2-(N-morpholino) ethanesulfonic (MES) acid solution. Subsequent concentrations of nickel solutions were obtained by dilution of the stock solution.

6.2.2.4 Sensing system setup

The sensing system schematic is shown in Figure 6-1. The sensor is interrogated by a broadband light source and the transmission spectrum of the sensor is monitored continuously by an optical spectrum analyzer (OSA).

Figure 6-1: Sensing system scheme

6.3 Experimental results and discussion

6.3.1 Sensing Principle and sensor calibration

The sensing system schematic is shown in Figure 6-1. The sensor is interrogated by a broadband light source and the transmission spectrum of the sensor is monitored continuously by an optical spectrum analyzer (OSA). On fusion splicing, the air holes collapse along a short length of the PCF, resulting in the independent excitation of core and cladding modes in the PCF segment [200]. The excited modes travel along the PCF section at different phase velocities and interfere with an accumulated phase difference at the second collapsed region of the PCF.

The transmission spectrum of the sensor exhibits periodic wavelength shifts because of the intermodal dispersion induced wavelength dependency of phase velocity and phase difference and can be expressed by Eq. (1) [201]. The transmission of the PCF sensor is $T(\lambda)$, the intensities of the core and cladding modes are I_{core} and $I_{cladding}$ respectively, the difference between effective refractive index (RI) changes of core and cladding modes is Δn_{eff} , the operating wavelength is λ and the length of the PCF segment is L .

$$T(\lambda) =$$

$$I_{core}(\lambda) + I_{cladding}(\lambda) + 2\sqrt{I_{core}(\lambda) \cdot I_{cladding}(\lambda)} \cdot \cos \frac{2\pi \cdot \Delta n_{eff} \cdot \lambda}{\lambda} \quad (6.1)$$

From Eq.(1) it can be seen that the transmission spectrum of the PCF sensor can be approximated to a sinusoidal function of λ and the simplified 2 mode interference

model can be applied to this PCF sensor. When $2\pi\Delta n_{eff}/\lambda = 2m\lambda$, where m is an integer, the transmission spectrum reaches a minimum and the transmission spectral dip wavelength λ_m can be expressed as [202]

$$\lambda_m = \frac{\Delta n_{eff}L}{m} \quad (6.2)$$

As seen from Eq. (6.2) any change in the effective refractive index causes a change in the dip wavelength λ_m and subsequently a shift in the interference spectrum. The changes in external environment perturb the cladding modes only and the core mode propagates undisturbed. Hence any change in the RI at the sensor-ambient interface induces a change in the effective refractive index of the cladding modes and thereby changing Δn_{eff} , resulting in a phase difference and shifting the transmission spectrum of the sensor.

The response of the sensor to RI changes was conducted by immersing the PCF portions in glycerol solutions of various concentrations and is shown in Figure 6-2 (inset). It is observed that the interference spectra dips shift towards longer wavelengths as given by Eq. (6.2). The dip shift vs refractive index plot is shown in Figure 6-2, and it is observed that the sensor exhibits a non linear response with change in refractive index. The refractive index response can be divided into two linear regions: a low refractive index region (1.33-1.3958) and a high refractive index region (1.40-1.4242). The corresponding refractive index sensitivities are determined to be 185.84 nm/RIU for the low RI region and 397.47 nm/RIU for the

high RI region. The RI sensitivity of the proposed sensor is comparable or relatively higher than other reported interferometric sensors [203-206].

Figure 6-2: Sensor spectral response to RI changes (insets) and sensor linear sensitivity regions.

6.3.2 Characterization of the nickel adsorbed chitosan film by energy dispersive X-ray spectroscopy and scanning electron microscopy (SEM)

The thin films of pure chitosan and nickel adsorbed chitosan were examined with energy dispersive X-ray spectroscopy (EDX) and the corresponding micrographs are presented in Figure 6-3(a) and (b) respectively. It is observed that in the pure chitosan sample no significant spike is observed for nickel whereas in the nickel adsorbed chitosan sample a strong spike is observed for nickel confirming the adsorption of nickel by chitosan. The SEM micrographs of pure chitosan and

nickel adsorbed chitosan are presented in Figure 6-4 (a) and (b) respectively. It is observed that the surface of pure chitosan is relatively smoother compared to that of the nickel adsorbed chitosan surface. The rough surface of the nickel adsorbed chitosan can be attributed to the chelation of nickel by the amino and hydroxyl groups of chitosan resulting in fold like structures.

Figure 6-3: EDX micrograph of (a) Chitosan (b) Nickel adsorbed chitosan

Figure 6-4: SEM micrograph of (a) Chitosan (b) Nickel adsorbed chitosan

6.3.3 Ni-ECH-CS functionalized sensor response to Ni²⁺ ions: Sensitivity and limit of detection (LOD)

The sensor was functionalized with chitosan crosslinked with ECH and imprinted with Ni²⁺ ion and the functionalization scheme is presented in Figure 6-5. The spectrum of the PCF sensor as monitored continuously throughout the functionalization process and is shown in Figure 6-6. It is observed that after the functionalization of the nickel adsorbed chitosan film the spectrum of the sensor shifts to longer wavelengths due to the increase in the ambient RI around the PCF. The spectrum shifts further on crosslinking with epichlorohydrin (ECH) due to the crosslinking of the hydroxyl groups of chitosan. The sensor was finally immersed in 1mM HCl solution for removing the Ni²⁺ ions and the spectrum can be seen to be shifting to the shorter wavelengths presumably due to the voids created in the polymer matrix on removal of Ni²⁺ ions.

Figure 6-5: Ni^{2+} imprinted chitosan – Preparation scheme

Figure 6-6: Sensor responses during functionalization of Ni -ECH-CS film

The Ni-ECH-CS functionalized sensor was immersed in Ni^{2+} solutions of different concentrations ranging from $10\mu\text{M}$ to 1mM and the spectral response was continuously monitored and recorded. Figure 6-7 shows the spectra of sensor with change in concentration of Ni^{2+} ions and it is observed that the spectral dip shifts to the longer wavelength with increase in concentration of Ni^{2+} ions. This can be attributed to the adsorption of Ni^{2+} ions onto the imprinted sites of the crosslinked chitosan leading to an increase in the ambient RI around the PCF surface. Increase in concentration of Ni^{2+} ions results in more binding sites being occupied and a further increase in ambient RI leading to a shift in the spectral dips to longer wavelengths.

Figure 6-7: Ni-ECH-CS functionalized sensor response to Ni^{2+} ions.

The sensor dip shift vs concentration curve is presented in Figure 6-8 and the linear sensitivity curve of the sensor is shown in Figure 6-8 (Inset). The sensor exhibits a

linear response in the range of 0-50 μM with a Ni^{2+} detection sensitivity of 0.0604 nm/ μM .

Figure 6-8: Ni-ECH-CS functionalized sensor: Ni^{2+} ions calibration curve and (inset) linear response.

For evaluating the limit of detection (LOD) the experimental dip shift vs concentration plot was fit into Langmuir isotherm model. The Langmuir isotherm model is expressed by the following equation,

$$\Delta\lambda = \Delta\lambda_{max} \left(\frac{KC}{1+KC} \right) \quad (6.3)$$

Where $\Delta\lambda$ is the spectral dip shift wavelength due to adsorption of Ni^{2+} ions onto the Ni-ECH-CS coated PCF sensor surface, $\Delta\lambda_{max}$ is the maximum wavelength shift due to complete monolayer formation, C is the concentration of Ni^{2+} in solution and K is the equilibrium binding constant under steady state conditions. Langmuir

isotherm based non linear regression model is used for determining values of K and $\Delta\lambda_{max}$. Figure 6-9 shows the Langmuir model fit of the wavelength shifts vs Ni^{2+} concentration plot and the Langmuir parameter values are determined to be $\Delta\lambda_{max} = 6.12 \text{ nm}$ and $K = 0.01633 (\mu\text{M})^{-1}$.

Figure 6-9: Ni-ECH-CS functionalized sensor response to Ni^{2+} ions and the associated Langmuir fit.

The resolution of the OSA is the major factor affecting the limit of detection of the proposed sensing system. Hence the value of $\Delta\lambda$ is made equal to the OSA resolution (0.02 nm) and the limit of detection C_{LOD} is expressed as

$$C_{LOD} = \frac{\Delta\lambda}{K(\Delta\lambda_{max} - \Delta\lambda)} \quad (6.4)$$

Using K and $\Delta\lambda_{max}$ values obtained from the Langmuir model fit, limit of detection of the sensor is calculated to be **0.2008 μM**.

6.3.4 Effect of crosslinking agent concentration on sensor response:

The effect of the molar ratio of ECH to chitosan on the performance of the sensor was investigated by treating the CS-Ni film functionalized sensor with ECH in the molar ratios (ECH to chitosan) of 5:1, 10:1 and 15:1 respectively for 2 hours followed by treatment with 1 mM HCl for 2 hours. The sensors were then tested with Ni^{2+} solutions of various concentrations and the respective dip shift vs concentration curves are presented in Figure 6-10. It is observed that the sensor with a molar ratio of 10:1 exhibits the highest sensitivity to Ni^{2+} detection. In the case of lower molar ratio (5:1) there is a decrease in sensor performance is presumably due to weak mechanical integrity of the Ni-ECH-CS film due to lesser degree of cross linking and hence resulting in slightly weaker adherence to the sensor surface [114]. In the case of higher molar ratio (15:1), the performances of the sensor degrades due to the extensive and increased cross linking of the different chitosan chains resulting in a reduced swelling state and reduced accessibility for the Ni^{2+} ions.

Figure 6-10: Effect of ECH concentration on Ni-ECH-CS functionalized sensor response to Ni^{2+} ions (Molar ratio of ECH to CS-5:1, 10:1 and 15:1).

6.3.5 Sensor specificity

To highlight the specificity performance of the sensor, an PCF sensor functionalized with pure ECH crosslinked chitosan (ECH-CS) was examined with 50μM concentration solutions of nickel and copper ions and the sensor responses are shown in Figure 6-11. It is observed that the sensor shows a slightly greater dip shift towards Cu^{2+} ions than Ni^{2+} ions since pure chitosan has higher affinity towards Cu^{2+} ions than Ni^{2+} ions. Next the Ni-ECH-CS sensor was examined in 50μM solutions of Ni^{2+} , Cu^{2+} , Na^+ and Ca^{2+} ions. The responses of the sensor to each of these metal ions is shown in Figure 6-12 and it is observed that the sensor shows the greatest dip shift for Ni^{2+} ions. Sodium and calcium being alkali and

alkaline earth metals are not adsorbed by the nickel ion imprinted chitosan and hence the sensor displays negligible response to these ions. Additionally the Ni-ECH-CS sensor shows a significant decrease in response towards Cu^{2+} ions compared to that of the ECH-CS sensor presumably due to the imprinting of chitosan with Ni^{2+} which drastically improves the affinity of chitosan to Ni^{2+} ions. This proves the proposed sensor's specificity towards Ni^{2+} ions over other heavy metal ions like Cu^{2+} and alkali and alkaline earth metal ions.

Figure 6-11: ECH crosslinked chitosan (ECH-CS) functionalized sensor response to 50 μM Ni^{2+} and 50 μM Cu^{2+} solutions.

Figure 6-12: Ni^{2+} imprinted chitosan (Ni-ECH-CS) functionalized sensor response to $50\mu\text{M}$ Ni^{2+} , $50\mu\text{M}$ Cu^{2+} , $50\mu\text{M}$ Ca^{2+} and $50\mu\text{M}$ Na^+ solutions.

6.4 Chapter conclusion

A photonic crystal fiber based MZI interferometric fiber sensor functionalized with Ni^{2+} ion imprinted chitosan and ECH as crosslinker for highly sensitive and specific detection of Ni^{2+} ions is proposed and demonstrated. The sensor exhibits a Ni^{2+} detection sensitivity of $0.0604 \text{ nm}/\mu\text{M}$ in the linear range and a limit of detection of $0.2008 \mu\text{M}$. The sensitivity of the sensor is comparable to the other heavy metal ion sensors discussed in the previous chapters. Additionally the sensor displays better specificity towards Ni^{2+} ions compared to Cu^{2+} , Ca^{2+} , and Na^+ ions. This is a marked improvement in specificity compared to the [CS/PAA] functionalized sensor which suffers from cross-sensitivity to other divalent transition metal ions. The mechanical strength of the chitosan film was further

improved by crosslinking the hydroxyl groups of chitosan with ECH and preserving the amine groups of chitosan which are essential for binding the Ni^{2+} ions. This sensing methodology could be further extended to selectively detect a variety of heavy metal ions by synthesizing imprinted chitosan films sensitive to the metal ion of interest by choosing the desired metal ion to be the template ion. The promising results of this sensor make it a viable alternative to the conventional methods of heavy metal ion detection.

Chapter 7

Conclusions & future works

7.1 Summary of the thesis work

In this work, a heavy metal ion sensor based on chitosan/PAA bilayer functionalized MZI sensor was first reported. A fiber-optic interferometric sensor for Ni^{2+} detection has been developed and experimentally demonstrated. The proposed sensor has many advantages such as ease of fabrication, compact structure, fast response, low cost and a relatively wide measuring range. The fiber was functionalized with electrostatically self-assembled multilayers $[\text{CS/PAA}]_{10}$ for effective Ni^{2+} adsorption and the detection sensitivity was determined to be $0.05537 \text{ nm}/\mu\text{M}$. The kinetic profile of Ni^{2+} adsorption onto the sensor was further analyzed by the Langmuir isotherm parameters and a lower detection limit of $0.1671 \mu\text{M}$ was achieved. The specificity and the lower detection limit of these heavy metal ion sensors could be further improved by immobilizing a suitable cation receptor (ligand) onto the polymer matrix [101], which would be the focus of future work. The promising results of this sensor encourage it to be employed in many potential applications such as monitoring of heavy metal ions in drinking water.

The previous work showcased the potential of chitosan for the chelation of Ni^{2+} ions. This part of the thesis extends on this concept and uses a chitosan-nickel film as the substrate for binding hexa-histidine tagged proteins. A CS-Ni film based

fiber-optic interferometric sensor for label-free detection of polyhistidine tagged proteins has been proposed and experimentally demonstrated. By monitoring wavelength shift of the sensor spectrum, the proposed biosensor was able to detect His-MccS effectively with sensitivity of 0.0308 nm/(ng/ml). The kinetics of His-MccS adsorption onto the CS-Ni functionalized NCF sensor was analyzed by fitting the sensor response to Langmuir isotherm model and the sensor's limit of detection was determined to be 0.8368 ng/ml. The selectivity of the proposed sensor towards histidine tagged proteins was comparatively evaluated with a full protein–trypsin and it was concluded that specificity mainly depends on the affinity of His-Tag to CS-Ni film. The proposed biosensor combines the advantages of small size and high sensitivity of NCF sensors and exhibited rapid response compared to existing techniques such as SDS-PAGE and western blot. The positive result of this sensor makes it a viable candidate to be used for high throughput screening in bioprocesses.

The third work of this thesis proposes an alternative method for countering the instability issues encountered in polymer based heavy metal ion sensors. A novel fiber optic intermodal sensor for detection of Ni^{2+} ions has been demonstrated. A very specific ligand to Ni^{2+} , Meso-Tetra(4-carboxyphenyl)porphine ($\text{C}_{48}\text{H}_{30}\text{N}_4\text{O}_8$) is directly immobilized onto the NCF sensor through covalent bonding and acts as binding sites for Ni^{2+} ions. Sensitivity of the sensor to Ni^{2+} ions was evaluated to be 121.03 nm/mM and the sensor is a viable alternative to polymer based fiber optic heavy metal ion sensors. The positive results prove the feasibility of covalent immobilization strategy of ligands directly on to the fiber optic sensor surface. In

this study we had used a commercially available ligand which is specific for Ni^{2+} ions. We propose to utilize a similar strategy to immobilize other novel ligands directly onto the intermodal sensor surface and use it subsequently for the detection of target molecules. The advantages of using this type of sensors are it is compact, easy to fabricate and can be used for testing a wide range of ligand-target species interaction. The sensing system also facilitates the study of binding events using adsorption isotherm models as shown in the preliminary study. Further the sensing system can be utilized to evaluate the selectivity of the synthesized ligands to target species in the presence of other competing species.

The final work in this thesis discusses a method to use chitosan for heavy metal ion sensing but without the major disadvantage of non-specificity to target metal ions. A photonic crystal fiber based MZI interferometric fiber sensor functionalized with Ni^{2+} ion imprinted chitosan and ECH as crosslinker for highly sensitive and specific detection of Ni^{2+} ions is proposed and demonstrated. The sensor exhibits an Ni^{2+} detection sensitivity of $0.0604 \text{ nm}/\mu\text{M}$ in the linear range and a limit of detection of $0.201 \mu\text{M}$. The sensor also shows a good specificity to Ni^{2+} ions compared to Cu^{2+} , Ca^{2+} , and Na^+ ions.

In summary, heavy metal ion and protein detection based on chitosan functionalized inline optical fiber interferometric sensors have been presented. The heavy metal ion sensors are based on MZI interferometric sensors implemented through NCF and PCF. The first work combines the high RI sensitivity of a NCF based MZI sensor and metal chelation property of chitosan along with its polycationic property for developing a optical fiber chemical sensor for sensitive

detection of Ni^{2+} ions. This MZI heavy metal ion sensor is easier to fabricate, is compact in size and could be used for continuous monitoring of Ni^{2+} ions. Hence this sensing technique could be a viable alternative to existing detection techniques like ICP-MS, atomic adsorption spectroscopy etc. The second work uses the metal binding affinity of chitosan to act as an immobilization matrix for Ni^{2+} ions on the NCF sensor surface and using the immobilized Ni^{2+} ions to detect histidine tagged proteins. This sensor exhibits relatively faster response times than other detection techniques like SDS-PAGE and Western Blot and hence could potentially be developed into a high throughput screening system for bioprocess. The third work involved the development of a sensor based on direct immobilization of the ligand onto the optical fiber sensor surface is presented. This work eliminates the limitations attributed to polymer based optical sensors by presenting a novel and direct immobilization strategy for covalently binding the ligand to sensor surface. The promising results of this work pave the way for development of polymer-free optical fiber chemical sensors and eliminate the limitations associated with polymer based optical chemical sensors. The final work involved the development of a molecularly imprinted chitosan based PCF MZI sensor for the selective detection of Ni^{2+} ions. The promising results of this sensor can lead to the development of highly selective optical fiber interferometric sensors based on molecular imprinted chitosan for detection of a variety of chemical species by suitably changing the template ions.

7.2 Future works

Microfluidic system for detection of chemical/biological species

In this thesis work we have successfully implemented optical fiber sensors for detection of chemical species (heavy metal ions) and biological species (histidine tagged proteins). Chitosan being a versatile sensing polymer, chemical modifications can be performed for addition of new functional groups to the polymer chain or modification of the existing functional groups to detect a wide range of chemical/biological species [207, 208]. Future work is suggested for developing a microfluidic based sensing system utilizing a microfluidic chip integrated with chitosan based interferometric sensors for a variety of applications like sensing of chemical species and biological species. The scheme of the system is shown in Figure 7-1. The proposed sensing system utilizes microfluidic principles for handling the samples and the associated buffers. The fiber optic sensor functionalized with chitosan can be embedded into the micro channel and examined with various samples. All the solutions are circulated through the system using a micro pump. This microfluidic sensing system with chitosan functionalized optical fiber could offer a perfect method for portable and continuous detection of chemical/biological species. In the case of protein detection, this system can be developed into a full scale high throughput screening system and can be applied for screening of proteins in bioprocesses.

Figure 7-1: Microfluidic sensing system scheme

Author's publications

Journal publications

1. **Raghunandhan, R.**, et al., *Chitosan/PAA based fiber-optic interferometric sensor for heavy metal ions detection*. Sensors and Actuators B: Chemical, 2016. **233**: p. 31-38.
2. **Ravikumar, R.**, et al., *Chitosan-nickel film based interferometric optical fiber sensor for label-free detection of histidine tagged proteins*. Biosensors and Bioelectronics, 2018. **99**: p. 578-585.

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1. **Ravikumar, R.**, et al. *Chitosan-hydrogel-based fiber optic sensor for heavy metal ion detection*. in International Conference on Optical Fibre Sensors (OFS24). 2015. International Society for Optics and Photonics.
2. **Ravikumar, R.**, et al. *Fiber Optic pH Sensor Based on Electrostatically Self-Assembled Polymer Multilayer*. in Workshop on Specialty Optical Fibers and Their Applications. 2015. Hong Kong: Optical Society of America.
3. **Raghunandhan, R.**, L.H. Chen, and C.C. Chan. *Fiber optic nickel ion sensor based on direct ligand immobilization*. in 2017 25th Optical Fiber Sensors Conference (OFS). 2017.

Co-authored publications

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1. Yang, J., L.H. Chen, Y. Zheng, X. Dong, R. Raghunandhan, P.L. So, and C.C. Chan, *Heavy metal ions probe with relative measurement of fiber Bragg grating*. Sensors and Actuators B: Chemical, 2016. **230**: p. 353-358.
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