Towards the Early Malaria Detection Based on Magneto-Optical Methods in Specialty Optical Fiber

by

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Vers le dépistage précoce de la malaria basé sur des méthodes magnéto-optiques dans une fibre optique spéciale

Saeed AZAD

RéSUMÉ

La malaria est l'une des maladies transmises par vecteurs les plus graves et une préoccupation majeure pour la santé publique dans le monde entier. Cette maladie potentiellement léthale est transmise par les moustiques dans les régions tropicales. Lors d'une piqûre de moustique, les parasites unicellulaires du plasmodium sont transportés par des moustiques infectés et entrent dans le sang humain. Le Plasmodium falciparum est le parasite responsable de la forme la plus grave de la malaria. Au niveau mondial, 300 à 500 millions de personnes sont infectées par la maladie chaque année et 1,5 à 2,7 millions de personnes en décèdent. La plupart des techniques de détection de la malaria dépendent de l'expertise, d'essais sophistiqués ainsi que de processus longs et fastidieux. Le sujet général de cette thèse concerne les capteurs à fibres optiques basés sur des fibres à cristaux photoniques (PCF) et des fibres spéciales (SF). Nous voulons connaître les avantages de l'utilisation de PCF et SF par rapport aux techniques de détection conventionnelles. En particulier, nous sommes intéressés par la détection du pigment de la malaria aux premiers stades de l'infection.

Le pigment de la malaria, également connu sous le nom de hemozoin (Hz), est un sous-produit de la maladie formé pendant le cycle de croissance des parasites. Ces pigments ont une structure cristalline triclinique avec une morphologie variée selon l'espèce de parasite. Ils ont généralement une forme allongée en forme de tige, avec une longueur allant de 300 nm à 1 µm. Ces micro-cristaux sont inertes, insolubles dans les solutions aqueuses, de couleur marron foncée et ont une caractéristique paramagnétique.

Chez une personne en bonne santé, Hz n'est pas présent. Par conséquent, Hz sert de biomarqueur diagnostique de l'infection à la malaria. L'importance et les singularités de ce biocristal ont incité les scientifiques à l'étudier comme cible potentielle pour les diagnostics et les thérapies contre la maladie à l'aide de techniques innovantes. En gros, l'extraction de Hz naturel (nHz) est un processus long et coûteux qui nécessite des installations biomédicales spéciales ainsi que l'expertise pour infecter des hôtes vivants (par exemple, des rats) avec la malaria et obtenir le nHz. Par conséquent, il existe un fort argument en faveur de la promotion de nouveaux progrès de la recherche dans ce domaine pour travailler avec la forme synthétique de Hz (SHz), également appelée β-hématine. Cette imitation de Hz a une structure qui est comparable physiquement et chimiquement à son homologue naturel.

Plusieurs travaux antérieurs ont étudié la synthèse de Hz, tandis que d'autres ont examiné les caractéristiques physico-chimiques de la production de SHz. Cependant, il reste des lacunes
dans la littérature scientifique sur la façon de contrôler la taille des composés SHz afin d'obtenir des dimensions similaires à celles de Hz naturellement présentes.

Le but principal de cette thèse est de détecter SHz en utilisant des fibres à cristaux photoniques (PCF) basées sur les caractéristiques magnétiques de Hz. À cet égard, une procédure systématique pour produire des SHz avec des caractéristiques de taille contrôlables compatibles avec nHz a été démontrée. De plus, une caractérisation physique détaillée a été effectuée sur les échantillons obtenus, en utilisant divers diagnostics tels que la magnétométrie par échantillon vibrant (VSM), la microscopie électronique à balayage (SEM) et la mesure de l'indice de réfraction (RI).

À notre connaissance, aucune étude n'a produit de SHz de taille comparable à ceux naturels tout en considérant les propriétés structurelles, optiques, chimiques et magnétiques dans une seule étude. Cette thèse est classée en trois parties différentes. Dans la première étape, la capacité du capteur proposé à détecter la présence de particules magnétiques dans une solution aqueuse a été considérée. Dans cette étape, les trous d'air des PCF infiltrés avec un fluide magnétique (MF) et la puissance transmise des PCF ont été surveillés en présence d'un champ magnétique. Dans la prochaine étape, nous nous sommes concentrés sur la synthèse de l'imitation de nHz à travers une procédure simple suivie d'une caractérisation détaillée. Enfin, nous avons considéré les performances du capteur pour détecter le SHz dans une solution aqueuse à différentes concentrations.

**Mots clés :** Paludisme, hemozoin, paramagnétique, détection rapide, capteur à fibres optiques, fibre cristalline photonique
Towards the Early Malaria Detection Based on Magneto-Optical Methods in Specialty Optical Fiber

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ABSTRACT

Malaria is one of the most serious vector-borne diseases and a major public health concern worldwide. This potentially lethal disease is spread by mosquitoes in tropical areas. During a mosquito bite, single-celled plasmodium parasites are carried by infected mosquitoes and enter the human bloodstream. Plasmodium falciparum is the parasite that causes malaria's most severe form. Globally, 300 to 500 million people are infected with the disease every year and 1.5 to 2.7 million people die as a result. The majority of malaria detection techniques rely on expertise, sophisticated assays as well as time consuming processes.

The general subject of this thesis pertains to optical fiber sensors based on photonic crystal fibers (PCF) and specialty fibers (SF). We want to know what are the benefits of using PCF and SF instead of conventional sensing techniques. In particular, we are interested in the detection of malaria pigment in the early stages of infection.

Malaria pigment also known as hemozoin (Hz) is a byproduct of the disease formed during the growth cycle of the parasites. These pigments have triclinic crystal structure with various morphology depending on the parasite species. They usually have an elongated rod-like shape with a length ranging from 300 nm to 1 µm. Malaria pigments are inert, insoluble in aqueous solution with dark-brown color and a paramagnetic feature.

In a healthy individual, Hz is not present. As a result, Hz serves as a diagnostic biomarker of malaria infection. The significance and oddities of this biocrystal have prompted scientists to investigate it as a potential target for diagnostics and therapies against the disease using innovative techniques. Basically, extracting natural Hz (nHz) is a time-consuming and costly process that needs special biomedical facilities as well as the expertise to infect living hosts (e.g. rats) with malaria and obtain the nHz. Therefore, there is a strong argument for promoting new research advances in this area to work with the synthetic form of Hz (SHz), aka β-hematin. This Hz imitation has a structure that is physically and chemically comparable to its natural counterpart.

Several prior works have studied the synthesis of Hz while others investigated the physicochemical characteristics of SHz production. However, there are still gaps in the scientific literature on how to control the size of SHz compounds so as to obtain similar dimensions to the naturally occurring Hz.

The main goal of this thesis is to detect SHz employing PCF based on the magnetic feature of Hz. In this regard, a systematic procedure to produce SHz with controllable size features that
are compatible with nHz was demonstrated. In addition, a detailed physical characterization was performed on the resulting samples, using various techniques including vibrating sample magnetometry (VSM), scanning electron microscopy (SEM), and refractive index (RI) measurements.
To the best of our knowledge, no study has produced SHz of comparable size to natural ones as well as considering structural, optical, chemical, and magnetic properties in one study.

This thesis is structured in three different parts. In the first step, the ability of the proposed sensor to detect the presence of magnetic particles in aqueous solution was considered. In this step the air holes of PCF infiltrated with magnetic fluid (MF) and the transmitted power of the PCF was monitored in presence of magnetic field. In the next step, we focused on synthesizing the mimic of nHz through straightforward procedure followed by detailed characterization. Finally, we considered the sensor performance to detect SHz in aqueous solution with various concentrations.

Keywords: Malaria, hemozoin, paramagnetic, rapid detection, optical fiber sensor, photonic crystal fiber
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<td>PCF</td>
<td>Photonic crystal fiber</td>
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<td>SF</td>
<td>Specialty fiber</td>
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<td>Hz</td>
<td>Hemozoin</td>
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<td>nHz</td>
<td>Natural hemozoin</td>
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<td>VSM</td>
<td>Vibrating sample magnetometry</td>
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<td>SEM</td>
<td>Scanning electron microscopy</td>
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<td>RI</td>
<td>Refractive index</td>
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<td>MF</td>
<td>Magnetic fluid</td>
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<td>XRD</td>
<td>X-ray diffraction</td>
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<td>FTIR</td>
<td>Fourier-transform infrared</td>
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<td>HRSEM</td>
<td>High-resolution scanning electron microscopy</td>
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<td>IOT</td>
<td>Internet of things</td>
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<tr>
<td>AC-PCF</td>
<td>Annular core photonic crystal fibre</td>
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<tr>
<td>RDT</td>
<td>Rapid diagnostic test</td>
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<td>HRP-2</td>
<td>Histidine-rich protein 2</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>S3M</td>
<td>Secondary speckle sensing microscopy</td>
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<td>MNPs</td>
<td>Magnetic nanoparticles</td>
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<tr>
<td>eFBG</td>
<td>Etched fiber Bragg grating</td>
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<tr>
<td>FBG</td>
<td>Fiber Bragg grating</td>
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<td>TCF</td>
<td>Thin core fiber</td>
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<td>POF</td>
<td>Polymer optical fiber</td>
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MZI  Mach–Zehnder interferometer
SNS  Single mode-nocore-single mode
FEM  Finite element method
RBCs Red blood cells
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<tr>
<td>T</td>
<td>Temperature</td>
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<tr>
<td>( \lambda )</td>
<td>Wavelength</td>
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<td>S</td>
<td>Spin number</td>
</tr>
<tr>
<td>L</td>
<td>Length</td>
</tr>
<tr>
<td>( \omega )</td>
<td>Frequency</td>
</tr>
<tr>
<td>S</td>
<td>Spin number</td>
</tr>
<tr>
<td>( \theta )</td>
<td>Angle</td>
</tr>
<tr>
<td>c</td>
<td>Speed of light</td>
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<tr>
<td>H</td>
<td>Magnetic field vector</td>
</tr>
<tr>
<td>( H_{cn} )</td>
<td>Critical magnetic field strength</td>
</tr>
<tr>
<td>E</td>
<td>Electric field vector</td>
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<tr>
<td>( \alpha )</td>
<td>Fitting parameter</td>
</tr>
<tr>
<td>( n_0 )</td>
<td>Refractive index of surrounding environment</td>
</tr>
<tr>
<td>( n_1 )</td>
<td>Refractive index of liquid</td>
</tr>
<tr>
<td>( n_{cld} )</td>
<td>Effective index cladding of fiber</td>
</tr>
<tr>
<td>( n_{eff} )</td>
<td>Effective refractive index</td>
</tr>
<tr>
<td>( n_{cld} )</td>
<td>Effective index cladding of fiber</td>
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<tr>
<td>( n_{MF} )</td>
<td>Refractive index n of MF</td>
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<tr>
<td>( n_s )</td>
<td>Saturated value of the RI of MF</td>
</tr>
<tr>
<td>( \varepsilon_{eff} )</td>
<td>Effective complex permittivity</td>
</tr>
<tr>
<td>( c_{SHz} )</td>
<td>Volume concentration of SHz</td>
</tr>
<tr>
<td>( \varepsilon_d )</td>
<td>Permittivity of water</td>
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\( \mu_{SHz} \)  Relative permeability of the SHz
\( \sigma \)  Radius of Gaussian beam
\( \Lambda \)  Air hole pitch
\( d \)  Air hole diameter
\( N \)  Number of air hole ring
\( C_j \)  Normalized amplitude coupling coefficients
\( \alpha \)  Fitting parameter
INTRODUCTION

Motivation

With the advent of the Internet of things (IoT), wearable sensors and personalized medicine, there is a growing demand for compact and reliable sensors to provide biosensing and environmental feedback to users and/or artificially intelligent beings. The general subject of this thesis pertains to optical fiber sensors based on PCF and SF.

In particular, we are interested in the following application scenarios of such novel sensors: PCF/SF functionalized with internal fluids (MF, aqueous solution of SHz) for magnetic field sensing and detection of malaria pigment. A specific target of this review is to find recent methods and techniques to perform the optical detection of malaria in a more compact manner.

Relation between Magnetic field sensing and Malaria detection

Basically, during malaria infection process, the spin state of Fe ions would change which leads to variation in magnetic properties of infected blood (Butykai et al., 2013). In infected individuals, the blood would contain malaria pigment with paramagnetic properties which respond to external magnetic field. In another point of view, any liquid containing magnetic particles can be applied for magnetic field sensing application. So, in this thesis we initially tried to design an optical sensor based on PCF and MF not only to detect magnetic field but also as an ideal model towards malaria detection. In this step we achieved some results that confirm using PCF in conjunction with aqueous solution of magnetic particles can detect magnetic field in a fraction of second employing small amount of MF (2 µl).
The identified research problem and its potential solutions

Early malaria diagnosis and treatment are essential to stopping the disease's spread and lowering patient death. Conventionally, the diagnosis of malaria is made through empiric/syndromic diagnosis, antibody-based rapid diagnostic tests, microscopic analysis, serological analysis using different components (figure 0.1).

![Figure 0.1 Schematic illustration of reported malaria diagnostic sensors' components (Ragavan et al., 2018)](image)

Malaria has a high death and morbidity rate among poor population with hard-to-reach health care facilities. In particular, if an infected person is identified quickly, treatment is almost always successful when properly administered and prescribed (Bigaillon et al., 2005). Consequently, in order to eradicate malaria, it is essential to recognise and treat the affected population.

Optical microscopy and rapid diagnostic tests (RDT) based on antibodies are the two approaches that are utilised the most frequently. For more than a century, the gold standard for diagnosing malaria has been light microscopy of blood smears (Conway, 2007; Makler et al., 1998). It is the most trustworthy technique currently in use for determining infections caused by any malaria parasite. However, while using powerful microscopes is required, it is labor-intensive, expensive, and low throughput (Butykai et al., 2013; Makler et al., 1998). Since the initial demonstration, species-specific RDTs based on various proteins and biomarkers have
been developed. For example, RDTs detecting the presence of Plasmodium lactate dehydrogenase (specific to P. ovale, P. vivax and P. malaria) histidine-rich protein 2 (HRP-2) (specific to Plasmodium falciparum) have been established (Bigaillon et al., 2005; Moody, 2002). The stability of the reagent(s) is, nevertheless, a crucial problem in a low-resource context, as it is for any antigen-based diagnosis (Ashley et al., 2009). Additionally, there have lately been concerns raised regarding the precision of these antibodies in the diagnosis of malaria (WHO, 2017). Therefore, there is an obvious need for and interest in creating a malaria screening method that is free from user mismanipulation.

An indication of the disease is at the core of every diagnostic procedure. Initially known as the pigment for malaria (Egan et al., 2006), hemozoin is a parasite by-product made of magnetic micro crystals (Ambele et al., 2013; Newman et al., 2008). It is an excellent biomarker since it is specific to malaria. Notably, hemozoin has substantial paramagnetic activity, reacting to weak magnetic fields, unlike all other naturally occurring compounds in the blood. Consequently, hemozoin is a sign of malaria infection if it is discovered in a patient's blood. Hemozoin's form varies depending on the parasite species. The specificity of hemozoin as a malaria indicator has led to the development of a variety of detection techniques. Raman spectroscopy and laser desorption mass spectrometry are two of the most used techniques that may reliably identify concentrations of hemozoin that are relevant for therapeutic use (Demirev et al., 2002; Hobro et al., 2013). These approaches, like traditional smear optical imaging, need a lot of time and equipment, as well as substantial user training in order to yield a reliable diagnostic.

A hemozoin imitation known as \( \beta \)-hematin has been developed concurrently with the creation of these detection technologies, enabling the study of hemozoin without handling samples that have been infected with malaria (Sandlin et al., 2016). Hemozoin from P. falciparum and \( \beta \)-hematin share the same magnetic and optical characteristics as well as the same unit crystal structure (Jaramillo et al., 2009).
Utilizing these distinctive characteristics of β-hematin, we have demonstrated straightforward synthetic procedure compatible with hemozoin, which rarely considered in previously reported literatures.

Since our proposed β-hematin sensor operates using specialty optical fibers, to achieve optimum sensor performance, we focused on optical wave guide features. Basically, infected blood is a dark liquid contains magnetic particles similar to magnetic fluid. So, in first step, we fabricated magnetic field sensor which can be applied in sensitive applications such as in oil and gas exploration and biomedical diagnostic instruments that are compatible with magnetic resonance imaging scanners. The most popular techniques rely on fluxgate, magneto-resistive, magneto-transistor and Hall effects to detect magnetic fields (Langfelder & Tocchio, 2013; Mancoff et al., 2000; Snoeij et al., 2016). These sensors exhibit some drawbacks related to their cost, power consumption, miniaturization, reduced multiplexing potential and remote monitoring capabilities. Moreover, environmental electrical field sources are prone to introduce noise via electromagnetic interference to the metallic circuits (Ripka & Janosek, 2010). Compact size, immunity to electromagnetic interference, remote and multiplexing capabilities via optical networks modalities, high reliability and sensitivity are some of the primary advantages that optical fibre based magnetic field sensors have over conventional sensors. In order to do this, annular core photonic crystal fibre (AC-PCF) with a large sensing area and MF (infiltrated into the nanoholes) was used. According to our findings, this arrangement allows for great magnetic field sensitivity and quick response and recovery times.

**Objective of the research**

The objective of this research work is to propose rapid and miniature synthetic hemozoin sensor. The effective understanding of PCF characteristics is critical for stable guidance of transmitted power. This could open new windows for biosensing applications compatible with living body. Recently, reported optical based sensors require large amount of sample (in the order of ml), while PCF based sensors only need a few µl while offering much more length of
interaction of the light with analytes. This unique advantage can provide rapid and sensitive detection of analyte.

The primary objectives of the research project represented in this thesis are conceptually classified as:

1. The first objective to design and simulate a novel magnetic field sensor toward stable transmission of power in absence of magnetic field followed by rapid detection of magnetic field. This goal was the cornerstone of designing PCF based malaria detection.

2. Second objective is to synthesize and characterize the β-hematin with dimensions similar to hemozoin. This characterization is essential in order to identify structural, optical and magnetic properties of β-hematin for optical sensing applications. Obviously, it helps in exploiting malaria diagnostic techniques with much less cost and time.

3. The final objective is to fabricate and engineer hemozoin sensor, that enable rapid malaria detection in early stages of infection. This kind of diagnostic platform has the potential to be ideally suited for mass testing in low resource environments.

Thesis outline

The thesis has been structured as follows:
In chapter 1 of this thesis, we considered the literature review. In this chapter the most popular malaria diagnostic techniques with pros and cons were investigated. Then, the process under which the malaria pigment was formed during infection process was mentioned, followed by the description about magnetic feature of hemozoin. Then magnetic fluid as an ideal model of infected blood was introduced in conjunction with optical fibers using for magnetic field sensing applications.
The chapter 2 is an article published in Scientific Reports in 2022. In this article we demonstrated rapid and sensitive magnetic field sensor based on photonic crystal fiber with magnetic fluid infiltrated nanoholes. Here in, output transmitted optical power was simulated and compared with experimental results.

In chapter 3 the second article entitled: dimensional controlled synthesis of malaria pigment: key study for diagnosis of the most severe form of malaria was presented. In this article we focused on the synthesizing the mimic of hemozoin and characterized that through different chemical and physical analysis.

In chapter 4 we, demonstrated a novel β-hematin sensor with a detection threshold well below the clinical relevance (50-100 parasite/µl) using very small liquid sample (less than 0.5 µl). The results of this study are submitted to the journal.
CHAPTER 1

BACKGROUND AND LITERATURE REVIEW

In this chapter the advantages of optical fibers compare to conventional sensing platforms were considered. More specifically, specialty optical fibers are introduced, and their applications were investigated. With respect to the magnetic feature of malaria pigment, as a basic model of infected blood, we used magnetic fluids in conjunction with specialty optical fibers. In this regard, magnetic field sensors are also reviewed. Finally, conventional methods to detect malaria were studied and their pros and cons were considered.

1.1 Optical fiber technology in sensing applications

Over the past two decades two major technological revolutions occurred due to the development of the fiber optic communications and optoelectronics industries. On one hand the optoelectronics industry has created significant products as bar code scanners, laser pointers, compact disc players and printers. On the other hand, fiber optic industry has literally revolutionized the telecommunications by providing reliable telecommunication with higher-performance. In parallel with these developments, many of the elements associated with these industries were progressed for fiber optic sensing applications. In addition, subsequent mass production of optoelectronic devices come to support sensor industries. By notable reduction in component prices and quality enhancement, the eligibility of optical fiber sensors to displace traditional sensors for electric and magnetic field measurement, chemical measurements, rotation, strain, acceleration, temperature, viscosity, pressure, acoustics, vibration, and a host of other sensor applications has been improved. The inherent advantages of fiber optic such as their ability to be lightweight, passive, very small size, immunity to electromagnetic interference, high sensitivity, high bandwidth and compatibility with living being renders it as an applicable candidate for high-tech based sensors (Yin et al., 2017).
1.1.1 Demand for optical fibers in bio photonic

The past decade has witnessed rapid development of biomedical diagnosis, therapy, monitoring and surgery. Photonics employ photons as an alternative for electrons to transmit, store and process data and therefore offer tremendous capabilities and speed in designed devices. The main issue in biophotonics is related to the collection and transmission of low-power (in the order of nanowatt) emitted light to a detector, how to focus a wide range of optical power to a certain tissue during different categories of treatment, and how to approach a diagnostic area inside a living being utilizing an optical detection probe in the least invasive route. The unique aspects of fiber optics enable them to resolve such implementation issues. Accordingly, various kind of optical fibers are possessing widespread applications in biophotonics technology for life sciences as well as research and clinical applications. Any optical fiber structure has certain advantages and limitations for specific uses in various spectral bands. Consequently, it is necessary to employ specialty fibers that is best suited for specific application (Keiser, 2022a).

1.2 Conventional and specialty optical fibers

With respect to extensive research activity for telecom networks, conventional solid-core silica-based optical fibers are extremely reliable and are accessible in different core sizes. These fibers are employed worldwide in telecom networks and in many industrial applications. It is composed of a cylindrical silica-based core surrounded by a glass cladding with lower refractive index (Kasap & Sinha, 2001). This is the key point that enables light travels in the core and reflect totally at the interface with the cladding, which is the physical mechanism that guides light signals along a fiber.

Specialty optical fibers are custom-designed for applications such as manipulating light wave signals to acquire some kind of signal-processing function or sensing a physical parameter, such as pressure or temperature. Incorporation of such properties into fibers is attained through either structural features or material compositions (Méndez & Morse, 2011). For instance, in
biophotonics functions, the main specialty fiber kinds are photosensitive fibers for preparing internal gratings (Pal et al., 2004), fibers insensitive to bending for circuitous routes within bodies (Keiser, 2022b), and polarization-preserving optical fibers for imaging and for fluorescence analyses in spectroscopic systems. Also, photonic crystal fibers are a very widely used category of specialty fibers that offer many degrees of freedom in their design to attain a number of peculiar properties. Basically, they are regular arrays of air holes along the entire length of the fiber as shown in figure 1.1 (Rifat et al., 2019; Sharma et al., 2019). A PCF is a type of microstructured fiber whose optical characteristics depend on the diameter of air holes, distance between holes and their arrangement that define how light is guided either by bandgap effect or total internal reflection. Compared to conventional SMF, PCFs have showed a high resistance to darkening phenomena via nuclear radiation and ability to transmit high optical power (Keiser et al., 2014). In addition, they have ability to deliver light in mid-IR with broadened window.

Figure 1.1 a) Standard PCF and b) Annular core PCF (Rifat et al., 2019; Sharma et al., 2019)
1.3 Magnetic field sensors

Magnetic field sensors are extensively employed in a variety of consumer products (Herrera-May et al., 2016). It can be seen in scanners, printers, flat panels and cameras. Navigation is one of the rapid growing applications of magnetic sensors in smartphones and tablets. Further, magnetic field sensors are employed in numerous industrial applications as contactless current sensing, rotation, angular and linear position sensing (Lenz & Edelstein, 2006a). In biological application there is high demand for sensing small magnetic field. For instance, example, the presence of specific molecules can be detected employing magnetic tag (Lenz & Edelstein, 2006b). As an another example we can mention to detect DNA hybridization by magnetic microbeads as labels in biosensors(Miller et al., 2001a). Further, giant magnetoresistance embedded on a micro-fabricated chip can detect the presence of beads(Miller et al., 2001b). It is worthy to note that eye lids vibration and tong movement during speech can be measured utilizing magnetic sensors (Sonoda, 1995). Future research moves toward designing compact, cost-effective and high sensitivity magnetic sensor employing nanotechnology in conjunction with photonic devices for diagnosis high risk diseases such as malaria and diabetes.

1.4 Conventional method of malaria diagnosis

Increasing drug resistance of the parasites strongly acts against the global malaria control. Notable enhancement could be acquired via the development of cheap diagnostic methods accurate even at the early stage of the infection malaria parasites (Rosenthal, 2013). Currently, in practice, microscopic observation of blood smears is the most reliable and sensitive able to detect parasitemia associated with 5–10 parasites in 1 µl blood, which is costly as requiring expertise and high-powered microscopes (figure 1.2) (Mathison & Pritt, 2017).
Rapid diagnostic tests that work based on Antigens and offer a cheaper alternative detection are widely used (figure 1.3). Presently, these techniques have serious drawbacks. Perhaps the two major limitations are: a) their sensitivity threshold being around 100 parasites/μl which is not enough to detect early-stage infections. b) the tests are not accurate enough to detect distinction between levels of infections.
Among various techniques reported for detection of malaria parasites, the most sensitive is the molecular biology-based method. These assays are sensitive enough to detect 1 parasite/μl. In this route a large number of target DNA is copied which simplifies the detection. The practical use of PCR assays on the field is limited due to requirements of sophisticated technology and expertise.

In recent years, the demand to develop new diagnostic methods has been driving extended research and a large diagnostic scheme has been proposed. Malaria pigment (hemozoin) is a by-product of the disease formed during the intraerythrocytic growth cycle of the parasites. Malaria parasites digest hemoglobin of blood which leads to the accumulation of monomeric heme. Since it is highly toxic to the parasites, they transform heme into an insoluble crystallized form (A. F. Slater et al., 1991). During this process low-spin diamagnetic Fe$^{2+}$ ions change into high spin (S=5/2) paramagnetic Fe$^{3+}$ ions in hemozoin. Hemozoin has a triclinic crystal structure with various morphology depending on the parasite species. They usually have an elongated rod-like shape with a length ranging from 300 nm to 1 μm. In addition, the natural formation of hemozoin inside the parasites, different methods have been established for its artificial synthesis (Bohle & Helms, 1993; Gluzman et al., 1994). Synthetic grown version is usually called β-hematin exhibited similar crystal structure optical and magnetic properties to hemozoin. Magnetic properties which are highly specific to malaria parasites can be applied as a key feature to design applicable sensors. As shown in figure 1.4, in absence of magnetic field the crystals in the suspension are randomly oriented (Butykai et al., 2013).

In presence of a magnetic field, the hard axes of the crystals start to align perpendicular to the magnetic field vector (McBirney et al., 2018).
Figure 1.4 Response of hemozoin crystals to magnetic field
(Butykai et al., 2013)

1.5 Magnetic fluid

Magnetic liquid, namely magnetic fluid (MF), have been developed for a diversity of applications in the broad fields owing to the controllable rheological characteristics under the applied electromagnetic field (Philip et al., 2008; Zahn, 2001). It is a kind of homogeneous colloidal magnetic nanoparticles such as Fe₃O₄ coated with the aid of surfactants, amine, carboxyl, hydroxyl, sulfur, in a suitable carrier phase of liquid metals, hydrocarbons and water which prevent particles from sedimentation. MF possesses both fluidity of liquid material and magnetism of solid magnetic material. Thanks to its unique optical properties, especially the RI tunability under magnetic field, MF has been widely employed for magnetic field and current sensing in conjunction with various optical fiber structures. Therefore, it can be predicted that the characteristics of ferrofluid will be enhanced and the applications of ferrofluid-based optical fiber sensors will be improved rapidly and maturely. MF firstly synthesized successfully by means of ball milling grinding with a low production efficiency (Stephen, 1965). Next in 1975, the enhancement in manufacturing process through chemical precipitation technique increase MF popularity (Kelley, 1977). Regarding applications of ferrofluid in integrated devices and sensing technology, the developments in optical devices are especially prominent, such as coarse wavelength division multiplexing, optical switches,
tunable optical gratings, modulators and optical fiber sensors. This chapter will that the RI tunability of MF which is mainly used in optical fiber sensors.

1.5.1 Relation between external magnetic field and RI of MF

The unique magneto-optical properties of MF are related to the microstructure configuration of magnetic particles under external magnetic field. Without applied external magnetic field, the nanoparticles disperse in the carrier liquid randomly and uniformly. When the magnetic field is applied, the nanoparticles undergo Brown and Néel relaxation (Kötitz et al., 1995), which will lead to aggregation of the magnetic particles to form chains along the direction of magnetic field. In the beginning and when the applied magnetic field intensity is weak, chainlike clusters are not clear, as the magnetic field intensity increases, they will form as parallel lines along magnetic field (Lv et al., 2014).

(Hong et al., 2004) investigated the refractive index of magnetic fluid versus temperature, concentration of magnetic particles and angles of applied magnetic field. It was observed that the variation in RI of MF is attributed to the column formation, Langevin function can be applied to describe refractive index \( n \) of MF as shown in equation (1.1).

\[
n_{MF}(H,T) = [n_s - n_0] \coth \left( \frac{\alpha (H-H_{cn})}{T} \right) - \frac{T}{\alpha(H-H_{cn})} + n_0, \text{ for } H > H_c
\]  

(1.1)

where \( H_{cn} \) is the critical field strength under which the refractive index of MF starts to change, \( n_0 \) is the refractive index of MF under fields lower than \( H_{cn} \), \( n_s \) denotes the saturated value of the RI of MF, and \( \alpha \) is a fitting parameter.

1.6 Optical based malaria sensors

There are many different literatures published for malaria sensing. In this report we consider the latest and optical based kinds. A new technique named ‘’secondary speckle sensing microscopy’’ (S3M) based on tracking of speckle patterns monitored for healthy and infected
red blood cells (Cojoc et al., 2012). They used fuzzy logic ruling patterns generated employing illumination of red blood cells with a laser and investigating them under a microscope. Then, using fuzzy logic ruling, notable quality of distinction between healthy and infected red blood cells was achieved in initial experiments. The S3M setup is depicted in figure 1.5 which consists of a custom inverted microscope in which the sample is illuminated by a tilted laser beam (Ar⁺ 514.5 nm). Additionally, an on-axis white light fiber optic illuminator is used for reference imaging and alignment purposes only. Time consuming sample preparation and many statistical analysis for detection process as well as high tech microscope make it impossible to use that in low based countries which are the main region for malaria parasite.

A portable, magneto-optic device for early stage malaria diagnosis was demonstrated by (McBirney et al., 2018). As shown in figure 1.6, they launched light to various samples containing different concentrations of malaria parasite and recorded output intensity through photodetector. They monitored the change in optical power before and after a magnetic field
is applied. Sensor was able to distinct early stage of malaria based on the detection of the malaria pigment, hemozoin. An artificial form of hemozoin known as β-hematin which possesses similar structural and optical properties was used. They demonstrate detection limits of <0.0081 μg/mL in 500 μL of whole rabbit blood with no additional reagents required.

This level corresponds to <26 parasites/μL. In practice the red light with wavelength 635 nm from laser diode (ThorLabs, CPS635) passes through a poly methyl methacrylate microcuvette containing 500 μL of sample to a photodetector (ThorLabs, S120C). To perform detection, output light intensity was measured in presence and absence of magnetic field. Because the magnet pulls all of the magnetic nanoparticles out of the beam path, the difference between the two signals is directly related to the nanoparticle concentration. This strategy is elegant in its simplicity as it reduces the effect of sample-to-sample variation, which is inherent in human specimens.

Figure 1.6 Schematic of portable sensor with 10 pounds weight
(McBirney et al., 2018)

Figure 1.7 exhibit the characterization of the working range of proposed sensor. It was able to detect hematin concentrations as low as 0.0087 μg/mL, the signal to noise ratio was small
which is not reasonable for accurate detection. In addition, it takes several minutes to reach the stable state that can be improved in future research.

Figure 1.7 Dynamic response of the portable malaria sensor (McBirney et al., 2018)
CHAPTER 2

RAPID AND SENSITIVE MAGNETIC FIELD SENSOR BASED ON PHOTONIC CRYSTAL FIBER WITH MAGNETIC FLUID INFILTRATED NANOHOLES

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2.1 Abstract

A fast response time (0.1 s) magnetic field sensor has been demonstrated utilizing a photonic crystal fiber with nano-size air holes infiltrated with polyethylene glycol based magnetic fluid. The effect of magnetic nanoparticles concentration in the fluid on the magneto-optical sensor performance and its dependence under varying magnetic-field loads was investigated in detail. In particular, the sensor response was analytically modelled with a Langevin function with a good fit (R≥0.996). A threshold sensing point as low as 20 gauss was recorded and a detection range of 0–350 gauss was demonstrated by means of optical transmission measurements. The experimental results were validated by theory using a waveguide light transmission model fed by finite-element method simulations of the principal guided modes in the infiltrated fiber sensor. The simple interrogation scheme, high sensitivity and quick response time makes the proposed hybrid fiber-optic magneto-fluidic probe a promising platform for novel biochemical sensing applications.
2.2 Introduction

With the advent of the internet of things, wearable sensors and personalized medicine, there is growing demand for compact and reliable sensors to provide biosensing and environmental monitoring to users and artificially intelligent beings. Among various kinds of optical fiber-based sensors, specialty fibers infiltrated with magnetic fluid has recently attracted scrutiny towards the development of highly sensitive and compact magnetic field sensors. Magnetic field sensors have been extensively used in electric current measurements, in metallurgy, power industry, in biomedical detection, for the oil and gas exploration as well as aviation industry (Kurosawa, 2014; Němec et al., 2016; Shi et al., 2015). The most common methods rely on magneto-transistor, magneto-resistive, fluxgate or the Hall effect to detect and measure magnetic fields (Langfelder & Tocchio, 2013; Mancoff et al., 2000; Snoeij et al., 2016). These sensors exhibit some drawbacks related to their power consumption, limited multiplexing, cost, miniaturization and remote monitoring capabilities. In addition, surrounding electrical field sources are prone to introduce noise via electromagnetic interference to the electronic circuits (Kapsalis, 2017). Compared to conventional sensors, optical fiber based magnetic field sensors offer promising key benefits such as a compact size, immunity to electromagnetic interference, remote monitoring and multiplexing capabilities through optical networks modalities, high reliability and sensitivity. Initial fiber-optic magnetic field sensors reported in the last four decades employed magnetostrictive materials in conjunction with Mach–Zehnder interferometry while other schemes exploit changes on the state of polarization of the light (Horng et al., 2000; Koo & Sigel, 1982; Rashleigh, 1981; Sedlar et al., 2000). Meanwhile with the growth of nanotechnology and the advent of liquids functionalized with nanoparticles, emerging applications of so called magnetic fluids (MF) are studied in the sensing field. A MF is a liquid typically composed of single-domain magnetic nanoparticles (MNPs) coated with surfactant in suspension within a liquid carrier, and with engineered physicochemical properties including magnetic susceptibility, polydispersity, and dipolar interactions. Owing to its customizable magneto-optical properties, MFs have been applied in a variety of photonic devices, including optical gratings (Horng et al., 2000), optical switches (Horng et al., 2004), modulators (Chieh et al., 2007), couplers (Dutt et al., 2011) and magnetic field sensors (W.
Wu et al., 2019). The ability to exhibit a magnetic-field-dependent refractive index (RI) (Jin et al., 2018; Philip & Laskar, 2012), that is attributed to the microstructural distribution of MNPs inside the MF-is a key parameter used in many sensing applications. Accordingly, different configurations of optical fibers in conjunction with MF have been well studied as magnetic field sensors.

They can be used in three different configurations, initially in the form of the MF thin film at the end facet of optical fiber cross section, as the cladding of an etched/tapered fiber (in the middle section) and finally as the filling material inside the fiber. For the first configuration, several Fabry–Pérot based sensors that incorporate MF within a section of optical fiber were reported (Hu et al., 2010; Lv et al., 2014). This technique suffers from sensitivity to thermal expansion and a complicated process to calculate and fabricate the correct cavity dimensions. Those problems were solved in etched tapered fibers (Azad et al., 2018; Zhao et al., 2015). The thinned fibers are very fragile owing to their low mechanical strength. Finally, by injecting the MF inside the fiber, the original microstructured features are not only preserved but the whole fiber also offers an extended interaction area that enhances the sensitivity (Gao et al., 2013).

In this work, we present a special photonic crystal fiber (PCF) with nanometer-scale air holes infiltrated with MF. Sensor performances including sensitivity, threshold and saturation points, response/recovery times were studied in detail for various concentrations of the MF. This work is organized as follow: section “Fabrication process and operation principle” describes the PCF infiltration process and sensing principle. In section “Results and discussion” the experimental investigation on the effect of MF concentrations in sensor responses are considered. Also, numerical simulations regarding output power, which is then followed by a comparison between the experimental and simulation results were done. The proposed functional sensor with features such as compact size and fast response/recovery time may find applications in future biochemical and industrial sensing.
2.3 Fabrication process and operation principle

Figure 2.1a,b depict the schematic experimental setup for filling the PCF with the MF (ferromagnetic MNP of 10 nm average size in polyethylene glycol solution from Ferrotec, USA). The flat-cleaved end of a 15 cm PCF was immersed perpendicularly in 2 ml sample vial containing the MF. The MF was successfully infiltrated into the air holes and throughout the whole length of the PCF based on Poiseuille law (Pfitzner, 1976) under which the induced pressure gradient between the two ends of the fiber results in a laminar flow of MF [Fig. 2.1c,d].

Figure 2.1b shows the SEM cross-section of the used PCF which features a holey cladding made of air holes arranged in a hexagonal lattice pattern with 1.4 mm pitch and average hole diameter of 480 nm. In order to apply a uniform magnetic field on the side of the MF-infiltrated PCF, a plate shaped magnet (KJ Magnetics, USA) was placed next to the sensing region at precisely defined distances from the fiber. A Hall probe-based magnetometer (KOSHAVA 5 model, Wuntronic GmbH) was employed to monitor the magnetic field strength and calibrate the magnetic field fiber-optic sensor. The output beam profile as well as transmitted power at the output of optical fiber were monitored via a CCD camera and optical power meter respectively. As shown in Fig. 2b, when the infiltrated PCF was exposed to the magnetic field the spatial distribution of MF transforms from randomly homogeneous to an ordered field dependent pattern. The MNPs tend to agglomerate and form chain-like clusters along the direction of magnetic field (Kötitz et al., 1995) owing to Néel and Brownian relaxation. This phenomenon induces a RI change of the MF that depends on the exerted magnetic field strength (Horng et al., 2003; Yang et al., 2002).
Figure 2.1 (a) Schematic of experimental setup for filling the PCF, (b) Cross-section SEM image of PCF, (c) Optical microscope image of Bare PCF and (d) infiltrated side views of the PCF

2.4 Results and discussion

It is well known that MFs possesses high optical absorption in the visible spectrum (Hoffmann & Köhler, 2003) as well as a high absorption band at 1470 nm wavelength. The absorption band is related to the orbital transition process in magnetite particles (Inaba et al., 1989). Therefore, to benefit from the RI tunability of MF in sensing applications, the geometry of the optical waveguide plays an important role. For example, in the case of a PCF with large air holes, a larger fraction of optical power would be absorbed resulting in very high optical loss. In this work, the use of a special PCF with very small air holes enabled light transmission in the range of 800–1000 nm. The experimental testing setup is depicted in Fig. 2.1a. The incident light from a near infrared laser source (\(\lambda = 976\) nm, Thorlabs, Pigtailed Butterfly Package) was coupled into the PCF through a combination of objective lenses. The transmitted light power and output beam profiles were recorded via an optical power meter and CCD camera, respectively. A linear polarizer was tuned so as to optimize the interaction of light with the MF: when the direction of E-field is parallel to the direction of exerted magnetic field (H) the induced change in optical absorption is almost twice that of the case when the E-field is perpendicular to the direction of applied H-field (Inaba et al., 1989).
2.5 Influence of MF concentration

The proposed sensor operates based on RI variation and this phenomenon strongly depends on the volume fraction of magnetite particles and the liquid carrier (water, organic solvent, etc.). In this regard, the used polyethylene glycol-based MF with superparamagnetic properties offers higher colloidal stability compared to water based MFs. In this experiment, three different concentrations of MF with 5.9, 8.8 and 11.8 Vol.% of magnetic particles were considered. As shown in Fig. 3 the transmitted optical power through the infiltrated PCF exhibits a strong dependence with the applied magnetic field strength. The sensor saturation point (identified by square markers in Fig. 2.3) increased with the concentration of MNPs, which is attributed to the saturation magnetization $M_s$ of the MF that follows a linear dependence with the concentration. Generally, the magnetization of superparamagnetic materials is described by a Langevin function under magnetic field (Bean & Livingston, 1959). Correspondingly, the experimental results in Fig. 2.3 were fitted with a Langevin function with a good degree of confidence ($R \geq 0.996$). The proposed sensor exhibited a limit of detection $\leq$
16 gauss within the effective sensing range (i.e. below saturation point). This demonstration of the magnetic-field modulation of transmitted light via the superparamagnetic response of magnetite NPs points to the potential of using the proposed MF-infiltrated PCF waveguide in magneto-optical sensing applications.

Figure 2.3 Transmission loss versus magnetic field for samples with various Vol.% concentration of MNPs

Figure 2.4 presents the CCD camera images related to the output beam pattern intensity of the infiltrated PCF with various concentrations of MF and submitted under varying magnetic field strengths. This figure provides a clear visual support to the fact depicted in Figure 2.3 that transmission losses increase with the applied magnetic field for all three MF concentrations investigated. In the absence of an external magnetic field, individual magnetite particle can be described as a single domain magnetic dipole with permanent moment. While in the presence of low magnetic field (20 gauss), the sensor containing the highest concentration of MNPs showed distinctive pattern changes highlighted by green dashed rectangles in Figure 2.4i,j. An explanation is that at higher volume percentages of MNPs, because the free distance between the centers of two dipoles is lower the portion of attractive polar energy is larger than the thermal energy such that dipole-dipole interactions dominate(Zhu et al., 1996). The latter phenomenon results in small RI changes, which in turn leads to the observed variation in the output beam pattern.
Figure 2.4 Output beam pattern intensity related to the PCFs infiltrated with:
(a–d) 5.9 Vol.%, (e–h) 8.8 Vol.% and (i–l) 11.8 Vol.% concentrations of MNPs under applied magnetic fields of 0, 20, 230 and 330 gauss

2.6 Dynamic response of the sensor

The reaction time in sensor applications is an important parameter. In order to evaluate the dynamic response of our sensor, equal length of infiltrated PCFs with different concentrations of MF were exposed to constant magnetic field of 250±8.7 gauss. To ensure the stability and repeatability of sensor responses, the samples were exposed to magnetic field consecutively for three repetitions. The samples containing the lowest concentration of magnetic particles (5.9 Vol%) exhibited very fast response time (time interval during which the transmitted optical power changes from 90 to 10% of its variation) of 0.1s and recovery time (in reverse of response time definition) of also 0.1s as shown in Figure 2.5.

Sample with 8.8 Vol. % showed 0.16s response/recovery time. MF with the highest concentration (11.8 Vol.%) showed a longer response time of 150s and recovery time of 9s. The latter observed long response time is explained by the growing short-range repulsive forces
that increase at high concentration levels of MNPs and which, in turn, slow down the attractive dipole–dipole interactions responsible for the formation of chainlike clusters [depicted in Fig. 2b]. It should be noted that the response and recovery times were not affected by the strength of the applied magnetic field. In Table 2.1 we summarized the performance of the main sensing specifications reported in the recent literature in comparison to the present work. The compiled results show that the proposed PCF sensor compares favorably in terms of sensitivity and response time.

![Dynamic response of the infiltrated PCF with 5.9 Vol. % MF in H=250±8.7 gauss, (b) Close-up view with finer resolution of the response time region](image)

Figure 2.5 (a) Dynamic response of the infiltrated PCF with 5.9 Vol. % MF in H=250±8.7 gauss, (b) Close-up view with finer resolution of the response time region
Table 2. Critical parameters in sensing performance based on optical fiber in conjunction with MF for sensing magnetic field. a Etched fiber Bragg grating. b Uniform fiber Bragg grating. c Thin core fiber. d Photonic crystal fiber. e Polymer optical fiber. f Mach–Zehnder interferometer. g Photonic crystal fiber. h Single mode-nocore-single mode

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<td>Wavelength shift</td>
<td>eFBG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Synthesized MF based on Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0–25</td>
<td>15</td>
<td>Not mentioned</td>
<td>(Dai et al., 2011)</td>
</tr>
<tr>
<td>Cladding mode intensity</td>
<td>FBG&lt;sup&gt;b&lt;/sup&gt;/ TCF&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Ferromagnetic Particles</td>
<td>7–15</td>
<td>30</td>
<td>1539-1546</td>
<td>(Tian et al., 2017)</td>
</tr>
<tr>
<td>Wavelength shift</td>
<td>Tapered PCF&lt;sup&gt;d&lt;/sup&gt;</td>
<td>EMG 507</td>
<td>10–60</td>
<td>30 min</td>
<td>1550</td>
<td>(Zhao et al., 2015)</td>
</tr>
<tr>
<td>Evanescent waves</td>
<td>POF&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td>2.5-150</td>
<td>0.7</td>
<td>550</td>
<td>(Azad et al., 2018)</td>
</tr>
<tr>
<td>Transmitted power variation</td>
<td>MZI&lt;sup&gt;f&lt;/sup&gt;</td>
<td>EMG 605</td>
<td>0–40</td>
<td>Not mentioned</td>
<td>1550</td>
<td>(J. Wu et al., 2014)</td>
</tr>
<tr>
<td>Optical loss</td>
<td>PCF&lt;sup&gt;g&lt;/sup&gt;</td>
<td>PBG 300, 0-35mT</td>
<td>0.1s</td>
<td>976</td>
<td>Present study</td>
<td></td>
</tr>
<tr>
<td>Wavelength shift</td>
<td>SNS&lt;sup&gt;h&lt;/sup&gt;</td>
<td>EMG 605</td>
<td>4–10</td>
<td>Not mentioned</td>
<td>1530-1560</td>
<td>(Y. Chen et al., 2013)</td>
</tr>
</tbody>
</table>

2.7 Sensor output modelling

We also modeled the principal waveguiding mechanism of the infiltrated PCF. Due to the small RI contrast between the silica glass fiber structure and the MF, we expect leakage of the guided light into the holey cladding region, as evidenced by the recorded output intensity patterns in Fig. 4. In order to model this peculiar waveguiding, finite-element method (FEM) simulations using COMSOL Multiphysics were performed. A uniform 480 nm diameter of PCF holes was assumed along with a pitch value of 1.4 μm inside the 125 μm diameter silica PCF coated with acrylate protective jacket (250 μm diameter). Moreover, the refractive index of different MFs was measured via a digital refractometer (Kruss DR301-95) at 589 nm wavelength. It was observed that the RI increased linearly with increasing concentration of samples. That is for 5.9, 8.8 and 11.7 Vol. % MF the measured RI were 1.4276, 1.4707 and 1.493, respectively. The latter values of RI were used in the simulations since changes in RI of the MF at 589 nm compared to a wavelength of 976 nm are negligibly small as well as exhibit a similar trend with respect to changes in MF concentration. The first five principal guided modes were
selected for each value of applied magnetic field, and the corresponding $n_{\text{eff}}$, loss ($\alpha$), E-field as well as H-field components were calculated with the FEM mode solver. Subsequently, the transverse E-field distribution at the output facet of the fiber sensor of length $L$ was modeled as the coherent superposition of the N guided modes as described by the following equation:

$$E_{\text{output}}(x,y,\omega) = \sum_{j=1}^{N} C_j \cdot E_j(x, y, \omega) \cdot e^{i\omega(n_{\text{eff},j})L} e^{-\frac{\alpha_j L}{2}}$$  \hspace{1cm} (2.1)$$

where $E_j = (E_j^x, E_j^y)$ are the $x$ and $y$ transverse field components, while $n_{\text{eff},j}$ and $\alpha_j$ denote the real effective index and the power loss coefficient of the $j$-th guided mode at a given frequency $\omega$. The variable $C_j$ stands for the normalized amplitude coupling coefficients calculated from the overlap integral of the input Gaussian beam and overlap integral of the respective modal distributions of the $j$-th mode:

$$C_j = \frac{1}{4} \int \left[ E_{\text{input}}^* (x,y) \cdot H_j^y (x,y) + E_j^x (x,y) \cdot H_{\text{input}}^* (x,y) \right] dxdy$$ \hspace{1cm} (2.2)$$

where the modal fields were properly normalized to unit power via $\frac{F}{\sqrt{\frac{1}{2} \int \text{Re}(E_{\text{input}} H_{\text{input}}^*)dxdy}}$, where $F$ stands for the ($E$ or $H$) field component of the electromagnetic field vector. The used 976 nm laser is linearly polarized such that an $x$-polarized Gaussian beam of radius $\sigma = 7 \, \mu$m was considered as the input source with optical power $P$ in the simulations:

$$E_{\text{input}}(x,y) \approx \hat{x} \cdot \sqrt{\frac{2P}{\pi \sigma^2 n_{\text{clad}}}} \exp\left[-\frac{(x^2+y^2)}{2\sigma^2}\right]$$ \hspace{1cm} (2.3)$$

$$H_{\text{input}}(x,y) \approx \hat{y} \cdot \sqrt{\frac{2P n_{\text{clad}}}{\pi \sigma^2}} \exp\left[-\frac{(x^2+y^2)}{2\sigma^2}\right]$$ \hspace{1cm} (2.4)$$

Therefore, by using Eq. (2.2) an expression can be derived for the transmitted power in the infiltrated PCF sensor:
where \((x_0, y_0)\) denotes the coordinates of the PCF cross-section center. In order to model the transmitted power in Eq. (2.5) of our fiber sensor, we used the FEM-simulated first five dominant guided modes that carry \(\geq 90\%\) of the transmitted power. Another key simulation parameter that was considered relates to the changes in the refractive index of the magnetic fluid (nMF) to implement in our simulations when the applied magnetic field is \(H > 0\). This relationship between nMF and \(H\) is a priori unknown. But knowing that the value of nMF obeys a decreasing Langevin function behavior (Bean & Livingston, 1959; Zhao et al., 2014) and the fact that we measured the value of nMF at \(H=0\) gauss, we performed a series of FEM simulations that allowed us to find the best fit between the simulated and experimental sensor output transmission loss in Fig. 2.6b using the model for nMF in Eq. (2.6) and shown in Fig. 6a. We note that the model in Eq. (2.6) was derived for a MF concentration of 5.9 Vol.% for which we observed the highest sensor performance. Consequently, all simulations were performed for this specific concentration.

Figure 2.6 (a) Modeled nMF versus applied magnetic field, (b) Comparison between simulation and experimental data regarding the optical transmission loss in the MF-infiltrated PCF as a function of applied magnetic field
\[ n_{MF} = 1.4109 + 0.0189 \times \left[ \coth \left( \frac{H - 263.5}{-33.81} \right) + \frac{33.81}{H - 263.5} \right] \]  

(2.6)

The discrepancies between the experiment and simulations are owed to additional optical scattering within the PCF that are not accounted for in the simulations which assumed a perfectly smooth PCF structure.

### 2.8 Conclusion

The exquisite precision of mature optical fiber technology in combination with functional fluids tailored with fine magnetic particles makes a hybrid fiber-optic magneto-fluidic probe design desirable for emerging biochemical and environmental sensing applications. In this work, we propose and demonstrate a new type of magnetic field fiber-optic sensor based on a special type of photonic crystal fiber (PCF) with very small submicron-sized air holes infiltrated with a functional magnetic fluid (MF). The resulting fiber-optic probe enabled us to demonstrate a highly sensitive (0–350 gauss), fast (0.1 s response time) and compact magnetic field sensor that can be driven using cost-effective near-infrared laser diodes. The experimental results were well fitted using a Langevin function and explained by a magnetic-field and mode-dependent optical transmission model that was validated by finite simulations. This demonstration provides another step towards novel hybrid magneto-fluidic fiber-optic sensing approaches for biochemical and environmental sensing applications.
CHAPTER 3

DIMENSIONAL CONTROLLED SYNTHESIS OF MALARIA PIGMENT: KEY STUDY FOR DIAGNOSIS OF THE MOST SEVERE FORM OF MALARIA

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3.1 Abstract

Infectious diseases pose a serious threat to human health over the world, especially malaria, with 2-3 million deaths cases annually caused by Plasmodium infection. Though for the diagnosis of malaria, conventional analytical techniques are frequently used, the majority of them are not affordable in areas with limited resources where malaria outbreaks are common. So, there is a high demand to provide novel recognition materials. Malaria pigment known as hemozoin (Hz) can be served as a biomarker in malaria diagnosis. It’s a complicated production process to navigate researchers to apply the synthetic form of that (SHz) known as β-hematin. Its highly physicochemical resemblance to natural hemozoin (nHz) appeared upon infection of red blood cells by Plasmodium falciparum, making it an efficient alternative for research applications. However, when performing functional studies, SHz's larger size and some main magneto-optical features, including magnetization, susceptibility, and refractive index (RI), are not available. The present work focuses on synthesizing SHz with controllable dimensions, resulting in SHz samples with properties similar to nHz via a cost-effective and straightforward process. Besides, spectroscopical, morphological, structural, and magneto-optical aspects were investigated. Findings revealed that SHz with the highest resemblance to
the hemozoin behaves like paramagnetic materials with susceptibility equal to 1.4E-5 and RI of 1.7035 along the preferred crystallographic growth direction at wavelength 635 nm. The findings of this study provide new insights into the field of opto-magnetic biomedical sensing, and in particular, towards the early-onset rapid diagnostic of malaria.

**Keywords:** Malaria pigment, hemozoin, magnetic and optical properties

### 3.2 Introduction

Despite great advances toward the elimination of malaria, it is still a serious mosquito-borne infectious disease throughout the world, particularly in the African continent (World Health Organization, 2021). Humans can be infected with five Plasmodium (P.) parasite species: *P. falciparum, P. oVale, P. VNax, P. malaria*, and occasionally *P. knowlesi*. Of these, *P. falciparum* causes the highest fatality rate particularly in sub-Saharan Africa, while the most vulnerable groups are pregnant women and children under age five (Snow et al., 2017). Rapid malaria infection diagnosis and point-of-care treatment can greatly lower the likelihood of acquiring the most severe forms of this disease. During malaria infection, Plasmodium parasites feed from red blood cells (RBCs) as a source of amino acids. During this process hemoglobin from RBCs is digested, leading to a continuous release of heme, which is as a toxic byproduct for the parasites (Bhat et al., 1984). The parasite thus converts heme into an insoluble, inert, reddish-brown crystalline derivative known as Hz, i.e. malaria pigment, in order to avert toxicity of heme and survive (Fitch, 2004; Sullivan Jr, 2005). In a healthy individual, Hz is not present. As a result, Hz serves as a diagnostic biomarker of malaria infection (Coronado et al., 2014). The significance and oddities of this biocrystal have prompted scientists to investigate it as a potential target for diagnostics and therapies against the disease using innovative techniques. One approach for advancing malaria medications and diagnosis is to study the hemozoin molecule. Preparing Hz is a time-consuming and costly process that needs special biomedical facilities as well as the expertise to infect living hosts (e.g. rats) with malaria and subsequently extract the nHz product. Therefore, there is a strong argument for promoting new research advances in this area to work with the synthetic form of
Hz (SHz), aka β-hematin, mainly produced in an inert acidic medium (A. F. Slater et al., 1991). The term β-hematin refers to a pigment that has been chemically manufactured in vitro, while Hz refers to the biosynthetic malaria pigment. This Hz imitation has a structure that is physically and chemically comparable to its natural counterpart. Several prior works have studied the synthesis of Hz (Ambele et al., 2013; Egan et al., 1994; Jaramillo et al., 2009) while others investigated the physicochemical characteristics of SHz production (Fescenko et al., 2019; Pagola et al., 2000; Pauling & Coryell, 1936). However, there are still gaps in the scientific literature on how to control the size of SHz compounds so as to obtain similar dimensions to the naturally occurring Hz, as well as what is the RI and magnetic susceptibility of Hz which are key points for magneto-optical based sensors. This work demonstrates a systematic procedure to produce SHz with controllable size features that are compatible with nHz. Subsequently, a detailed physical characterization was performed on the resulting samples, including vibrating sample magnetometry (VSM), scanning electron microscopy (SEM), and RI measurement. These latest results can provide key data and methods relevant to future novel detection and treatment techniques.

3.3 Materials and Methods

3.3.1 Materials

Highly crystalline hemin powder, Chloro(protoporphyrinato)iron (III), was acquired from Sigma-Aldrich LLC. Other chemicals, including propionic acid, methanol, sodium hydroxide, and bicarbonate sodium, were obtained from Thermo Fisher Scientific Chemicals, Inc. and used as is without further purification.

3.3.2 Synthetic hemozoin (SHz) arrangements

As shown in figure 3.1, SHz was obtained by dissolving NaOH (0.4 gr) in pure water (100 ml). The aqueous solution was degassed with nitrogen for 30 min to provide an efficient reaction medium. Then, hemin (500 mg) was added and gently stirred for 20 min at room temperature.
The pH of the solution was monitored (via Fisherbrand accumet pH meter) while adding propionic acid. At the starting point of this procedure, the pH of the mixture was 12.44 and progressively reduced by adding acid drops under mild stirring for 40 min. The samples were prepared in different pH solution values of 3.3, 4.1, and 4.7. Noteworthy, the synthesis of SHz failed at higher pH values (in neutral and basic medium). The well-stirred mixture was placed into an oil bath and heated at 70°C for 17h. After cooling, the solid precipitation was separated using a micropipette and washed extensively with NaHCO₃ (0.1M), methanol and water. The samples were then dried in an oven at 40°C for 48h over phosphorus pentoxide.

Figure 3.1 The synthesis process of synthetic hemozoin
3.4 Characterization Results and Discussion

3.4.1 Fourier-transform IR(FTIR) spectroscopy

FTIR spectroscopy was used to explore the chemical structure of hemin, followed by synthesized SHz at different acidic solutions (Figure 3.2(a)). To obtain IR spectra, 1mg of each sample (hemin and SHz) was located on the diamond crystal of the spectrometer (Thermo Fisher Scientific Inc, Nicolet 6700 FTIR) and compressed. The Spectra were recorded in reflection/transmission mode, 2000-650 cm\(^{-1}\) at a resolution of 2 cm\(^{-1}\). SHz spectra exhibited specific sharp bands absent in the hemin (the spectra are shifted vertically for clarity). These extremum bands at 1209 cm\(^{-1}\) and 1660 cm\(^{-1}\) are assigned to coordinated carboxylate (C—O) and carbonyl (C=O) stretching vibrations, respectively (Egan et al., 1994; Tempera et al., 2015) which are the main peaks in nHz (Jaramillo et al., 2009). The hydrogen-bonded carboxylate group, which is considered to connect dimers in the extended porphyrin array, is assigned to the band at 1707 cm\(^{-1}\) (Wood et al., 2004). Figure 3.2 (b) exhibits the FTIR spectra related to the samples prepared at different pH. It is observable by pH reduction that all three spectra exhibit a similar spectrum, confirming that pH variation during synthesis does not affect the chemical structure of SHz. However, the spectrum intensities reduced specifically between samples with pH 4.7 and pH 3.3, which can be attributed to the growth mechanism of Hz crystals.
3.4.2 X-ray powder diffraction (XRD)

The XRD patterns were obtained via a Bruker AXS D8 advance diffractometer, utilizing a copper-based radiation source (Cu Ka, λ=1.5406 Å) within the range of 2θ= 5-30°. Figure 3 presents the XRD patterns of hemin and SHz. Sharp Bragg diffraction peaks corresponding to the crystalline materials appeared in all recordings, indicating that the proposed synthesis technique of SHz yielded samples with a high crystalline structure. The XRD patterns clearly show that during the SHz synthesis process, the multi-peak structure changes completely compared to hemin, which corresponds to the formation of a new lattice configuration. Each unit cell of the SHz is occupied by two heme molecules that are symmetry-related in a triclinic lattice structure(Bohle et al., 1997). Samples with different pH showed similar growth directions with priority in the (100) plane at around 2θ = 7°.
3.4.3 High-resolution scanning electron microscopy (HRSEM)

SEM images and energy dispersive spectroscopy (EDS) were acquired using a Hitachi SU-8230 SEM. Prior to the measurements, the samples were Au-sputter deposited using a magnetron target assembly (model: K550x). The deposition was done at a pressure of $1 \times 10^{-1}$ mbar with a current of 30 mA for 2 minutes, resulting in a thin film of Au with 20 nm thickness. SEM was carried out to observe changes in the morphology of hemin powder after reactions and the effect of pH on the dimensions of SHz, as shown in Figure 3.4. At relatively low magnification (Figure 3.4 (a)), hemin crystal appeared to consist of large angular sheets with a length of 6.7-58.9 μm and a thickness of 1-4 μm (shown in the magnified right side image). It would seem that these large sheets transform into micro-crystals with a needle-like appearance.
Figure 3.4 HRSEM images of (a) Hemin powders, SHz prepared in different pH solutions (b) 3.3, (c) 4.1 and (d) 4.7. The pictures on the right-side exhibit lateral dimensions with higher magnification.

The inset in part (b) exhibits a side facet of crystal in 200k-magnification.
In order to facilitate the comparison between the SHz Samples, SEM images with higher magnification were provided with a scale bar of 1 µm. The SHz with pH 4.7 exhibited the largest size laterally and longitudinally. In the published literature, SHz mainly were prepared in pH 4.1 medium, resulting in crystals with bigger size and less uniformity than nHz. In the present work, SHz samples prepared in pH 3.3 exhibited the highest compatibility with nHz in terms of size and morphology (Noland et al., 2003). Higher magnification (200k) was considered to identify the SHz formation process. It was observed that the micro crystals packed together along the side facet with a thickness of ~ 20 nm, depicted in the inset of Figure 3.4(b), highlighted by the red square.

Over a hundred crystals were identified from SEM images, and their dimensions were analyzed via Image J software to evaluate the average size and uniformity of the various manufactured samples. The increase in pH from 3.3 to 4.7 increased the average length from 517 nm to 2218 nm along with a larger standard deviation from the average dimensions, thus confirming the crystals' lower uniformity with increasing pH, as seen in Figure 3.5.

![Figure 3.5 Average length, diameter and RI of samples prepared in solution with different pH 3.3, 4.1, and 4.7](image)

The spatial distribution of inorganic elements connected with SHz was collected by the EDS mapping option of the state-of-the-art HRSEM. As shown in Figure 3.6(a), EDS mapping was
combined with a SEM image and confirm the uniform distribution of elements along the SHz crystals and demonstrate the purity of products. The related spectrum exhibiting characteristic elements of SHz including oxygen (O), carbon (C), nitrogen (N), and iron (Fe) was recorded (Figure 3.6 (b)).

![Figure 3.6 EDS elemental mapping (a) combined with SEM image of SHz and (b) overall spectrum](image)

3.4.4 **Refractive index measurement**

The access to a measured value for the RI of SHz is of fundamental and technological importance for further magneto-optic biomedical sensing research. To measure the RI of SHz, first the RI of aqueous SHz solutions was measured by employing a simple optical setup containing: a He-Ne laser, a rotating stage, a quartz cuvette, a digital vernier caliper, and a photodetector. Then the RI of SHz was derived through calculations based on the effective-medium theory. Thus, an aqueous solution of SHz with a volume concentration \(c_{SHZ}\) of \(1.7 \times 10^{-5}\) was prepared and poured into a minuscule (2 mm inner thickness) quartz cuvette. When the He-Ne laser beam incident obliquely on the surface of the cuvette and passes through the cell, the propagation axis of the transmitted beam would be displaced from that of the incident ray. The displacement allows us to calculate the liquid's RI (Nemoto, 1992; Sengupta & Ung, 2019). In the next step, to extract the RI of SHz from the measured RI of the heterostructure, we applied an electrodynamic calculation of a dielectric medium with a given
size (Belyaev & Tyurnev, 2018). Empirical effective medium models such as Maxwell Garnett (Garnett, 1904) and Bruggeman (Bruggeman, 1935) are valid for low volume concentrations (< 10^-6) of particles in the heterostructure. Their principle is based on the quasi-static approximation that does not valid for structures, such as SHz, with a size higher than the applied wavelength. We applied equation (3.1) to figure out the effective permittivity of SHz (\(\varepsilon_{SHz}\)) (Belyaev & Tyurnev, 2018).

\[
\varepsilon_{eff} = \frac{H_e + i\sqrt{-H_e^2 - 8\varepsilon_{SHz}\varepsilon_dJ(k_{SHz}a)}}{4}
\]

\[H_e = (2 - 3c_{SHz})\varepsilon_d - (1 - 3c_{SHz})\varepsilon_{SHz}J(k_{SHz}a)\]

\[J(x) = 2\frac{1 - x\cot\alpha}{x^2 + x\cot\alpha - 1}\]

\[k_{SHz} = \sqrt{\varepsilon_{SHz}\mu_{SHz}\omega/c}\]

Where \(\varepsilon_{eff}\) is the effective complex permittivity of the heterostructure, \(c_{SHz}\) stands for volume concentration of SHz in the dielectric medium (DI water), \(\varepsilon_d\) is the permittivity of water and \(\mu_{SHz}\) is the relative permeability of the SHz (\(\approx 1\) for nonmagnetic medium). As shown in equation 1 the \(\varepsilon_{eff}\) depends on the size of particles defined by "a". We considered the size distribution of each sample in this regard to calculating the RI of SHz. On the other hand, since the RI of Hz is different along various crystal axis (Coronado et al., 2014) we consider that for two directions. Firstly, the preferred crystallographic growth direction was considered, which consists of rods with different lengths. Equation 3.1 was applied for the various lengths of SHz to determine the average RI of samples with respect to the size distribution. In addition, the RI is calculated along the diameter of the rods. Since in practice, we have a mixture of randomly distributed rods we did average over the crystallographic growth direction and perpendicular to that (along diameter). As shown in figure 3.5 these results indicate that a reduction in the size of SHz samples translates into an increase in their RI value.

3.4.5 UV-Vis-NIR spectroscopy

The optical absorption spectra were recorded using a UV-Vis-NIR spectrophotometer (PerkinElmer, lambda 750) equipped with deuterium and tungsten halogen light sources. All measurements were carried out for aqueous SHz samples in the range of 250-800 nm and
employing a 1 cm path length quartz cuvette. The Uv-vis absorption spectrum related to different samples shown in Figure 3.7 contains a Soret band in the UV region and Q bands in the visible region (Bohle et al., 1994; Wood et al., 2004). The latter with a specific peak at 649 nm is the characteristic peak attributed to hemin aggregation as determined previously by photoacoustic spectroscopy (Balasubramanian et al., 1984) and micro-spectrophotometry (Morselt et al., 1973). By Increasing the pH, a red shift occurred in the absorbance spectrum, which agrees with the growth in the dimension of synthesized samples.

Figure 3.7 UV-vis absorption spectra of SHz synthesized in pH: 3.3, 4.1 and 4.7

3.4.6 Vibrating sample magnetometry (VSM)

The magnetization curve (Figure 3.8) was measured using a EV9 vibrating sample magnetometer (VSM) from ADE Technologies. The obtained powders were weighted and exposed to a maximum field of 2 T. Then, the magnetization (M) properties were measured as the field lowered to zero and increased again in the opposite direction. Figure 8 presents the M-H curves obtained for SHz. In order to calculate the magnetization, a value of 1.44 g/cm³ was assumed as the density of our hemozoin samples (Coronado et al., 2014).
Magnetic susceptibilities were calculated from the slope of M-H curve for different samples and exhibited in the inset of figure 3.8. The positive susceptibility value confirms the paramagnetic nature of SHz, which arises from the presence of unpaired electrons in Fe$^{3+}$ ions and is not affected seriously by the size of SHz.

![Figure 3.8 M-H curve related to SHz synthesized in different pH values](image)

**3.5 Conclusion**

This study has shown that the SHz prepared in an aqueous acidic medium under the physiological condition of temperature 70°C and pH 3.3 have similar dimensions and identical physicochemical structure to nHz (Buller et al., 2002; Jaramillo et al., 2009). Characteristics analyses such as XRD, FTIR, and UV-Vis-NIR confirmed that changes in pH do not affect samples' properties and primarily affect the dimension of SHz. Two main magnetic and optical properties that play a critical role, specifically in optical sensing applications, which were not addressed previously, were investigated in the present work. Prepared samples exhibited paramagnetic features with a minor reduction in magnetization.
and susceptibility for SHz prepared in the highest pH of 4.7. The RI of samples along different crystallographic directions were calculated, and the results revealed an inverse relationship between the size and RI. Finally, the results obtained in this study can offer applicable data set for future diagnostic techniques for malaria detection, specifically for those magneto-optical based sensors that has high potential for rapid detection of malaria.
Magneto-Optical Detection of Synthetic Malaria Pigment in Photonic Crystal Fiber

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4.1 Abstract

The necessity to develop new technologies for high-sensitivity malaria diagnosis has sparked a global effort in medical and integrative sciences. Most developing procedures rely on research-grade instruments, sophisticated assays, or on expertise. In this work, we propose an alternative optical methodology using a compact and user-friendly apparatus based on a photonic crystal fiber (PCF). Malaria pigment known as hemozoin is an insoluble reddish brown microcrystalline. These crystallites stand out from other blood components in terms of their exceptional magneto-optical features. Consequently, they can function as spinning entities in suspension in response to the external magnetic field. Here Synthetic hemozoin (SHz) was obtained in a forthright way with a high yield of 75\%. In addition, the prepared sample was characterized morphologically and structurally. The PCF’s nanoholes were filled with the aqueous suspension of SHz with various concentrations and transmitted power recorded in response to the magnetic field. We demonstrate a sensor with a detection threshold of 7.2 parasite/µl well below the level of clinical relevance (50-100 parasite/µl) at a very small liquid sample (less than 0.5 µl). The results of this investigation may provide new light on potential medicinal and sensor applications.
Keywords: Malaria pigment, Diagnosis Malaria Sensor, Photonic Crystal Fiber

4.2 Introduction

Plasmodium falciparum (P. falciparum) causes the greatest and the harshest form of Malaria among the five protozoan species with 90% of total deaths. It is an infection that strikes between 300 to 500 million people globally and causes 1.5 to 2.7 death annually (Jaramillo et al., 2009). It remains the most common vector-borne infectious disease despite global attempts to eradicate it, including preventive measures and pharmacological regimens. The global effort to eliminate malaria is severely hampered by the parasites' growing medication resistance, and climate change may potentially bring back malaria mosquitoes in areas that were previously free of them. The creation of inexpensive diagnostic techniques that are reliable even at an early stage of infection could lead to a major improvement. The most accurate and sensitive diagnostic technique currently in use is the microscopic examination of blood smears to evaluate parasitemia, which is defined as 20 parasites in 1 µl of blood (Ragavan et al., 2018). This test is costly and time-consuming because it calls for special training and powerful microscopes. On the other hand, rapid diagnostic tests (RDT) that use antigen-based detection of malaria parasites are not only quick but also more affordable (Bell et al., 2006; Wilson, 2012). However, the sensitivity threshold for RDTs is limited to around 100 parasites/µl; which is not sensitive enough to reliably detect early-stages of infections. Although polymerase chain reaction (PCR) analysis is sensitive enough to detect even 1 parasite/µl among molecular biology-based techniques, the practical application of PCR analysis in the field is limited due to the requirements of advanced technology and expertise. Extensive study and a vast array of diagnostic schemes have been offered in the last few years because of the necessity to develop new diagnostic procedures. Some instances include selective microscopic detection of infected blood cells such as third harmonic generation microscopy (Bélisle et al., 2008), magnetic deposition microscopy (Zimmerman et al., 2006), photoacoustic spectroscopy (Custer, 2011) and cell microarray chips (Yatsushiro et al., 2010). On the other hand, a growing number of approaches use malaria pigments as the target molecules based on their magnetic properties, including magneto-optical detection with polarized light (Mens et al., 2010; Newman et al., 2008), enhanced resonance Raman spectroscopy using magnetic field enriched surface (Yuen
& Liu, 2012), magneto immunoassays (de Souza Castilho et al., 2011) as well as intensity based malaria screening (McBirney et al., 2018). Malaria pigment known as hemozoin is a byproduct of the disease formed during the intraerythrocytic growth cycle of the parasites. Malaria parasites digest hemoglobin of blood which leads to the accumulation of monomeric heme. Since it is highly toxic to the parasites, they transform heme into an insoluble crystallized form (A. Slater et al., 1991). During this process, low-spin diamagnetic Fe\(^{2+}\) ions change into high spin (S=5/2) paramagnetic Fe\(^{3+}\) ions in hemozoin. Hemozoin has a triclinic crystal structure with various morphology depending on the parasite species. They usually have an elongated rod-like shape with a length ranging from 300 nm to 1 \(\mu\)m. Natural hemozoin (nHz) preparation is a time-consuming process that requires competence to infect the living being and extract the product. As a result, researchers prefer to deal with synthetic hemozoin (SHz) or \(\beta\)-hematin, different methods have been established for its artificial synthesis (Bohle & Helms, 1993; Gluzman et al., 1994). Synthetic grown version exhibited similar crystal structure (Pagola et al., 2000), optical and magnetic properties to natural hemozoin (Bohle et al., 1998; Frosch et al., 2007). There are various publications that address the synthesis of SHz and its physicochemical properties (Ambele et al., 2013; Fescenko et al., 2019; Pagola et al., 2000; Pauling & Coryell, 1936), to the best of our knowledge no study has produced SHz of comparable size to natural ones as well as measuring the refractive index (RI) of aqueous solution of SHz and assessed the susceptibility of that all in one study.

In this work, we present a novel method for the rapid monitoring of synthetic malaria pigment crystals. Which in contrast to the majority of the previously described emerging approaches, it might be realized as a compact diagnostic tool. In addition, the controlled procedure that enables to produce SHz samples with similar dimensions to the naturally occurring Hz provided through a straightforward process. Finally, The detection threshold of our instrument for aqueous solution of SHz is 0.8pM to a level of parasitemia 7.2 parasite/\(\mu\)l that is below clinical relevance (<50-100 parasite/\(\mu\)l) (K. Chen et al., 2016; Newman et al., 2008). Clinical trials will be required to prove this, but the detection limit achieved is already a significant improvement over RDTs.
4.3 Materials and Methods

4.3.1 Materials

Hemin crystalline powder known as Chloro(protoporphyrinato)iron (III), was purchased from Sigma-Aldrich. Other chemicals including sodium hydroxide (NaOH), methanol (CH₃OH), propionic acid (C₃H₆O₂), phosphorus pentoxide (P₂O₅), and bicarbonate sodium (NaHCO₃) were obtained from Thermo Fisher Scientific Chemicals, Inc.

4.3.2 Mimic of Hemozoin: Synthesis Process

Synthetic Hemozoin (SHz) was obtained by dissolving NaOH powder (0.4 gr) in deionized (DI) water (100 ml). The aqueous solution was degassed with nitrogen (N₂) for 30 min. In the next step, 500 mg of hemin was added slowly and stirred gently at room temperature for 20 min. Then propionic acid was added via micropipette drop wisely with mild stirring for 40 min. The solution acidity was adjusted by propionic acid and monitored by a pH meter (Fisherbrand accumet) during adding acid. Initially, the pH was 12.44 which was reduced to 3.3 with the acid addition. The well-stirred suspension was heated in an oil bath at 70°C for 17h to provide uniform and stable reaction conditions. The achieved mixture was cooled at room temperature and decanted via micropipette. To purify the product, it was washed extensively with NaHCO₃ (0.1M), methanol, and water three times. The SHz product was finally dried in the oven at 40°C for 48h over P₂O₅. The applied technique yielded more than 75 percent of the product.

4.4 Characterization of SHz

4.4.1 Fourier-transform IR(FTIR) spectroscopy

To investigate the chemical structure of SHz, FTIR spectroscopy was employed. To acquire IR spectra small portion of SHz powder was positioned on the diamond crystal of the
spectrometer (model: Nicolet 6700 /Smart ITR, Thermo Scientific) and compressed. The Spectra were recorded in reflection/ transmission mode over the range of wavenumber 2000-650 cm\(^{-1}\), at the resolution of 2 cm\(^{-1}\) with 128 coadded scans. As shown in figure 4.1 SHz spectra exhibited specific sharp bands at 1660 cm\(^{-1}\) and 1209 cm\(^{-1}\) which were assigned to coordinated carbonyl (C=O) stretching vibrations and carboxylate (C—O), respectively (Egan et al., 1994; Tempera et al., 2015).

4.4.2 X-ray powder diffraction (XRD)

The D8 advance Bruker AXS diffractometer was used to acquire the XRD patterns of SHZs, using a copper-based radiation source (Cu K, 1.5406 ) over a range of \(2\theta = 5\text{-}30^\circ\). Figure 4.1 displays the SHZ's XRD pattern. The presence of sharp Bragg diffraction peaks confirms a high crystallinity structure. The SHZ's unit cells are made up of two heme molecules each with symmetry-related interactions in a triclinic lattice structure (Bohle et al., 1997). The obtained sample exhibited different growth directions with priority at \(2\theta = 7^\circ\) which corresponds to the (100) plane.

![Figure 4.1 Fourier-transform IR spectra and X-ray powder diffraction pattern of SHz](image)
4.4.3 High-resolution scanning electron microscopy (HRSEM)

The morphology of SHz was investigated using HRSEM. Basically most SHz possesses higher dimensions compared to natural hemozoin. Here in SHz samples prepared at lower pH (3.3) resulted in samples with dimensions comparable their natural counterpart. As Shown in figure 4.2 (a) needle like microcrystals with length less than one micron appeared. To assess the average size and uniformity of the obtained samples more than a hundred crystals were chosen from SEM images, and dimensions were analyzed using Image J software. As shown in figure 2(b) the length of microcrystals mostly distributed between 400nm to 600nm with average length and diameter of 517 nm and 130 nm respectively.

![Figure 4.2 (a) High-resolution scanning electron microscopy image of synthetic hemozoin, distribution histogram of synthetic hemozoin: (b) length, (c) diameter.](image)

4.4.4 Vibrating Sample Magnetometry (VSM)

The magnetization hysteresis loop was measured using EV9 VSM from ADE Technologies. The obtained powders were weighted and exposed to an increasing magnetic field (up to 2 T) Then the field lowered to zero and increased again in opposite direction. In order to calculate the magnetization, the value of 1.44 g/cm³ was considered as the density of hemozoin (Custer, 2011). As shown in figure 4.3 the M-H curve presents the recorded values. The positive susceptibility of 0.14E-5 confirms the paramagnetic feature of SHz which arises from the presence of unpaired electron in Fe³⁺ ions.
4.4.5 Refractive index (RI) measurement

In order to model the transmitted light in the infiltrated photonic crystal fiber (PCF) it is crucial to define the proper RI that corresponds to different concentration of SHz. In this regard, several aqueous solutions of SHz with different concentrations were prepared and poured into the quartz cuvette. When the He-Ne laser beam is incident at a small angle on the facet of the cuvette and passes through the cell, the propagation axis of the transmitted beam becomes displaced through refraction. The displacement (Δ) allows us to calculate the liquid's RI based on the equation 1 (Nemoto, 1992; Sengupta & Ung, 2019).

\[ n_1 = n_0 \sin \theta \sqrt{1 + \left[ \frac{\cos \theta}{\sin \theta - \frac{\Delta}{d}} \right]^2} \]  

(4.1)

Where \( n_1 \) stands for the RI of liquid, \( n_0 \) is the RI of surrounding environment (air), \( \theta \) the angle of incidence between the laser beam and the facet of cuvette, while \( d=10 \) mm represents the internal length of cuvette. The measured refractive index values as a function of concentration of SHz samples are plotted in figure 4.4.
4.5 Detection principle

Hemozoin microcrystals possesses magnetic signature as well as opaque intrinsic feature that offer key parameters for fabricating magneto-optical based sensors. Their paramagnetism is caused by the presence of high spin unpaired electrons in Fe$^{3+}$ ions. When such crystallites suspended in a liquid upon exertion of an external magnetic field, they start to form chain like clusters and co-align along the direction of magnetic field to gain magnetic energy (Butykai et al., 2013) under this phenomenon the refractive index of liquid would change. Here, we propose a PCF infiltrated with aqueous solution of SHz that provide miniature sensor with ability to guide most portion of light in the silica core and small changes in refractive index in response to external magnetic field affect the output power as well as the beam pattern which can detect small fraction of Hz in solution. Figure 4.5(a) shows a schematic of the diagnostic setup, He-Ne laser source was chosen because light absorption is weaker for water in the visible band compared to near-infrared band. When the E-field of the guided light is parallel to the direction of the external magnetic field, optical absorption increases in fluids containing magnetic particles (Inaba et al., 1989). In practice, using polarized beam splitter not only
improve the interaction of light with SHz but also reduce the fluctuation in output power and offer higher signal-to-noise ratio. In the next step the collimated laser beam coupled to the infiltrated fiber via 20x objective lens. To fill micro holes of PCF we exploited Poiseuille law (Pfitzner, 1976). In this method, one end of the fiber is submerged in the solution and kept under high pressure (3 bar), while the other end is exposed to ambient pressure. The pressure difference enables the PCF to be filled with the liquid sample. Figure 4.5(b) exhibit the SEM cross section image of PCF which contains holes with average diameter of 1.2 µm and spaced with a period of 1.6 µm. The middle section of fiber placed between two magnetic discs made of NdFeB that provided a uniform magnetic field of 1 T. To monitor the changes in output power and beam pattern at the same time during exertion of magnetic field, the guided light on the other end of fiber collimated and divided via 20x objective lens and beam splitter respectively.

Figure 4.5 (a) Schematic illustration of experimental setup, (b) SEM cross-section image of PCF
Figure 6(a) presents the output power changes related to the PCF infiltrated with different concentrations of SHz upon exertion of external magnetic field (1T). As expected, changes in power correspond to the concentrations of SHz in suspension. Furthermore, a sample consisting of sole distilled water was used as the reference (shown with solid black line). We observe that the reference sample did not affect the transmitted power. The sample with the lowest percentage of SHz, 0.8 pM (which corresponds to 7.2 parasite/µl that is in agreement with the relevant clinical concentration (Ragavan et al., 2018)), exhibits detectable changes in power compared to the background noise with a signal-to-noise ratio of 2.84. The slight fluctuation in power is ascribed to the magnetophoretic movement of SHZ micro crystals and random particle-particle interactions. The experiments were repeated several times and the average value of power changes and corresponding error bar for each concentration is shown in figure 6-(b). Measurements indicate that the response time increased from 1 min to 4.6 min for samples with 0.8 pM to 16 pM concentration of SHz, respectively.

Figure 4.6 (a) Response time of the proposed sensor in presence of external magnetic field (1T) and (b) Average changes in output power for different concentrations of SHz
4.6 Conclusion

The fine precision of advanced optical fiber technologies in combination with functional fluids can offer desirable diagnostic tool for emerging bio medical sensing applications. In this work, in the first step we synthesized the mimic of malaria pigment via straightforward technique with a yield reaches of 75%. The RI of aqueous solution of SHz with different concentrations was measured which rarely considered in literatures. In addition, the positive magnetic susceptibility of 1.4E-5 was determined from magnetometry measurements which confirms the paramagnetic property of SHz. We also demonstrate a novel magneto-optical malaria sensor using PCF with submicron air holes infiltrated by SHz suspension with ability to detect SHz. The proposed configuration requires solely a tiny amount of liquid sample (0.5μl) for the detection of pigment crystals. The threshold detection limit was 7.2 parasite/μl with full order of magnitude below the clinical level. Simple magneto-optical configuration scheme and ability to detect in point of care indicate a promising alternate technique for malaria detection compared to conventional methods.
CONCLUSION

Early detection of diseases with optical fibers has the potential to provide a new tool for clinicians in low-resource communities owing to their compact size, low-power, ease of use and cost-effectiveness. Advanced optical fibre technology, when combined with functional fluids, can provide a useful diagnostic tool for upcoming biomedical sensing applications. In this research thanks to the unique feature of photonic crystal fibers that offer interaction of light with analyte without affecting the fiber structure, we were able to design rapid and sensitive sensors. Initially, we applied magnetic fluid in combination with submicron-sized air holes that resulted a highly sensitive (0–350 gauss), fast (0.1 s response time) and compact magnetic field sensor. The transmitted optical power upon exposure of magnetic field were recorded in the lab and validated by theory using a waveguide model fed by finite-element method. The applied configuration can be considered as a promising platform to detect malaria pigments. Basically, during malaria infection process some micron-sized crystals with paramagnetic properties created. These by products can be considered as biomarkers to detect malaria in early stages. Owing to magnetic property and high absorption they have great potential to be detected using magneto-optical based sensors. One of the main hurdles slowing the research in magneto-optical biomedical detection projects is related to extracting the precious malaria pigments. In this regard a lot of literature addressed synthesizing mimic of malaria pigment, known as β-hematin or synthetic hemozoin. But mostly possesses higher dimension than natural hemozoin and there is not enough data related to their magnetic or optical properties e.g. magnetic susceptibility and refractive index.

This research found, SHz generated in an aqueous acidic medium under physiological conditions of 70°C and pH 3.3 have similar dimensions and physicochemical structure to natural counterpart (Saeed, Bora, 2023a). We measured the RI of SHz in aqueous solutions at various concentrations, which is rarely discussed in literature. Analysis of characteristics using XRD, FTIR, and UV-Vis-NIR techniques indicated that variations in pH had no effect on the properties of the samples. In the current study, two key magnetic and optical features that are crucial, particularly in optical sensing applications, but have not previously been addressed, were examined. Samples that were prepared showed paramagnetic characteristics with a slight
decrease in magnetization and susceptibility to SHz when they were prepared at the maximum pH of 4.7. Calculating the RI of samples along various crystallographic orientations, the results showed an inverse correlation between size and RI. In the next step we demonstrated a novel magneto-optical malaria pigment sensor utilising PCF (Saeed, Bora, 2023b). In comparison with similar techniques the proposed configuration only requires 0.5 μl of sample for detection, which is important in diagnostic tools. The detection limit was 7.2 parasite/l, which was an order of magnitude lower than the clinical level. In contrast to existing methods, a straightforward magneto-optical configuration strategy and the capacity to detect at the point of care suggest to a viable alternative technique for malaria detection.
SUGGESTIONS FOR FUTURE STUDIES

Detection of Malaria in early stages of infection via user friendly and cost-effective sensors applicable in highly condensed tropical countries with low resources is vital. Here we propose some suggestions for further improvements of malaria pigment detection.

a) Basically, paramagnetic feature of hemozoin offers rapid response to magnetic field. In the meantime, the environment temperature can reduce the magnetization. Consequently, there is still a need to study the temperature dependence of the detection method.

b) Current techniques apply constant magnetic field in the order of 1T that results in slow movement of hemozoin in liquid (water or blood). While in a slowly rotating magnetic fields the hemozoin crystallites respond as micro-rotors. Based on this idea, one could explore in the future novel mechanical ways to manipulate the infected blood so as to provide a higher signal to noise ratio.

c) Here in this work, we used a PCF with air holes comparable to the size of hemozoin pigments. In the future, one could explore other variations of the same PCFs with higher number/diameter of holes that could improve the sensitivity.

d) PCF has potential to abide propagation of guided modes with certain effective index called bandgap effect. Since the RI of infected blood change upon exposure to magnetic field so the PCF waveguide can be designed in a way that offer significant reduction in output intensity.


Saeed, Bora. (2023a). DIMENSIONAL CONTROLLED SYNTHESIS OF MALARIA PIGMENT: KEY STUDY FOR DIAGNOSIS OF THE MOST SEVERE FORM OF MALARIA.

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