UNIVERSITY OF NAIROBI

OPTO-MAGNETIC DETECTION OF MALARIA: A POTENTIAL LOW-COST, RAPID, AND SENSITIVE MALARIASCREENING METHOD

BY

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of degree or publication. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

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DEDICATION

This thesis is dedicated to my family.

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ABSTRACT

Malaria is a sickness transmitted by a bite of female Anopheles mosquito which carries a *Plasmodium* parasite. Malaria is prevalent in Sub-Saharan Africa, parts of South America, Asia and portions of Central America. In the year 2017, close to 219 million people were infected with malaria with an estimate of 435000 deaths globally according to the WHO report. The malaria deaths are mainly associated with late diagnosis and lack of screening kits as most of these places are remote and less resourced. The commonly used screening methods include: PCR (polymerase chain reaction), RDT (Rapid Diagnostic Tests) and Giesma microscopy which are expensive and require specialist to operate.

This work involved fabrication of a portable and rapid malaria screening device. The device composed of an LED, lenses, a pair of disc magnets, a photo-diode and a screen. This battery powered device was applied in the detection of hemozoin (malaria pigment) first when suspended in de-ionized water and later in rat's blood. The absorption spectra (measured using a spectrophotometer) of hemozoin suspensions were obtained.

Prominent absorption bands were observed at around 377, 421 and 449 nm assigned to SoS2 transitions (Soret/B-band) 551, 665, 723 and 866 nm assigned to So-S1 transitions (Q-band) in hemozoin. Transmittance of LED light emitting at 450 nm and 667 nm decreased with concentration of hemozoin. This was due to absorption by hemozoin. These LEDs were used in the malaria screening device. The voltage difference detected by the photodiode indicated nonlinear relationship with concentration. The transmittance was also measured using the malaria device in presence and absence of magnetic field.

When graphs of voltage against concentration were plotted, the fit equation was obtained and the trend was equation of the form y=kLn(x)+ C which can be equivalently expressed as $e = \frac{y-c}{k}$ In this relation, y and x represented the voltage and hemozoin concentration respectively. The later equation was used as malaria diagnostic algorithm as it (x) in the sample. The R^2 values obtained for the curve in the presence of magnetic field was 0.983 and was higher than that without the magnetic field which was 0.971. This indicated that taking the measurements in the presence of magnetic field was better and makes the device more sensitive. This also supported the idea that the magnetic field influences the orientation of hemozoin nanocrystals such that majority are aligned in a certain way.

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LIST OF ABBREVIATIONS, ACRONIMS AND SYMBOLS

ACC	Automated Blood Cell Counters
A. U	Arbitrary units
AUC	Area Under Curve
B	Magnetic Field
C_{4V}	Hard Symmetric Axis (z-axis)
CE	Cotton Effect
DNA	Deoxyribonucleic Acid
DUV	Deep Ultraviolet
FCM	Flow Cytometry
FPIX	Iron Protoporphyrin
Hb	Hemoglobin
HD	High Definition
Hz	Hemozoin
IFA	Immunofluorescence Antibody
IMCI	Integrated Management of Children Illness
L	Focusing Lens
LDMS	Laser Desorption Mass Spectrometry
LED	Light Emitting Diode
N-S	North – South pole of a magnet
PCR	Polymerase Chain Reaction
PD	Photodiode
POC	Point of Care
PT	Presumptive Test
PZ	Polarizer
QBC	Quantitative Buffy Coat
RBC	Red Blood cells
RDTs	Rapid Diagnostic Tests
UV	Ultra Violet
WBC	White Blood Cells

WHO	World Health Organization
WP	Wave-plate

CHAPTER 1: INTRODUCTION

1.1 Overview

Malaria is a sickness transmitted by a bite of female *Anopheles* mosquito which carries a *Plasmodium* parasite. Malaria is prevalent in Sub-Saharan Africa, parts of South America, Asia and portions of Central America. In the year 2017, close to 219 million people were infected with malaria with an estimate of 435000 deaths globally according to the WHO report(Chan et al., 2013) The data shows that 90% amongst the fatalities emanates from Africa. *Plasmodium* m*alariae, Plasmodium vivax Plasmodium falciparum* and *Plasmodium ovale* are the main malaria parasites with *Plasmodium falciparum* being the most fatal. They are transmitted by a female *Anopheles* mosquito during bite (Alumasa & Paul D. Roepe, 2010) This illness affects commonly children and women with deaths reported every minute(Alumasa & Paul D. Roepe, 2010). Reports show that a child dies after 45 seconds daily in sub-Saharan Africa due to this disease(Chan et al., 2013)

1.1.1 The Malaria disease

Malaria history can be drawn back to about 30 million years discovered in mosquito remains(Joy, 2003). It was attributed by a Greek philosopher to poisonous waste called miasma which was purportedly transmitted through air henceforth miasma concept. The word malaria was derived during the medieval period from the Italian language "Mal Aria" meaning "bad air." Alternatively, it was commonly known as marshy fever because of its link to marshy zones and humid places. (Poinar, 2005). Dr. Charles Louis Alphonse performed the first study of malaria in a French military hospital. He detected malaria parasite pigment in a patient's fresh unstained blood in WBC. This parasite was later detected in Red Blood Cell (RBC)(Jani et al., 2008). The device used was a X400 magnification dry focus lens. Later in the 19th century, the mosquitos were discovered to be the transmission agent.(Bousema et al., 2010). Later studies showed that the current human malaria is triggered by the 4 parasitic protozoan species mentioned above (Padial et al., 2005). It has also been found that the *Plasmodium falciparum* virus is the deadliest and affects childrenand expectant mothers. A female *Anopheles* mosquito which serves as an agent reproduces and moves the parasite from host to host. (Poinar, 2005).

1.1.2 Malaria incubation period

After a human has been bitten by a mosquito, the parasite takes an incubation period of about 7 days or longer depending on the species (WHO, 2016). Any serious illness, therefore, developing in less than 1 week after the first possible bite is not malaria. *Plasmodium falciparum (P. falciparum)* causes the most fatal form of this disease with a diversity of symptoms comprising of abdominal pain, high body temperature, diarrhea chills, muscular aching, headache, vomiting and cough. The initial signs may be minor and hard to classify as malaria disease. In most cases, the likelihood of malaria is deliberated during these cases of baffling fever whose onset is between seven days later after the first possible exposure to malaria and 90 days later after last conceivable acquaintance (WHO, 2015). Anybody who records higher temperature during such periods ought to instantly undertake diagnosis and definite action of medical care or notify health practitioner of whichever likely exposure to malaria infection. If the malaria case turns to be *falciparum* form, then it may be lethal if not treated within one day after the inception of proven signs. This is most prevalent to infants, expectant mothers and the elderly(Karnad et al., 2018)

Malaria triggered by the rest of *Plasmodium* (*P*) species results in major illness but is occasionally fatal. Circumstances of serious *P. vivax* malaria is experienced in sub-tropical countries(Howes et al., 2016). This species of *Plasmodium* has an ability of being dormant in the hepatic cells for long time but still possess malaria risk.

P. ovale just *like P. vivax* may be indolent in the hepatic cells which may degenerate due to persistently living in the liver in the form of hypnozoites which may appear months or years later but rarely severe (Rojo-Marcos *et al.*, 2014). Infection with *P. malariae* may be existing in the human body for many years without being life-threatening to the host (Wellems *et al.*, 2009). Therefore, it may not require urgent and swift actions when detected.

P. knowlesi is prevalent amongst people residing or available in forest zones such as parts of southern and eastern Asia(Wesolowski et al., 2015). Most cases have been reported from different travelers and workers showing infections of *P. knowlesi*. Although this parasite is able to infect other primates like monkeys (Collins, 2012) most humans in this regions (Asia) have been infested with this 'monkey malaria' parasite when residing in the rain forests. Other areas prone with this *P. knowlesi* include: China, Indonesia, parts of Brunei Darussalam, Philippines, Vietnam

Singapore, Malaysia, Cambodia, Myanmar, Singapore, Thailand and Lao People's Democratic Republic (WHO, 2015). *P. knowlesi* parasite has an incubation period of 24 hours and can cause daily fever spikes happening within 12 days after exposure showing typical malaria symptoms(CDC, 2021) *P. knowlesi* may cause organ failure and periodic fatal consequences.

1.1.3 Malaria control approaches

A few anti-malarial methods have been utilized since the recognition of the ailment. These methodologies can be ordered by and large into three classes including: control, diagnosing and treatment. The control methodology involves lessening the quantity of *Anopheles* mosquitoes and by instructing individuals on the sickness through courses and coordinating it in the national educational plan.

It additionally incorporates the dissemination of treated mosquito nets, clearing bushy zones, spraying, among others, with bug sprays (Pluess *et al.*, 2010). On account of poverty in most influenced places, it is hard to purchase malaria sprays and nets.

Consequently, diagnosis and treatment are the subsequent strategies. The most recent symptomatic procedures used incorporate stain microscopy of Giemsa, chain response of the polymerase (PCR), fluorescence microscopy, and Rapid Diagnostic Tests (RDTs)(Jain et al., 2014)

1.1.4 Malaria detection and diagnosis.

Some malaria detection methods are presently deployed in different countries in the world to reinforce malaria control toward malaria eradication. For this objective to be achieved, the detection method used needs to be specific and more accurate. Most of these current detections leads to making decisions that heavily relies on microscopic diagnosis. Sensitive diagnosis tool that is affordable is required. Some of these methods are control measures and not detection. They target at eradicating or decreasing the number of the mosquito insects and bites through educating societies concerning the ailment in workshops and assimilating the program to the nationwide education system. In addition, it includes dissemination of insect side treated bed nets, eradicating hatching locations of the mosquitos, distribution of pesticides to kill the parasites among many other methods(Pluess B, et al., 2010).

The currently used detection and diagnostic methods includes: PCR (polymerase chain

reaction), fluorescence microscopy and RDTs (rapid diagnostic tests) and Giemsa stain microscopy(Steenkeste et al., 2009). These methods are profound with capability of detection. PCR has a detection of 5parasites / microliter of blood and is capable of differentiating between parasitespecies but limited as discussed in the table 1.1 below. It clearly reveals how difficult it is to deploy them in the field with highly skilled personnel required in the rural setups.(Bensch Staffan et al., 2009)

Although RDTs are self-administered and portable, they have short shelve life. RDTs are not capable of detecting malaria at early stages and require cold storage materials especially during transportation.(WHO, 2000). Giemsa stain microscopy is capable of detecting malaria plasmodium DNA(Cho et al., 2012). It involves application of microscopes of high power resolution in order to identify and determine infected RBC in a blood smear when Gemsa dye is applied. The method can sense 5-10 parasites/microliter of blood. Nevertheless, this method is a bit slow (45 minutes), which calls for an expert to control costly and not easy to deploy to outdoor field for use

Therefore, Giemsa microscopy is rendered inappropriate to poor regions which are vulnerable. RDTs on the other hand, has an ability to identify 100 parasites/microliter of blood in less than half an hour of blood though with short shelve life besides not being suitable for early stage malaria detection.

Presumptive Test (PT) is an alternative and extensively applied technique mainly in poor rural clinics while studying symptoms (Padial et al., 2005). This involves study or taking note of the symptoms presented by the patient and comparing with the known ones in malaria patients. Treatment is then initiated but the method has disadvantages as it can lead to misdiagnosis as there is overlap of symptoms with other diseases. Besides, wrong drug administration may lead to drug resistance by the parasite. These methods are discussed in details in section 2.7

1.2 Statement of the problem

1.2.1 Malaria; a killer disease

Malaria is a killer sickness causing deaths of masses of individuals globally every year. Throughout Africa and Asia, most affected regions are economically unstable with no electric

connectivity. The available traditional screening machines are predominantly done in the laboratory, they are huge, costly and slow. In this thesis we are advancing a technique for malaria screening which is portable, fast, cheaper and field deployable.

1.2.2 The economic and public health burden of malaria in Kenya

Where malaria thrives, there is a negative impact on economic growth. The nations distribution of per-capita gross domestic product indicates a outstanding relationship between malaria and poverty, and malaria-endemic countries also have lower rates of economic growth(Sachs & Malaney, 2002). In Kenya, malaria has impeded development in terms of worker productivity where most average Kenyans depend heavily on small scale farming. Sick farmers suffering from malaria boycott their duties hence lowering the production. Absenteeism from places of work has lowered the production at work places hence affecting the income., absenteeism, premature mortality and medical costs. According to the Kenyan ministry of health, billions of money is pumped in the sector to curb malaria by developing programs and policies.

The economic impact of malaria on households and individuals is increasingly becoming a subject of considerable interest to researchers and policy makers in Kenya. Available evidence from Kenya welfare monitoring survey show that in Kenya, malaria is the leading cause of morbidity, accounting for about 19 per cent of hospital admissions and 50% of outpatient cases in public health institution(Ministry of Planning Report, 2014)

1.3 Main objective

The main aim of this project is to advance an affordable, simple, compact and sensitive method that is able to screen malaria based on simple materials of light-emitting diodes, magnetic field source, photodiodes and is battery driven. The gadget uses hemozoin's optical and magnetic properties (a by-product of the malaria parasite digestion of hemoglobin in red blood cells).

1.4 Specific objectives

i. To measure the absorption spectra, identify the prominent absorption bands of hemozoin in suspensions in de-ionized water and use the information to select the appropriate excitation LED
ii. To analyze the transmission spectra of selected LED lights through different hemozoin concentrations and determine a diagnostic equation using data fit equation.
iii. Design, fabricate, assemble and test a portable malaria diagnostic device.

1.5 Justification

Alarming global malaria infection rates can be reduced, particularly among expectant mothers and children in Africa and other regions if a low-cost system of malaria screening is established to be used during infection. The appropriate method for this situation should be compact, flexible, user-friendly and can be used in arrears without power supply.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

As mentioned in the foregoing chapter, there exists several methods already to detect and diagnose malaria but each has a number of weaknesses. Most of them are costly, non-portable and take long time before results are obtained. As the results are being awaited, the disease advances and may even cause more deaths. We believe that if a less costly and portable device that is easy to use was available; a lot of deaths can be reduced due to rapid screening. In this work we report a portable device that is battery powered and involves an LED, a magnet and electronic circuits.The device is designed to detect hemozoin (HZ), one of the by-products produced when malaria parasite consumes hemoglobin of the RBC. The hemozoin produced is an inorganic crystal that results from heme detoxification process. This crystal is released from the food vacuole into circulation during erythrocyte lysis in the blood stream. The hemozoin has some optical and magnetic properties which are capable of defining existence of malaria in the body when utilized well. The composition of the device involves a system of optical detectors which operate on basis of the opto-magnetic properties of HZ.

Preceding research, have revealed that light emitting diode of specific wavelength can be combined with photodiode as detector to show presence of Hz crystals(Butykai et al., 2013). Several improvements have been made on the device especially on detection where transmitted light through the sample held in cuvette was detected by the photodiode and displayed by a multi-meter. The device operation is based on a photodiode for identifying strength of transmitted beam of light and modified recording and exhibition system. The power of the device involves use of a battery which are lighter hence portable and easily deployed in places without main electricity.

2.2 Malaria infection.

This is an infectious disease transmitted by mosquito and affects humans, primates and other animals in particular. This disease is caused by single-celled *Plasmodium* group microorganisms whose symptoms include nausea, exhaustion, diarrhea, and headaches. These symptoms usually start 10 to 15 days once an infected mosquito has bitten them.

If it is not handled within 24 hours, *P. falciparum* malaria, one of *Plasmodium's* species, may develop a severe disease that usually leads to loss of lives. Children having extreme malaria develop severe anemia, breathing problem relative to metabolic acidosis, or cerebral malaria on a regular basis. Multi-organ problems are also common to adults. Some partial immunity can be experienced in malaria prone areas leading to asymptomatic infections to occur(Lindblade et al., 2013).

2.3 History of Malaria

Malaria is still the main killer of humans globally among other diseases affecting more than 30 %of global population (Guinovart et al., 2006). Studies reveal that humans have been ailing malaria for the past 5000 years(Cox, 2010). It is a worldwide protozoan parasite infection. The *Plasmodium* that causes malaria include: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae* spread by a bite of female Anopheles *mosquito*(Alumasa & Paul D. Roepe, 2010). It's commonly detected in sub-tropical areas of Africa, India and her neighbors and parts of America(Chan et al., 2013). Children and expectant mothers are more vulnerable and loss lives every minute(Alumasa & Paul D. Roepe, 2010). Studies show that malaria claims live of an innocent child in every minute in Africa(Chan et al., 2013).

History of this disease is backdated to millions of years ago in mosquito vestiges(Joy, 2003). The scholars accredited this to poisonous smell referred to us miasma and believed to be transmitted by air thereafter miasma concept. –Malaria name has Italian origin –Mal Aria which translates to "bad air" in middle age period. Owing to connection of this problem with marshy land and tropical zones(Poinar, 2005), malaria was otherwise known as marshy fever. The initial examination and detection of this ailment was performed by Dr. Charles Louis Alphonse. This was carried out in a military hospital in France. The examination was carried out in fresh unstained WBC of malaria victim which was later confirmed in RBC(Ita, 2013). A dry lens was used as an examination device. Later research done in late the 19th century revealed that malaria was transmitted by an insect agent known as mosquito. Successive studies have revealed the four parasitic protozoan species aforementioned.(Padial et al., 2005). It

has also been found that *Plasmodium falciparum* species *is* the deadliest. This parasite lives in human host and mosquito host which acts as an agent(Poinar, 2005).

2.4 Malaria disease challenges.

In most populations for tropical and sub-tropical countries, malaria is a major challenge. The ailment presents a major problem for the well-being and economic development of tropical nations and has been described by the poorest regions of third world nations as a primary obstacle to sustainable development(Ricci, 2012). Malaria's highest prevalence is certainly foundin tropical African nations and is a source of premature death and suffering, endorsing financial hardships in most families that stagnate economic growth and lower the living standards of their respective families(Mackey et al., 2014). Hence high numbers of consultations and admissions according to the Ministry of Health are caused by a large population of people, particularly in Kenya(Hay et al., 2002). It is also revealed that many infants are vulnerable from childbirth(Craig et al., 1999)

Infection with malaria varies from region to region and from age to age (Graves et al., 2009). Many survivors are treated according to the presumptive diagnostic technique. The illustrated gold standard is a medical examination in which blood smears are used in combination with the microscope. This approach requires a high level of experience that may be lacking in most areas vulnerable to malaria (Ita, 2013). Many mothers and care givers treat patients and buy drugs on their own without understanding the risk of taking wrong medication. Therefore, most important conditions diagnosed and treated may not be successful. The main objective of this work is to provide an effective rapid detection and diagnosis of malaria that can help to reduce deaths from malaria and the possibility of incorrect diagnosis and medication.

2.5 Malaria parasites and transmission

Malaria is transmitted by female Anopheles mosquito. Various *Anopheles* groups (30 to 40 species) serve as human disease vectors. Many physiological, behavioral and ecological features determine the effectiveness of different species of Anopheles as malaria vectors(Issa Lyimo & Heather Furguson, 2009). This infection contact between the *Plasmodium* parasite and the host immune system strikes a poor balance.

The association can trigger shielding immunity or damaging immune responses. This composite relationship made it difficult for people to discover defensive mechanisms. A better understandingabout existence of these parasites would probably provide significant understandings into new ways of eradicating malaria.

2.5.1 Types of malaria parasites

2.5.1.1 P. falciparum

Plasmodium falciparum is the most lethal in the world which causes a lot of deaths due to malaria(Cho et al., 2012). The disease has approximately 14 days of the shortest life cycle with sudden signs which can be detected after screening, identified and treated due to presence of malaria cure.

Table 2.1: Different stages of plasmodium falciparum and their characteristic. (Cho et al., 2012)

Parasitic stage in blood	**Characteristics of RBC**	**Parasite appearance**
Ring	-Normal size -Rings appear on the periphery of the RBC	-Mild cytoplasm -Small chromatin dots
Trophozoite	-Usual shape and size	-Hard to be observed in blood; compact-Darkish pigment
Schizont	-Regular size and shape	- 8-24 small merozoites in mature schizonts -Darkish pigment clumped in one mass. -Rarely seen in marginal blood..
Gametocyte (8-10 days to mature)	-Destroyed by parasite	-Semi-circular or sausage shape

2.5.1.2 P. ovale

Plasmodium ovale is not a common parasite mostly found in west African countries and parts of America, however there are less effects and better recovery(Kang & Yang, 2013).

Table 2.2: Different stages of plasmodium ovale and their characteristic.

(Kang & Yang, 2013).

Parasitic stage in blood	**Characteristics of RBC**	**Parasite appearance**
Ring	-Round to oval in shape	-Tough cytoplasm -Enlarged chromatin
Trophozoite	-Appears round or oval	-Compact -Enlarged chromatin -Dark-brownish pigment
Schizont	-Round to oval	-6-14 merozoites with enlarged centers -Bunched mass -Dark-brownish pigment
Gametocyte (3-4 days to matur	-Appears round or oval	-Round to oval -Almost fill RBC -Scattered brown pigment

2.5.1.3 P. malariae

Plasmodium malariae can exist in the host for decades. This is alluded to its longer life cycle where symptoms are seldom pronounced. This hinders clinical diagnosis. The extensive life cycle of this parasite may lead transmission to another human during blood transfusion process.

Table 2.3: Different stages of plasmodium *malariae* and their characteristic.

(Kang & Yang, 2013).

Parasitic stage in blood	**Appearance of RBC**	**Appearance of Parasite**
Ring	Normal size	-Large chromatin
Trophozoite	Normal size	-Enlarged chromatin -Dark-brownish pigment
Schizont		-Enlarged nuclei, -Dark-brown pigment
Gametocyte **(**6- 8 days to mature**)**	Normal size	-Appears round or oval -Almost fill RBC -Brownish pigment

2.5.1.4 P. vivax

Plasmodium vivax is the most widespread species in India and its neighborhoods and sections of African sub-tropics and has an extensive life span of about 3 years in the body after which any distressing effects are detected(Cho et al., 2012)

Table 2.4: Different stages of *Plasmodium vivax* and their characteristic.

(Cho et al., 2012)

Parasitic stage in blood	**Characteristics of RBC**	**Appearance of parasite**
Ring	-Relatively enlarged	-Pronounced cytoplasm -Enlarged chromatin
Trophozoite	-Enlarged size -May be distorted	-Enlarged cytoplasm -Lager chromatin -Yellowish pigment
Schizont	-Enlarged size -May be shapeless	-Large (almost fill RBC) -Yellowish-brown,
Gametocyte (3- 4 days to mature)	-Enlarged size -May be distorted;	-Appear round or oval -Almost fill RBC -Brown pigment

As shown above, some morphological key features of infected RBCs and parasites may be used todirect the diagnosis of the four *Plasmodium* species that infect humans. These features are by no means absolute, however. The final diagnosis for the different characteristics should focus on the collective results. The likelihood that either of the above *Plasmodium* will occur depends on its availability and existence as discussed in this thesis

2.5.2 Parasite transmission

Malaria disease is triggered by a mosquito parasite that exists in humans (host) and in mosquitoes(host) in some cases. This is attributed to the easy way of transmission by female *Anopheles* mosquitoes, which are mobile parasite transporters that have gained global recognition(Phillips, 2001). Mosquito-to-human transmission of malaria occurs when sporozoites are injected into the skin during blood feeding from the mosquito's salivary gland. Then the parasites move to the hepatic cells in which they replicate and a single sporozoite produces a lot of merozoites that cause manifest infection(Teboh-Ewungkem et al., 2021).

At any given time, a quarter of the global population suffer from the disease (Chan et al., 2013). Out of these numbers, at least an African child loses life in a span of 45 seconds(Vo,

2014). Globally, there exist more than 100 species of *Plasmodium.* Although the ones responsible for the malaria ailment in different animals, birds and people are mainly *P. vivax, P. malariae, P. ovale and P. falciparum*(Hoang, 2010) Among the species, each has varied characteristics symptoms while inside the body.

2.5.3 Malaria parasite life-cycle

Two groups of hosts, namely humans and female *Anopheles* mosquitoes, are constantly infected by malaria parasites. Sporozoites are fed into human blood d system by a female *Anopheles* mosquito during bite for blood. Sporozoites invade in the liver and develop to become schozonts which later release merozoites(Cho et al., 2012). During this phase, the *Plasmodium* species of *vivax* and. *ovale* can be able to stay in the hepatic cells and cause relapses by entering the bloodstream after a longer period of time ranging to months or even years afterwards, at a dormant stage (hypnozoites).

At this phase, the parasites undertake asexual reproduction in the erythrocytes (erythrocytic schizogony) later after this primary replication in the liver (exo-erythrocytic schizogony). The merozoites then infect red blood cells. The trophozoites of the ring stage mature into schizonts which become independent of merozoites. Many species differ in the sexual erythrocytic (gametocyte) stages. At this stage, the parasites can easily be detected in the blood stream as malaria manifestation. Male and female gametocyte i.e. microgametocytes and macro gametocyte respectively are ingested by the mosquito when feeding on human blood meal. The two multiply in the *Anopheles* mosquito's belly in the process known as sporogonic cycle. The sporogonic cycle generates zygotes. Zygotes are free and appear elongated (ookinetes) which later occupy themosquito's midgut wall and develop and become oocysts. The oocysts then mature and develop into sporozoites which maneuver and find their way into salivary glands of the mosquito. Sporozoite enter into another human host and starts another life cycle of malaria.

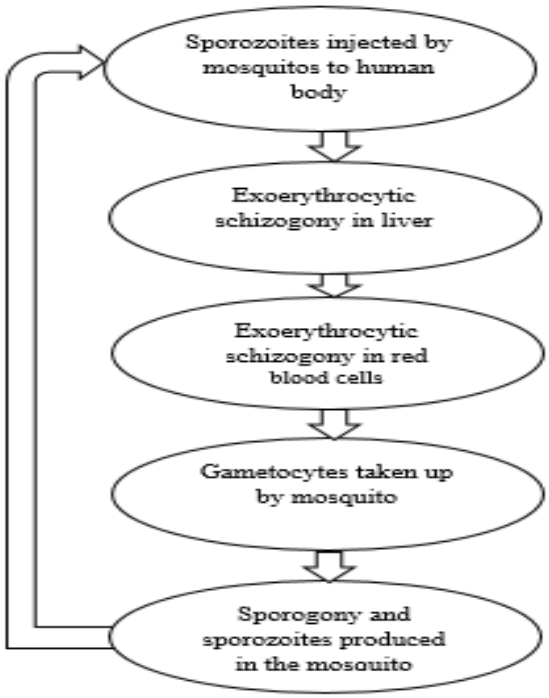

Figure 2.1: Life cycle of *Plasmodia* that cause malaria in humans

The parasites emerge and replicate quickly in the liver cells in the first host and then in the blood'sred cells. Inside the red cells, successive young parasites develop and kill them in the blood, releasing daughter parasites ("merozoites"), which continue the process by invading other red cells. The ruptured red blood cells release a hemozoin-known by-product that is an indication of a person infected with malaria. Parasites in the blood stream are those that cause malaria symptoms.

When a female Anopheles mosquito picks up certain types of blood-stage parasites known as gametocytes during a blood meal, they begin another, different growth cycle and multiplication in the blood stream

The parasites (sporozoites) are found in the salivary glands of the first host (mosquito) after 2 to 3 weeks. When the *Anopheles* mosquito bites the human host, the sporozoites are introduced inwith the saliva of the mosquito and when they invade the liver cells, and commence the infection to the person.

As a result, the mosquito passes the infection from person to person (acting as a "vector"). Contrary to human beings, the mosquito only serves as a vector bad does not suffer from malariaas the effect. For each *plasmodium*, the length of each step mentioned above is

unique as shown intable 2.5.

Table 2.5: Duration of each *Plasmodium* malaria parasite species at the indicated stages(WHO, 2015), (Siciliano & Alano, 2015)

Stage	**Plasmodium species**			
	P. vivax	***P. ovale***	***P.malariae***	***P. falciparum***
Pro-erythrocytic phase	6-8 days	9 days	14-16 days	5-7 days
Erythrocytic cycle	48 hours	50 hours	72 hours	48 hours
Incubation period	12-17 days	16-18 days	18-40 days	9-14 days
Sporogony	8-10 days	12-14days	14-16 days	9-10 days

2.6 Malaria prevalence in tropical countries

Across tropical and subtropical regions around the equator, the sickness is boundless(Caraballo & King, 2014). It incorporates Sub-Saharan Africa, Asia and Latin America's extension. As indicated by Owen, 216 million instances of malaria were enrolled worldwide in 2016, bringing about an expected 445,000-731,000 deaths out of which about 90% of the cases happened in Africa(Owens, 2015).

Malaria sickness is normally connected to poverty and has a significant unfortunate effect on financial development(Worrall et al., 2005). Proof has uncovered that about US$ 12 billion is lost every year because of expanded human services costs, malaria treatment, diminished capacity to work, and negative impacts on the travel industry. Universally, this sickness cases will in general be on the ascent because of the expanded danger of transmission in regions where disease control has fizzled, expanded event of medication, safe parasite strains, enormous increments in worldwide travel and relocation in a moderately low malaria cases. Most of these diseases are shown in Figure 2.2 on the global map.

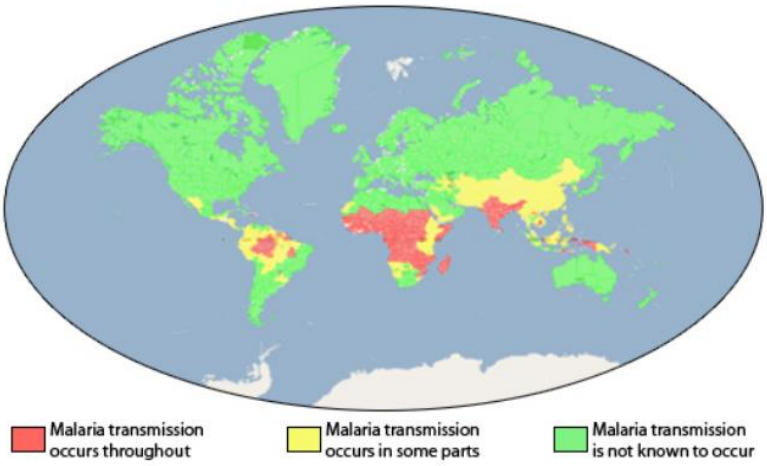

Figure 2.2: Distribution of malaria in the world

Extracted from Centre for Disease Control and Prevention*(CDC, 2021)*

2.7 Methods of malaria detection and diagnosis

Several methods have been discovered and used to minimize malaria deaths globally. The methods employ various scientific techniques to combat the disease but discovered at different times and at different technological advancement stages. Some of them are discuss together with their limitations.

2.7.1 Clinical diagnosis of malaria

This is the most established treatment strategy for malaria. In spite of the fact that it is additionallyviewed as a possible procedure(Osei-Yeboah *et al.*, 2016) it is the most affordable most generally utilized. A clinician makes a determination and the malaria treated depending on the symptoms articulated by the patients. This methodology may not be effective in light of the fact that it is frequently not one of a kind to the earliest manifestations of malaria sickness The greater part of the manifestations is fever, migraine, myalgia, chills, dazedness, stomach torment, the runs, queasiness, spewing, anorexia, and pruritus(McMorrow et al., 2008) (Waitumbi et al., 2010). Such side effects may cover with other basic diseases like pneumonia and the common cold. The closeness of malaria with other tropical infections complicates diagnostic precision, which can encourage the unpredictable utilization of antimalarial medications and underrate the nature of care in endemic territories for patients with non-malarial fever(McMorrow et al., 2008). The Integrated Management of Children's Illness (IMCI) has created clinical calculations for research facility testing, especially among

newborn children, because of the absence of satisfactory lab apparatuses in most developing nations. In these low-asset conditions, professionals utilize this strategy to treat child malaria. An investigation directed by Tarimo and partners shows that the utilization of the clinical calculation IMCI brought about an over diagnosis of malaria at 30 percent(Tangpukdee et al., 2009). This suggests improved and exact jungle fever analysis requires a blend of this technique and some other particularly parasite basedstrategy(Kyabayinze et al., 2008).

2.7.2 Laboratory diagnosis of malaria

Rapid and powerful treatment of malaria assuages the transmission and relieves pain. Clinical finding is assumptive as indicated by Bhandari and can prompt over-or under-treatment or even erroneous treatment(Bhandari et al., 2008). Malaria laboratory treatment requires an assortment of methods including: customary microscopic assessment of slight and thick fringe smears of blood(Bharti et al., 2007), (Ngasala et al., 2008). To conclude on malaria sickness and its illness status, this strategy requires a trained personnel to recognize uninfected and infected blood smears.

Little has changed after Dr. Charles Louis Alphonse first found the procedure in a French military emergency clinic where the parasite color was recognized in White Blood Cells (WBC) in a patient's new impeccable blood and later in Red Blood Cell (RBC). The instrument utilized was a dry X400 magnification objective lens (McKenzie et al., 2005). This was later extended in the late1800c by Romanowsky (Horobin, 2011). Giemsa-stained thick blood films are as of now being utilized for testing to distinguish the parasite's presence while thin blood films are being utilized tovalidate the malaria infection. This methodology is perceived globally as the diagnostic technique of the gold standard laboratory(Bharti et al., 2007).

This can be because of its moderately low-cost, minimal effort and ease of use. Regardless of staining and interpretation are labor-intensive and require extensive aptitude and talented medicinal services laborers health care worker, particularly in the early stages of accurate identification of organisms. The strategy is additionally generally less resistant, particularly at low degrees of parasites(Payne, 1988).

Microscopy requires a skilled and qualified specialist. Subsequently, microscopy is

impractical in remote country zones, peripheral medical centers with no electricity and medical facilities(Erdman & Kain, 2008).

The other laboratory technique is quantitative buffy coat (QBC) to upgrade microscopic parasite distinguishing proof and advance the procedure(Clendennen et al., 1995). The technique fundamentally includes staining deoxyribonucleic acid (DNA) with fluorescent dyes such as acridine orange in micro-haematocrit tubes and its subsequent detection by epi-fluorescent microscopy. In numerous research centers, the method has demonstrated more prominent affectability to malaria diagnosis(Adeoye & Nga, 2007)**.**

Work has indicated that it is conceivable to utilize convenient portable microscopic instruments utilizing LED in combination with arranged glass slides(Tangpukdee et al., 2009). While the QBC strategy is straightforward, precise and easy to understand, it requires explicit, increasingly costly instrumentation. Rapid diagnostic tests (RDTs) are the other laboratory process. WHO built up this way to deal with microscopy deficiencies.

A drop of blood is taken from a finger in RDT and put on a test strip right away. A couple of drops ofthe solution are applied and a red line develops on the strip a couple of moments later. At the point when two red lines show up, the *falciparum* malaria is certain

Apparently, this test can recognize just the dangerous type of *falciparum* malaria. Comparable tests may likewise be accessible later to distinguish the less destructive type of vivax malaria. The advantage of this method is that learning is simple, there is no requirement for a laboratory and it just takes 15 minutes to get the outcome. A few scientists have portrayed this strategy for diagnosing malaria as probably the best choice for diagnosing malaria(Kyabayinze et al., 2008) (Ratsimbasoa et al., 2008) Nonetheless, Moody(Moody, 2002), Bell and co-workers (Bell et al., 2006) and Murray and co-workers(Murray et al., 2003) points out extensive variations in sensitivity and its execution has been curtailed by poor device operation.

Insufficient strategies are available to assess item quality and absence of focus and capacity to manage these featured setbacks. Nevertheless, in order to exploit their potential it is significant that RDT devices be made accessible, work consistently and precisely under field conditions This is beyond the realm of imagination because of financial imperatives,

particularly in developing nations(Bell et al., 2006), (Murray et al., 2003). In spite of the fact that RDTs seem, by all accounts, to be an exceptionally significant strategy, it must be utilized as affirmation method for the results got in combination with different techniques(Murray et al., 2003).

In the long run, in the asexual stage in the blood, serological tests are among the laboratory diagnostic method that depend on the parasite antibodies. Immunofluorescence antibody testing (IFA) is one of the most recent serological tests. (She et al., 2007). Doderer characterizes IFA as the gold standard for serology screening for malaria(Doderer et al., 2007). IFA is particularly suitable for screening prospective blood donors to avoid malaria parasite transfusion of blood(Mungai et al., 2001).

The method strategy is additionally used to test non-immune casualties for recent infection. As per this method, antibodies are created 14 days after disease infection, which can keep going for around 5months after the parasite has been clear(Tangpukdee et al., 2009).

IFA is easy to use, fast and sensitive, but it takes time and cannot be optimized to limit the numberof detections per day. Special storage conditions are additionally required because the crude antigen prepared on a slide must be stored at a temperature of below -30 °C until it is used(Tangpukdee et al., 2009). It also includes expert technicians and fluorescence microscopy. In contrast, the lack of IFA reagent testing makes repeated use of blood transfusion centers in general difficult.

2.7.3 Molecular diagnostic methods

New development has been on the rise in molecular biological technologies. Many widely used diagnostic methods are molecular in nature. One of these methods includes reaction to the polymerase chain (PCR), which includes molecular malaria diagnosis. PCR can detect most cases of malaria with low parasitemia (Morassin et al., 2002).

The PCR technique is capable of identifying malaria even in regions with malaria drug resistance regions.(Chotivanich et al., 2007) and has proved to be more sensitive than RDTs(Makler et al., 1998). Moreover, PCR can be automated to work on large numbers of tests (Leowattana & Krudsood, 2005).

Research conducted by Cox-Singh and co-workers and Luchavez and co-workers revealed that the PCR approach is most widely accepted for *P. knowlesi* detection(Cox-Singh et al., 2008). While PCR appears to have overcome the two key hitches of malaria diagnosis in terms of sensitivity and specificity, it has complicated methodologies and is very costly (Luchavez et al., 2008). In addition,highly trained specialists are required. For this reason, in developing countries that lack financial skills and experts, PCR is not much available(Mens et al., 2008).

Another novel technique for in-vitro detection of malaria parasites has been recorded for mass spectrometry(Demirev et al., 2002). This requires a process for washing whole blood samples and then performing direct ultraviolet laser desorption mass spectrometry (LDMS). The main purpose ofthis LDMS concept is to identify specific biomarkers in clinical samples. Hemozoin heme is the parasite-specific biomarker of importance in malaria detection. This method (LDMS) is fast, high, and automated. But very expensive compared to other approaches.

Wongchotigul and colleagues study Flow cytometry (FCM) testing a malaria diagnostic process(Wongchotigul et al., 2004). The technique involves the identification of hemozoin produced on the hemoglobin of the host's red blood cells during feeding of the parasite. In the acid food vacuole, toxic heme is formed that is converted into hemozoin(Ginsburg et al., 1998). The depolarization of laser light can detect hemozoin in phagocytes as cells move through a cytometer flow stream(Hawkes & Kain, 2007). The system of flow cytometry is highly sensitive and precise(Padial et al., 2005). This form, however, is labor intensive, needs trained staff to work, and it is very necessary

2.7.4 Merits and demerits of current malaria screening methods

Table 2.6: Merits and demerits of current malaria screening methods (Butykai et al., 2013), (WHO, 2016), (Cho et al., 2012)

Method	Function	Merits	Demerits
RDT (Rapid Diagnostic Test)	-Stain parasites RNAwith Acridine	-Portable Comparatively faster	-Unable of detecting parasites at initial stages- Short shelve live
Clinical Diagnosis (Presumptive)	-Study of symptoms	Cheap Fast	-Inaccurate diagnosis -Can lead to wrong drugs insemination
Giemsa	-Parasite DNA staining	-Sensitive -Can identify parasite species	-Slow -Expensive -Requires an expert to operate -Not fielddeployable
Wide field and confocal fluorescence microscopy		-Can give images of infected RBCs. -Study live infected RBCs	- Expensive - RequireTrained personnel,
PCR (Polymerase Chain Reaction)		-More sensitive	-Expensive -Need trained personnel. -Not field deployable

2.7.5 New methods under exploration.

Several methods are under research while others crop up occasionally. most of them are advancement of the previous methods while others are novel. Some of these methods are discussed here.

2.7.5.1 Mass spectrophotometry

This method has a sensitivity of about 10 parasites/µl of blood. It applies ultraviolet laser desorption mass spectrometry (LDMS). It is a rapid method and is specific to a particular biomarker and can analyze a sample in less than 60 seconds (< 1 min) (Scholl et al., 2004). It involves processing of individual spectra a written software and matched-filter algorithm to detect heme. The algorithms calculate a score that represents a similarity of the test spectrum to that of heme. The laser desorption mass spectrum of hemozoin exhibits more than five structurally characteristic heme fragment ions(Demirev et al., 2002). This is due to the fact that HZ can strongly absorb ultraviolet laser light resulting in the vaporization and ionization of individual heme molecules.(Lvova et al., 2016).

2.7.5.2 LAMP method

A newly developed molecular technique that amplifies nucleic acid under isothermal conditions is the loop-mediated isothermal amplification process (LAMP)(Ocker et al., 2016).This approach detects *P. falciparum's* retained ribosome RNA gene. This technique is a simple and cheap molecular diagnostic test for malaria(Zerpa et al., 2008). By being simpler and quicker, LAMP sought to solve two drawbacks of PCR and offers a high level of accuracy. The working theory is based on a set of four specifically designed primers to identify six different regions of the target DNA as well as polymerase used for DNA synthesis of auto- cycling strand-displacement. In isothermal conditions, amplification is achieved(Ocker et al., 2016). If a water bath is replaced, the process can be made less costly(Poon et al., 2006). On the other hand, to maintain low temperatures, the reagents used require special storage devices. This method, according to Hänscheid, validates the feasibility and clinical utility in clinical trials(Hänscheid et al., 2001).

2.7.5.3 Automated blood cell counters (ACC)

This method detects malaria pigment (hemozoin) in monocytes using a Cell-Dyn ® 3500 unit. It is an automated process that makes it possible to diagnose malaria by detecting malaria pigment in white blood cells during daily blood counts(Hänscheid et al.,

2001)(Hänscheid, 2001). In other cases, the system is used to detect malaria infection among combination with depolarized laser light (DLL). This approach has not been fully exploited and generally is not used in the clinical laboratory. More studies are therefore needed to improve and validate the development of better technology for the instrument.

2.7.5.4 Bio-Sensing Technology

A label-free identification of red blood cells contaminated with *P. falciparum* using an improved platform for gold nanoparticle (GNP) is used in this process(Kumar et al., 2016). Biosensors are self-contained analytical instruments capable of analyzing complex matrices such as blood and urine without the need for further processing or reagent(Krampa et al., 2017). The gold nanoparticle is deposited electrode to form a well-controlled matrix on screen-printed electrodes that serve as the dual role of antibody immobilization and signal enhancement. Infected red blood cells are identified byanalyzing changes in electrical parameters due to their binding to cell-reactive antibodies immobilized on the electrode.

The test is then used to show tolerance and linear reaction between the resistance of the electron transfer and the logarithm of the number of infected red blood cells observed over a cell concentration per milliliter.

2.7.6 Identification of diagnostic biomarkers

Such biomarkers may be markers for genomics, transcriptomy, proteomy, or metabolomy (Foxman, 2010). Such approaches contribute to the quantification and study of plasmodium and parasitemia bacteria. It is also possible to determine the immune response and the key sample used is blood. Nevertheless, research is currently underway showing opportunities to use other body fluids such as urine and saliva as samples(Ray et al., 2011).

2.8 The hemozoin

Hemozoin (malaria pigment) is one of the most significant biomarkers for malaria in the blood. The malaria parasite creates these organic, insoluble nano-crystals.

2.8.1 Hemozoin the malaria pigment

After digestion of the host's RBCs, the parasite first produces heme, which is later converted to hemozoin chemically known as Fe3+ protoporphyrin with the formula; $C_{272}H_{240}N_{32}O_{32}Fe_8$ (Hackett et al., 2009). Heme is a ferrous compound while hemozoin is crystalline, non-toxic

and poisonous to the parasite. Hemozoin is paramagnetic and will align these nano-crystals when suspended in a fluid along the magnetic field while applying magnetic field(Butykai et al., 2013). Hemozoin crystals have refractive indices that are dependent on the direction of light polarization and propagation, an optical property known as birefringence and therefore birefringent or refractive(Pirnstill & Coté, 2015). Upon exposure to a magnetic field, they are aligned along a certain axis with the resultant alteration of their optical properties relative tothose oriented randomly (i.e. no magnetic field). In order to understand the optical and magnetic properties of malaria pigment, we must analyze its structure first and then the origin of its special magneto-optic properties.

2.8.2 Hemozoin production

The parasites feed on the hemoglobin, which is the key source of protein through a complex structure called a cytostome, during the development of malaria parasite in the human host (Goldberg et al., 1990). This cycle removes hemoglobin and heme that is contained within the digestive vacuole of the cell in the form of crystals(Adams et al., 1996). For parasite development and maturation, hemoglobin is broken down into amino acids(Zarchin et al., 1986). It is estimated that between 25% and 75% of hemoglobin is depleted in an infected erythrocyte, according to Morrison and co-workers(Morrison & Jeskey, 1948).

The action of the cytostome activates free heme (Fe2+-protoporphyrin IX). The Fe2 + is oxidized to Fe3 + forming hematin (a heme dimer) when there is an excess of free heme without connecting the globin chain. Where a heme is bound by bonds between the carboxylate oxygen of one heme and the next heme's central ferric ion(Slater et al., 1991). As a consequence of the detoxification cycle, the heme units accumulate into insoluble crystals called hemozoin (Fe3 +-protoporphyrin IX)(Kim et al., 2012).

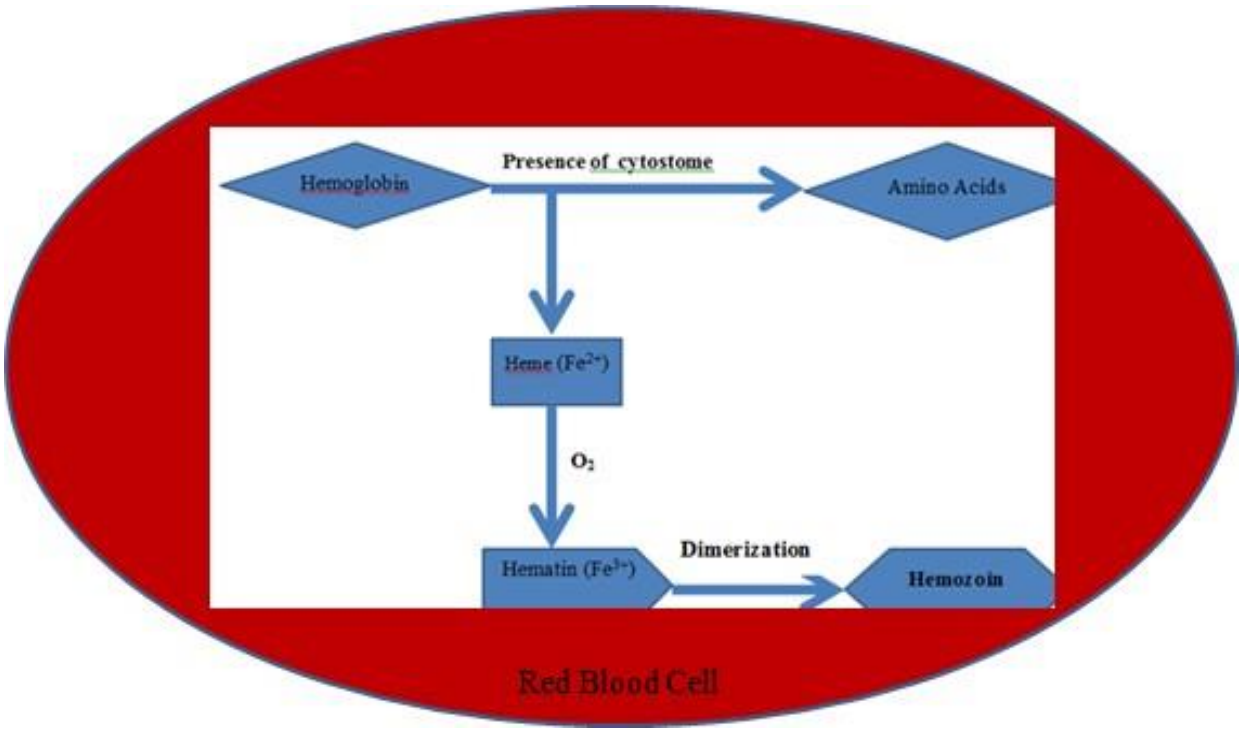

Figure 2.3: Hemoglobin degradation by *Plasmodium falciparum* in RBC

According to Pagola and his colleagues, hematin is a nano-crystalline dimer of ferriprotoporphyrin IX (Fe(III)PPIX))(Pagola et al., 2000). Hemozoin is similar to a synthetic hematin pigment called beta-hematin(Fitch & Kanjananggulpan, 1987) chemically and structurally. The compound's iron ion makes a magnetic compound of the hemozoin.

2.8.3 Structure of hemozoin

The heme (chemically known as ferri-protoporphyrin IX[Fe(III)PPIX)] is released after hemoglobin degradation by the malaria parasite *(Plasmodium falciparum*), which is a microcrystalline cyclic dimer(Hoang, 2010). This compound is toxic to the parasite, thus, as shown in Figure 2.5, the parasite transforms it into crystalline and harmless hemozoin. The hemozoin has structured bonds that bind with the neighboring hemes and ends through the hydrogen bonds as shown in Figure 2.4(Bonaventura et al., 1975).A study conducted by Slater and partners utilizing infrared spectroscopy, UV/visible spectroscopy and broadened fine-structure X-ray ansorbtion(Slater et al., 1991) reveals that a crystalline heme (Hz) comprises of an iron molecule encompassed by a cyclic ring of nitrogen and carbon particles alongside an oxygen atom attached to the atom of the atmosphere. A long needle-shaped cristals with magnetic moment long the iron-oxygen bond is the subsequent structure. The ironic crystals therefore

display paramagnetic properties.

Figure 2.4: Intermolecular hydrogen bond which links one heme to another.

Adapted from: (Vanderesse et al., 2016)

The chemical formula for hematin is $C_{34}H_{33}FeN_4O_5$(Solomonov et al., 2007). Two hemes are linked together by hydrogen bond to form β-hematin also referred to as hemozoin(Vanderesse et al., 2016).

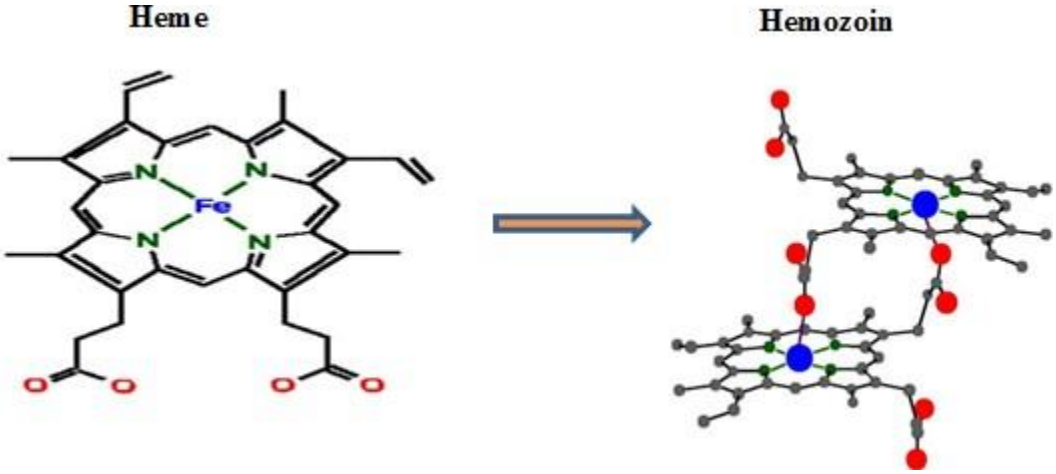

Figure 2.5: Chemical structure of heme transforming to hematin (heme dimer).

Adapted from: (Fong & Wright, 2013).

This compound of hemes is encircled by particles of carbon. Studies including optical properties of hemozoin in bulk arrangements are uncommon on the grounds that the greater part of them

include stained blood smear that is costly, complex and not reasonable for use in fields. This research aims at utilizing hemozoin's magnetic and optical properties in making a malaria diagnostic device dependent on a light emitting diode (LED). Chan and partners recently exhibited a comparative device utilizing just hemozoin's optical properties(Chan et al., 2013) and reported low levels of detection. The consideration of hemozoin's magnetic propertiesas one of the reason for recognition in this procedure significantly improves its sensitivity.

2.8.4 Optical and magnetic properties of hemozoin

Hemozoin compound is paramagnetic in nature and its refractive index relies upon the direction of light polarization and propagation, otherwise called birefringent or birefractive. At the point when placed in a liquid, the hemozoin atoms have spinning properties. At the same time these properties can be utilized as magnetically small micro-rotors (cutting edges) and in suspension turning polarizers(Butykai et al., 2013). The heme groups are dimerized by iron carboxylate contacts during the conversion of the monomeric heme(single heme) into hemozoin by the parasite.(Chan et al., 2013).

As a result of the cycle, iron changes the valence hence its local coordination transforms into high spin (s= 5/2) paramagnetic Fe3 + ions in crystals from low diamagnetic Fe2 + spins in the oxyhemoglobin(Butykai et al., 2013). In a hemozoin crystal, the local symmetry of five-fold coordinated iron almost maintains a four-fold axis of rotation, C4v. The angle spanned by the hard axis of the elongated crystal indicated as the C4v axis of the crystallographic c-axis is $\delta \approx 60^{o}$ with the c-axis pointing out of the plane as indicated in figure 2-6

Figure 2.6: Spinning symmetry of heme.

The local symmetry of five-fold coordinated iron in heme nearly preserves a four-fold rotation axis, C4v. The angle spanned by this C4v axis (hard axis of the magnetization) and the crystallographic c-axis (fore-axis of the elongated crystals) is $\delta \approx 60°$, where the c-axis points out of the plane of the figure. Extracted from(Butykai et al., 2013).

Hemozoin's low crystal symmetry demonstrates that it is incredibly anisotropic paramagnetic with different magnetic resistance values for each of the three major crystallographic axes shown in figure 2-6 above. Nevertheless, Fe3 + ions centrally located in porphyrin rings are subjected to lower local symmetry because they are immediately perpendicular to the rotation axis. The magnetic properties of hemozoin are in this manner determined basically by Fe3 + particles, which shows the symetry of axis (C4v) and hemozoin either as an easy axis or as a simple plane paramagnet. Past studies by (Sienkiewicz et al., 2006) proposed that the S = 5/2 spins of Fe^{3+} ions in the malaria pigment can be described by the Hamiltonian equation suggested that the S = 5/2 spins of Fe3+ particles in the malaria pigment can be depicted bythe Hamiltonian equation(Butykai et al., 2013)

$$H = D\overline{\left(s_z^2 - \frac{s(s+1)}{3}\right)} + E(s_x^2 - s_{X)+\mu Bg\ BSBg}^2 \qquad \textbf{(2.1)}$$

D is the zero-field splitting associated with an axial anisotropy in the first term, the symmetry of C4v is diminished by the addition of E that is negligible. When such a hemozoin crystal is suspended in a liquid and exposed to an external magnetic field, as shown in figure 2.7, they incline and co-align in the direction of the field to receive magnetic energy U. This energy is demonstrated at room temperature as stated in equation 2.2 (Butykai et al., 2013).

$$U = -\frac{1}{2}\frac{B^2}{\mu o} cos\,\theta(x_{zz} - x_{xx})V \qquad \textbf{(2.2)}$$

Where the magnetic resistance of hemozoin along the hard axis and in the easy plane respectively is expressed by χ_{zz} *and* χ_{xx}. The elevator represents the angle between the axis direction and the magnetic field and is the liquid volume. The crystal orientation is altered bythe thermal variations that cause the crystals to be randomly focused. Butka and colleagues(Butykai et al., 2013)showed that magnetizing crystals to point along the hard axis is a field and temperature function. It has been found that magnetization often depends heavily on the angle of inclination θ.

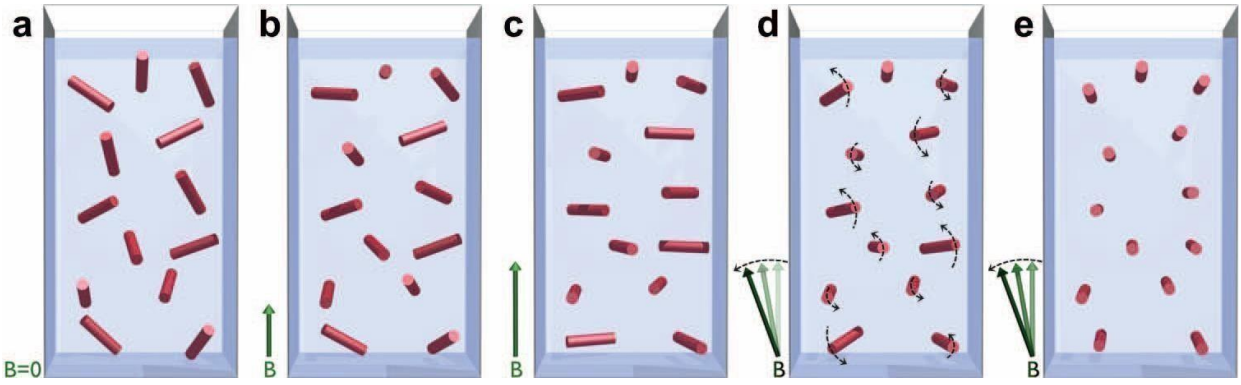

Figure 2.7: Magnetic orientation of hemozoin in different magnetic fields.

Adapted from: (Chan et al., 2013)

The red cylinders in Figure 2-7's schematic diagram reflect hemozoin suspension in a liquid (blood). The cylinders ' axes reflect the strong magnetic axis. The hemozoin crystals are distributed randomly if the external magnetic field is absent (B=0) as shown in (a). Nonetheless, the hard axes of the hemozoin crystals start to align normally with the magnetic field vector B after the application of the external magnetic field as shown in figure (b).

High thermal fluctuations can impede the orientation of crystals but, as shown in figure (c), when the field strength is increased, the crystal alignment is saturated along the normal plane to the ground. If the magnetic field is slowly rotated, the fluid's viscosity allows the hard poles to converge at high frequencies perpendicular to the rotational axis. The cylinders will therefore stop spinning as shown in figure (3d and 3e).

If linearly polarized light beam exists on such dichroic crystals, light will be absorbed differently by relation to the materials optical axis according to the direction of the polarization vector. Therefore, if the attenuation of polarized light is measured along the length and breadth of the crystal.

The device can be used in vivo to replace the LED light source with a low-cost laser pointer. LEDs powered by simple dry cells are used as the light source that passes through the hemozoin-containing blood. This improves malaria detection capacity. The opto-magnetic malaria detection method will also be cheap and affordable

CHAPTER 3: THEORETICAL BACKGROUND

3.1 Introduction

As mentioned earlier in this study, by feeding on hemoglobin in red blood cells, malaria parasite produces insoluble nano-crystals known as hemozoin. Consequently, identification of this compound in the blood is a positive indicator of malaria infection, and its concentration can correlate with the rate of parasitemia in a manner that is not yet understood. Scanning electron micrographs indicate that hemozoin crystals are formed in a distinct rectangular shape by the malaria parasite(Noland et al., 2003). Once suspended in a liquid, such as blood, the long axes of the hemozoin crystals are arbitrarily oriented in three dimensions, so that the suspension does not reflect a desired optical absorption path in on interrogation using linearly polarized radiation.

Nevertheless, when a magnetic field is applied, the paramagnetic crystals will become weak bar magnets with a torque that tends to guide them along the line of the applied field(Badescu et al., 2009). The light will be attenuated in a specific direction once all crystals are completely aligned with the magnetic field. This phenomenon is analogous to the Cotton- Mouton effect where, when tested in different directions, a material in a magnetic field shows different optical and magnetic properties(Badescu et al., 2009).

3.2 Optical absorption and transmission of hemozoin

According to beer-lamberts law, the amount of light absorbed is affected by both concentration and solution path length. It was also noted that if the light beam is allowed to encounter the solution for a longer distance, high absorbance was expected to result in a low percentage of transmittance and a high percentage of absorbance; whereas, if the beam was allowed to encounter the solution for a shorter distance, a high percentage of transmittance and low absorbance was expected.. For light travel at a constant speed, $c= 3.0x\ 10^8$ m / s implies that as discussed above, the absorbance should also be proportional to the beam's path length throughthe sample. There was also directly proportional light absorbance to intensity. Such considerations pave the way for the following relationship leading to beer lamberts law to be inferred:

$$A = klc \quad \textbf{(3.1)}$$

where,

k = Proportionality constant,l = path length, (cm)

c = Concentration of hemozoin suspensions equation 3.1 reduces to Beer-Lamberts law 3.2

$$A = \varepsilon lc \quad \textbf{(3.2)}$$

Where ε is the extinction coefficient

Equation 3.2 above may be used to measure the absorption of a given hemozoin suspension and to infer the suspension concentration from that measurement as the hemozoin absorption wavelength is established from the discussions in section 5.1. Nonetheless, very high absorption occurs at high concentrations, resulting in poor signal. It supports the argument in the literature that does not apply at high concentrations under the Beer-Lambert law.

3.3 Optical detection of hemozoin

As discussed in the literature, the main problem in the fight against malaria is the correct diagnosis at an early stage and it is therefore necessary to develop a proper diagnostic method. This novel method is heavily dependents on hemozoin's optical and electromagnetic properties, some of which are discussed below.

3.3.1 Optical Dichroism.

As a paramagnetic material, only in the presence of an applied magnetic field, hemozoin crystal has a magnetic moment. This paramagnetism comes from the iron III ion($Fe^{3+)}$ unpaired electrons. The random unpaired spins line up to boost the field when an external magnetic field is applied. Such unpaired spins are easily randomized by thermal agitation when the applied magnetic field is removed(Coronado et al., 2014).

The hemozoin crystal can cause it to spread differently from its crystal lattice by incident light.(Coronado et al., 2014). This hemozoin property was developed to detect infections with malaria(Newman et al., 2008). Therefore, within the digestive vacuole of live parasites, a strong magnetic field of about 0.5Teslas can guide the hemozoin crystals. Hemozoin crystals can be magnetically oriented because they are paramagnetic and form as rods(Chan et al., 2013).We also exhibit magnetic anisotropy, thus assembling the so-called "simple" axis of the crystal along directions with greater magnetic susceptibility as shown in figure 2.6.This

anisotropy provides a torque of orientation that keeps it compatible with the ground. This process may take some time to respond due to the viscosity of the surrounding fluid (around 100μs for an applied field of 1 tesla,)(Coronado et al., 2014). Nonetheless, according to Newman and his colleagues (Newman *et al.*, 2010), the field strength should be controlled in such a way that a very strong field of about 1 T can cause hemozoin crystals to spin and can impede crystallization, while weekly magnetic fields do not produce sufficient torque on crystals to overpower the rotational distribution caused by natural thermal agitation(Coronado et al., 2014). When a very weak field is used, it may not be able to orient the hemozoin againstthermal diffusion.

3.3.2 Paramagnetism of β-hematin

The paramagnetism of β-hematin has been exploited in a number of ways, including the separation of infected red blood cells from uninfected cells. This hemozoin pigment is key in curbing the disease in various aspects of the development of antimalarial drugs.(Alumasa & Paul D. Roepe, 2010). Once analyzed from its shape, physiochemical structure and optical properties, this by-product of hemoglobin degradation plays a crucial role in malaria diagnosis. Such bio- cristal results gave researchers a framework for deliberating on it as a target for any new antimalarial or experimental approaches to malaria diagnosis and therapy. New thoughts could use all the current data on this macromolecule's unique chemical and biophysical properties to create new ways to fight the disease(Spadafora et al., 2011). This property enables the hemozointo be attracted up a magnetic gradient toward the pole of a strong permanent magnet.(Paul et al., 1981).

3.4 Absorption spectrum

Previous studies and measurements carried out by silva and colleagues in isolated aqueous solutions using acrylic cuvettes with an optical path of 1 cm showed absorption spectra at wavelengths of 670 nm, 430 nm, 540 nm and 540 nm (Silva *et al.*, 2017a) Low concentrations ofapproximately 0.05 g / L were observed, which was agreed with other studies carried out by Zijlstra(Zijlstra & Buursma, 1997). There was a high linearity of 0.97 and 0.99 between hemozoin and concentration(Silva et al., 2017a). The hemozoin absorption spectrum is similar to β- hematin spectrum with similar dominant peaks at 375nm, 430nm, 550nm, 655nm and 870nm. (Frosch *etal.*, 2009)

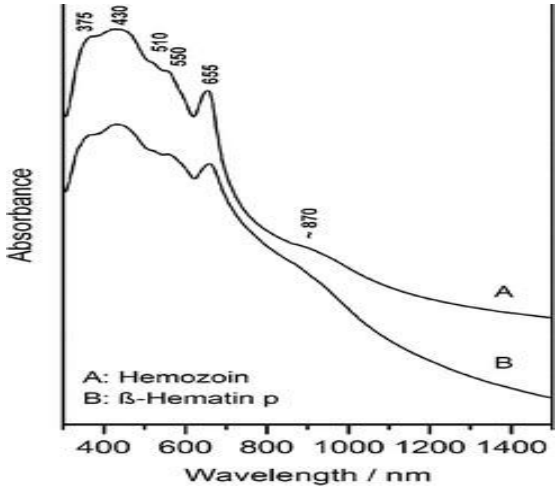

Figure 3.1: The hemozoin and β- hematin absorption spectrum*(Slater et al., 1991).*

3.5 Synthetic Hematin-Crystals

Hemozoin and beta-hematin are identical in terms of structure, spectroscopy and chemistry. Recently, β-hematin and hemozoin extracted have been investigated and show different patterns for different wavelengths of excitation(Frosch et al., 2009) (Jones et al., 2006). A wide range of hemozoin and beta hematin Raman spectra were compared and it was observed that β-hematin is the synthetic hemozoin analog(Frosch et al., 2009).

3.6 LED Light Polarization

Light being an electromagnetic wave, it propagates parallel to each other with magnetic and electrical fields. On the other hand, polarized light is a wave in which the waves happen in one direction. The polarization rate is determined by the transition between the magnetic and

electrical fields. A phase of λ between the fields makes the light linearly polarized(Jones et al., 2006). This makes 100% light alignment with the magnetic field at every moment. This alignment makes it easier to detect signals as the light is in line with the crystals. Hemozoincrystals are known to be micro-rods(Jones et al., 2006) with a magnetic moment acting on them and pointing along the long axis as discussed in the literature. This may be counter-affected by the hemozoin-natural Brownian motion and the particle thermal agitation that makes themrandomly align in a fluid(Wesolowski et al., 2015). Coronado's research showed that the power of the crystals U obeys equation 3-3 under this condition

(Coronado et al., 2014):

$$U = -\bar{\mu} \cdot \bar{B} \quad \textbf{(3.3)}$$

View μ as the crystal's magnetic moment and B as the magnetic field. Because of this effect, the crystals that rotate are imposed a torque and they acquire minimal energy that makes them stop spinning(Griffiths, 1999). This will essentially align the crystals along the light path and produce a simple polarizer. Hemozoin being dark brown in color will absorb, rather than reflect,any incident light(Moore et al., 2006). The dichroic particles absorb light differently depending on the orientation of the particles(Kara et al., 2021).

CHAPTER 4: MATERIALS AND METHODOLOGY

In this chapter the materials, equipment and methods used are discussed and how they were used towards achieving different objectives of the study. Most of them were available in the labswhile others were made in the course of research.

4.1 Characteristic absorption and transmittance spectrum of hemozoin

Most of the materials and equipment used for determining the absorption and transmittance properties of hemozoin were available in the Department of Physics as described in the subsequent sections.

4.1.1 UV-VIS-NIR spectrophotometer solidspec-3700 DUV, Shimadzu

This instrument (ultraviolet-visible-near infrared UV-VIS-NIR Solidspec-3700 DUV, ShimadzuCorporation) spectrophotometer was used to obtain the hemozoin spectrum and determine the dominant peak where hemozoin absorbs the light and at what range. This information was important especially determining the LED to use as the point source.

The instrument uses the beam of light from the UV-VIS-NIR source (D2 lamp) which splits into two beams i.e. sample and reference beams using the half mirror. The reference beam is set to pass through empty cuvette (air) which provides 0% absorbance and 100% transmittance while the other beam passes through the sample (hemozoin suspensions) . The ratioof the two beam intensities (reference and sample signals) were then evaluated using the two detectors (photodiodes), with the aid of a computer connected to the system . The computer was installed with UV probe 2.221 software for better data collection. The UV probe thus helped in optimizing wavelength range between 200-900nm.

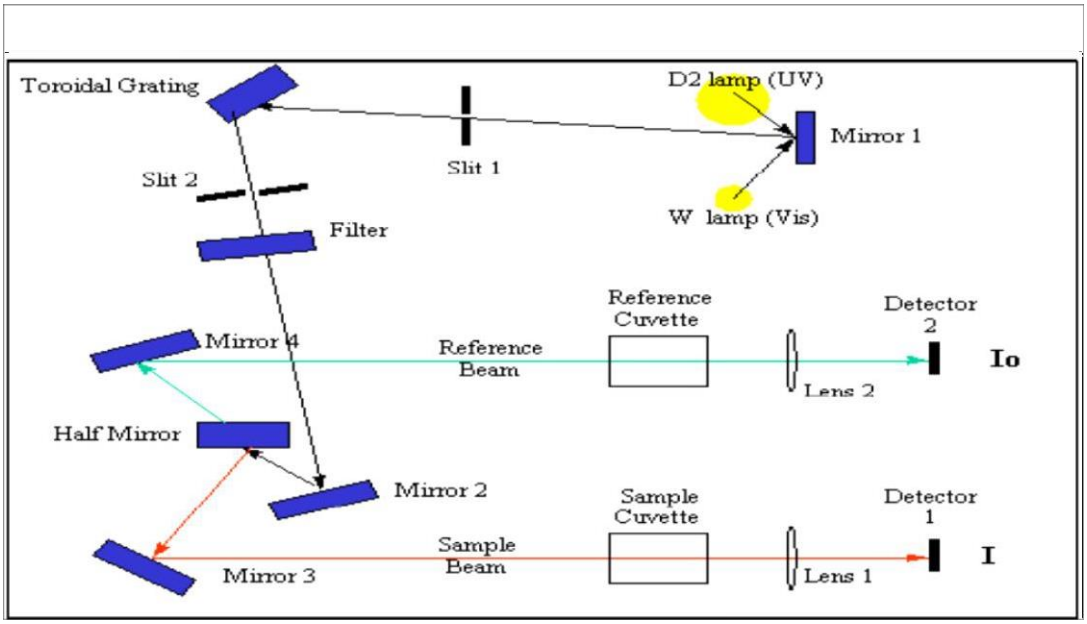

Figure 4.1: Internal structure of UV-VIS-NIR Spectrophotometer solidspec-3700 DUV, shimadzu.

The spectrophotometer was started by logging in the computer to activate the UV-Probe software which comes up with command that help to connect with the spectrophotometer. The hemozoin suspension were inserted into the sample compartment and a blank cuvette into the reference side. The program prompted to save several data in the disk for further analysis

4.1.2 Storage vials and, cuvettes

The storage vials were acquired from local supplier (Mani gate Research Suppliers, Nairobi, Kenya). The transparent plastic vials used were visible and easy to clean. The transparency was also necessary in order to check the levels while making concentration and clear viewership. The vials had airtight lids. These containers were safely stored in a vials rug that could hold a dozen of them. Each of the vials with given concentration of hemozoin in suspension were labelled accordingly before storage. For absorption and transmission measurements quartz cuvettes with path lengths of 10mm were used.

4.1.3 Preparation of homogeneous hemozoin suspensions

For a uniform suspension of hemozoin, Branson sonicator was used. The samples in

the vials were first put in a bath sonicator (Power Sonic 405) for 60 seconds to shake the crystals that might have stuck at the base of the vials. The hemozoin samples in the vials were then inserted inthe beaker full of ice (to maintain the low temperatures) then sonicated using Brason sonicator for 5minutes to obtain a more homogeneous dispersion of the crystals using ultrasound

4.2 Preparation of samples and instruments

. Most of the materials used required initial preparations and fabrication before use. The preparations included calculation and making of concentrations, measurement of masses and building of the malaria detection device.

4.2.1 Hemozoin suspensions

Hemozoin suspensions were prepared by mixing 5mg of crystals purchased from Invivo Gen in 10ml distilled water to produce the stoke solution of concentration 500µg/ml. Different concentrations were obtained after further diluting the stoke solution by adding distilled water using the concentration formula:

$$C_1V_1=C_2V_2 \quad \textbf{(4.1)}$$

Where,

C_1 = The stock concentration

V_1 = Starting volume stock from the stockC_2 = the new desired concentration

V_2= Total volume needed

The 200µg/ml was computed as follows

$$V_1 = \frac{200\mu g/mL}{500\mu g/mL} \text{ x } 5mL = 2mL \quad \textbf{(4.2)}$$

Therefore, 2ml of the stoke solution was mixed with 3ml of distilled water to obtain a / solution. The process was repeated for several concentrations needed and stored in different vials.

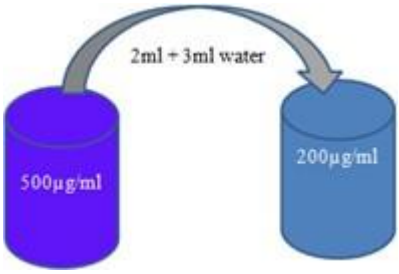

Figure 4.2: Serial Dilution process and computation

The suspensions were stored in tight vials at around -20^{o}C using the fridge. Before performingmeasurements, each of the samples were mixed thoroughly using a bath sonicator (Power Sonic 405) for 60s to ensure all the crystals are agitated then sonicated using Branson sonicator for 5minutes using ultrasounds.

4.2.2 Transmitted light intensity measurement using a spectrometer

In order to measure transmitted light intensities of chosen LEDs through various hemozoin suspensions,the USB 2000 spectrometer was used (ocean optics). The set up was as shown in figure 4.3 below. The transmitted intensity was expressed as a percentage (%T_λ) as:

$$\%T_\lambda = \frac{S_\lambda - D_\lambda}{R_\lambda - S_\lambda} X\ 100\% \qquad \textbf{(4.3)}$$

In this case:

S_λ = intensity of the sample at wavelength λ,

D_λ = intensity in dark at wavelength λ,

R_λ = reference intensity at wavelength λ.

The spectrometer is connected to a PC using a USB cable. The PC has an installed Ocean OpticsOOIBase32 application software.

Figure 4.3: A typical transmittance set up:

The light source (far right) sends light via an input fiber into a cuvette in a cuvette holder (bottom center). The light interacts with the sample. The output fiber carries light from the sample to the spectrometer (top center) which is connected to the PC (far left). Figure 0-3b –practical setup

The code was used to store the reference and dark measurements prior to taking measurements. The entire signal was on scale and by modifying the integration time, the reference signal strength was preserved at the maximum at about 3500 counts.
The reference spectrum was stored with the solvent cuvette (de-ionized water) then the dark spectrum recorded by using a dark opaque card to block the light path to the spectrometer. Through an optical fiber, the light from the light source (LED) was transmitted to the samplekept in the cuvette and placed in a cuvette holder. The light then interacted with the specimenand transmitted the output of the interaction to the spectrometer to another optical fiber that was collected and transmitted. The spectrometer measured the amount of light and converted the collected data into digital information. The spectrometer then passed the sample information to OOIBase32 software which compared the sample to the reference measurement and displayed the processed spectral information.

4.2.3 Cuvettes and cuvette holder.

Various concentration swere was used to test the absorbance and transmittance. The specimen was retained for measurements using transparent quartz cuvettes of path length 10 mm purchasedfrom Roithner LaserTechnik. Such cuvetes were appropriate as measurements of hemozoin showed near absorption in the far ultraviolet (below~250

nm). Therefore, this was a better option than plastic bowls. The quartz cuvettes also allowed the detector to reach a lot of light without much absorption. Because hemozoin absorbance (A) depends on the path length (l) according to Beer Lambert equation 3.2, the path length (l) of the quartz cuvettes used in this study was10 mm, which was normal to enable light to pass at different concentrations and enter the detector.

In order to make the cuvette be aligned well in the setup to determine transmittance and absorbance, we designed a cuvette holder. Measurements of the required size were made and designed using sketch up software in the electronics lab. The cuvette holder was screen printed using a 3D printer

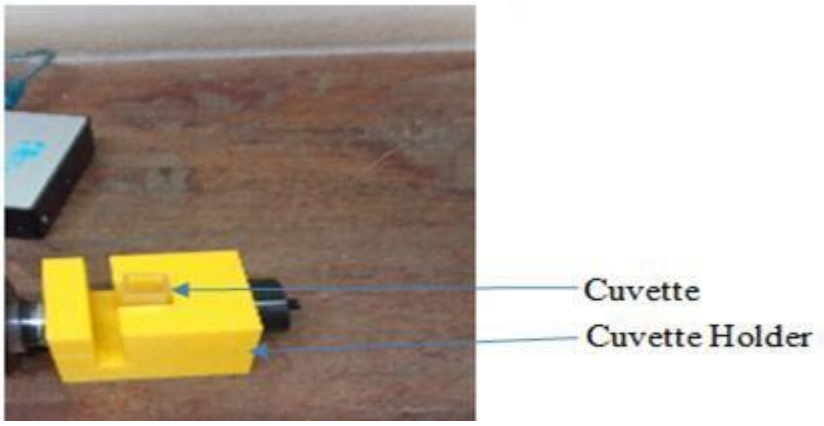

Figure 4.4: A cuvette holder with a cuvette.

4.2.4 LED power circuit

LEDs emitting at the centre wavelength of some prominent hemozoin absorption bands of about 395 nm, 450 nm, 660 nm were used for initial work to determine the required LED. They were powered by special circuit designed using a breadboard, resistors, jump pins and power supply. Unlike most electronics need a constant voltage source, LEDs require a constant current source.

For the LED to properly work, the power supply with high enough voltage was needed to turn it on and with a controlled constant amount of current. Therefore, the forward voltage V_f (voltage required to illuminate the LED) needed to be controlled using resistors to provide the required forward current I_f (Forward current is typically specified as a maximum current required to illuminate the LED). This was achieved

using equation 4.3 below because forward current is a function of forward voltage as shown in equation 4.2-5 below.

$$I_f = \frac{V_f}{R} \tag{4.4}$$

This current was maintained to avoid damaging of the LED. Nevertheless, the output varied withthe forward current i.e decreasing the forward current decreased the LED light output.

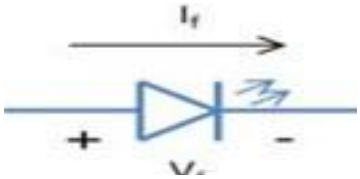

Figure 4.5: Circuit symbol of an LED with forward voltage and current.

4.2.5 LED current limiting resistors

It is very important to limit the current in an LED. An LED operates very differently from a circuit resistor. Unlike a resistor which acts linearly in accordance with Ohm's law; V= IR that is alinear relationship, an LED does not function in this manner but has a special characteristic I-V as shown in figure 4.7.

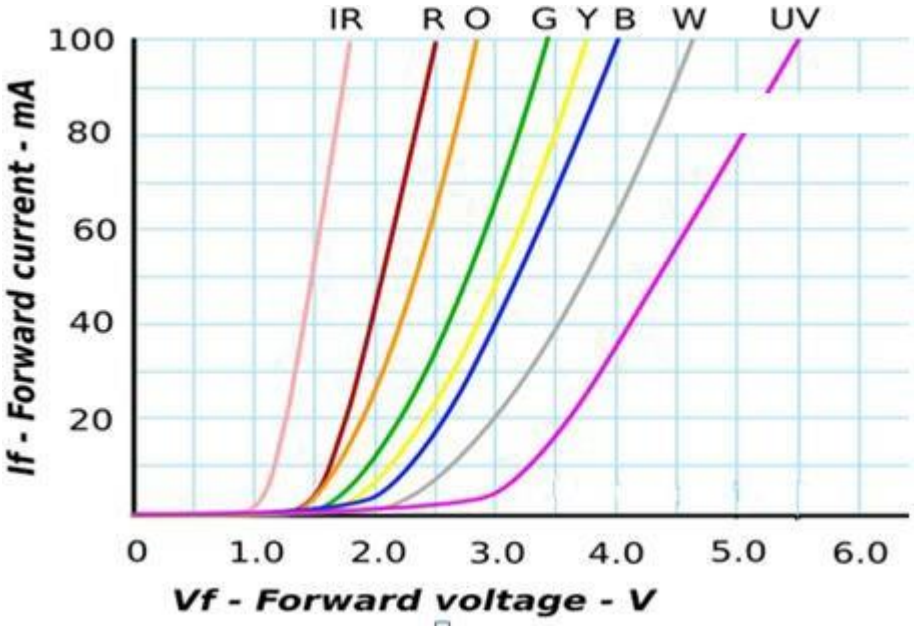

Figure 4.6: LED special characteristic I-V

In order to turn on the LED, the characteristic voltage must be reached. However when thisvoltage is exceeded, the LED's resistance quickly drops off. Hence, the LED will begin to draw a much current and it may blow off. Therefore, a resistor is used in series with the LED to keep the forward current at a specific level.

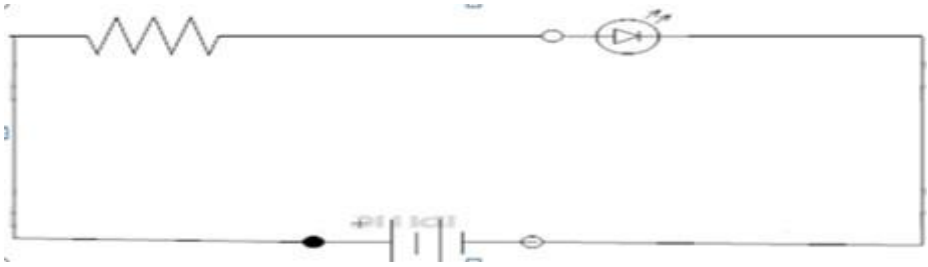

Figure 4.7: Simple LED Circuit with Current Limit Resistor

Using the circuit above, three values were noted in order to determine the current limiting resistor value.

I = LED forward current in Amps (found in the LED datasheet) V_f = LED forward voltage drop in Volts (found in the LED datasheet)

V_s = Supply voltage. The above values were obtained and the limiting resistance determined by the formula inequation 4.5

$$R = \frac{V_s - V_f}{I} \qquad (4.5)$$

The appropriate resistance was determined and the circuit board designed on the breadboard as shown in the figure 4.8a below.

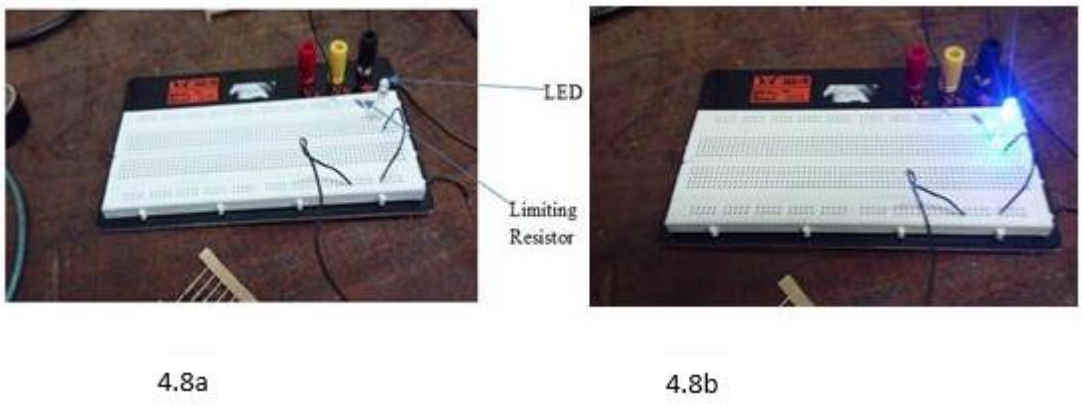

Figure 4.8: Simple LED Circuit with Current Limit Resistor. 4.8b- Illuminated LED using a limiting resistor

The source of power was from the mains which was converted to lower voltage using the power supply unit. The unit had different power terminals with varying voltages that were used accordingly. The setup in figure 4.8 was used in conjunction with the laptop with the spectroscopy software from ocean optics software

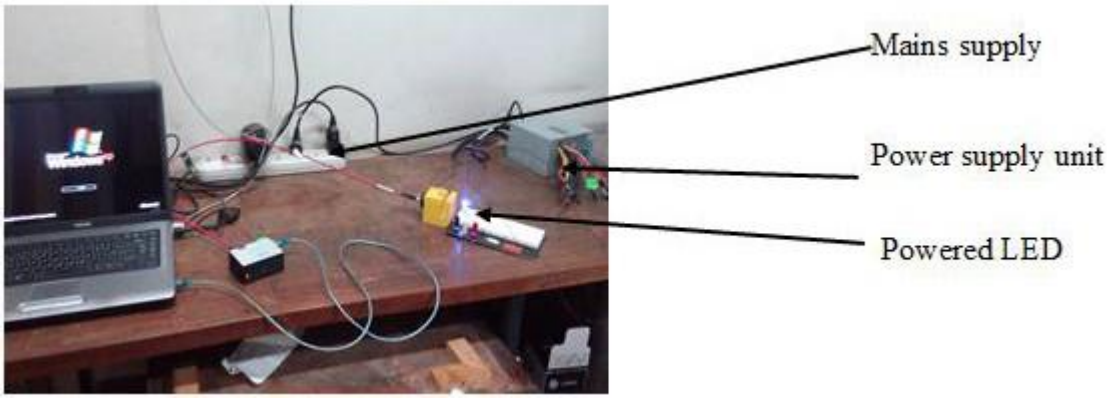

Figure 4.9: Set of the powered LED using the designed circuit and power supply unit

4.2.6 Intensity of transmitted LED light through human blood smear samples.

Light from LED was used to determine transmittance through human blood smears on glass slides. The slides were prepared using malaria cultured cells from malaria prone areas of Nyanza region of Kenya courtesy of Mr. Omucheni Dickson from the Physics department of the University of Nairobi. The slides made were thin smears which were cultured and harvested at different hours of culturing period. The slides stored in LASER laboratory of the Department of Physics of University of Nairobi were used in conjunction with the slide holder and aligned with the source of light and detection done by an Ocean Optics spectrophotometer. Measurements weretaken from slides harvested at different hours and the data compared.

4.2.7 Influence of magnetic field on the intensity of transmitted LED light through hemozoin suspensions.

Disc magnets were purchased from local vendors which provided the magnetic field. The radius and strength of the magnets were considered according to the literature of this paper. They were fitted in the side-slots of the malaria screening device. The strength of the disc magnets was about 0.5T therefore caution was taken while fitting them to avoid discs from clinging together. The light was then allowed to pass through the sample place between the magnets as shown in figure 4.11.

4.3 The portable opto-magnetic malaria detector.

4.3.1 Construction of malaria detector

The device was constructed using several materials. The prototype was designed using sketch up software on a PC. All measurements were accurately measured then screen printed in the electronics laboratory of University of Nairobi.

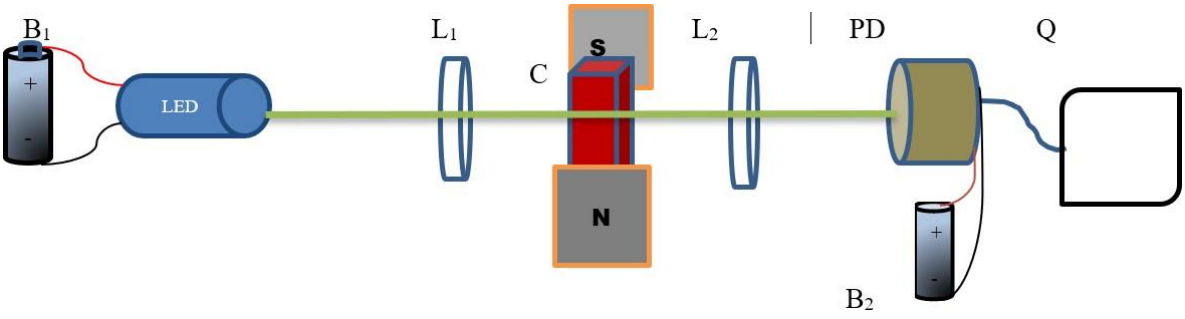

Figure 4.10: Schematic diagram of low-cost malaria screening device

T h e s c h e m e comprises of an LED light source (LED), collimating lens (L1), , a magnet (N-S), sample in a cuvette (C), a focusing lens (L2) and a photodiode (PD) connected to a display screen (Q)

The device was screen printed using plastic material and was made dark to avoid multiplereflection of light. The cells and magnets were purchased from local supplies but with consideration of the LED specification and expected light attenuation as discussed in the literature. A multimeter was used a display screen to obtained data from the photodiode. The photodiode, LED and plano-convex lenses were obtained from Thorlabs.

4.3.2 Malaria screening device setup and operation

The device is used to detect presence of malaria in blood sample using the optical and magnetic properties of hemozoin discussed elsewhere in this paper. The simple sketch diagram in figure 4.10 was used. An LED light of wavelength 450nm shines through a plano-convex lens L1 which in this case acts as a collimator to focus the divergent LED beam to the cuvette C holding blood sample or the liquid used in this experiment (deionized water) located at the center of the device. This beam is transmitted to sample of a specific concentration of which part of the light is absorbed proportion to the concentration. the transmitted light passes through the lens L2 which

focuses the beam towards a photodetector diode PD which transduces the photo signal to electrical signal to the display screen Q. this signal is noted and compared with the same signal in presence of the magnetic field induced by presence of permanent neodymium with surface field of about 0.3 T magnet and diameter of about 2.5cm shown in figure 4. The two cylindrical magnets are separated the strong plastic material which make up internal side pockets on the device with a smaller circular opening at the center and orthogonal to the sample holder to allow free field and light interaction in the sample. These pockets prevent the two magnets from pulling each. The cells which provide power system of the LED and photodiodes are housed at other pockets made at far internal corners of the device.

Figure 4.11: The low cost malaria screening device

4.3.3 Precautions

Just like any machines and devices, precautions are provided while using them in order to achieve the goals. For the UV spectrophotometer to give the accurate and required values, the baseline was zeroed by clicking on the ‒Baseline‖ button near the bottom of the screen. It was ensured that there were no samples in either the sample or the reference side while correcting the baseline. It was ensured that the covered was covered every time the sample were run

The sample holders were rinsed clean to avoid contamination with other sample.
Precaution was taken while cleaning glassware to avoid cuts and injuries. This was facilitated by use of protective gloves.

Proper alignment was required while taking measurement using the device so that the incident light is focused when with the photodiode to collect maximum transmitted light and minimize errors.

CHAPTER 5: RESULTS AND DISCUSSION

In these results we discuss β- haematin which is chemically and spectroscopically identical form of blood hemozoin. We demonstrate how concentration of β-haematin in deionized water and laterin blood is affected by presence of specific light wavelength with and without external magnetic fields

5.1 Hemozoin's characteristic absorption and transmittance spectrum

Figure 5.1 displays the absorption spectra of hemozoin suspensions at a concentration of 25µg/ml in deionized water. Within the 300 - 900 nm spectral range, peaks associated with the S_o- S_2 (377, 421 and 449 nm) and S_o-S_1 (551, 665, 723 and 866 nm) transitions usually referred as the Soret/B-band and the Q-bands respectively were prominent(Ali & Oppeneer, 2015) (Dragomir et al., 2007). This spectral profile was similar to the one theoretically calculated by Ali and Oppeneer(Ali & Oppeneer, 2015) and experimentally elsewhere(Rifaie-Graham et al., 2019) (Silva et al., 2017b). The intensities of these bands increased linearly with hemozoin concentration as expected according to the known Lambert-Beer's law (see Figures 5.2 and 5.3). This was supported by the area under curve values of these absorption spectra displayed in Figure 5.3.

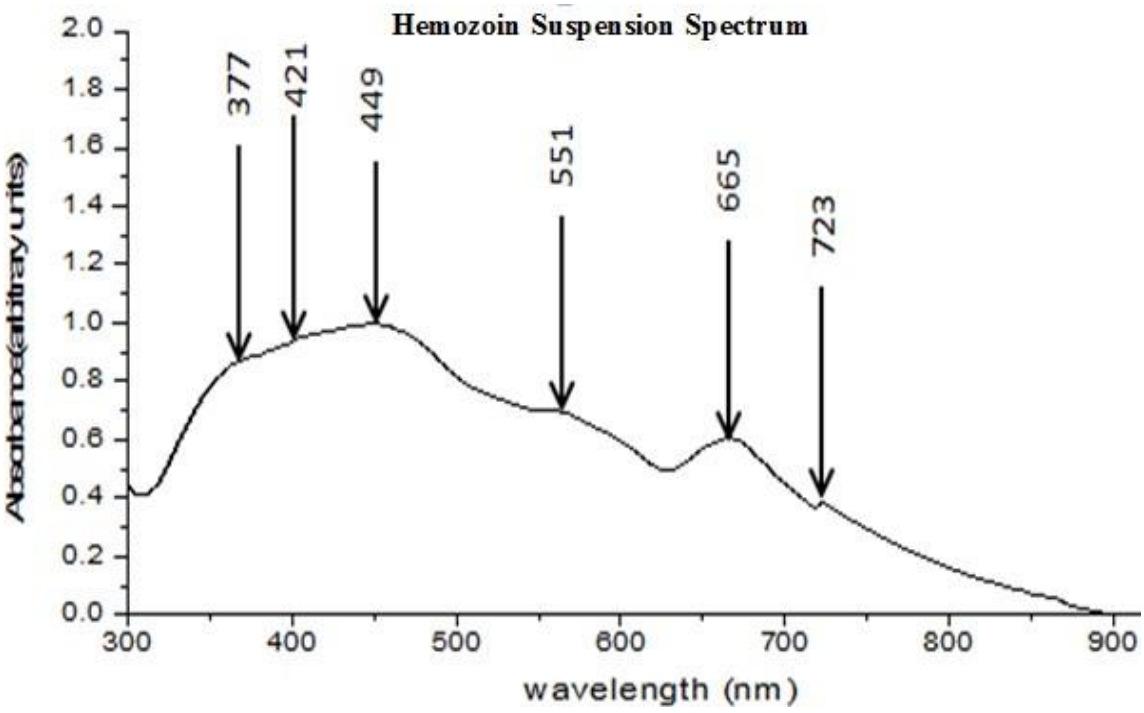

Figure 5.1: Characteristic absorption spectrum of hemozoin.

In other studies, it was observed that there exists a correlation between hemozoin and hemoglobin absorption spectrum upto around 550 nm but the band at around 665 nm was missing in the latter(Silva et al., 2017b). This band could be used in hemozoin detection in whole blood. The optical absorption intensity increased with concentrations and the area under curve (AUC) values gave a linear relationship with coefficient of determination R^2 of 0.9919 as displayed in Figure 5.3. The high R^2 values indicated a good correlation between intensity (or AUC) with hemozoin concentration. The observed prominent hemozoin absorption bands at 377 nm, 421 nm, 449 nm, 554 nm, 665 nm and 723 nm can, therefore, be used as hemozoin biomarker bands and can be utilized in the detection of malaria if an excitation beam with peak wavelength centered at eitherof the bands is passed through a sample containing hemozoin. The transmitted intensities of these beams are expected to decrease with increase in hemozoin concentrations. In this work, LEDs with emission centered at 430nm and 660nm were used.

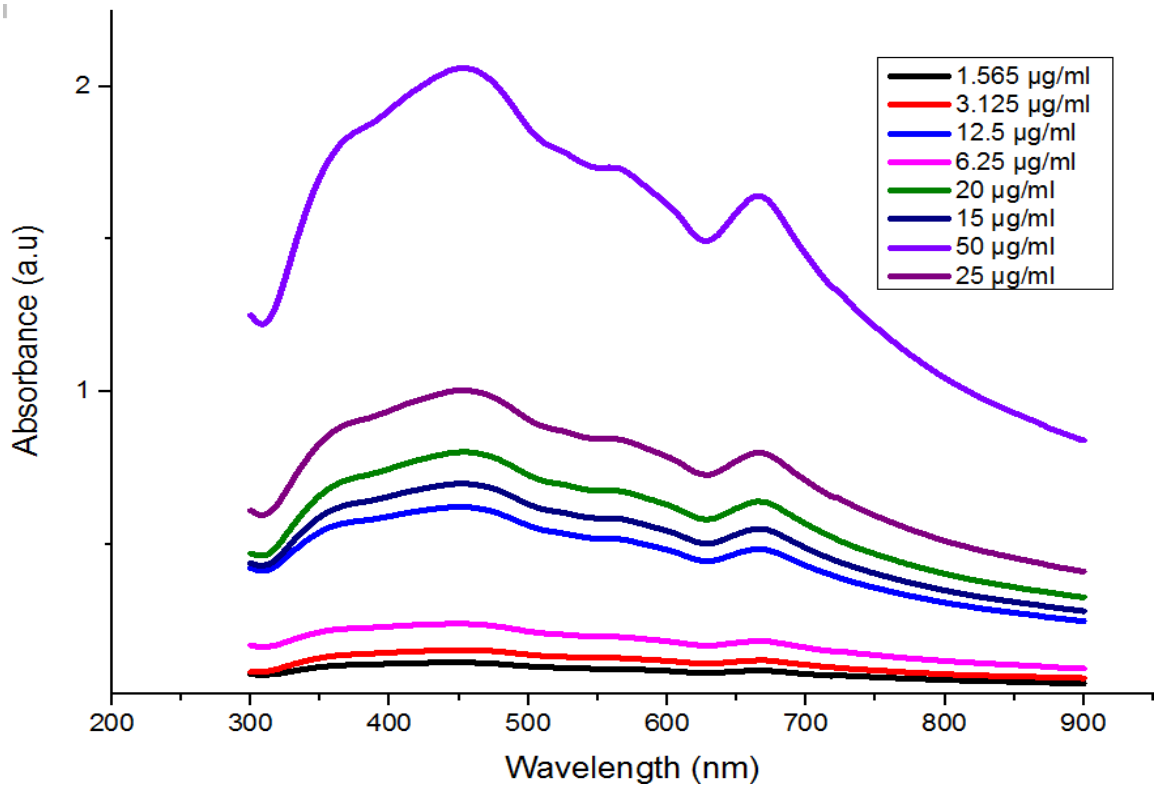

Figure 5.2: The absorption spectrum of hemozoin

The measurement was done in hemozoin suspensions at different concentrations in a cuvette taken in the spectral range 300 - 940 nm using DUV 3700 spectrophotometer

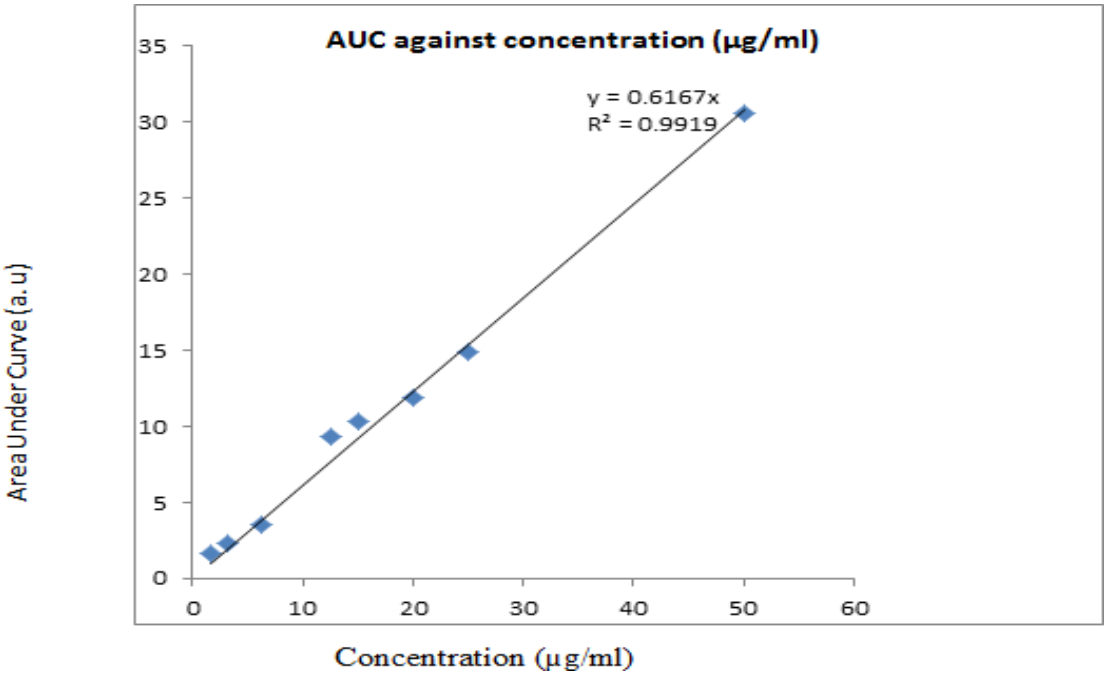

Figure 5.3: Graph of AUC Vs Hz Concentration

The transmittance spectra displayed in Figure 5.4 exhibited small minima at the absorption bands of hemozoin as expected. The higher the concertation the lower the transmittance. The inverse relationship is displayed by Figure 5.5 where selected absorption and transmittance spectra are plotted for hemozoin concentrations of 1.5 µg/mL and 25µg/mL.

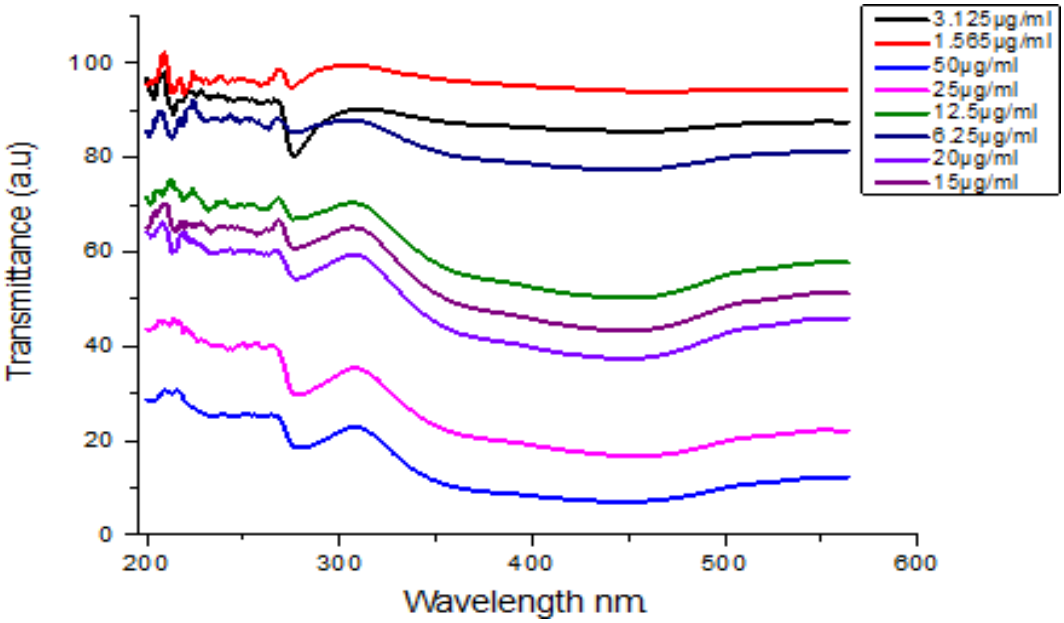

Figure 5.4: Transmittance spectrum of hemozoin suspensions using DUV 3700 spectrophotometer

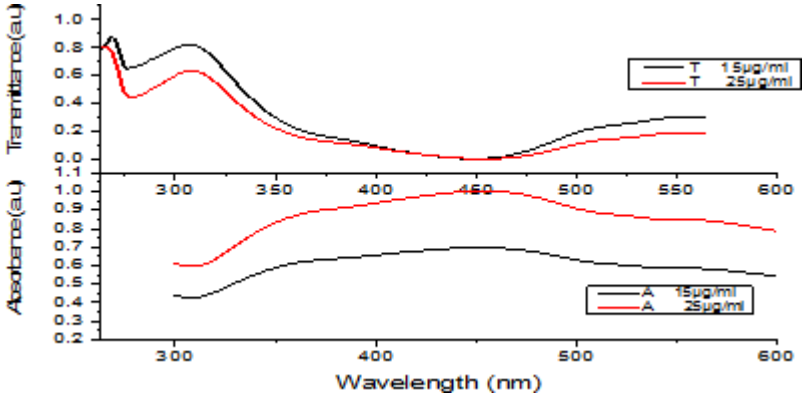

Figure 5.5: Comparison between transmittance and Absorbance of Hz

There was higher absorbance at the band centered at a wavelength of 450nm with a consequent low transmittance .

5.2 Intensity of transmitted LED light through various concentrations of hemozoin in de-ionized water.

In this work, LEDs emitting around 430 nm and 660 nm were used in studying hemozointransmissions around these wavelengths. The hemozoin was suspended in deionized water and their concentrations were varied. Since hemozoin is paramagnetic, the study was conducted in presence and absence of a magnetic field where a pair of disc magnets were positioned near the sample in a cuvette and the transmitted light was first dispersed in a USB Ocean Optics spectrometer and recorded. Later, the signals were detected by a photodiode and the resulting electrical signals (in volts) recorded using a multi-meter and values compared. Figure 5.6 displaysthe intensity of the LED light centered at 430 nm for different hemozoin concentrations.

5.3 Transmittance graph of hemozoin suspensions using a 430nm LED

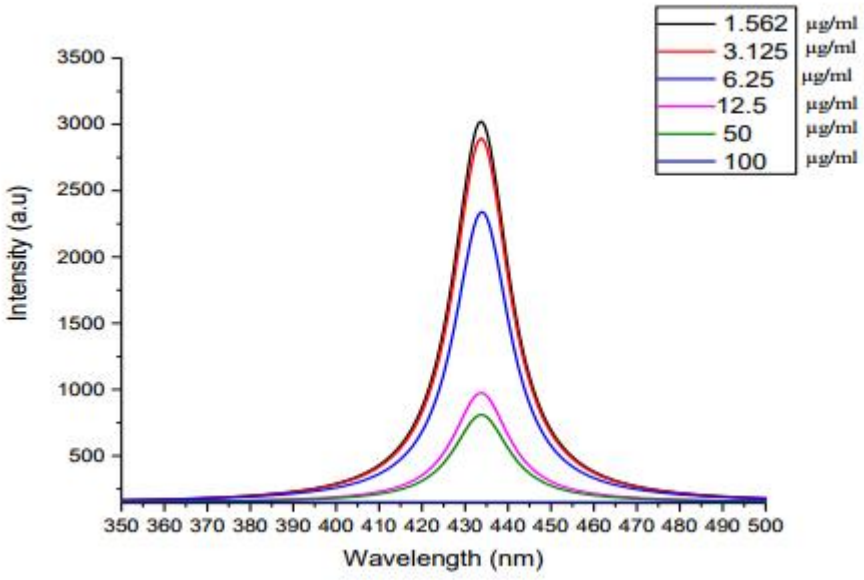

Figure 5.6: Transmittance spectrum of hemozoin using a 430 nm LED

From the spectrum 5.6, the transmitted intensity decreased with hemozoin concentration as expected according to Beer's law. A plot of AUC values against concentration is shown in Figure 5.7. The AUC values decreased exponentially according to the fit equation 5.1

$$y = a - b * Ln (x + c) \quad \textbf{(5.1)}$$

The fit equation 5.1 is similar to equation 5.2

$$AUC = a - b * Ln (Conc. + c) \quad \textbf{(5.2)}$$

Where a, b and c are constants. The obtained R^2 value was 0.99454.

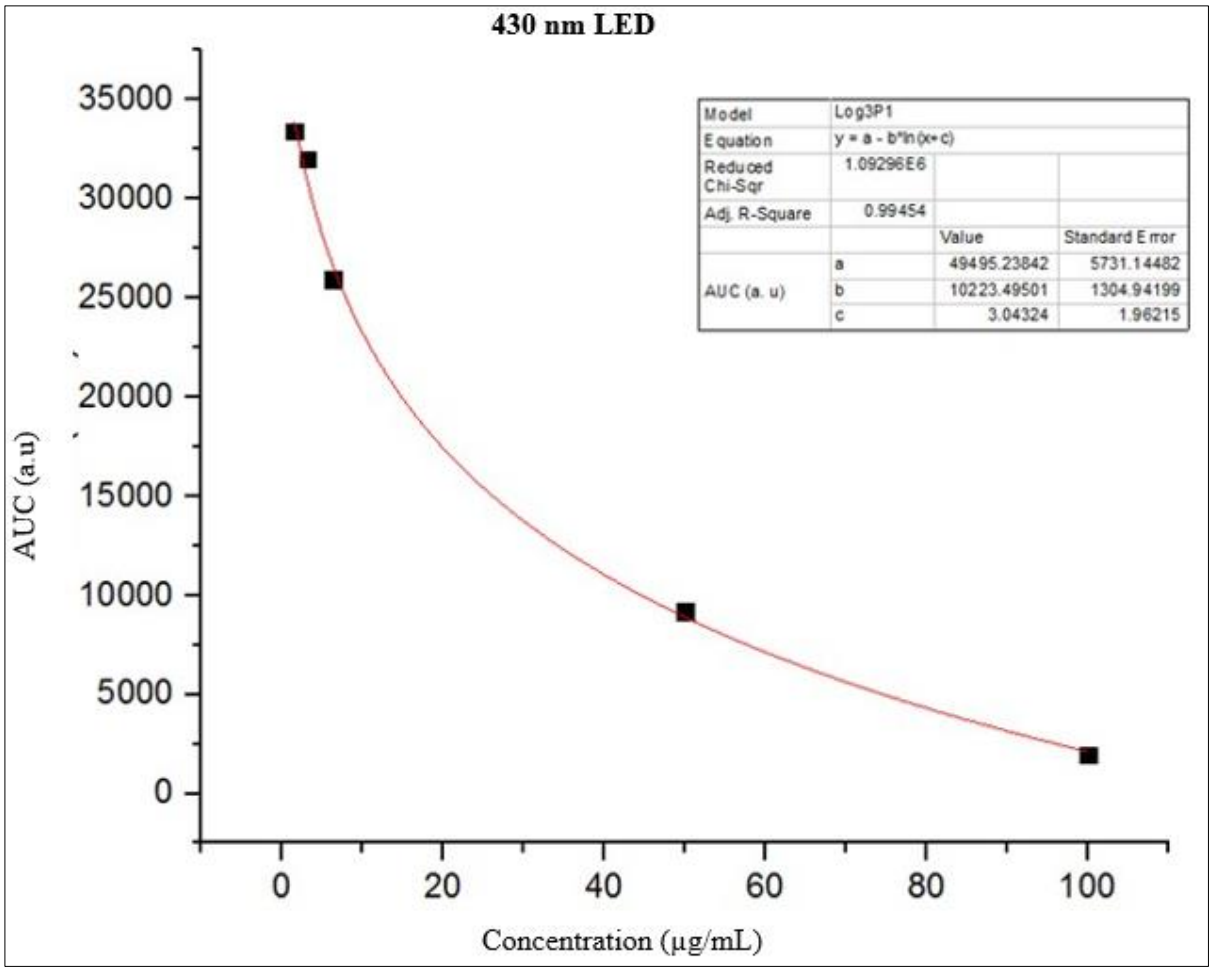

Figure 5.7: A plot of the AUC values Vs concentration of the transmittance spectrum displayed for the LED light beam centered at wavelength 430nm.

A similar transmittance spectrum was obtained for LED beam centered at wavelength 660 nm andthe AUC versus concentration plot was as displayed in Figure 5.8. The R^2 value obtained after fitting the data points with the relation identical to the one used above was 0.9593. The high R^2values for both the 430 nm and 660 nm LED beams indicated the great capability of the bands being used as hemozoin (and by extension malaria) detection bands with the fit equation acting as a diagnostic algorithm with 430 LED showing a comparatively higher R^2.

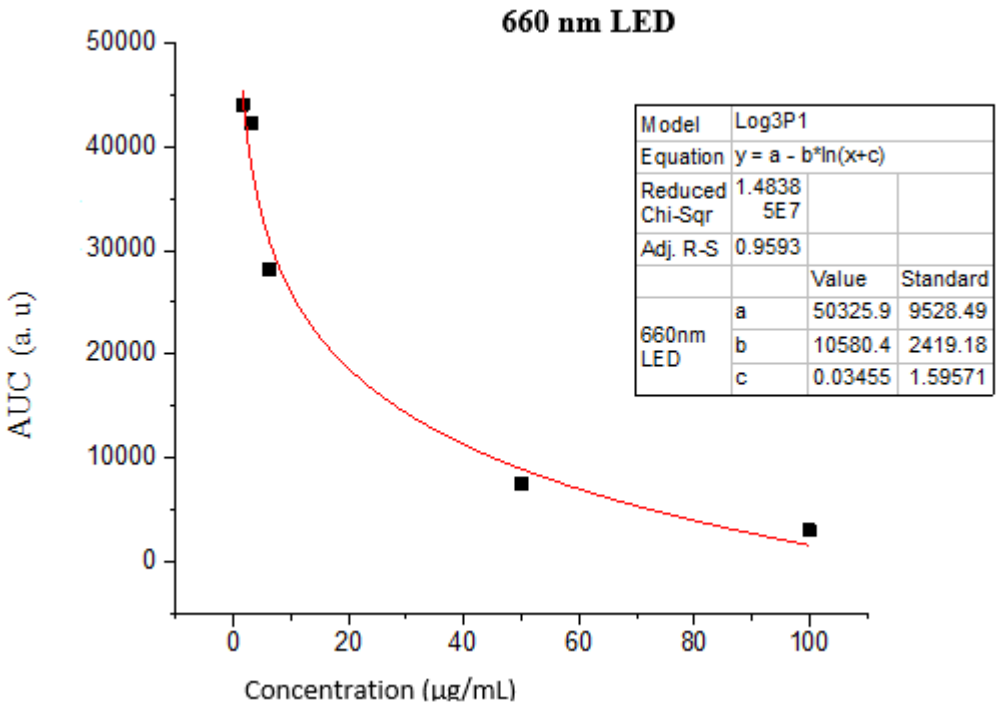

Figure 5.8: A plot of the AUC values Vs concentration of the transmittance spectrum for 660nm LED

The fit equation 5.1 which corresponds to equation 5.2 transforms to transmittance equation 5.3 in the form:

$$T = a - b * Ln\,(Conc. + c) \qquad \textbf{(5.3)}$$

Equation 5-3 is identical to the absorbance (A) equation expressed as

$$A = -\log\left(\frac{I}{I_0}\right) = -\log\left(\frac{\%T}{100}\right) = 2 - \log(\%T) \quad (since\ T\% = I/I_o x100). \qquad \textbf{(5.4)}$$

Where the ratio $\frac{I}{I_O}$ is the transmitted intensity (T) and %T is the percentage transmission or transmittance. It should be noted that according to Beer-Lambert's law

$$A = \varepsilon lc \qquad \textbf{(5.5)}$$

The symbol ε, l and c representing absorption coefficient, length or thickness and concentration of sample under consideration, respectively.

5.4 Influence of magnetic field on the intensity of transmitted LED light through hemozoin suspensions.

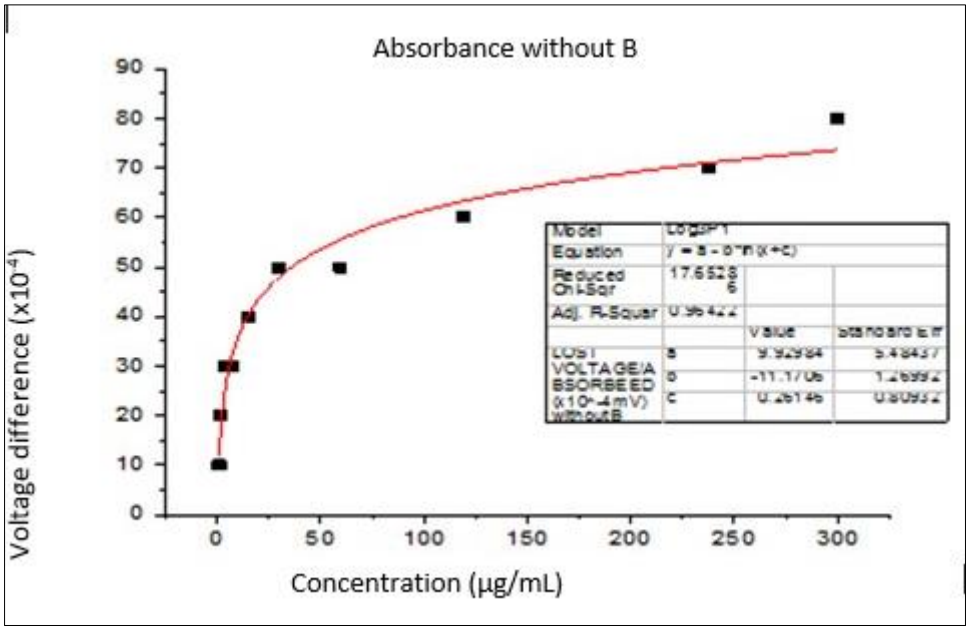

Figure 5.9: A plot of voltage difference Vs Hemozoin concentration in the absence of B-field.

The data was obtained using our opto-magnetic screening device.

Since hemozoin is paramagnetic, the presence of magnetic field is expected to influence these nanocrystals such that their orientation in a suspension would be influenced away from random. This is expected to in tern affects its optical absorption. For purposes of calibrating our designed portable malaria screening device, measurements of transmitted intensity through hemozoin suspensions in deionized water were done in presence and absence of magnetic field (B-field).The B-field used was from two neodymium disc magnets sandwiching the sample Quartz cuvette. The transmitted intensity was detected using a photodiode (PHD) and the resulting electrical signals in volts displayed using a multimeter. The value i.e. voltage through cuvette with deionized water and through cuvette with hemozoin suspensions were recorded and difference evaluated. The resulting difference voltage value (or lost voltage) is equivalent to the LED light energy absorbed by hemozoin and so is related to absorbance (A). Figures 5.9 and 5.10 displays plots of lost voltage versus hemozoin concentration in absence and presence of B-field, respectively. The voltage difference was obtained by subtracting voltage measure through an empty cuvette (without sample) and voltage through the cuvette with different hemozoin concentrations.

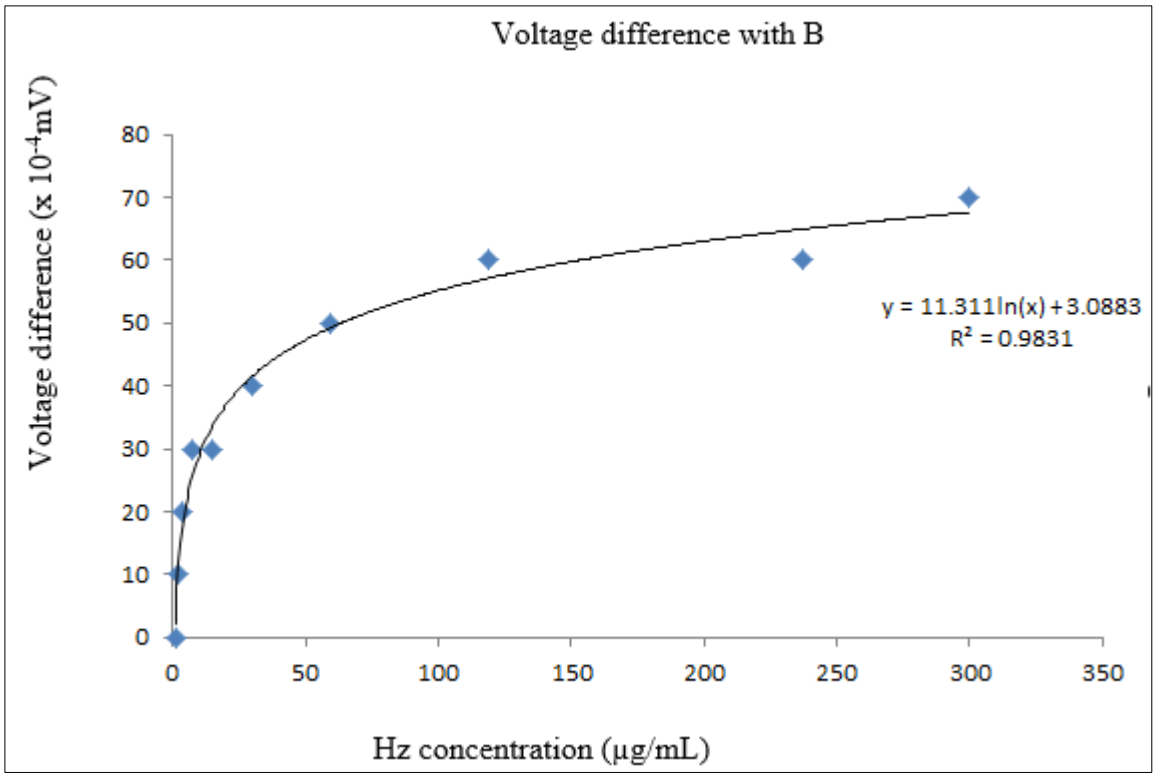

Figure 5.10: A plot of voltage difference versus Hemozoin concentration in the presence of B- field.

The data was obtained using our opto-magnetic screening device.

The plots exhibit an exponential relation similar to the Beer-Lambert's relation:

$$T = e^{\varepsilon lc} \qquad \textbf{(5.6)}$$

The fit equation that reproduced the trend was given by:

$$y = kLn(x) + c \qquad \textbf{(5.7)}$$

which can be equivalently expressed as:

$$\overline{e} = \frac{y-c}{k} \qquad \textbf{(5.8)}$$

In this relation, y and x represents the voltage difference and hemozoin concentration respectively.Equation 5.7 can be used as malaria diagnostic algorithm as it estimates hemozoin concentration(x) in the sample. Constants c and k can be evaluated. The R^2 values obtained for the curve in thepresence of magnetic field was 0.9831 and was higher than that without the B-field which was 0.9718. This indicated that taking the measurements in the presence of B-field was better and makes the device

more

sensitive. This also supports the idea that the B-field influences the orientation of hemozoin nanocrystals such that majority are aligned in a certain way.

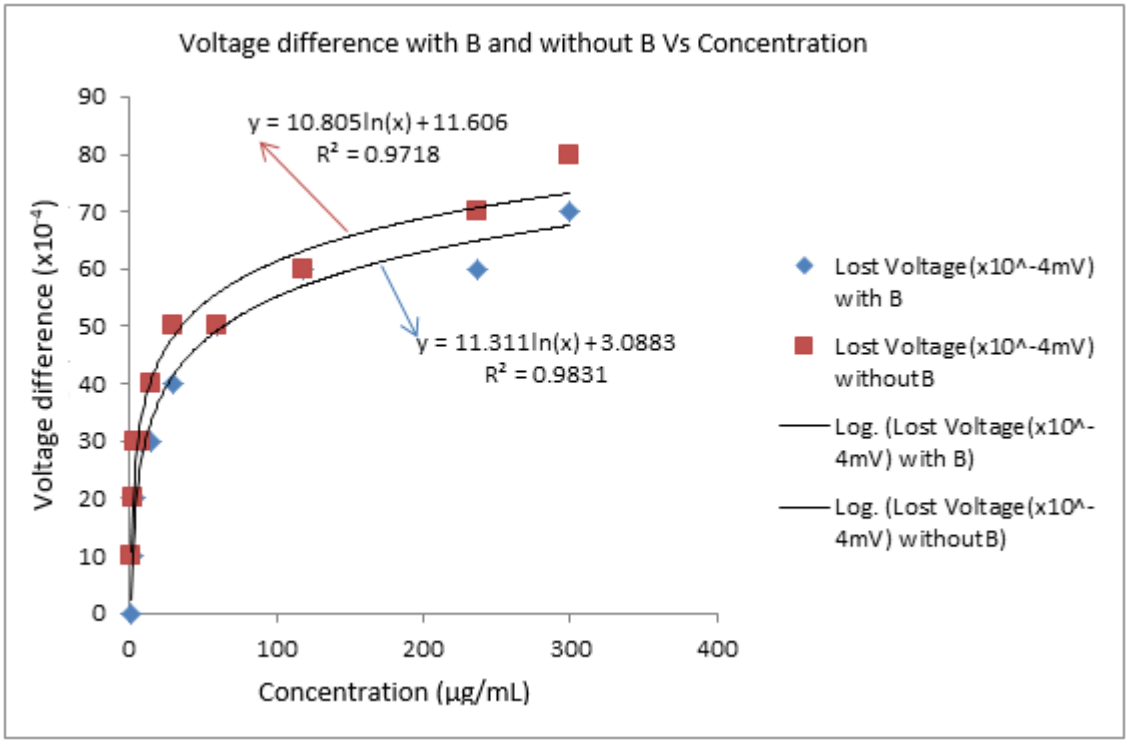

Figure 5.11: Hemozoin voltage difference using opto-magnetic screening device with and without B.

The two curves obtained in the presence and absence of B-field on the same axes. The two have identical trends. The device was also assessed on blood smears. The smears were prepared from human blood in which the malaria parasite *plasmodium falciparum* was cultured. The smears were made atvarious times or durations of culturing between 0 hours and 144 hrs. The intension was to establish a correlation between numbers of culturing hours and transmittance of the 660 nm light beam through the smears. It was thought that the higher the culturing duration, the higher the number of parasites and consequently an increased concentration of hemozoin in blood. Figure 5.12 displays the obtained transmission spectrum for LED beam centered at wavelength 660 nm.

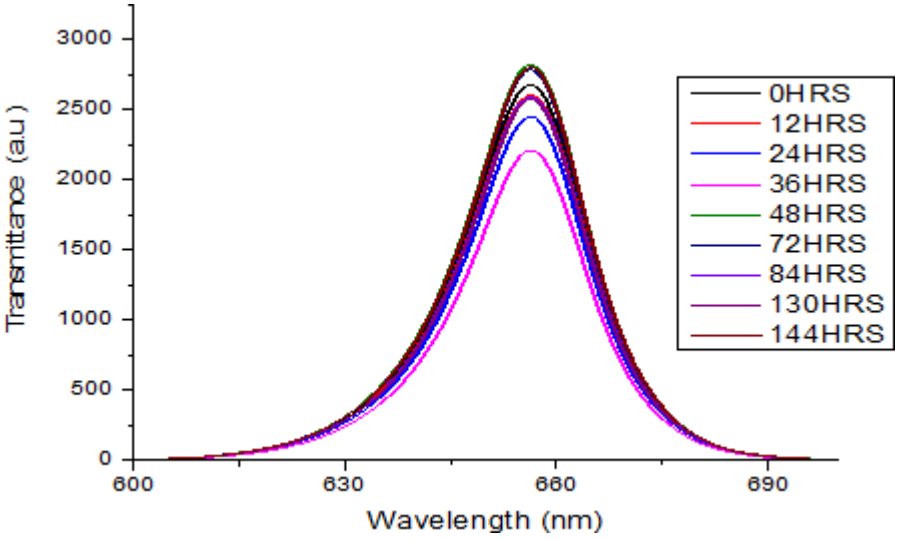

Figure 5.12: Intensity of transmitted LED light through human blood smear samples.

The AUC values of this spectrum plotted against the culturing hours exhibits a complicated profile as shown in Figure 5.13. The reason for the later observation could be attributed to the design of the culturing process where after 24 hours, culturing medium (blood) was added which meant that hemozoin concentration remained fairly the same or decreased every time fresh blood was added. The other reason could be that the samples had over-stayed or expired causing some hemozoin to fall off the glass slides hence influencing the measurement.

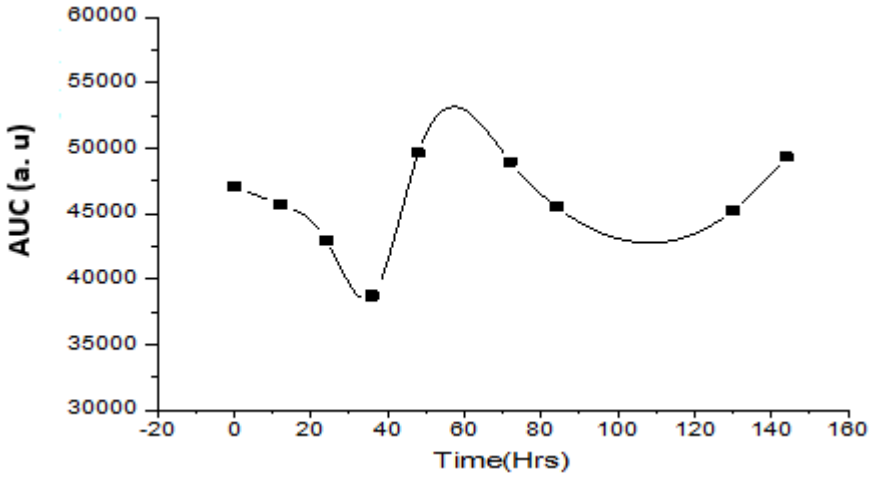

Figure 5.13: Transmittance spectrum of human blood smears

When known concentrations of hemozoin was prepared in Sprague Dawley rat's blood supplied from the department of medical physiology, University of Nairobi and their transmission measured using our opto-magnetic device, results as displayed in Figure

5.14 were obtained. The blood samples containing hemozoin were placed in a 1400 µL fluorescence cuvettes (Thorlabs). The LED beam used was the one with wavelength centered at 450 nm. The results were similar to those obtained from hemozoin suspensions in deionized water displayed in Figures 5.9 and 5.10 and so indicating the power of the device in hemozoin and hence malaria detection in blood. The R^2 value obtained was however lower 0.7795 due to possibly errors in the estimation of concentration of hemozoin in the prepared samples.

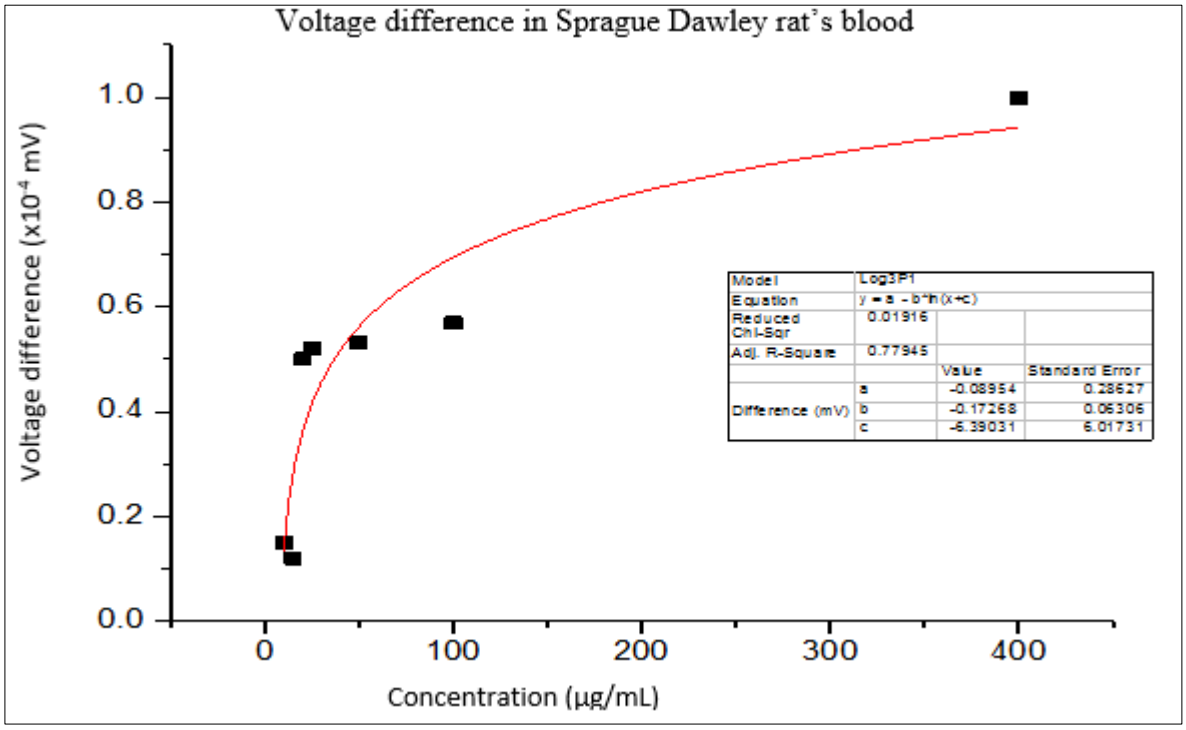

Figure 5.14: Voltage difference in Sprague Dawley rat's blood.

There was a similarity in the data collected using hemozoin suspended in deionized water and rat blood. Nevertheless, there was a higher regression in deionized water than in blood. This may be attributed to other blood components that might have interfered with the caused the difference.

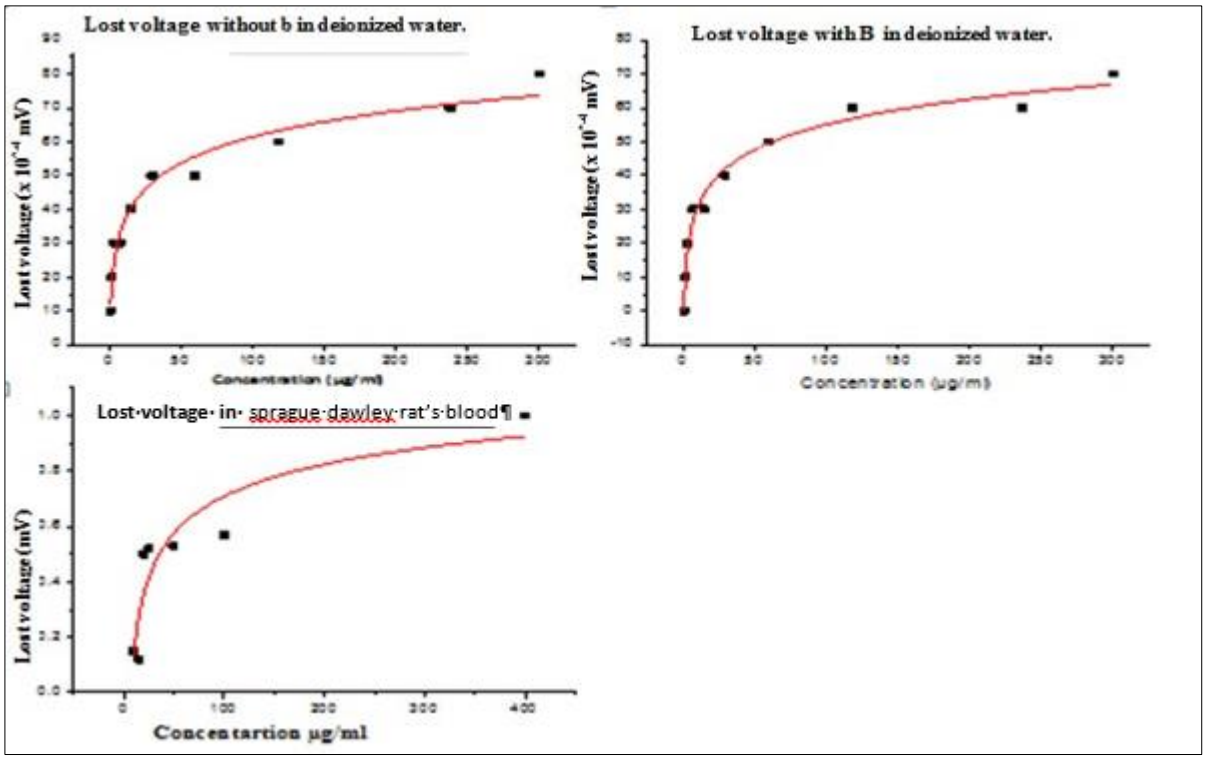

Figure 5.15: Comparison between lost voltage in rat blood and in de-ionized water

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CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Overview

The major conclusion that can deducted from this research is that the hemozoin crystals which areby-product of malaria infection can make a basis and potential tool for diagnosis of the deadly malaria infection. Its magnetic and optical properties offer a chance for more exploitation and better ways of curbing the disease. Another conclusion that can be drawn from the data and results is that that it would indeed be possible to use this thedata to create an ‒infection table‖ which would allow an unknown sample to be run, and that

signal could be used to infer parasitemia. Nevertheless, this might certainly require more testing, pparticularly sets of data would necessitate to be tested in whole human blood extracted from a vast population of persons so as to understand the practicability of this idea. When working on this, it has to be bearded in mind that the objective is to create a less costly and accurate malaria screening device just as discussed in this thesis nevertheless this device is less costly than other methods considering the materials used to fabricate it. Despite this research being able to achieve all the objectives, more has to be done in oder to to determine accurate the infection lavels and parastemia for every plasmodium species. The other setback of this devicewas tge detection screen which needs improvement for accurate results

6.2 Advantages of the device.

Several similar methods and devices have initially been advanced for the same course. Jones and co-workers(Jones et al., 2006) designed a comparable device which used a laser powered by 12v car battery. This device lacked portability and was expensive in operation and transportation. Our device requires batteries for the electrical power system which are extremely portable and cheaplyavailable even in villages with household with low income. The 650nm laser used could be dangerous to the user and patients hence a knowledgeable expert was required to operate it unlike our device which uses an LED light that is less dangerous and doesn’t require high level precautions during operations. Most of the machines and devices advanced earlier required rotating magnets to induce magnetic field. The rotation caused vibration of the sample and this could alter the vibration of the sample hence wrong values may be recorded. Our device uses still magnets which are permanent

and our LED are used without any physical disturbance. The device is handy and portable and easy for shipping without extra accessories like external power sources and display methods.

6.3 Device limitations

The device relies majorly on dry cells which are not 100% reliable in constant transmitting powerto the LED. This cause deviation in terms of the power dissipated at a given moment. The device operates on basis of comparing transmitted light in presence and absent of magnetic field hence the incident LED power should be of the same magnitude for accurate values. The other limitation is determining the relaxation time of the hemozoin crystals when exposed to magnetic field. This determines appropriate time to take the measurement. Newman and coworkers (Newman et al., 2008) approximated the relaxation time of hemozoin as 100μs which is the appropriate to take the measurement. This pauses a challenge of taking near-exact values. The display system also fluctuated depending on the strength of the detected light by the photodiode. A special and high definition (HD) screen that is sensitive would be better to get the readings.

6.4 Future prospects

The future prospects of exploring malaria diagnosis in relation to this research could be the use ofacoustic properties of hemozoin which also opens way for more research.

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