Palestine Polytechnic University

College of Engineering

Design of Non-Invasive Methemoglobin Concentration Measuring System

By

Imran Ebeido Asma Zeidat

Supervisor: Eng. Ali-Amro

Submitted to the College of Engineering

In partial fulfillment of the requirements for the

Bachelor's degree in biomedical engineering

Hebron, Dec 2018

Palestine polytechnic University

Hebron – Palestine

College of Engineering Electrical

Engineering Department

Design of Non-Invasive Methemoglobin Concentration Measuring System

Project Team:

Imran Ebeido Asma Zeidat

By the guidance of our supervisor, and by the acceptance of all members in the testing committee, this project is delivered to department of electrical engineering in the college of engineering and technology, to be as a partial fulfillment of the requirements of the department for the degree of B.sc.

Supervisor signature

Testing committee signature

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The head of department signature

جامعة بوليتكنك فلسطين

الخليل – فلسطين

كلية الهندسة

دائرة الهندسة الكهربائية

Design of Non-Invasive Methemoglobin Concentration Measuring System

فريق المشروع

اسماء زيدات **عمران عبيدو**

بناء على نظام كلية الهندسة وإشراف ومتابعة المشرفة المباشر على المشروع وموافقة أعضاء اللجنة المناقشة، تم تقديم هذا العمل إلى دائرة الهندسة الكهربائية. وذلك للوفاء بمتطلبات درجة البكالوريوس في هندسة الأجهزة الطبية.

توقيع المشرف

توقيع اللجنة المناقشة

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توقيع رئيس الدائرة

Abstract

The primary function of red blood cells, and erythrocytes, is to carry oxygen from the lungs to the body tissues, and carbon dioxide away from the tissues and back to the lungs. Hemoglobin (Hgb) is an important protein in the red blood cells.

Methemoglobinemia (MetHb) is a blood disorder in which an abnormal amount of methemoglobin is produced. Methemoglobin is a form of hemoglobin. With methemoglobinemia, the hemoglobin can carry oxygen, but is not able to release it effectively to body tissues.

Methemoglobin concentration is not measured directly in hospitals. The idea of this project is to design a device that can measure non-invasively by this device will facilitate doctors to diagnose diseases that related to increasing in percentage of methemoglobin in blood by measuring methemoglobin concentration directly.

The device, which is designed to measure methemoglobin in blood, is based on the Beer Lambert law. It sends a wavelength light absorbed by the methemoglobin through a person's finger. After that, the light is taken from the other end of the finger and analyzed to obtain methemoglobin in the blood.

الملخص

تتمثل الوظيفة الأساسية لخلايا الدم الحمراء , في نقل الأكسجين من الرئتين إلى أنسجة الجسم وتخليص الأنسجة من ثاني أكسيد الكربون باتجاه الرئتين عن طريق الهيموغلوبين.

الميتهيموغلوبينيميا هي اضطراب في الدم يتم فيه إنتاج كمية غير طبيعية من الميتهيموغلوبين , الميتهيموغلوبين هو شكل من أشكال الهيموغلوبين . مع وجود الميتهيموغلوبينيميا , يمكن أن يحمل الهيموغلوبين الأكسجين , لكنه غير قادر على إطلاقه إلى أنسجة الجسم بشكل فعال.

لا يتم قياس تركيز الميتهيموغلوبين مباشرة في المستشفيات , ان فكرة هذا المشروع هي تصميم جهاز يقوم بقياس تركيز الميتهيموغلوبين بشكل غير جراحي مما يسهل على الأطباء تشخيص الإمراض التي ترتبط بزيادة نسبة الميتهيموغلوبين في الدم عن طريق قياس تركيز الميتهيموغلوبين مباشرة.

يعتمد هذا الجهاز الذي تم تصميمه على مبدأ بير-لامبارد , حيث انه يرسل الضوء بالطول الموجي الذي يمتصه الميتهيموغلوبين من خلال اصبع الشخص ، ثم يتم التقاط الضوء الذي خرج من الطرف الآخر للإصبع وتحليله للحصول على نسبة الميتهيموغلوبين في الدم.

إهداء

إهداء

إلي كل من أضاء بعلمه عقل غيره

أو هدى بالجواب الصحيح حيرة سائليه

فأظهر بسماحته تواضع العلماء

وبرحابته سماحة العارفين

أهدي هذا العمل المتواضع إلى أبي الذي لم يبخل علي يوماً بشيء

وإلى أمي التي ذودتني بالحنان والمحبة

أقول لهم: أنتم وهبتموني الحياة والأمل والنشأة على شغف الاطلاع والمعرفة

وإلى إخوتي وأسرتي جميعاً

ثم إلى كل من علمني حرفاً أصبح سنا برقه يضيء

الطريق أمامي

أحبكم حبا لو مر على أرض قاحلة

لتفجرت منها ينابيع المحبة

الشكر

نشكر الله العلي القدير الذي انعم علي بنعمة العقل والدين. القائل في محكم التنزيل

" وفوق كل ذي علم عليم " ..... صدق الله العظيم

وقال رسول الله (صلى الله عليه وسلم) " من صنع إليكم معروفا فكافئوه, فان لم تجدوا ما تكافئونه به فادعوا له حتى تروا إنكم كافأتموه "

وأيضاً وفاءً وتقديراً واعترافاً منا بالجميل نتقدم بجزيل الشكر لأولئك المخلصين الذين لم يألوا جهداً في

مساعدتنا في مجال البحث العلمي , ونخص بالذكر

الدكتور: علي عمرو

صاحب الفضل في توجيهنا ومساعدتنا في تجميع المادة البحثية فجزاه الله كل خير

ولا ننسى ان نتقدم بجزيل الشكر

للمهندسة : فداء الجعافرة

للدكتور : رمزي القواسمي

اللذان قاما بتوجيهنا طيلة هذه الدراسة

فريق العمل

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List of Abbreviation

RBC: Red Blood Cell

WBC: White Blood Cell

Hb: Hemoglobin

MetHb: Methemoglobin

HbO_2: Oxygenated Hemoglobin

NRI: Near-infrared spectroscopy

LPF: Low Pass Filter

CBC: Count Blood Cell

LED: Light-emitting diode

Chapter One
Thesis Overview

Methemoglobinemia is a blood disorder in which too little oxygen is delivered to your cells. This disorder or disease has two types Congenital Methemoglobinemia and Acquired Methemoglobinemia, and the main symptoms experienced by those people who suffer from this disorder or disease cyanosis and chocolate-brown colored blood.

This project discusses the measure of methemoglobin concentration by non-invasive method the CBC measure the methemoglobin concentration by add diluent to blood sample and translate to WBC chamber and add lysis to damage the RBC membrane and measure the methemoglobin concentration. The disadvantage of this method that the measure not real time and required to take a blood sample.

1.1 Project Idea Description

The idea of the project is to design a measuring system that can measure non-invasively methemoglobin and directly depending on the wavelength of oxidized hemoglobin, total hemoglobin, and total methemoglobin.

1.2 Project Aims

The main objectives of this project can be summarized as follow:

- Design a non-invasive medical system that has the ability to diagnose methemoglobinemia by measuring methemoglobin percentage directly, by optical method through a specific wavelength.

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- Monitor the methemoglobin concentration by connecting finger probe to the patient

1.3 Project Motivation

In this project, the device will be design to measure methemoglobin concentration non-invasively, that means getting rid of the invasive measurement which has discomfort, pain, and infection, increasing the accuracy of the methemoglobin measurements by measuring its percentage directly (no need to Spo_2 calculation), also the device can be use easily, and the device relatively low cost.

1.4 Project Scope

Will be tackle the design problem as follows in Figure 1.1 :

Firstly, three LEDs are used to emit three wavelengths for oxyhemoglobin, deoxyhemoglobin, and methemoglobin, this wavelengths are going to finger, the part of wavelength that is not absorbed reach the receiver , the electrical signal processed to be a percentage of methemoglobin concentration, finally the value of methemoglobin concentration will displayed.

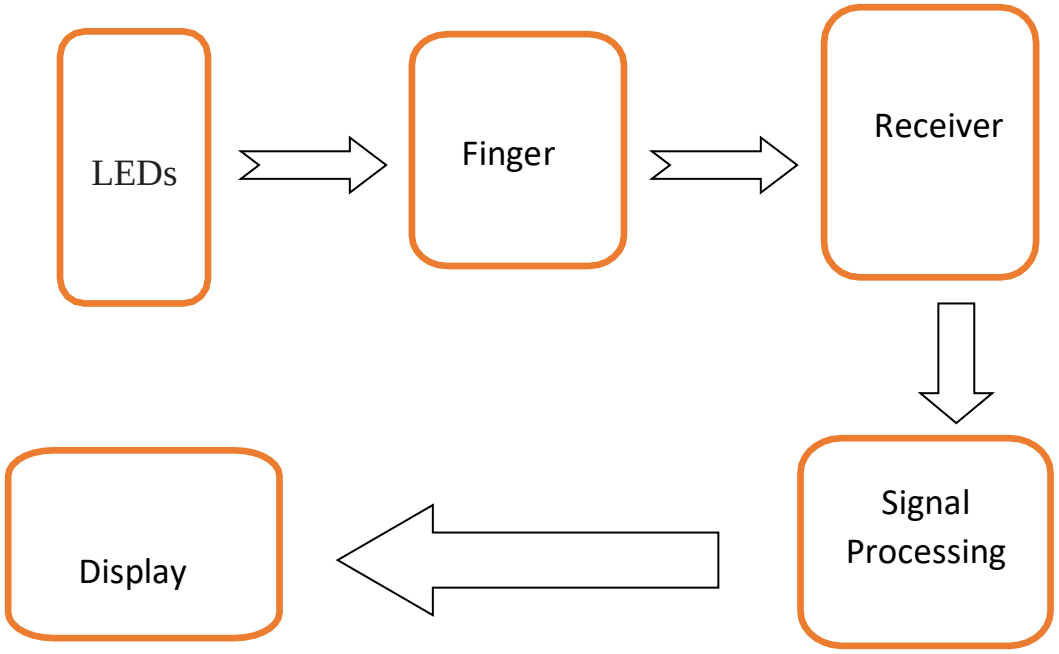


Figure 1.1: Block Diagram

1.5 Literature Review

A series of experiments was designed to assess the concentration of sodium nitrite that is necessary for the conversion of hemoglobin into methemoglobin in the range from 10% to 100%. Variable concentrations of methemoglobin were induced in normal blood samples by adding various concentrations of sodium nitrite prepared by diluting a 12.41 g/L sodium nitrite stock solution. An equal amount of diluted sodium nitrite was added to test tubes containing 1.0 mL of normal blood. The blood sample's methemoglobin concentration was measured using the method of Evelyn and Malloy. [1]

Red–dark brown blood was observed in the 10% to 100% methemoglobin blood samples. Freshly prepared series of methemoglobin samples were placed on a piece of white absorbent material and scanned within 1 minute using a Canon Scan LiDE 25 color image scanner (Canon Inc, Vietnam). The color value (from red to brown) of scanned samples was measured using an open source image processing and analysis program ImageJ 1.37v. [2**]**

Pre-measurement methods were to take blood samples and then to dilute them with distilled water and to measure the oxidized hemoglobin and then calculate the methemoglobin percentage which was obtained by oversaturation with potassium ferricyanide.

The result of the preceding studies are inaccurate because the contains a theoretical aspect which is the equations on which the calculations is based, and it is also expensive because the basis of measurement takes blood samples and waste time and effort.

1.6 Economical Study

This section present estimated the cost of the project components that will be used in implementation of the system, table 1.1 contains the main required hardware components of the project design, and it's cost.

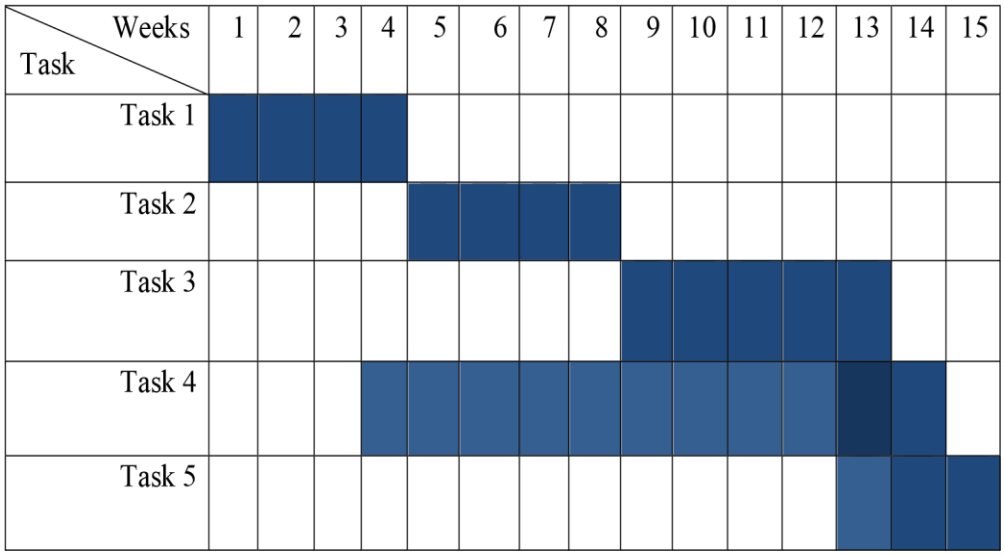
Table 1.1: Estimated Component Cost.

Components	**Unit**	**Costs per unit**
LEDs	3	5 $
Photo Detectors	3	100 $
Transimpedances	1	20 $
Amplifier	1	40 $
Micro Controller	1	20 $
LCD Display	1	5 $
Total	**10**	**130 $**

1.7 Schedule Time

In this section we make a plan for the predictive project tasks due to the time zone is made, this time plan shown in the table 1.2.

Table 1.2: Schedule Time



*** Task 1**: Data Collection

*** Task 2:** Literature Review

*** Task 3:** Design Methemoglobin Measuring System.

*** Task 4:** Documentation

*** Task 5:** Presentation Preparing

Chapter Two

Human Blood

The human body contains eleven organ systems. Each of them needs foodstuffs, such as nourishment, electrolytes, hormones, vitamins, antibodies, heat and oxygen to operate metabolic process. Moreover, they produce the nourishment and metabolic waste product. Therefore, they need transport for these products to its target. The blood has this function and others.

Blood is essential to life. it circulates through human body and delivers essential substances like oxygen and nutrients to the body's cells. It also transports metabolic waste products away from those same cells. There is no substitute for blood. It cannot be made or manufactured. Generous blood donors are the only source of blood for patients in need of a blood transfusion.

Blood performs many important functions within the body, including supply of oxygen to tissues (bound to hemoglobin which is carried in red cells),supply of nutrients such as glucose , amino acid , and fatty acid (dissolved in the blood or bound to plasma proteins (e.g., blood lipids),removal of waste such as carbon dioxide, urea, and lactic acid ,immunological functions (including circulation of white blood cells, and detection of foreign material by antibodies),the transport of hormones and the signaling of tissue damage ,regulation of core body temperature ,and hydraulic functions.

The Properties of blood is shown in the following table

Table 2.1: Properties of Blood

Amount	7-9% of total body weight
Volume	5-6 liters
Viscosity	3.5-5.5 times more than water
Specific gravity	0.45-1.065
PH	3-7.4 (slightly alkaline)
Temperature	38^0C (100.4F)
Osmotic pressure	25 mm Hg
Color	red due to hemoglobin

2.1 Composition of Blood

Blood is composed of plasma (the liquid portion) and blood cells.

Three types of cells are present in the blood, red blood cells (RBC), white blood cells (WBC), and platelets.

Figure 2.1 depicts these components, which will be discussed in the following sections.

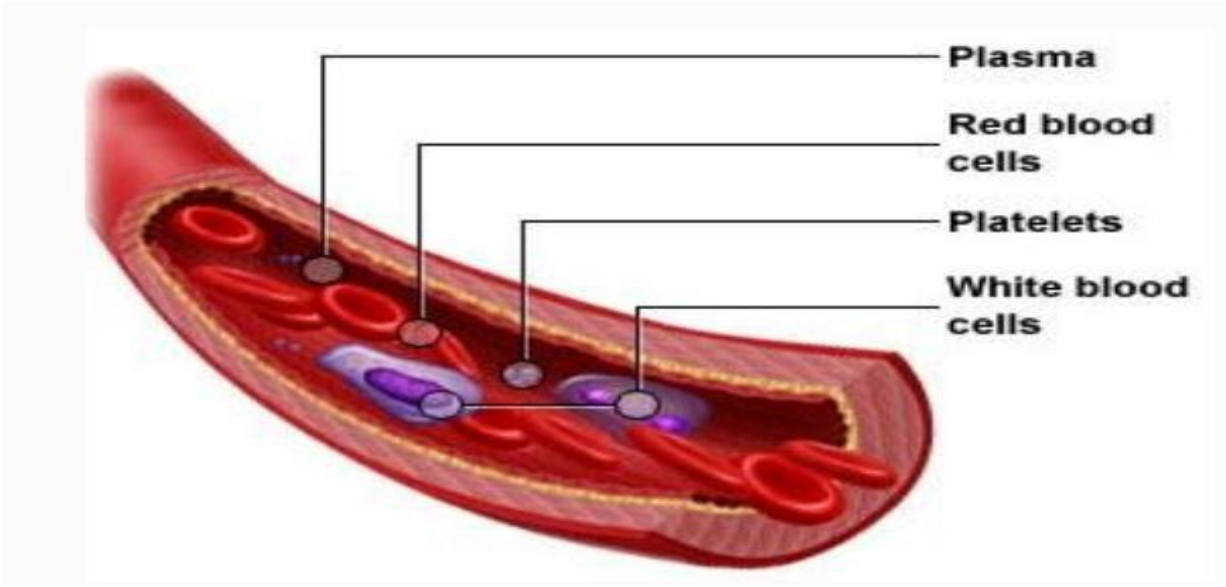


Figure 2.1: Composition of Blood

Plasma is the liquid component of blood is called plasma, a mixture of water, sugar, fat, protein, and salts. The main job of the plasma is to transport blood cells throughout your body along with nutrients, waste products, antibodies, clotting proteins, chemical messengers such as hormones, and proteins that help maintain the body's fluid balance.

Platelets **(**thrombocytes) helps the blood clotting process (or coagulation) by gathering at the site of an injury, sticking to the lining of the injured blood vessel, and forming a platform on which blood coagulation can occur. This results in the formation of a fibrin clot, which covers the wound and prevents blood from leaking out. A higher than normal number of platelets can cause unnecessary clotting, which can lead to strokes and heart attacks; however, thanks to advances made in antiplatelet therapies, there are treatments available to help prevent these potentially fatal events. Conversely, lower than normal counts can lead to extensive bleeding.

White blood cells (leukocytes) protect the body from infection. They are much fewer in number than red blood cells, accounting for about 1 percent of the blood.

The most common type of white blood cell is the neutrophil, which is the "immediate response" cell and accounts for 55 to 70 percent of the total white blood cell count. Each neutrophil lives less than a day, so your bone marrow must constantly make new neutrophils to maintain protection against infection. Transfusion of neutrophils is generally not effective since they do not remain in the body for very long.

The other major type of white blood cell is a lymphocyte. There are two main populations of these cells. T lymphocytes help regulate the function of other immune cells and directly attack various infected cells and tumors. Lymphocytes make antibodies, which are proteins that specifically target bacteria, viruses, and other foreign materials.

As methemoglobin is found in Red Blood Cells, red blood cells will be discussed in the following section.

2.2 Red Blood Cells (erythrocytes)

Known for their bright red color, red cells are the most abundant cell in the blood, accounting for about 40 to 45 percent of its volume. The shape of a red blood cell is a biconcave disk with a flattened center - in other words, both faces of the disc have shallow bowl-like indentations (a red blood cell looks like a donut).

Production of red blood cells is controlled by erythropoietin, a hormone produced primarily by the kidneys. Red blood cells start as immature cells in the bone marrow and after approximately seven days of maturation are released into the bloodstream. Unlike many other cells, red blood cells have no nucleus and can easily change shape, helping them fit through the various blood vessels in your body. However, while the lack of a nucleus makes a red blood cell more flexible, it also limits the life of the cell as it travels through the smallest blood vessels, damaging the cell's membranes and depleting its energy supplies. The red blood cell survives on average only 120 days.

Red cells contain a special protein called hemoglobin, which helps carry oxygen from the lungs to the rest of the body and then returns carbon dioxide from the body to the lungs so it can be exhaled. Blood appears red because of the large number of red blood cells, which get their color from the hemoglobin. The percentage of whole blood volume that is made up of red blood cells is called the hematocrit and is a common measure of red blood cell levels.

Further details about hemoglobin will be discussed in the following section.

2.3 Hemoglobin

It is an oxygen/CO_2 carrier protein present in the red blood corpuscles of blood. Hemoglobin is a conjugated chromo-protein having heme as its prosthetic group. Heme is the prosthetic group, not only of hemoglobin but also of myoglobin, cytochromes etc.

2.4 Composition of Hemoglobin

Hemoglobin is consist of Heme, and Porphyrin

2.4.1 Heme

Heme is an iron porphyrin structure, synthesized in the reticuloendothelial cells (bone marrow) of adult human being. Erythropoietin produced in kidney stimulates the formation, maturation and release of erythrocytes by bone marrow.

Early stage of erythrocyte cells contains porphy•rin, during the course of their development, porphyrin is converted to heme by addition of iron and then to hemoglobin by addition of protein, globin. The type of porphyrin present in heme is protoporphyrin-III (also known as No. IX).

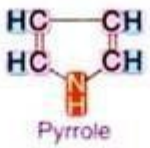
It is synthesized starting from glycine and succinyl-CoA. Given below is the diagrammatic representation of biosynthesis of Heme where 'A' stands for acetyl group, 'P' stands for propyl group, 'M' for methyl group, and 'V' for venyl group.

2.4.2 Porphyrin

Porphyrin is a complex compound with a tetrapyrrole ring structure. Pyrrole is a hetero•cyclic compound having the following structure.

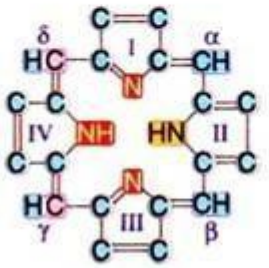
This porphin is substituted by different groups at positions numbered from 1-8 to form the porphyrin. Depending upon the groups (methyl, acetyl, propyl, butyl or venyl) present on these positions different types of porphyrins are identified, that will be seen during the synthesis of heme.

Porphyrins have several of properties; they act both as acids (-COOH) and bases (-NH_2), Their isoelectric pH is between 3-4.5, Porphyrins are fluorescent and coloured due to presence of alternating double bonds, Porphyrinogens are colourless. Figure 2.2 shows the structure of Porphyrins.

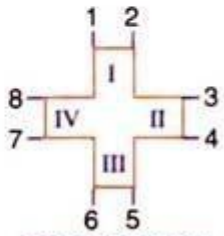


Pyrrole

4 pyrrole rings join together through methylidyne bridges (–CH=) to form a porphin.



Porphin



Fisher's projection formulae for a porphin

Figure 2.2: The structure of Porphyrins [3]

2.5 Hemoglobin Derivatives

There are some derivatives of normal Hb that arise due to metabolic changes in the RBC. The various hemoglobin derivatives are Oxyhemoglobin, Reduced Hemoglobin, Carbaminohemoglobin, Methemoglobin, Carboxyhemoglobin

Oxyhemoglobin (HbO_2)

The main function of hemoglobin is to transport oxygen from the lung to the tissues. In lungs the partial pressure of oxygen is 100 mm of Hg, at this pressure hemoglobin is 95-96% saturated with oxygen. On binding with O_2 in the lungs hemoglobin is converted to oxy-hemoglobin (Hb02). O_2 is bound to heme iron.

$Hb + O_2 \rightarrow HbO_2$.....[2.1]

Reduced Hemoglobin (HHb)

Oxy-hemoglobin moves to the tissue where the partial pressure of O2 is 26 mm of Hg due to which oxygen is released into the tissues and in turn H+ binds to Hb and forms reduced hemoglobin.

$HbO_2 + H+ \rightarrow HHb + O_2$ .....[2.2]

Carbaminohemoglobin:

Hemoglobin also binds to CO_2 in the tissues. CO_2 is bound to the α-amino group at the N-terminal end of each of the four polypeptide chains of hemoglobin to form carbaminohemoglobin. As one CO_2 binds O_2 is released.

Methemoglobin:

In RBC the iron of hemoglobin is normally in ferrous (Fe^{2+}) form, but it is readily oxidized to the ferric (Fe^{3+}) form by hydrogen peroxide formed by RBC cell metabolism, to yield met-hemoglobin. Ferric iron is incapable of binding O2 therefore the functions of hemoglobin are disturbed. Normally 1.7 to 2.4 % of total hemoglobin will be in the form of met-hemoglobin. Increase in the percent of met-hemoglobin is prevented by the peroxidase action of a naturally occurring peptide known as glutathione present in the RBC. Met-hemoglobin is dark brown in colour.

The percent of met-hemoglobin can increase if the person consumes drugs like ferricyanide, nitrite, qui-nines, hydroxylamine's, acetanilide and sulfonamide. Higher levels of met-hemoglobin is observed clinically in factory workers who inhale (or contact through skin) aromatic nitro and amino com•pounds and in patients taking large amounts of acetanilide and sulfonamides. The symptoms are cyanosis (blue skin) and dyspnoea (labored breathing)

Carboxyhemoglobin:

Oxy-hemoglobin can bind to carbon monoxide (CO). Even normal, non-oxygenated hemoglobin can bind with CO to form carboxyhemoglobin. [Hb + CO → HbCO]. CO has got an affinity of 200 times more than that of O2 towards Hb. Hemoglobin can bind more readily to CO than to O_2. Even if there is a little amount of CO in air, it can displace oxyHb to form carboxyHb. Due to this there will be tissue hypoxia because the oxygen binding capacity is reduced and there is also reduced O_2 releasing capacity i.e. it cannot release O_2 though it may be bounded to O_2.

City dwellers have at least 1% of carboxyhemoglobin which can increase to 8% depending upon the pollution. Over traffic can increase carboxyHb to 40% which leads to death. Clinically such patients show cherry red colour of skin. CO poisoning can be treated if high amount of O_2 is provided continuously at high pressure, then at such high concentrations and pressure HbCO is dissociated forming HbO_2 + CO .When treatment continues for 2 hours CO is expelled out.

2.6 Types of Hemoglobin

There are three types of hemoglobin's that are normally found in human beings, they are**:**

HbA;

Found in normal adult human beings – contains 2α and 2β chains.

HbA2:

Found in some human beings and is considered normal — contains 2α and 2β chains.

HbF:

Foetal hemoglobin — found in growing foetus — contains 2α and 2γ chains.

2.7 Methemoglobin

Methemoglobin (MetHb) is altered state of hemoglobin (Hb) in which the ferrous (Fe^{2+}) irons of heme are oxidized to the ferric (Fe^{3+}) state. The ferric hemes of MetHb are unable to bind oxygen (O_2). Thus, oxygen dissociation curve is left-shifted, making it more difficult to release O2.

2.8 Methemoglobinemia

Methemoglobinemia is a blood disorder in which too little oxygen is delivered to your cells. Oxygen is carried through your bloodstream by hemoglobin, a protein that's attached to your red blood cells. Normally, hemoglobin then releases that oxygen to cells throughout your body. However, there's a specific type of hemoglobin known as methemoglobin that carries oxygen through your blood but doesn't release it to the cells. If your body produces too much methemoglobin, it can begin to replace your normal hemoglobin. This can lead to not enough oxygen getting to your cells.

2.9 Types of Methemoglobinemia

There are two types of Methemoglobinemia, congenital methemoglobinemia, and acquired methemoglobinemia.

2.9.1 Congenital Methemoglobinemia

Methemoglobinemia can be congenital, which means you're born with the condition. Congenital methemoglobinemia is caused by a genetic defect that you inherit from your parents. This genetic defect leads to a deficiency of a certain enzyme, or protein. This protein is responsible for converting methemoglobin to hemoglobin. Congenital methemoglobinemia is much less common than the acquired form of the condition.

2.9.2 Acquired Methemoglobinemia

This is also known as acute methemoglobinemia. Acquired methemoglobinemia is the most common type of the condition. It's caused from exposure to certain medicines, chemicals, or foods. People who carry a genetic form of the condition have a higher chance of developing the acquired type. But most people who acquire this condition don't have a congenital problem. If acquired methemoglobinemia is not treated immediately, it can lead to death.

Previously, the method used and available to measure methemoglobin concentration by Calculations on the samples taken, while here will designed a device measure the methemoglobin concentration directly without samples.

The main symptoms are cyanosis, which describes a bluish color of the skin, especially the lips and fingers, and chocolate-brown colored blood.

As methemoglobin levels increase, symptoms continue to get more serious as shown in Table 2.2

Table 2.2 Symptoms with percentage of methemoglobin

Percentage of Methemoglobin	**Symptoms**
>15%	**Cyanosis**
>20%	**Headache, Anxiety, Dizziness**
>30%	**Dyspnea, Fatigue, Confusion**
>50%	**Seizures, Acidosis Arrhythmias, Death**

2.10 Methemoglobinemia Treatment

The first treatment is infusion with the drug methylene blue. This medication usually helps people quickly. But methylene blue can’t be used on people who have a congenital type of methemoglobinemia.

People who don’t respond to methylene blue may need a blood transfusion, where as People with type 1 hereditary methemoglobinemia may receive aspirin therapy.

Chapter Three
Theoretical Background

More than a century has elapsed since the first measurement of hemoglobin (Hb) for blood diagnosis, so that hemoglobin was among the first diagnostic blood test available to clinicians during the first decades of the 20th century, when medical laboratory was in its infancy. Today it is the most frequently requested blood test and is performed not only in the hospital laboratory but in a variety of healthcare settings, by a range of health care personal, using technology of diverse sophistication, in an intensive care and emergency room setting, for example hemoglobin is measured by nursing staff using technology incorporated into blood gas analyzers. There are many ways to measure hemoglobin but it falls under two main titles invasive and non-invasive.

3.1 Invasive Hemoglobin Concentration Measurements

Several types of invasive method are used to measure hemoglobin, such as spectrophotometry, hematology analyzer, blood gas analyzer, conductivity-based method. The following sections dive brief description about each type.

3.1.1 Spectrophotometry

This technique used in many clinical laboratory instruments that including the measurement of hemoglobin concentration. As shown in figure (3.1) the spectrophotometer consists of light source, wavelength selector, cuvette, and detector.

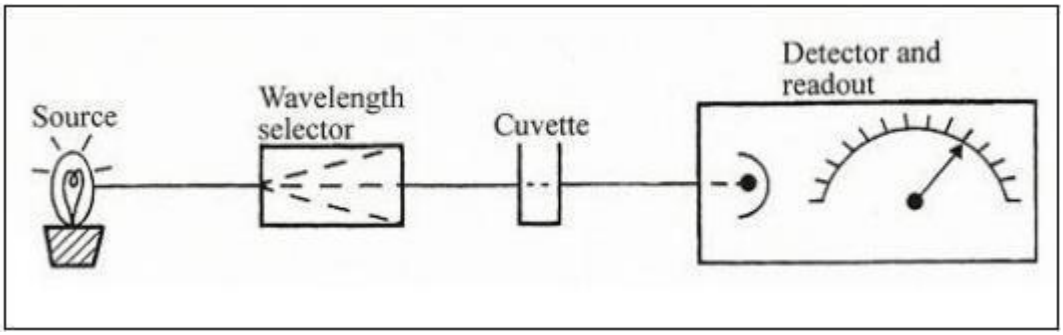


Figure 3.1: Block diagram of spectrophotometer [4]

The light source must give a wide range of wavelengths, so tungsten or xenon lamp has been used. After the light be emitted from light source, it enters to wavelength selector to select the appropriate wavelength for measure hemoglobin concentration.

The cuvette containing a blood sample, when the wavelength that has been selected passes through the cuvette the hemoglobin absorbed some of this light. while the other light transmitted and detected by the detector (photo-diode or photo-transistor). After that, the hemoglobin concentration calculated by using Beers law.

3.1.2 Hematology Analyzer

A Complete Blood Count (CBC) is a broad screening test used to check for certain disorders relating to the blood. Whenever a CBC is requested from the clinical laboratory, samples are processed on a hematology analyzer. A standard CBC includes: RBC count, WBC count, hemoglobin, HCT, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and platelet count. Using the Coulter Principle, the analyzer can electronically count and size the red blood cells. In addition to electronic particle counting, hematology analyzers use HiCN spectrophotometrically measure total hemoglobin and the dyshemoglobin content.

Blood samples are drawn by a phlebotomist, medical technologist or other qualified clinician in a collection tube containing an anticoagulant such as ethylenediaminetetraacetic Acid (EDTA). The samples are labeled, bar-coded and processed in the laboratory. Once arriving at the hematology analyzer, the samples are inserted into the analyzer and the automated measurement process is initialize by the lab technician.

3.1.3 Blood Gas Analyzer

Blood gas analyzers are devices used for the determination of PO_2, PCO_2, pH, sodium (Na^+), potassium (K^+), ionized calcium (Ca^{++}), chloride (Cl^-), glucose, lactate, total hemoglobin (tHb) and the dyshemoglobins in arterial, venous, and capillary whole blood samples where tHb is calculated. Additional CO-oximetry features of some analyzers allow the measurement of tHb, Fraction of oxygenated Hemoglobin (FO_2Hb), fraction of reduced hemoglobin (FHHb), The Bayer RapidLab 800 series of

blood gas analyzers is a family of analyzers that provide different functionality. The base models can measure tHb and FO_2Hb, as well as the standard blood gas measurements. More featured models, such as the RapidLab 865, have a separate CO-oximetry module that can measure the fractional components of the different hemoglobin derivatives. This CO-oximetry module spectrophotometrically measures hemoglobin. It contains an optics module and lamp, hemolyzer, and sample chamber. First the sample is pumped through the hemolyzer which uses ultrasonic sound vibrations to rupture the red blood cells and release the hemoglobin. The sample then enters the sample chamber for spectrophotometric measurement.

3.1.4 Conductivity-Based Method

The conductivity-based method measures the conductivity of the blood sample between two electrodes to determine the hematocrit. The measured conductivity is inversely related to the blood hematocrit. The hemoglobin concentration is calculated by the assumption that hemoglobin is approximately one-third of the total hematocrit.

Blood has a high temperature coefficient and it is essential to maintain a constant temperature during measurement. Conductivity-based devices have built-in thermostat-regulated temperature chambers to regulate the sample temperature. The most abundant electrolyte in plasma is sodium. Increases or decreases in sodium concentration will affect RBC volume and ultimately, the hematocrit measurement. Additionally, decreases or increases in the protein concentration of plasma can also alter results.

3.2 Non-Invasive Hemoglobin Concentration Measurements

Several types of non-invasive method are used to measure hemoglobin, such as optical methods, conductance methods, optoacoustic methods. The following sections dive brief description about each type.

3.2.1 Optical Methods

Hemoglobin has different forms in the blood. such as oxyhemoglobin, reduced hemoglobin, carboxyhemoglobin, and methemoglobin. Oxyhemoglobin (HbO2) and reduced hemoglobin (Hb) are main forms that are available in the blood. The other forms are available only in traces. Oxyhemoglobin is mainly available in arteries and

the reduced hemoglobin is available in veins, but in capillaries, both the forms are available the oxyhemoglobin and reduced hemoglobin have a different absorption of light at different wavelengths. Figure (3.2) shows the variation in molar extinction coefficient of light (μa) of the two hemoglobin forms with wavelength variation. The pulse oximetry method based on this property for finding the oxygenation of hemoglobin percentage (SaO2).

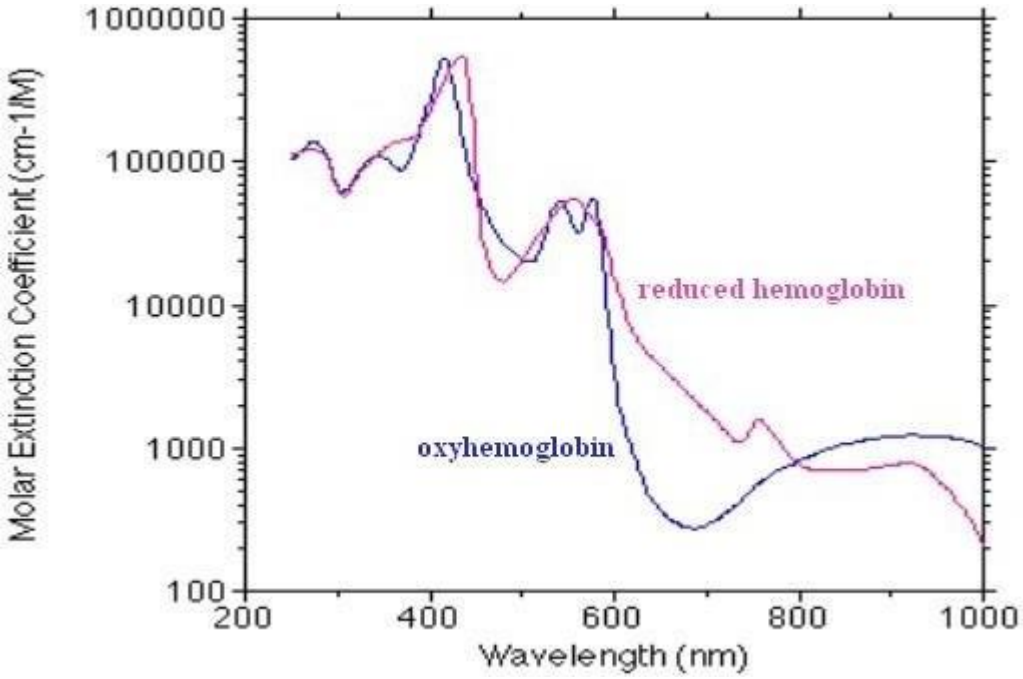


Figure 3.2: Molar extinction coefficient of light of HbO_2

3.2.2 Conductance Methods

This technique using an electrical admittance finger cuff. Electrodes are placed in the interior of an annular cuff, which is then filled with an electrolyte solution. With the finger inserted through the cuff, the change in blood volume in the finger translates to a change in electrical admittance (conductivity) of the finger. Submerging the finger in an electrolyte solution whose admittance is equal to that of the finger compensates for pulsatile variation in conductivity, after which the conductivity of the electrolyte solution can be related to the conductivity of arterial vessels and then correlated to the hemoglobin concentration.

3.2.3 Optoacoustic Method

The optoacoustic technique is based on the generation of ultrasound waves by short laser pulses. The rapid thermal expansion of the tissue through laser absorption creates an optoacoustic wave; hemoglobin has a higher absorption coefficient than surrounding tissue, enhancing optically induced thermal modulation.

The superficial radial artery is an effective location for optoacoustic stimulation in the NIR because it located close to the skin surface; this accessibility facilitates irradiation and optoacoustic wave detection. The absorption coefficient of blood depends on the hemoglobin concentration, oxyhemoglobin saturation, and laser pulse wavelength. In arteries, oxyhemoglobin saturation approaches 100%. Registration and analysis the optoacoustic signal generated from blood vessels yield information about the total hemoglobin concentration. Fig 3.3 shows the variation in optoacoustic signal with hemoglobin concentration in a tissue. [3]

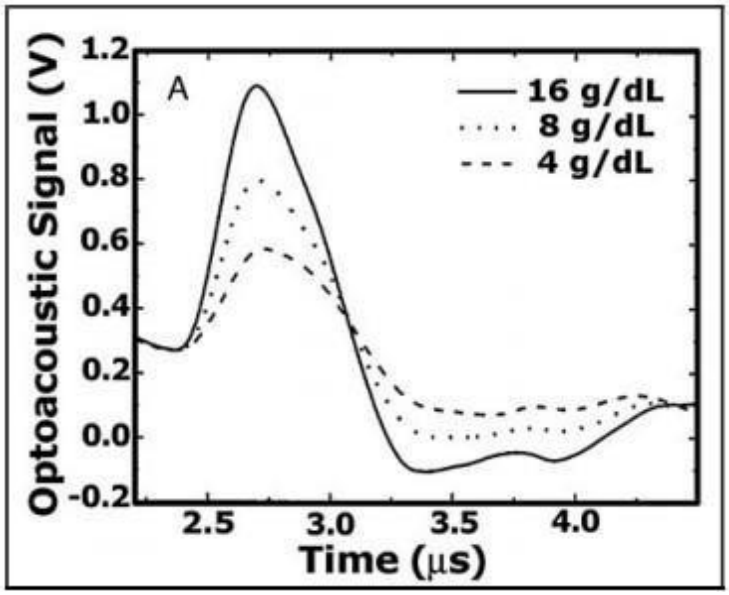


Figure 3.3: Typical optoacoustic signals [5]

3.3 Color and Absorption Spectroscopy

Spectrometers can accurately distinguish and quantify radiation in the ultraviolet, visible, and infrared regions of the spectrum.

The visible light is composed of a range of frequencies. The frequency of the radiation is proportional to its energy and the wavelength of the radiation is inversely proportional to the energy. Red is the lowest energy visible light and violet is the highest.

A solid object has color depending on the light it reflects. If it absorbs light in the red and yellow region of the spectrum, it will have a blue color.

Table 3.1 shows absorbed and perceived colors for different color wavelength.

Table 3.1 Absorbed and Perceived Color

Absorbed Wavelength (nm)	Absorbed Color	Perceived Color
400	Violet	Green – yellow
450	Indigo	Yellow
480	Blue	Orange
490	Blue – green	Red
530	Green	Purple
570	Yellow – green	Dark blue
600	Orange	Blue
650	Red	Green

3.4 Method of Methemoglobin Concentration Measurement

Methemoglobin (MetHb): is altered state of hemoglobin (Hb) in which the ferrous (Fe_2+) irons of heme are oxidized to the ferric (Fe_3+) state. The ferric hemes of MetHb are unable to bind oxygen (O_2). Thus, oxygen dissociation curve is left-shifted, making it more difficult to release O_2

This device consist of a photodiodes and photodetector, the 3 light emitting diode emit light of different wave lengths the light emitted by the diodes is absorbed by tissues and the amount of absorption is determined by the photo detector, the methemoglobin in arterial blood a waveform corresponding to the pulsatile flow in arterial vessels the light absorption of oxyhemoglobin is greater at wavelength of 800_1000 nm , the light absorption of deoxyhemoglobin is greater at wavelength of 600_800nm , the light absorption of methemoglobin is greater at wavelength of 550_650nm (is shown in figure 3.4), one light emitting diode emit light in the wavelength of 660 nm at which the light absorption of deoxyhemoglobin is greater than that of oxyhemoglobin the other diode emit light at a wave length of 940 nm , and the light absorption of methemoglobin is greater than deoxyhemoglobin and oxyhemoglobin the diode emits light at wavelength of 645 nm, the microcontroller analyze the light absorption of the tissue at each wavelength to determine the calculate concentrations of methemoglobin , deoxyhemoglobin and oxyhemoglobin .The result of the previous calculations is displayed in the following equation

$$\text{MetHb\%} = \frac{MetHb}{MetHb+HbO2+Hb} * 100 \qquad [3.1]$$

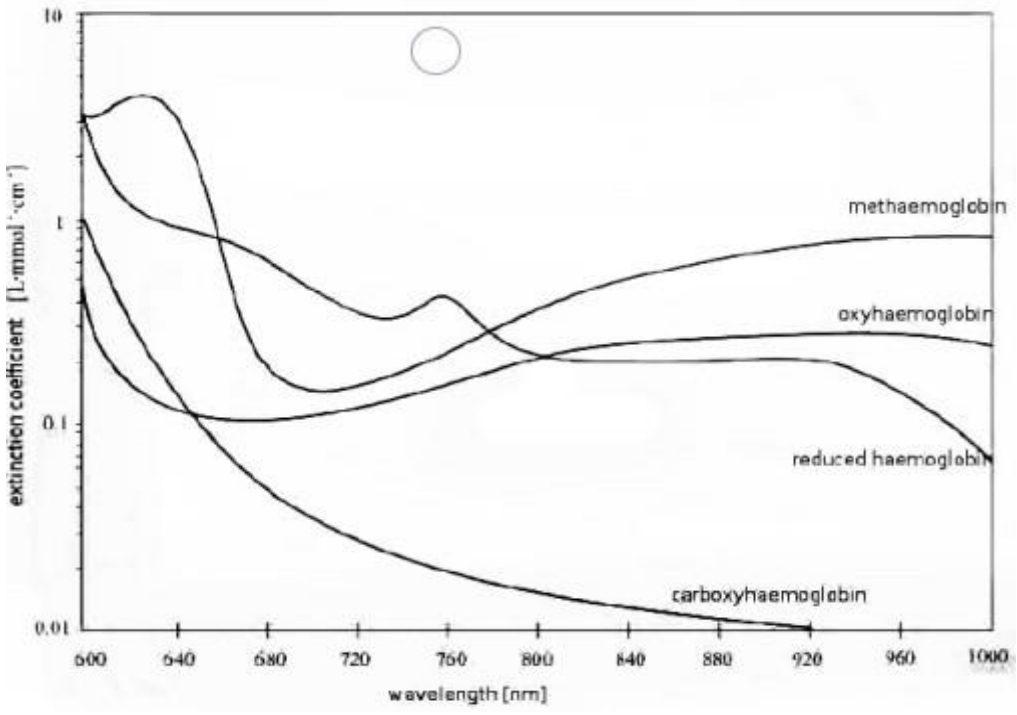


Figure 3.4 Absorptivity's of the four most common hemoglobin [6]

The signal had Ac and Dc components, the AC/DC ratio of both wavelength leads to equation (4.9)

$$R = \frac{A(t,645nm)}{A(t,660nm)+A(t,940nm)+A(t,645nm)}$$

So, the ratio is equal to:

$$\frac{\ln(I(Ac+Dc),645nm}{I(Dc,645nm)}$$

$$\frac{\ln(I(Ac+Dc),645nm+(Ac+Dc),660nm+(Ac+Dc),940nm}{I(Dc,645nm)+I(Dc,660nm)+I(Dc,940nm)}$$

This value express of ratio of methemoglobin absorption for the other types of hemoglobin at a specific wavelength.

The output signal has AC and DC component, the Ac signal generated by blood flowing, while DC component generated by constant component of figure as shown in figure (3.5)

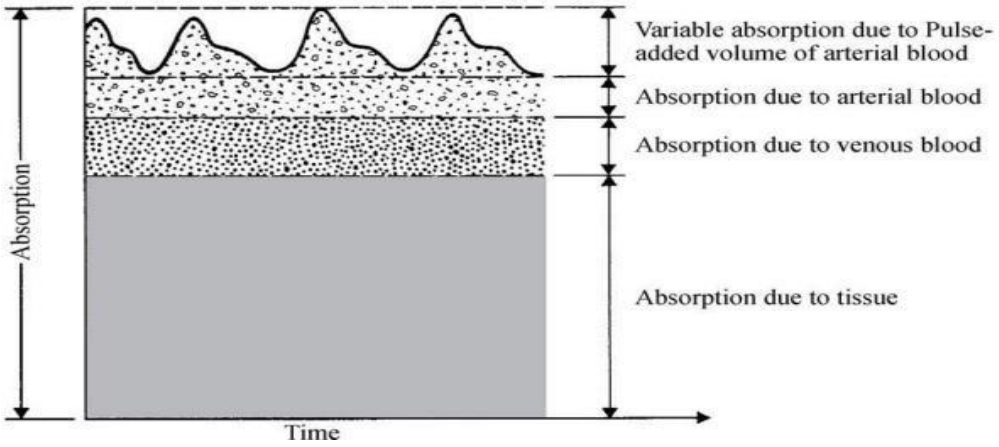


Figure 3.5: Component of Ac and DC [7]

Non-invasive methods for determining a person's total methemoglobin concentration as well as the concentration of the hemoglobin species which contribute to this total concentration, i.e., oxyhemoglobin, deoxyhemoglobin, and methemoglobin are described. The measurement comprises a ratio formed by dividing absorbance data at analyte wavelengths by absorbance data at total wavelengths.

The device is position so that photo detector and light emitting diode face each other(is shown in figure 3.6) with layers of tissue between them the photo diode turn on and off several hundred times per second to record the light absorption during pulsatile and non-pulsatile flow during positive flow the light absorption of arterial blood background tissue and venous blood is detected during non-pulsatile flow only the light absorption of background tissue and venous blood is detected the microcontroller compares the light absorption during pulsatile and non-pulsatile flow to isolate the light absorption of arterial blood and thus determine the arterial MetHb%

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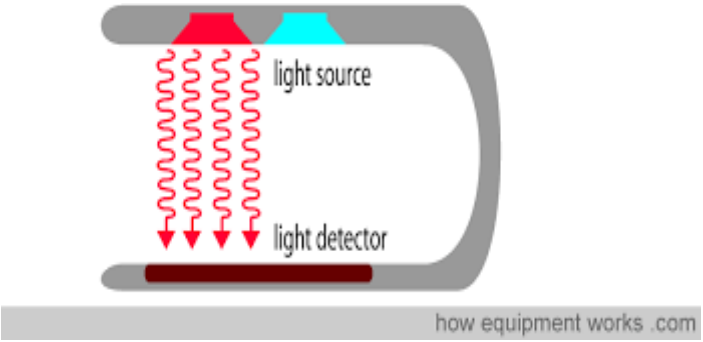

Figure 3.6: The sites of photo diode and photo detector for each other [8]

Chapter Four

System Design

This chapter describes the main elements of the system and their circuits, and the requirements to design system essential requirement, the framework is summarized in the following block diagram, as shown in figure (4.1).

Firstly, three LEDs are used to emit three wavelengths for oxyhemoglobin, deoxyhemoglobin, and methemoglobin, this wavelength are going to finger, the part of wavelength that is absorbed reach the photodiode, the electrical signal that is produced by photodiode must convert from current to voltage by transimpedance, then the signal go to band pass filter to pass frequencies within a certain range and rejects undesired frequencies outside that range, after that the signal go to the amplifier for amplification, the microcontroller is used to convert the signal from analog to digital and apply a certain equation for methemoglobin to acquire the percentage of methemoglobin concentration.

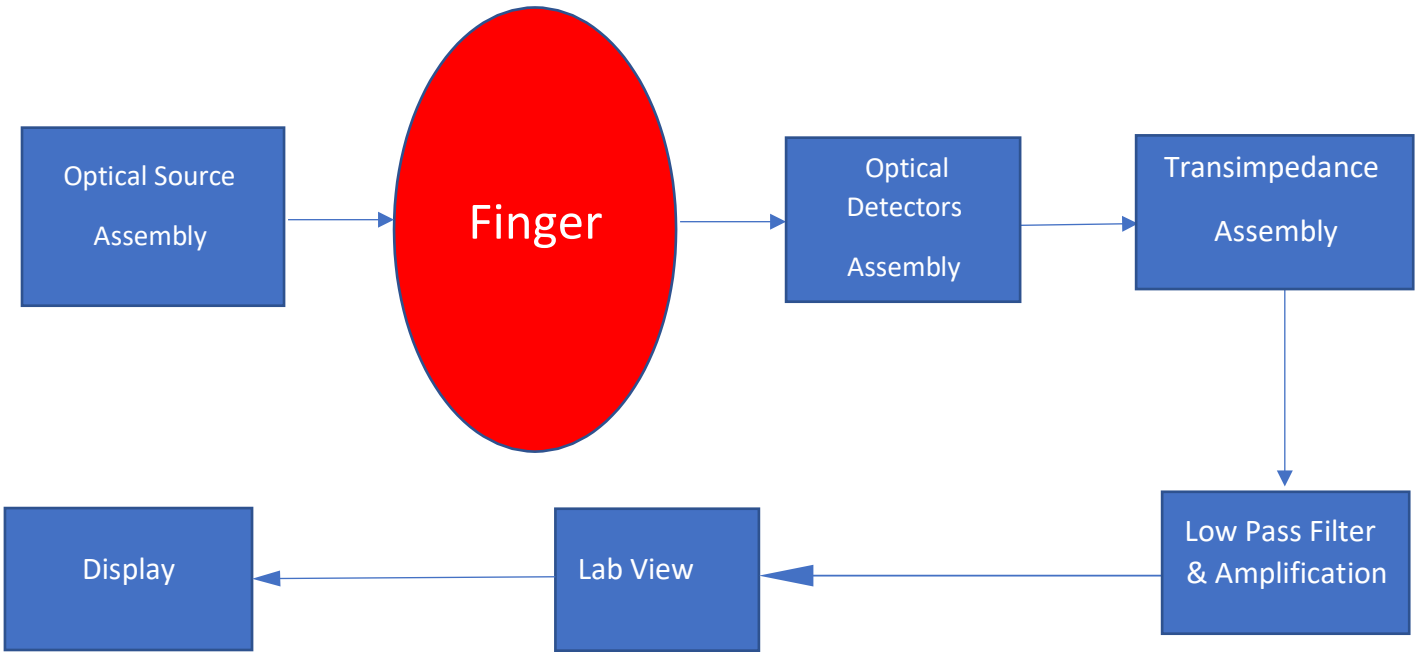

Figure 4.1: Block diagram of Methemoglobin concentration measurement

4.1 Optical Source Assembly

Three optical source with different wave length are required in this project, the first one is used to emit the oxyhemoglobin wave length which is 660 nm, the second one is used to emit the Deoxyhemoglobin which is 940 nm, and the third one is used to emit the methemoglobin which is used 645 nm.

LEDs assembly consist of 3 LEDs, the first LED is used to emit the oxyhemoglobin wavelength, the second LED is used to emit the deoxyhemoglobin wavelength, and the third LED is used to emit the methemoglobin wavelength.

The following table (Table 4.1) shows the type of each light source and its specification required to obtain the desired wave length

Table 4.1: Light Source Properties

Light source name	Light source type	Light source wave length	Light source specification
TLUR6400	Red	660 nm	If=20 mA
IR333-A	Infrared	940 nm	If=20 mA
TLUR6400	Red	645 nm	If=20 mA

The diode TLUR6400 (LED1) is used to emit 660nm wavelength, to maintain diode emit (590 - 750) nm and specific value 660nm, the current is 20mA(appendix1). The LED1 driving circuit shown in figure [4.2].

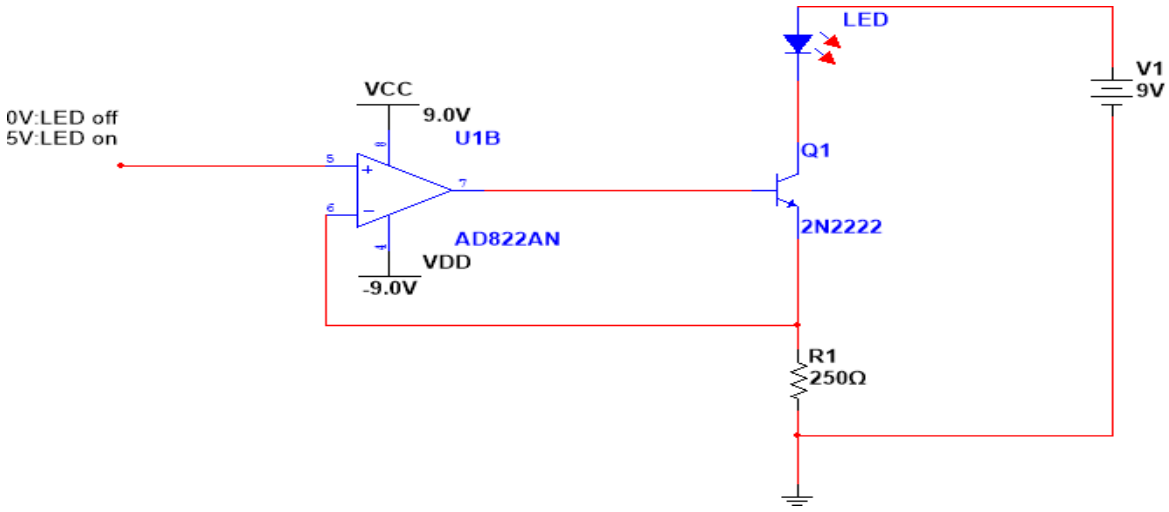

Fig 4.2: LED driving circuit

To maintain the current 20mA, the resistor R1 calculate by equation (4.1).

$$R = \frac{Vc}{I} \quad (4.1)$$

$$R1 = \frac{5}{20\ mA} = 250\ \Omega$$

By stander resistance value, the standard resistance is 250Ω.

The AD822 is a dual precision, low power FET input op amp that can operate from a single supply of 5 V to 30 V, or from dual supplies of ±2.5 V to ± 15 V. The op-amp AD822 is rail-to-rail op-amp, with CMRR=80dB, and its input impedance $=10^{13}\ \Omega$, with Offset voltage of 800 µV maximum. The input bias currents below 25 pA, 1.8 MHz unity-gain bandwidth and 3 V/µs slew rate. (appendix5)

The diode IR333-A (LED2) is used to emit 940nm wavelength, to maintain diode emit (880 – 1040) nm, and specific value 940nm, the current is 20mA. (appendix2)

The diode TLUR6400 (LED3) is also used to emit 645nm wavelength, to maintain diode emit (590 - 750) nm and specific value 645nm, the current is 20mA. (like LED1)

4.2 Optical Detector Assembly

A light detector circuit is required to detect the residual lights of the LEDs three photo diode with specific wavelength corresponds to LED1, LED2, LED3 are implemented in three transimpedance Amplifiers.

The photodiode S1227-66BR (photodiode1), with special response range (340-1000) nm and peak sensitivity 720nm, the output current at 660nm is 19µA. (appendix3)

The current must convert to voltage to process by transimpedance as shown in figure (4.3).

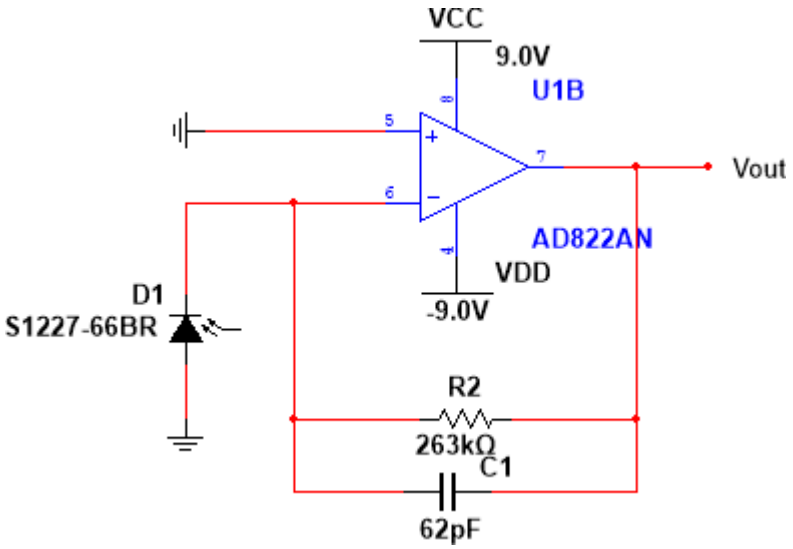

Fig 4.3: Photodiode1 driving circuit

The frequency of transimpedance (fp) is 10 kHz, the max o/p is 5V (appendix 7), and can be determine through equation (4.2).

$$\text{Vout} = \text{Ip}\,\frac{-R2}{1+\frac{1}{\beta}} \quad (4.2)$$

$$\beta = \frac{1}{1+R2C1} \quad (4.3)$$

β = 1

$$\text{R2} = \frac{Vmax-Vmin}{Imax} \quad (4.4)$$

$$\text{R2} = \frac{5v-0v}{19\mu A} = 263\text{k}\Omega$$

$$\text{C1} = \frac{1}{2\pi R2 fp} \quad (4.5)$$

$$\text{C1} = \frac{1}{2\pi * 263k * 10kHz} = 62\text{pF}$$

The photodiode SFH 205 F (photodiode2), with special response range (800-1100) nm and peak sensitivity 960nm, the output current at 940nm is 60µA (appendix4). figure (4.4)

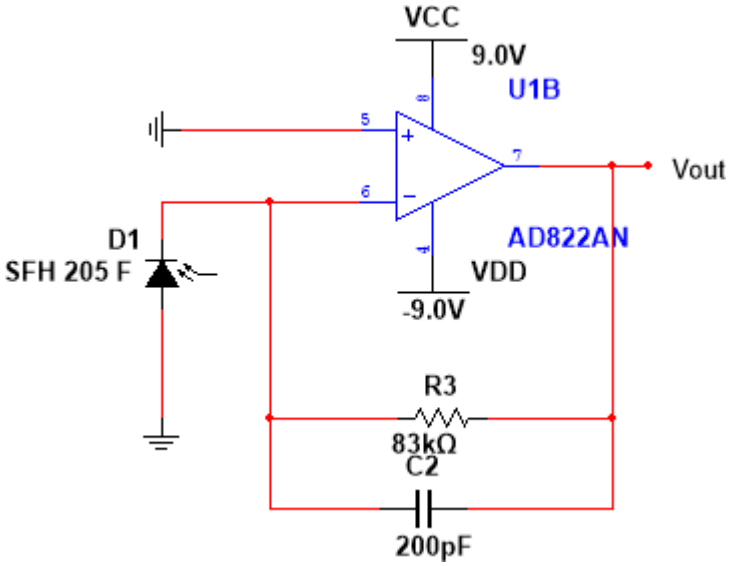

Fig 4.4: Photodiode2 driving circuit

R3=83kΩ

C2=200pF

The photodiode S1227-66BR(photodiode3), with special response range (340-1000) nm and peak sensitivity 720nm, the output current at 645nm is 19µA. (like photodiode1)

4.3 Low Pass Filter & Amplification Circuit

The output of the Transimpedance circuit is than fed into a Low pass filter. The Low Pass Filter, as shown in Figure 4.5. with the critical frequency equal 1.7 Hz.

Butterworth filter is used to amplify all harmonic component in the same quantity.

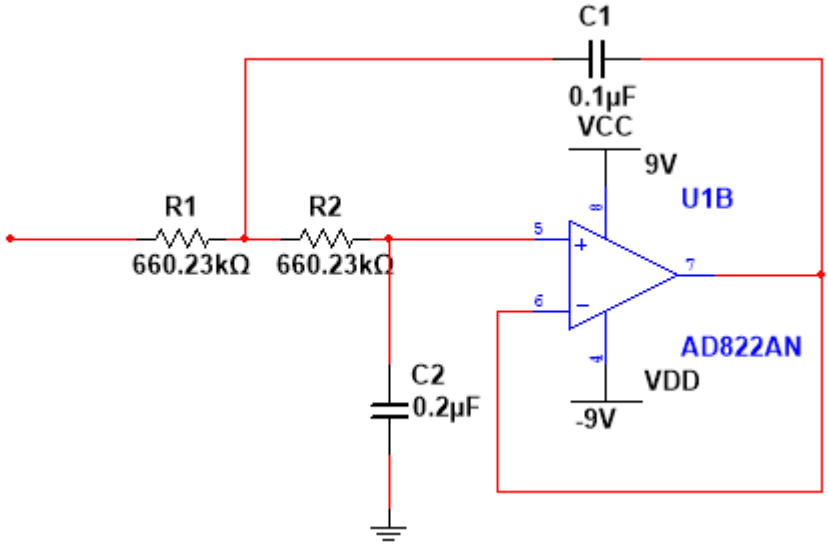

Figure 4.5: Butterworth Low Pass Filter

$$A(s) = \frac{A_o}{1+W_C R_C(3-A_o)s+(W_C R_C)^2 s^2} \qquad (4.6)$$

$$a_1 = W_C R_C(3 - A_0)$$

$$b_1 = (W_C R_C)^2$$

For Low Pass Filter, the cutoff frequency (fc) is set at 1.7Hz

R8=R9=R

$$C6=C5\frac{4b1}{a1^2} \qquad (4.7)$$

C5=0.1µF, C6=0.2µF

$$R=\frac{a1C2-\sqrt{a1^2C2^2-4b1C1C2}}{4\pi fcC1C2} \qquad (4.8)$$

R=660.23kΩ

Amplification is the stage that follows the filtering stage, as shown in figure 4.6.

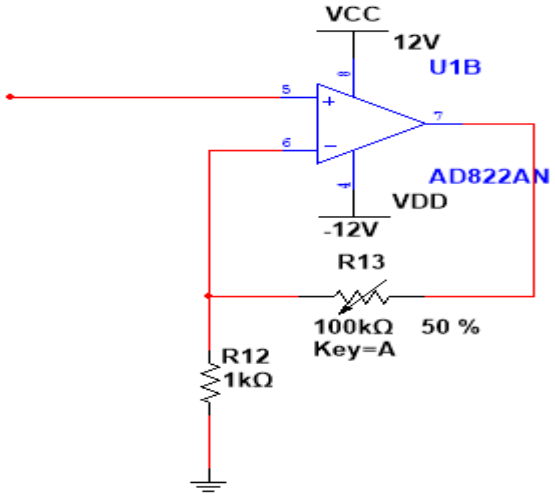

Figure 4.6: Amplification circuit

$$G1= 1+\frac{R13}{R12} \qquad (4.9)$$

Non-inverting amplifier has high input impedance.

The variable resistance is used to control the gain of the amplifier, the gain is change according to change in the absorption in the wavelength.

4.4 LABVIEW

LABVIEW (laboratory virtual instrument engineering workbench) also called virtual instrument of Vis, because their appearance and operation imitate physical instruments, such as oscilloscope and mustimeters. LABVIEW contains a comprehensive set of tools for acquiring, analyzing, displaying, and storing data as well tools to help your troubleshoot your cade. In this project, LABVIEW used to control LED1and LED2 and LED3 on and off time. at the same time, LABVIEW will acquire signals from three detectors, analysis signals and display result.

4.5 NI myDAQ

National Instruments myDAQ is an affordable data acquisition (DAQ) device that allows to analyze and measure live signals anytime, anywhere. NI myDAQ is portable and easy to transport , includes two analog inputs and two analog outputs at 200 kS/s and 16 bits, allowing for applications such as sampling an audio signal; eight digital inputs and output lines, providing power for simple circuits with +5, +15 , and -15 voltpower supplies; and a 60 V DMM to measure voltage, current, and resistance.

4.6 Signal process

Figure 4.7 shows the flow chart of processing the signal

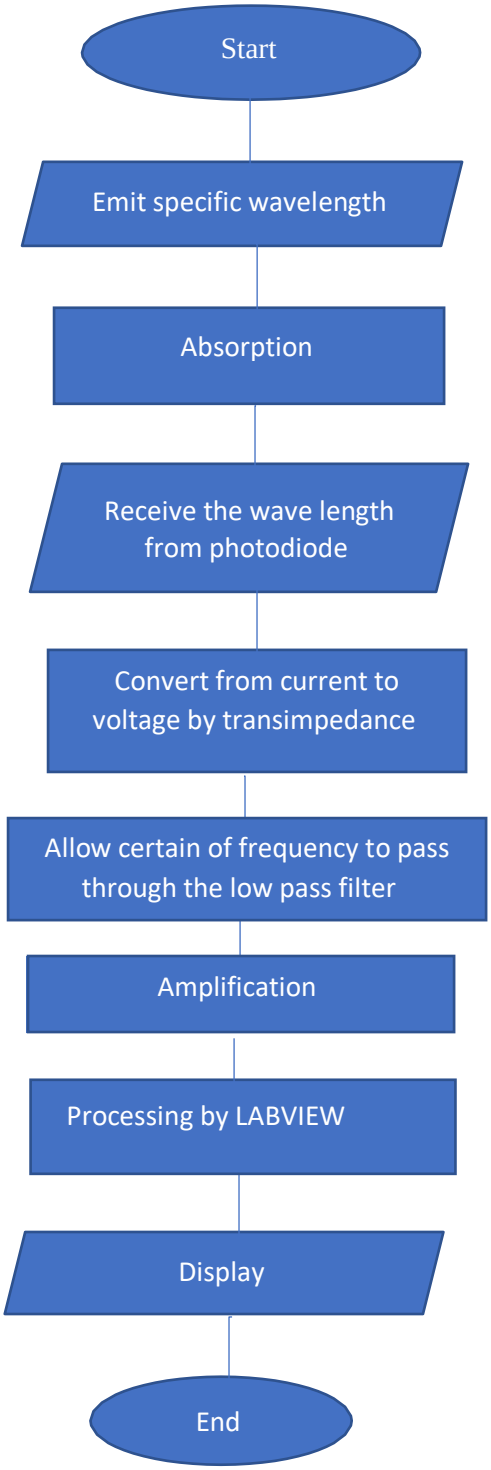

Figure 4.7: Signal process

Chapter Five

Results and Analysis

Chapter four described all necessary electrical component to determine the methemoglobin concentration by non-invasive method. This chapter describe the result and analysis of system.

5.1 System Implementation

First stage of this system is LED's and detectors driving circuit, figure 5.1 show LED's and detectors implement in prop.

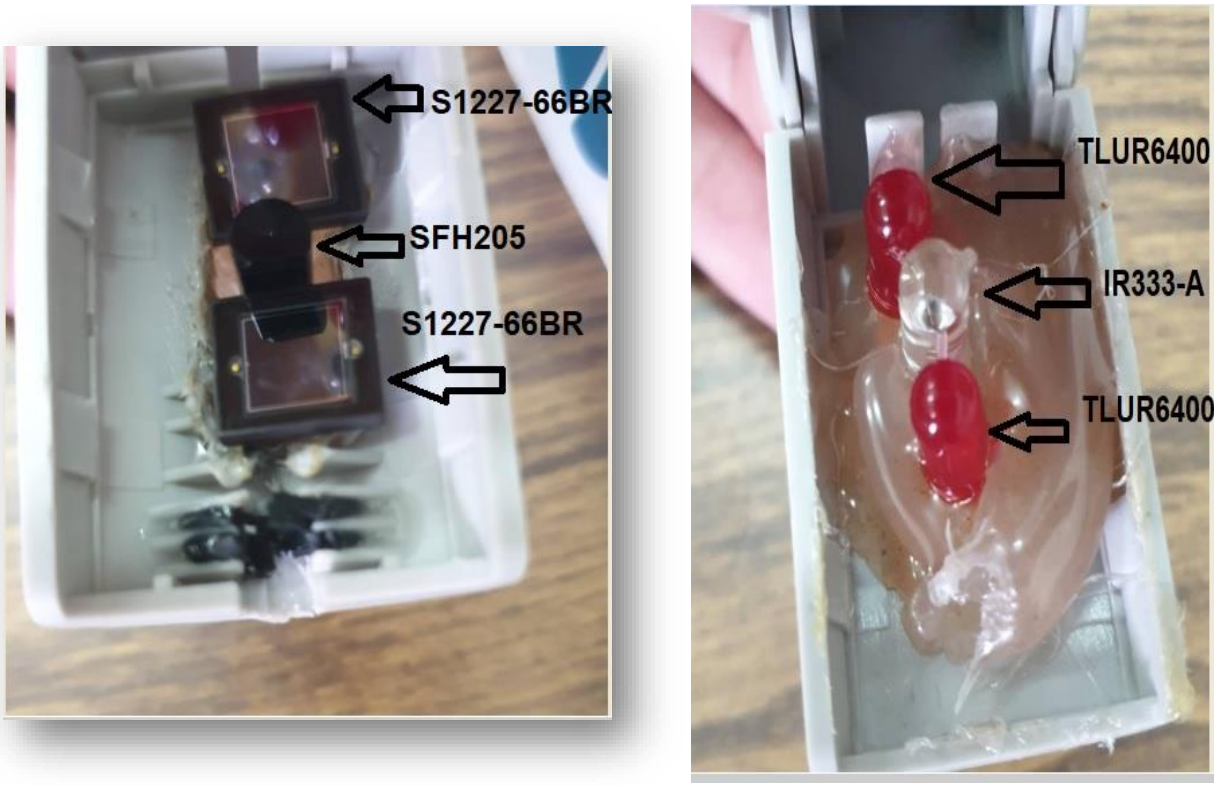

Fig 5.1: LEDs and detectors implementation

Figure 5.2 show IC implementation (LEDs circuit, Transimpedance detector circuit, LPF, and Amplifier circuit

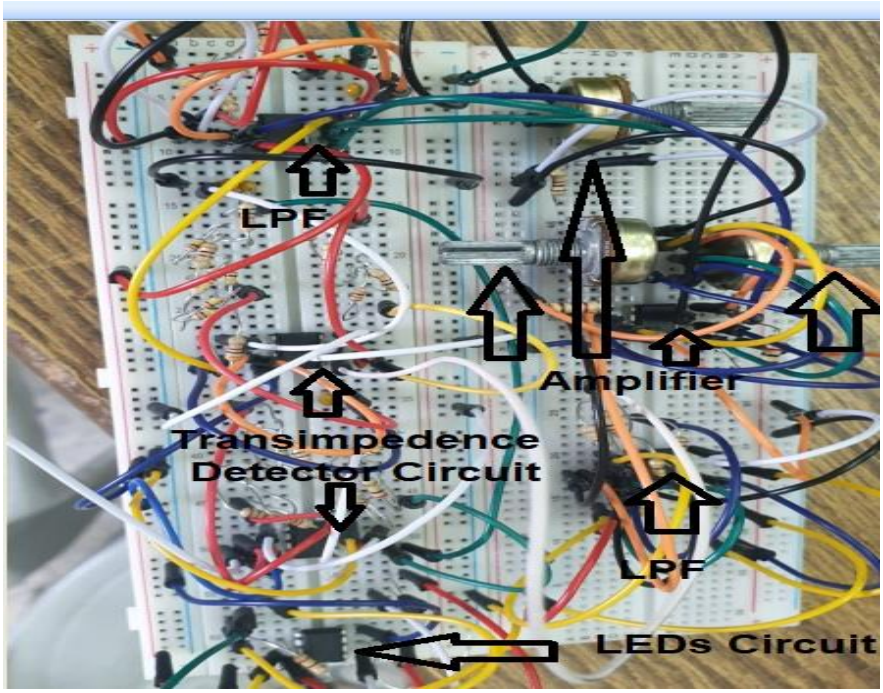

Fig 5.2: IC implementation

5.2 Results of Methemoglobin Concentration

When no light detect by photodiode S1227-66BR, the output as explain in figure 5.3

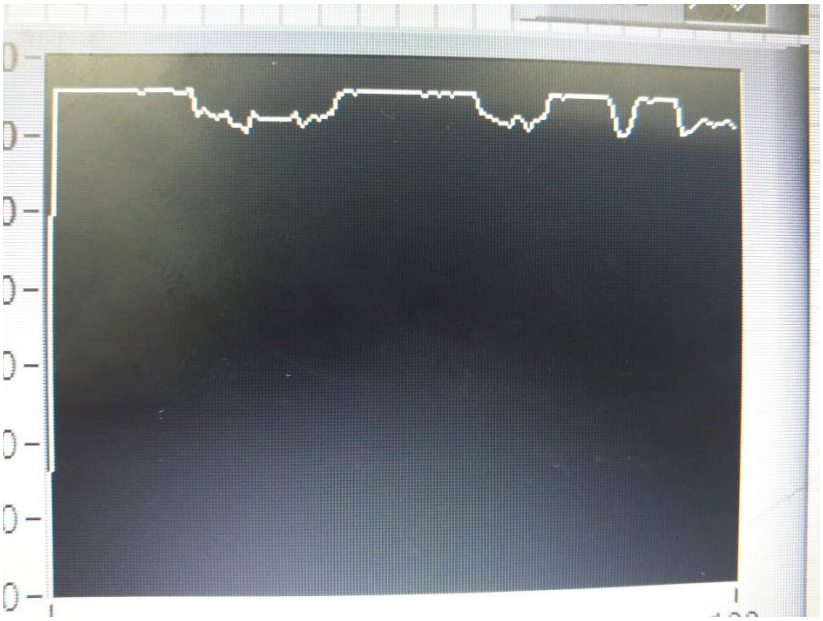

Fig5.3: output of detector1 when darkness

When full illumination light, the output of detector S1227-66BR as explain in figure 5.4

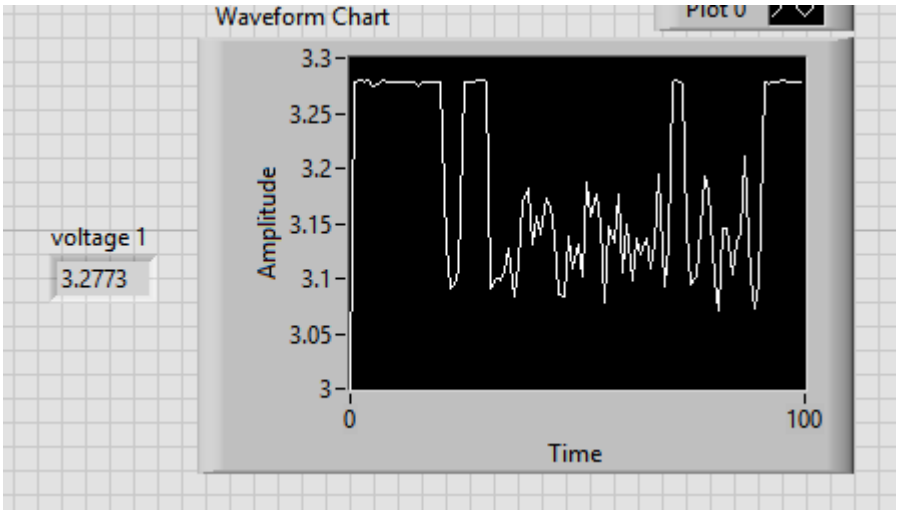

Fig 5.4: output of detector 1 when full illumination

When no light detect by photodiode SFH 205 F, the output as explain in figure 5.5

Fig5.5: output of detector2 when darkness

When full illumination light, the output of detector SFH 205 F as explain in figure 5.6

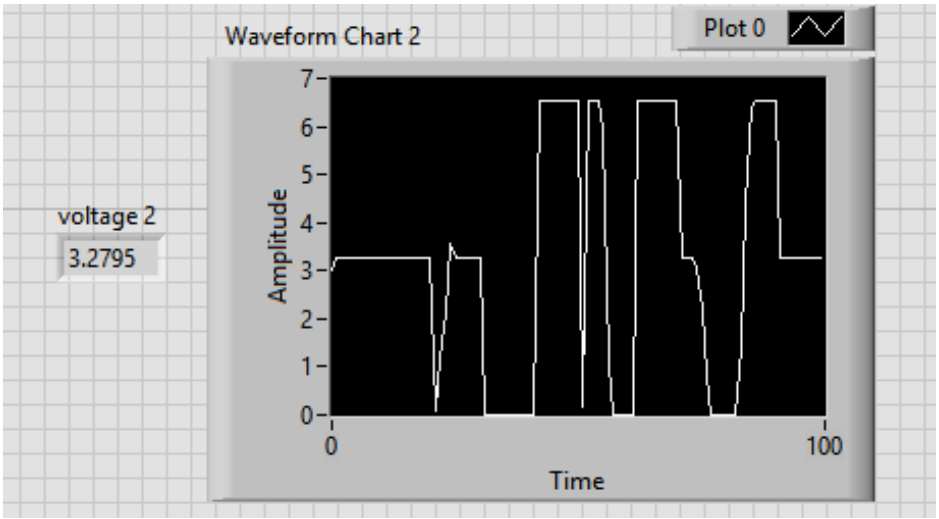

Fig 5.6: output of detector 2 when full illumination

When no light detect by photodiode S1227-66BR, the output as explain in figure 5.7

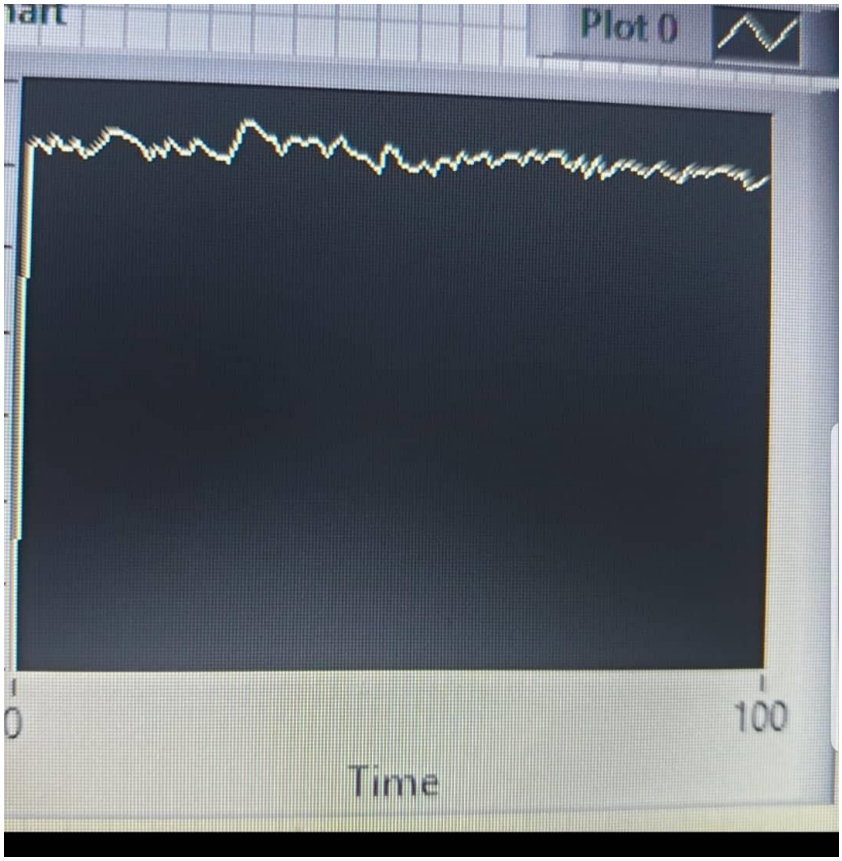

Fig5.7: output of detector3 when darkness

When full illumination light, the output of detector S1227-66BR as explain in figure5.8

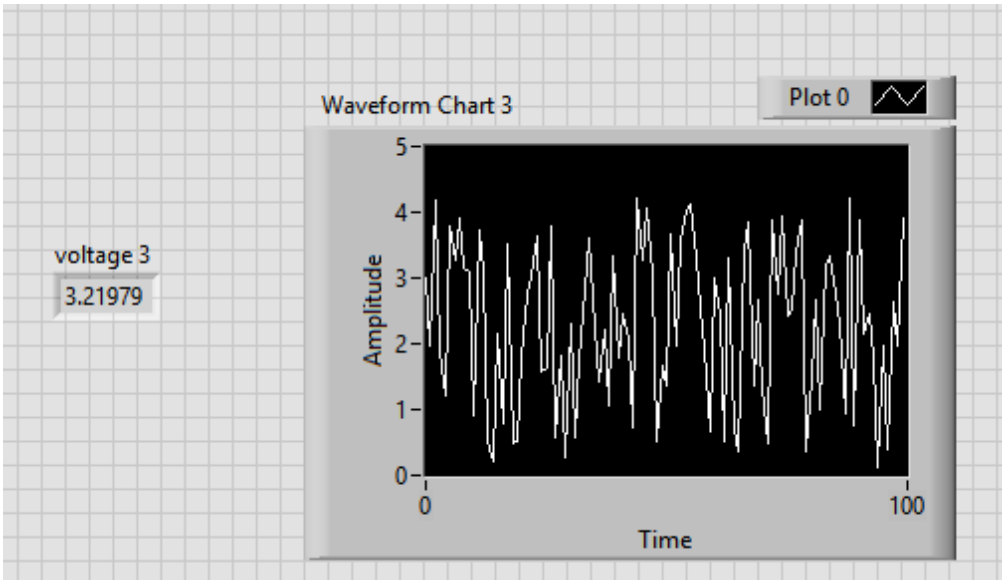

Fig 5.8: output of detector 3 when full illumination

As described in chapter three and four, the acquire signal well be process to find the methemoglobin concentration, figure 5.9 show in front panel of LABVIEW.

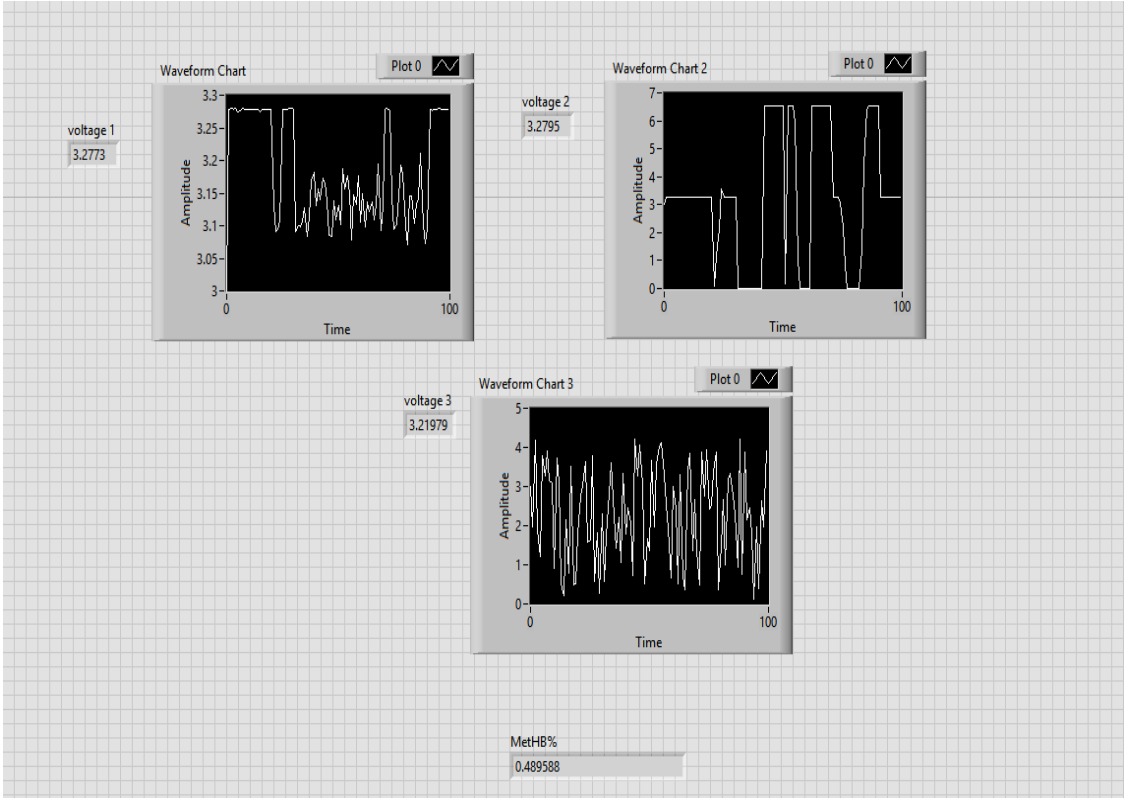

Fig 5.9: front panel of LABVIW

Chapter Six

Future work and recommendation

- Use microcontroller with high resolution
- Compare our result with CBC device, and correct the percentage of error.
- Real time monitoring the peripheral blood pressure and heart rate.
- Develop phone application to send a result and control sample time. In addition, save results.

Challenges:

- Chose spatial IC's, LED and detectors.
- Export electronics part take time.
- Sample taken

Appendix

1- https://www.vishay.com/docs/83171/tlur640.pdf
2- http://www.everlight.com/file/ProductFile/IR333-A.pdf
3- https://www.hamamatsu.com/resources/pdf/ssd/s1227_series_kspd1036e.pdf
4- https://html.alldatasheet.com/html-pdf/1039597/OSRAM/SFH-205-F/359/2/SFH-205-F.html
5- http://html.alldatasheet.com/html-pdf/48425/AD/AD822/43/2/AD822.html
6- http://roboromania.ro/datasheet/Arduino-Nano-roboromania.pdf
7- http://www.ti.com/lit/an/sboa268/sboa268.pdf?fbclid=IwAR3O5Dlh9xUKqhgiDuzq2_2wZ6ZrF33gimF-cw2eYu-6qWY9z3QE6zmC-mEc

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1- **Measurement of the methemoglobin concentration using Raman spectroscopy Mingzi Lu, Lian Zhao, Ying Wang, Guoxing You, Xuemei Kan , Yuhua Zhang , Ning Zhang, Bo Wang, Yan-Jun Guo& Hong Zhou, 2013**

2- **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2977536/?fbclid=IwAR3Bn7gXjYgV96rEdGgJrOH9oDnmRcCsTVHeIBbNkDlWpXcPExE4cxUxAp4**

3- **http://www.biologydiscussion.com/biochemistry/hemoglobin-introduction-composition-derivatives-types-and-abnormality/17385?fbclid=IwAR3XnDFMBugMM2D5rYZTv0TJjPeLvsZnCGRZZJoZQd20pLA7qZOKHewTVfg**

4- **A COMPARATIVE STUDY OF TOTAL HEMOGLOBIN MEASUREMENT TECHNOLOGY: NONINVASIVE PULSE COOXIMETRY AND CONVENTIONAL METHODS Jared S. Ruckman University of Connecticut, 2011**

5- **Design of Pulse Oximeters - Oliver Wieben 1997 - Light Absorbance in Pulse Oximetry, Chapter 4**

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