Non-invasive SPO2 & Blood Glucose Measurement Using Near Infrared Spectroscopy (NIRS)

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Declaration

I certify that this research work titled "*Non-invasive SPO2 & Blood Glucose Measurement Using Near Infrared Spectroscopy (NIRS)*" is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources, it has been properly acknowledged / referred.

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This thesis has been read by an English expert and is free of typing, syntax, semantic, grammatical and spelling mistakes. Thesis is also according to the format given by the university.

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Copyright Statement

Acknowledgements

I am thankful to my Creator Allah Subhana-Watala, who guided me throughout this work at every step and for every new thought which came into my mind to improve it. Indeed, I could have done nothing without precious help and shear guidance of Allah. Whosoever helped me throughout the course of my thesis, whether my parents or any other individual was because of Allah's will, so indeed none be worthy of praise but Allah.

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Dedicated to my exceptional parents whose tremendous support and cooperation led me to this wonderful accomplishment

Abstract

Diabetes mellitus (DM) is a chronic disease in which a body fails to produce sufficient amount of insulin required by the body. It is increasing across the globe at an unprecedented pace and has become a serious health concern these days. The conventional devices available in the market are invasive, pain causing, and require puncturing of skin every single time a person needs to take a glucose reading. Moreover, they are expensive because every time a new test strip and needle are required to check the glucose level. Diabetes is a cureless disease till the date. The only thing diabetic patients can do is to continuously monitor their blood glucose level and take insulin accordingly. Previously, much research has been done in the field of non-invasive glucose measurement by implementing various techniques. However, all previously invented glucometers suffer from inaccuracies due to the relatively weak absorption bands of the glucose in the near infra-red spectrum. They were not proven to be practical, precise, clinically approved and/or economically viable. All of them were either for investigational purpose or market awareness. That is why there was a need to propose a non-invasive approach to deal with all said issues. The proposed prototype is based on NIRS which undergoes photoplethysmography (PPG) and double regression analysis (DRA). DRA helped to increase the accuracy, overcome the deviations and get more reliable results. Near infra-red spectroscopy (NIRS) is most famous because it allows minimum attenuation while measuring the blood glucose level. NIRS helps to deal with the tissues having low absorption energy and permits glucose measurement up to few millimeters depth under the skin. In order to validate the results, a 3 day clinical trial is conducted, to perform in vivo analysis, in Holy Family Hospital, Pakistan, and a total of 132 diabetic and non-diabetic test specimens are analyzed. Non-invasive (NI) system's results are compared with the invasive Beckman Coulter AU-480 chemistry analyzer. The Clarke error grid (CEG) analysis of all 3 day results yields that 98.48% of the results fall in the clinically accepted zone A and the mean and median absolute relative differences (ARDs) values are 8.25% and 7.94%, respectively. The coefficient of determination R^2, depicts the goodness of a fit that how close the predicted values are to the reference glucose values. After the implementation of double regression model (DRM), the coefficient of determination R^2 gets improve to 0.9471.

Key Words: Non-invasive, Photoplethysomography, Chemistry analyzer, Clarke error grid (CEG), Double regression model (DRM) and Absolute relative differences (ARDs).

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CHAPTER 1: INTRODUCTION

The research work presented in this dissertation is about the design and development of a non-invasive prototype to measure pulse rate, blood oxygen saturation and glucose level in the arterial blood. People suffering from different cardiac diseases are advised to regularly monitor their pulse rate and oxygen saturation ratio of blood to ensure stable condition of human cardiovascular system. Literature suggests that for a diabetic patient, glucose must be measured 4 to 5 times in a day. The available conventional techniques are invasive in nature. These are very painful for patients as they involve pricking of finger, puncturing of skin and have chances for a patient to get infected that is why a non-invasive glucose nursing system is much needed. In this thesis research, a prototype is design and developed its viabilities and challenges are inquired. There is no cure for diabetes, till date. The most one can do is to keep an eye by continuous monitoring it. That is why accuracy plays a vital role when it comes to glucose measurement. Thorough results validation is being done, data is collected from different hospitals. Furthermore, result comparison and accuracy analysis is done by doing Clarke Error Analysis (CEG). This chapter focuses on significance of oxygen saturation, glucose concentration and diabetes, pulse oximetry, optical properties of blood, effect of glucose concentration on blood, hemoglobin, its structure and optical spectra, wavelengths used in pulse oximetry and diabetes measurements, Beer Lambert's law, its application in pulse oximetry and diabetes calculations, photoplythysomography (PPG), its principle, basic PPG wave form and its usage, types of diabetes, types of pulse oximeter probes, their advantages and disadvantages, glucose testing, glucose monitors, their working principle and accuracy of those glucometers and pulse oximeters.

1.1 Oxygen Saturation Importance

Oxygen (O_2) is necessary in human body for each cell to work properly. In human body, there are cells which have high metabolic rate and they can be damaged irreversibly if there is a lack of oxygen in the body. Hence, oxygen is an essential parameter of a person's health. Oxygen binds to hemoglobin, present in the blood, when it moves through lungs and it is transported throughout the body as arterial blood. Glen Allen Millikan, a young physiologist, made a light weight device for measuring arterial blood oxygen saturation non-invasively during World War II.

It was an ear oximeter specifically designed for pilots, fighting during war. Those pilots were used to fly at heights in hassled cockpits. In early 70's, an ear oximeter which was used commercially later on, was manufactured by the company named as Hewlett Packard (HP). It was the first extensively used device for measuring SaO_2 [1]. After this invention of non-invasive device, a term "oximeter" was coined to use it in aviation research to measure blood oxygen saturation level of pilots during high altitude flying [2]. Several methods have been developed after that but the basic concept of oximetry is to measure the absorbed sum of light in the body by oxygenated (HbO_2) and deoxygenated (Hb) hemoglobin molecules when the light is being transmitted. In human body, plasma consists of around 2% of oxygen and hemoglobin (Hb) present in red blood cells contains 98% of oxygen. HbO_2 is formed when Hb combines to O_2 likewise HbCO forms when Hb combines to carbon monoxide (CO). In body, a small volume of methemoglobin (MetHb) is existing which cannot combine with oxygen. Therefore, the measure of Hb in the arterial blood in the form of HbO_2 is the percentage oxygen saturation ($\%SaO_2$) and it can be defined as equation 1.1. Adult blood is usually classified into four main classes. They are hemoglobin, oxyhemoglobin (HbO_2), deoxyhemoglobin (Hb), methemoglobin (MetHb) and carboxyhemoglobin (COHb). Multi wavelength oximeters are capable of measuring all these four classes and their ratio is called as "fractional saturation" which is also known as "oxyhemoglobin fraction" or "hemoglobin %" [3]. And it is defined as equation 1.2.

$$\text{Functional } SaO_2\% = \frac{HbO_2}{HbO_2 + Hb} * 100 \tag{1.1}$$

$$\text{Fractional } SaO_2\% = \frac{HbO_2}{HbO_2 + Hb + COHb + MetHb} * 100 \tag{1.2}$$

1.2 Glucose Concentration and Diabetes Mellitus

In living organisms, glucose is the primary source of energy. Insulin, also known as the transporter, provides the key for glucose to enter into a cell and energize it. In body, pancreas are located in vertebrae behind the stomach and insulin is a hormone produced by cells in the pancreas. In this disease, body fails to produce insulin [4]. In the human body, there are two hormones i.e. insulin and glucagon, they maintain the requirement of glucose in the body. The amount of glucagon and insulin in the body, produced by the pancreas determines whether a person is a diabetic, suffering from hyperglycemia or any other glucose relevant issues. Figure 1.1 depicts the

normalization process of glucose in the body. As the amount of blood glucose level increases in the body, amount of insulin also increases and vice versa. Pancreas secretes glucagon into the body during periods, during exercise or between meals. This secretion releases the stored glucose to raise the blood up to the normal range in the body.

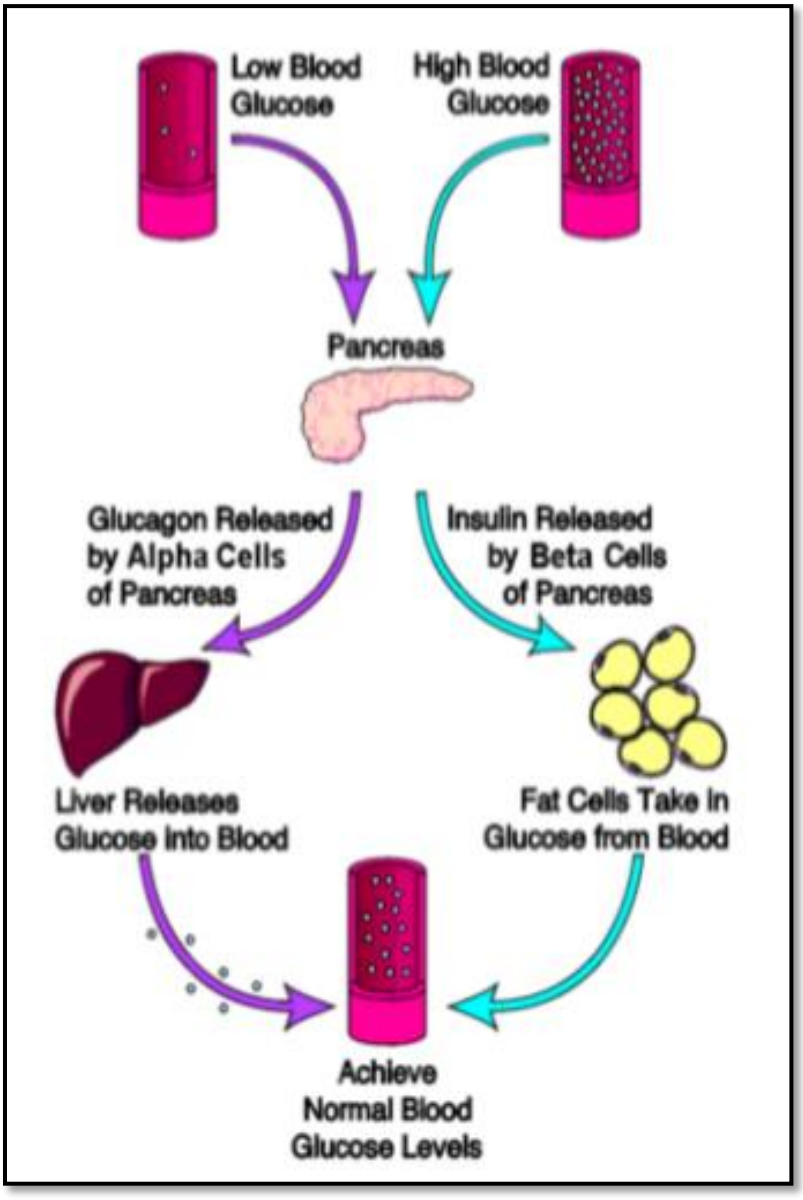

Figure 1.1 Normalization process of glucose in the body [5].

1.3 Pulse Oximetry

An expedient sensor used to detect the blood oxygen saturation is known as pulse oximeter and this phenomenon is called as pulse oximetry. Clinically available pulse oximeters are non-invasive in nature and they monitor the blood oxygen saturation level and the outcome provides the necessary evidence of the cardiorespiratory function [6]. A pulse oximeter uses two different light sources with different wavelengths and frequencies to measure (%) of hemoglobin in the blood whilst observing the light absorbance of tissue and arterial blood pulsation to distinguish the arterial blood absorbance and other absorbers at two wavelengths. And the percentage is called as "oxygen saturation", or SpO_2. Apart from measuring oxygen saturation ratio, it measures heart rate at the same time, too. Conventionally, two light wavelengths are used such as red (660 nm) and near infra-red (IR - 940 nm).

Pulse oximetry follows Beer Lambert's law to measure oxygen saturation ratio followed by an empirically calibrated equation. There are two main techniques in pulse oximetry depending upon the placement of light source and detector. Reflectance and transmittance oximetry, both have their own limitations, pros and cons. Transmittance pulse oximeters can only measure oxygen saturation from the peripheral parts of the body for example ear, toe or fingers etc. Whilst at the other hand, the reflectance oximeters determines the oxygen saturation virtually any part of the body. Since, accuracy of pulse oximeter is partial to low peripheral perfusion that is why there is need to design and develop a robust pulse oximeter which can be used in such physiological conditions.

1.4 Optical Properties of Blood

Human body consists of red blood cells which contain hemoglobin (Hb) that is the iron containing oxygen transport metalloproteinase. It plays vital role in light absorption at different wavelengths [7]. In adult human blood, mainly four species of hemoglobin are present [30]. They are as follows;

- **Oxyhemoglobin (HbO_2)**

  Oxyhemoglobin is the hemoglobin which is fully soaked with O_2.

- **Deoxyhemoglobin (Hb)**

Deoxyhemoglobin is the hemoglobin which is not completely soaked with O_2.

- **Methemoglobin (MetHb)**

Methemoglobin is the oxidized hemoglobin and it is formed when a free hemi iron (Fe+) gets oxidized.

- **Carboxyhemoglobin (COHb)**

Carboxyhemoglobin is the hemoglobin which is formed after its combination to carbon monoxide (CO).

The last two species are present in small concentration because the saturation of methemoglobin and carboxyhemoglobin as a proportion of hemoglobin is between 1% to 2% and 0% to 2.3%, respectively [8]. Due to some reasons like sulfa drugs and exposure to different anesthetics and nitrites, methemoglobin levels may get high apart from ingrown abnormality [9]. Furthermore, an increase level of CO (and COHb) can be observed in the blood of the people who smoke and work in underground garages, mines etc.

Light absorption and scattering characteristics of red blood cells (RBCs) are important in the development of some methods to analyze the blood such as "spectroscopic". The optical performance of human blood relies upon several physiological factors and oxygenation of hemoglobin has the importance of the back bone. Investigation of hemoglobin oxygenation plays significant role in the field of heart surgery, neonatology and rigorous care. It has major impact on the RBCs optical behavior. According to radiation transport theory, there are many factors effecting the RBCs optical properties and they are absorption coefficient μ_a and scattering coefficient μ_s.

The change in the hemoglobin's absorption spectra yields the difference in the blood oxygen saturation. Since, the hemoglobin absorption doesn't change with the SpO_2 level from 1300 nm to 2000 nm but unveils strong saturation dependence in the range of 250 nm to 600 nm. The scattering parameters tempted variations happen mostly from 250 nm to 1100 nm, although the saturation-induced vicissitudes of μ'_s arise from 250 nm to 600 nm [11]. The following figure 1.2 depicts the absorption spectrum of hemoglobin and deoxyhemoglobin molecules in the body.

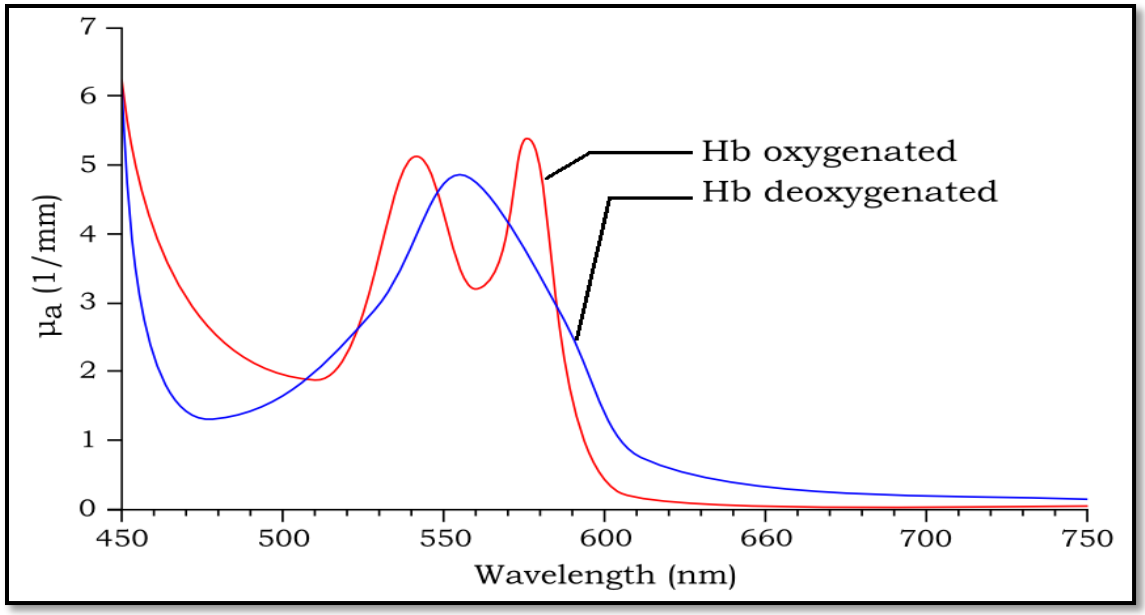

Figure 1.2 Absorption spectrum of hemoglobin and deoxyhemoglobin [11].

1.5 Effect of Glucose Concentration on Blood

Study of glucose concentration on blood is well explained by Caduff et al., they reported changes in the relative permittivity of red blood cells, known as erythrocytes, as a role of glucose in it [12].

The following figure 1.3 shows the hypothetical model mechanism. The glucose structure is divided into two extensive groups i.e. D-Glucose and L-Glucose. Since, Dexter means right in Latin so D-Glucose is also called as dextrose because its solution rotates polarized light to the right. As shown in the following figure 1.3, the glucose transporter GluT1 converts the D-Glucose to ATP and this process of conversion is known as metabolism. The ATP controls the channel for the conveyance of ionic particles i.e. Na+ and K+ [13]. As shown in figure 1.4, the capacitance readings are normalized to the capacitance for 0 mM glucose level. It can be seen clearly that as glucose level upsurges from 0 mM to 20 mM, the membrane capacitance also rises by approximately 30%.

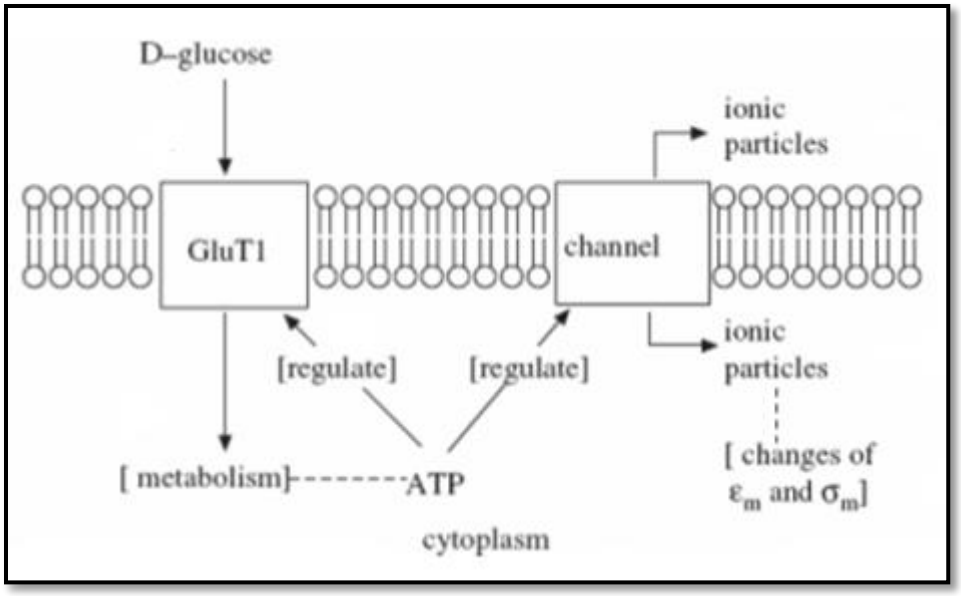

Figure 1.3 Hypothetical model mechanism of glucose [13].

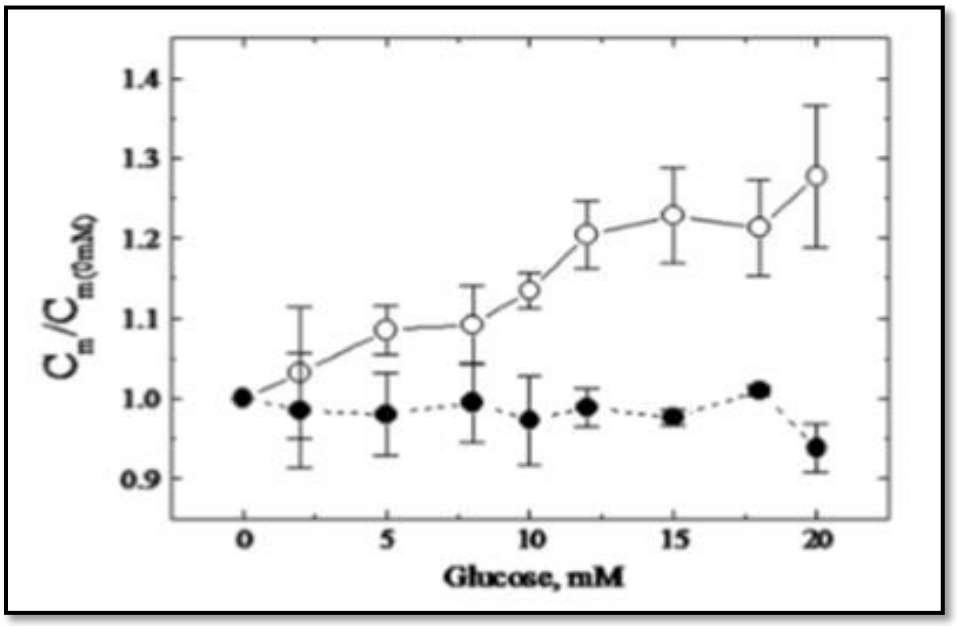

Figure 1.4 Membrane capacitance as a function of D-glucose (white circles) and L-glucose (black circles) concentration [13].

1.6 Hemoglobin (Hb)

In human body, hemoglobin (Hb) is a red protein which delivers the binding mechanism resulting to transport the oxygen in the body [14]. Hemoglobin carries oxygen from the lungs to

the different body parts. There are some iron molecules present in the hemoglobin which are helpful in maintaining the normal shape of red blood cells.

1.6.1 Structure of Hemoglobin

Hemoglobin consists of an iron-containing porphyrin, heme, shared with the protein globin. A porphyrin is a heme cluster consisting of an iron ion detained in a heterocyclic ring. The porphyrin ring is cyclically linked together with four pyrrole molecules. This ring contains an iron ion in the center as can be seen in figure 1.5. A fifth bond is attached to the globin part of the molecule while at the other hand the sixth one is for oxygen. Since, there are 4 iron atoms in every hemoglobin molecule that is why four heme groups are also there. When oxygen gets in surplus, there is a co-operation between these four heme groups [14]. Others slightly change their shape and their attraction to oxygen increases when oxygen drags to any of the clusters.

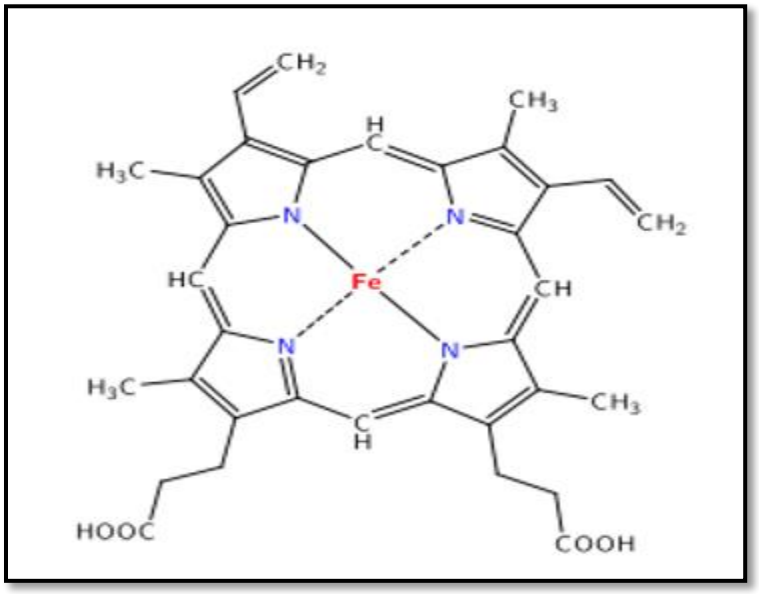

Figure 1.5 Structure of the heme group showing the iron (Fe) atom attached to four pyrrole groups by covalent bonds [15].

1.6.2 Hemoglobin Absorption Spectra

The following figure 1.6 demonstrates the extinction coefficients at the 660 nm and 940 nm wavelengths of the four types of hemoglobin. Methemoglobin absorbs light at both wavelengths equally whereas the absorption of the red light by carboxyhemoglobin is alike to

oxyhemoglobin. Isopiestic point is the point where the extinction coefficients of both hemoglobin species are equivalent. Actually, the isopiestic point is the wavelength at which extinction coefficients of oxyhemoglobin and deoxyhemoglobin are identical and that is 805 nm. The extinction coefficients of carboxyhemoglobin and oxyhemoglobin are same for red light i.e. 660 nm. When it comes to infra-red region, it is almost transparent. At 940 nm, extinction coefficient of methemoglobin remnants higher than oxyhemoglobin whilst it absorbs more light at 660 nm [16].

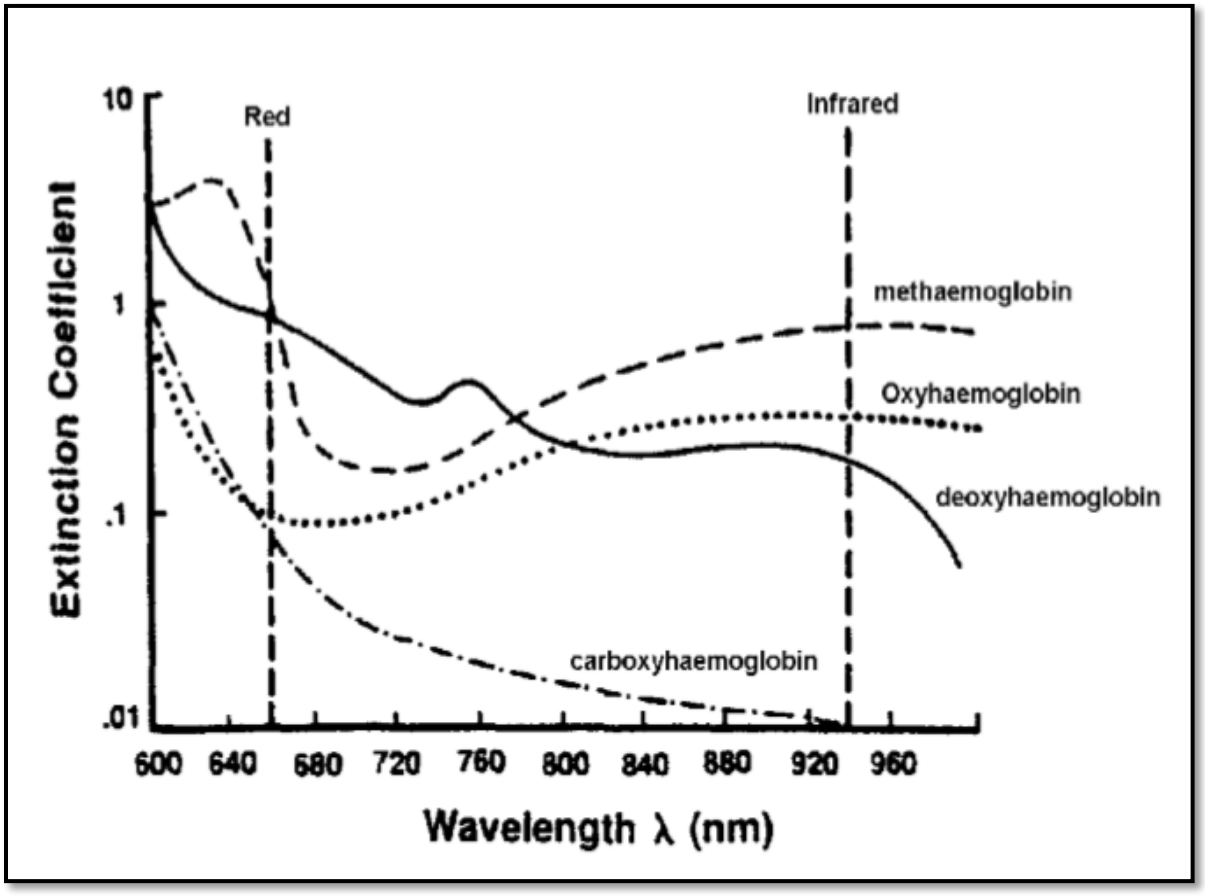

Figure 1.6 Hemoglobin absorption spectra, showing the extinction coefficients ($Lmmol^{-1}cm^{-1}$) of the four types of hemoglobin (reproduced from [2]).

1.7 Cardiovascular System

Oxygen combined with hemoglobin transports around the body by the blood to different cells and tissues which require oxygen for life [20]. Cardiovascular system is the complex system

consisting of the heart, the pulmonary system and the systemic circulatory system and it is responsible for transportation of blood in the whole body [79].

1.7.1 Heart

The human heart is muscular double pump, consisting of four chambers, can be seen in figure 1.7. The upper left and right chambers are identified as atria, and the lower ones are recognized as ventricles. There are four valves situated on each end of the two ventricles. These valves avert the regressive course of blood. When the heart muscles contract and relax, the valves get open and close, respectively. In result, blood flows into the atria and ventricles alternatively. The figure 1.7 shown below depicts the anatomy of the heart.

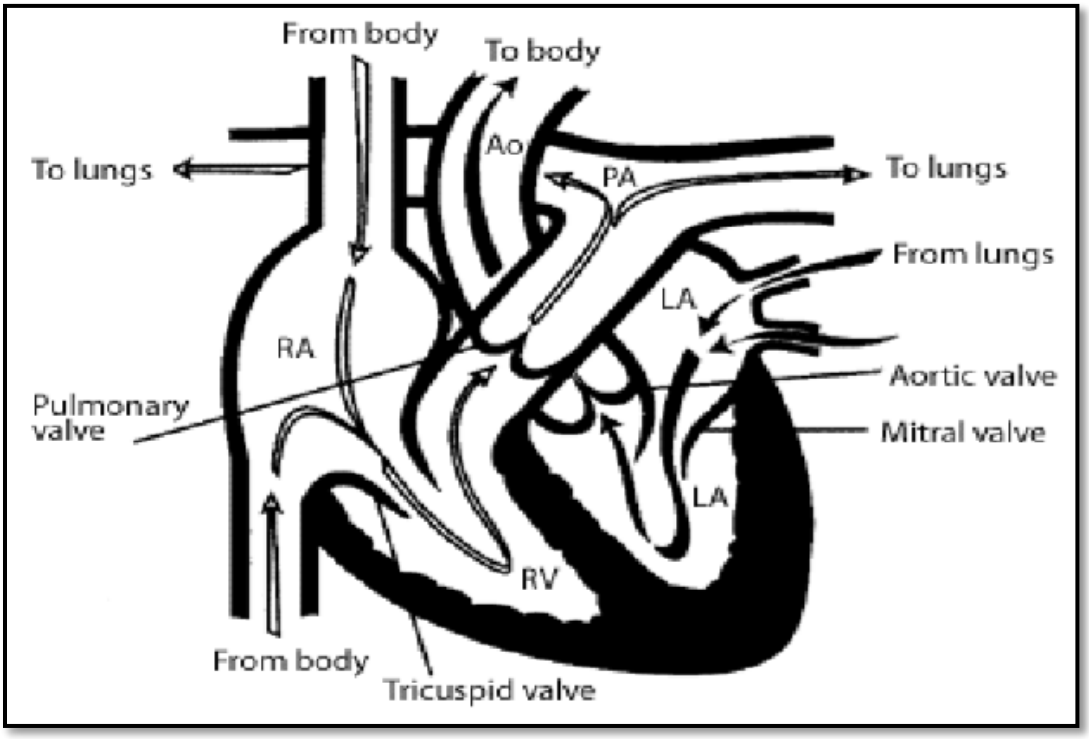

Figure 1.7 Basic anatomy of the heart [81].

1.8 Blood Pressure at Different Parts of the Circulation

Blood flows through the blood vessels along a pressure ramp because any fluid experiences pressure when it is driven by a pump with the help of a circuit of some closed channels. Therefore, the dynamics of the blood flowing in vessels is no exception. Blood in the body, always flows

from higher pressure areas to lower pressure areas. Resistance opposed flow causes that pressure [80]. The following figure 1.8, shows the blood pressure in different parts of the systematic circulation [80]. The systematic circulation is highest in the aorta and decreases till it reaches 0 mm Hg in the right atrium.

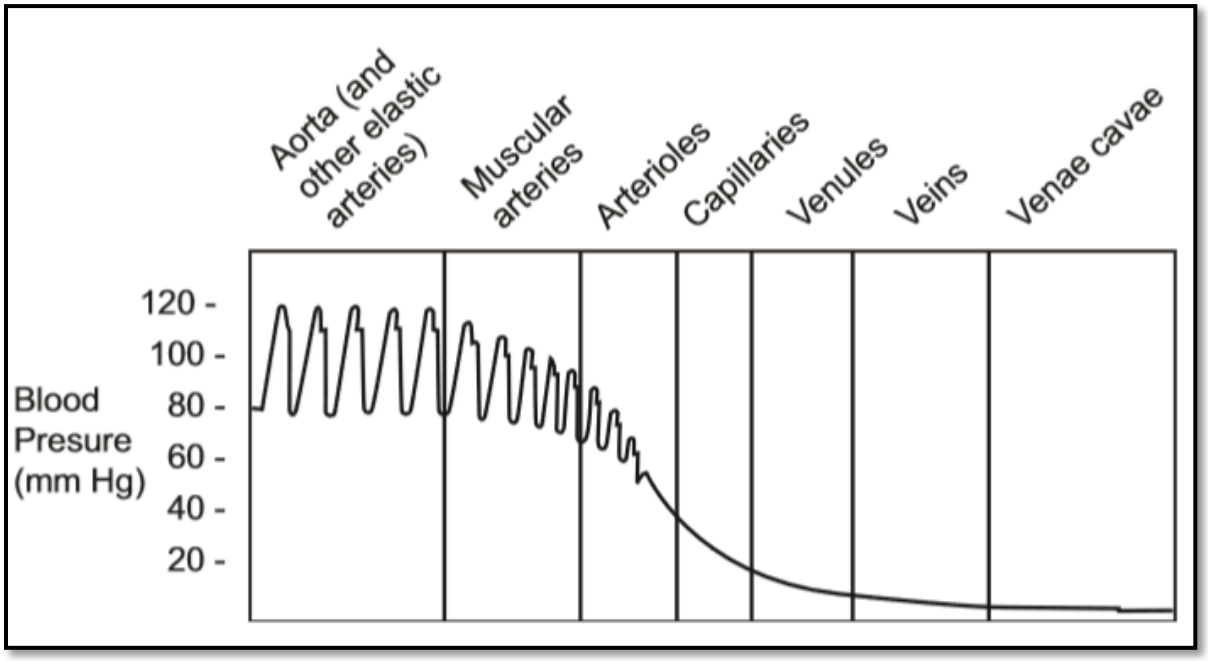

Figure 1.8 Blood pressure in different parts of the systematic circulation [80].

1.9 Blood Flow Velocity

The velocity of blood varies as blood voyages through systematic circulation. Blood flow velocity and cross sectional area of vessels are inversely proportional that is why aorta possess more blood velocity than the other capillaries do [82]. Blood flow rate in the aorta is 40-50 $\frac{cm}{s}$ and it's 0.03 $\frac{cm}{s}$ in the capillaries. Since, tolerable time is always required for exchanges in between tissue cells and blood that is why slow capillary flow is always favorable [83]. The change in the velocity of blood is from 10 to 30 $\frac{cm}{s}$ in vessels, normally [80].

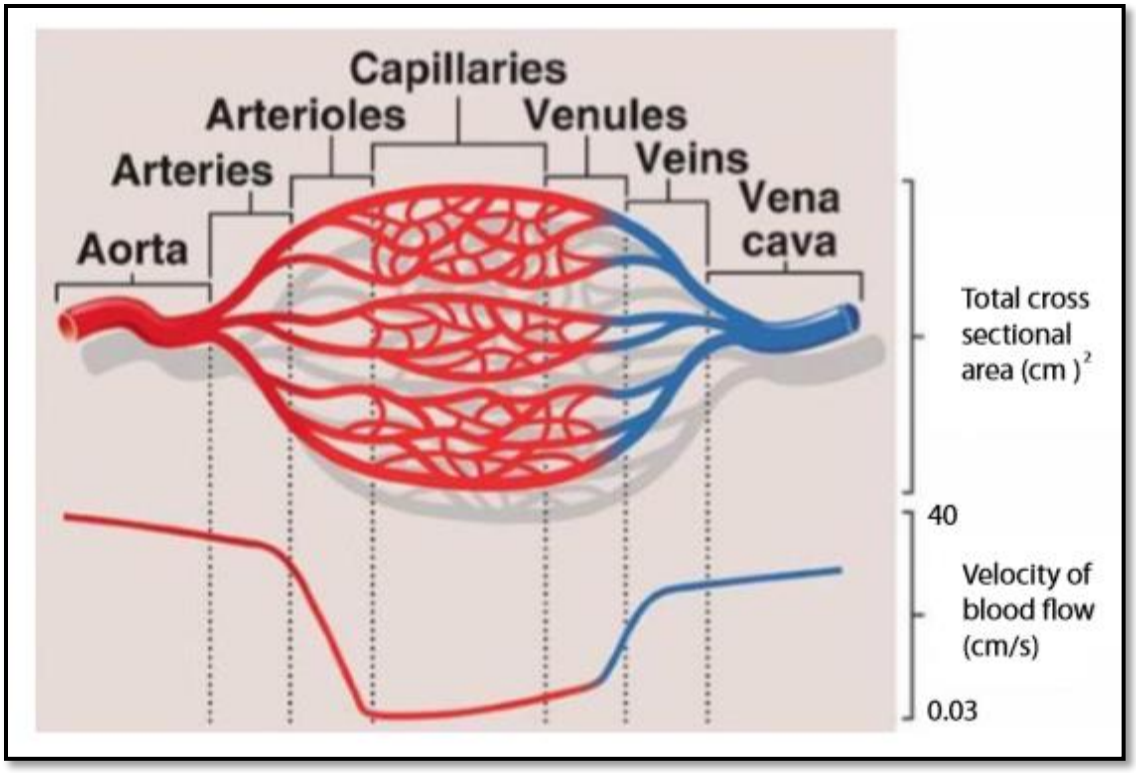

Figure 1.9 Velocity relationship of cross sectional area and the blood flow of the systematic circulation [80].

1.10 Wavelengths Used in Pulse Oximetry

Blood oxygen saturation level is measured by the light absorbed by the tissues at 660 nm and 940 nm wavelengths by a pulse oximeters. For this purpose, pulsatile nature of arterial blood is used to distinguish between arterial blood absorbance and some additional absorbers. These can be human bone, skin and venous blood etc. In pulse oximetry, a good choice of wavelength matters a lot because that is where the huge alterations in the extinction coefficients of deoxyhemoglobin and oxygenated hemoglobin occur [17]. In pulse oximetry, the mostly used wavelengths are the red and near infra-red (660 and 940 nm) as shown in figure 1.10. Minor difference in the peak wavelength makes slight variation to calibration because absorption spectra of oxyhemoglobin and deoxyhemoglobin are reasonably flat at 940 nm wavelength. That is why choice of the near infra-red 940 nm is much easy and feasible for measurements. A huge disparity is observed for absorption spectra of deoxyhemoglobin and oxyhemoglobin at 660 nm. Therefore, selection of red light i.e. 660 nm is much beneficial because it yields visible fluctuations in the absorption with minor alteration in oxygenation [18].

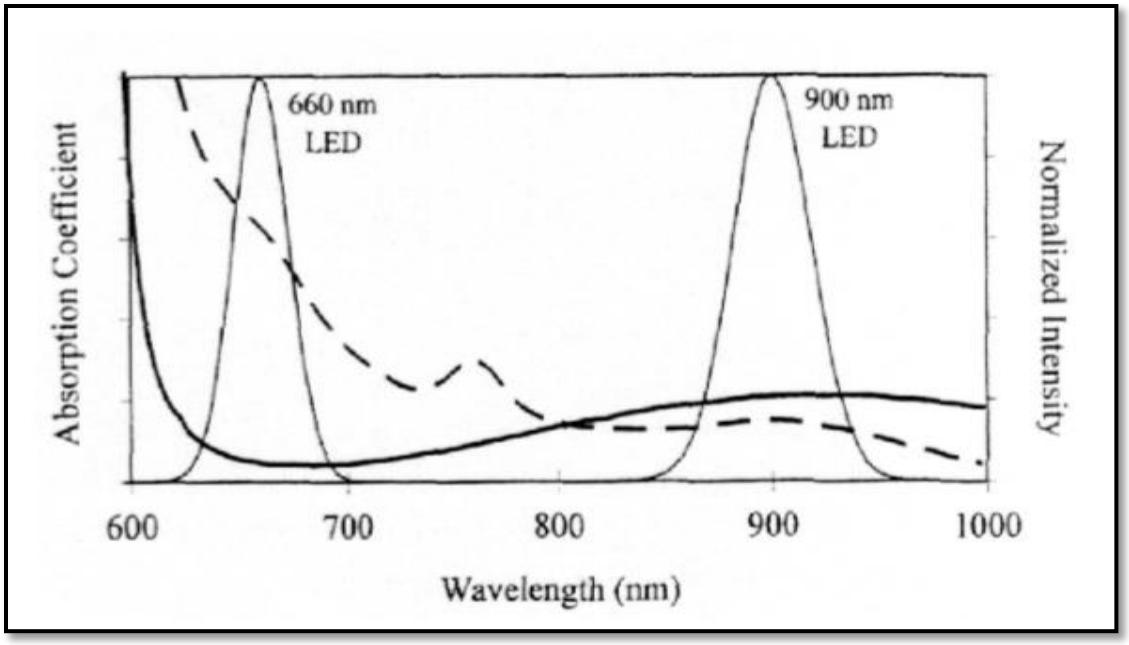

Figure 1.10 Absorption coefficient plot versus wavelength of oxyhemoglobin (solid) and deoxyhemoglobin [18].

1.11 Wavelengths Used in Diabetes Mellitus Measurement

For glucose, to deal with tissues having low energy radiation, normally the near-infrared spectroscopy (NIRS) is used with the wavelength ranges from 750 nm to 2500 nm. In order to get precise results for glucose level, selection of correct wavelength plays a vital role. The range, 700 to 1100 nm wavelength is usually termed as therapeutic window because it lets to measure the glucose up to few millimeters of depth under the skin. Glucose has absorption peaks at 939 nm, 970 nm, 1197 nm in higher overtone region, 1408 nm, 1536 nm, 1688 nm, 1925 nm in first overtone region and 2100 nm, 2261 nm, 2326 nm in combination region [19]. For proposed method, near infra-red LED having wavelength 940 nm is used. The advantage of using this wavelength is that minimum attenuation is offered by some constituents present in human body such as plasma, hemoglobin and deoxyhemoglobin molecules and water etc. to the emitted light signal which yields to find better results. The following figure 1.11 depicts the absorption spectra of body analytes.

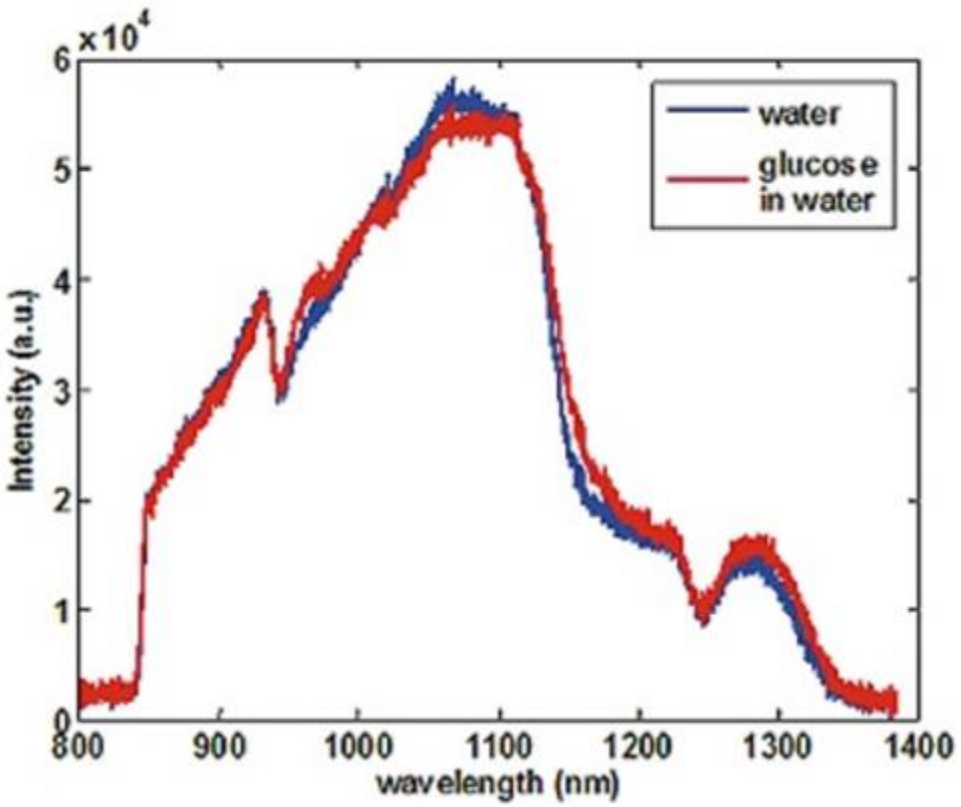

Figure 1.11 Absorption spectra of water and glucose in the body [19].

1.12 Beer Lambert's Law

Determination of hemoglobin oxygen saturation in-vitro by using spectrophotometry was done by Matthews, back in 1930 [2]. This method was based on Beer Lambert's law and referring to the law when a monochromatic incident light intensity passes though the fingertip, some of the light is absorbed in it and some of the part is transmitted through it. Actually, this law defines the light attenuation in a uniform medium through which a light is passing by. The transmitted light intensity I_{out} traversing though the medium decreases exponentially with the distance as shown in equation 1.3, below [2].

$$I_{out} = I_{in} e^{-\varepsilon(\lambda) c d} \tag{1.3}$$

Where $\varepsilon\ (\lambda)$ is the absorbing substance absorptivity or extinction coefficient at a particular wavelength, c is the absorbing substance concentration, and d is the optical path length through that medium. The units of concentration of "c" are $mmol\ L^{-1}$ and $L\ mmol^{-1}\ cm^{-1}$ for extinction

coefficient. The transmittance (T) of light is expressed as the ratio of transmitted light intensity I_{out} to the incident light intensityI_{in}, as shown in equation 1.4 [20].

$$A = - ln\ (T) = - ln\ (\frac{I_{out}}{I_{in}}) = \epsilon cd \quad (1.4)$$

When more than one substance absorbs the light then the Beer's law properties are valid. Beer Lambert's law allows to determine *n* various absorbing substances concentration in a standardized medium and it is possible when the extinction coefficients are known and the light absorbance is measured at *n* different wavelengths. The mathematical depiction of the manifold absorber system is a superposition of the discrete absorbing procedures. Total absorbance of light "A_t"can be expressed as written below in equation 1.5, with *n* absorbing substances is the totality of their *n* self-governing absorbance [2].

$$A_t = \sum_{i=1}^{n} \epsilon_i\ (\lambda)\ c_i\ d_i \quad (1.5)$$

1.12.1 Application of Beer's Law in Pulse Oximetry

Beer Lambert's law of light absorption gets vital importance when it comes to theoretical descriptions of pulse oximetry. But somehow this law is incomplete because it fails to account for the scattering effects of light, adequately. Practically, the scattering light effects are dealt with the empirical calibration of pulse oximeter and different sensors etc. But it is not a perfect way since it works up to a certain point [20]. Some conventions are made during an empirical calibrations and they become unacceptable under extreme conditions because they are limited to a particular range of oxygen saturation values. Nevertheless, the Beer Lambert's law is widely used in pulse oximetry to derive the forms of different equations because when it is a non-scattering absorbing solution, the attenuation of light can be written as a linear equation of the absorbance from every donor [18]. Figure 1.12 describes the absorption and transmitted light in vivo. And the AC and DC component analysis through venous and arterial blood can be seen very clearly. A continual light absorption by the skin correlates to the arterial blood in the body. Referring to figure 1.12, by applying the equation 1.4 at one point in time (at t_1 = diastole), the attenuation effects of all the DC photoplethysmogaphic constituent of tissues can be observed for example pulsating arterial blood, non-pulsating venous blood, and other tissues and bones. By the application of equation 1.4

again at a different time (at t_2 = systole), the AC photoplethysmographic component of the arterial blood is observed because of pulsatile nature of the blood [20].

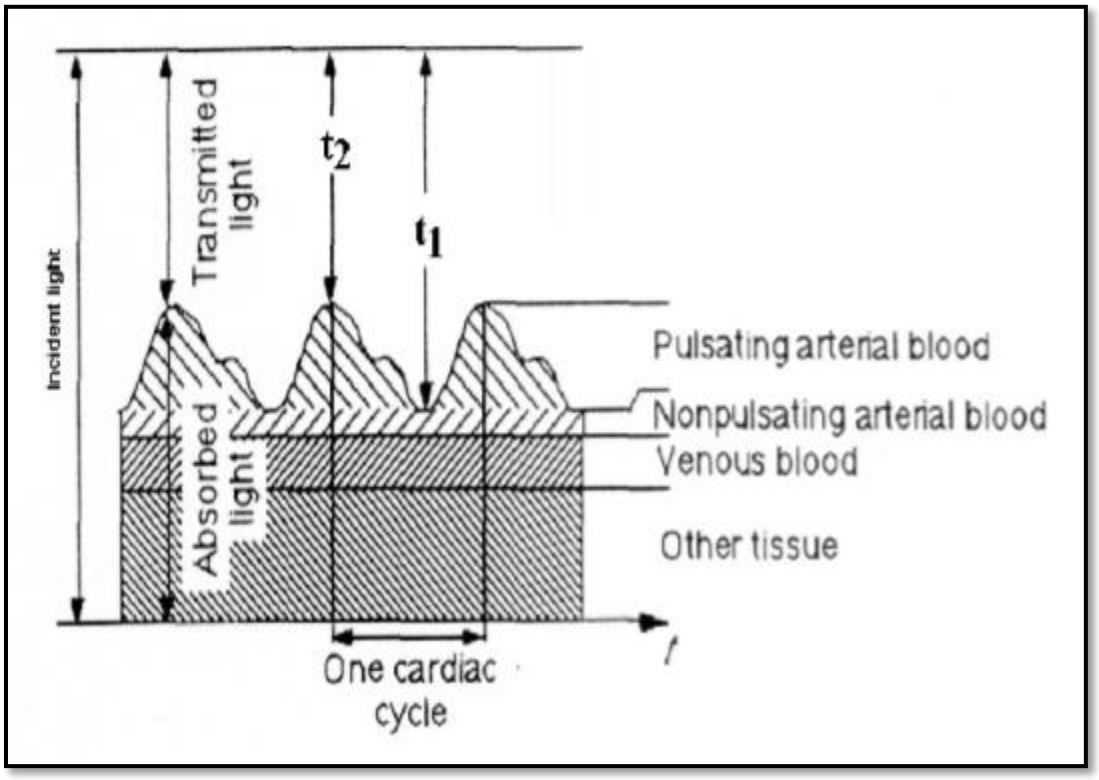

Figure 1.12 Arterial and venous blood configuration in the body [20].

1.13 Photoplethysmography

Photoplethysmography (PPG) is an optical technique which is widely used to sense volumetric changes in the arterial blood circulation. It is a less expensive optical non-invasive technique that is used to determine the oxygen saturation by measuring at the surface of the skin [21]. The photoplethysmographic waveform is basically comprises of an AC physiological waveform accredited to volumetric variations in pulsatile arterial blood. Moreover, it is superimposed on a DC signal. It is observed that generally pulses are generated inside the arteriolar vessels at the point of extreme pulsation [22]. Photoplethysmography is built on the optical absorption properties of vascular tissues when it is trans-illuminated by the light. There is a possibility for tissues to be straight trans-illuminated where the source and the receptor both are on the opposite side (transmittance mode) or where the light source and receptor both are on the same side (reflectance mode). The transmission mode has some restrictions such as it can only be

used for the areas for example the finger, the ear lobe, or the toe, whereas the reflectance mode allows measurements virtually on any skin area [23]. In either reflectance or transmittance, the intensity of the light reaching to the receptor is evaluated and the differences in the receptor's current are related to the blood volume deviations [24, 25]. These distinctions are amplified and further logged as the photoplethysmographic signal to find the blood oxygen saturation level. Pulse oximeters operate on the principle of photoplythesmography by illuminating vascular tissue with visible red and near infra-red light. The pulsatile photoplethysmographic (AC PPG) signal is attributable only to the arterial blood component because of the oxygenated and deoxygenated hemoglobin light absorption at red and infra-red light. The amplitude of both lights is sensitive to deviations in the oxygen saturation ratio [26]. Thus, pulse oximetry is a method which counts on the occurrence of a tolerable peripheral arterial pulse [27].

1.13.1 Principle of Photoplethysmography

When light is absorbed in the body, it passes through the biological tissues. In the body, blood absorbs more light than the surrounding tissues that is why a PPG sensor detects the changes in blood flow as change in intensity because the voltage signal is relative to the amount of blood flowing through the vessels. Although, it cannot measure the quantity of blood but minor fluctuations in blood are noticeable. In arterial blood, volumetric changes in the venous blood volume modulates the PPG signal. Since, mostly blood flow occurs in the arteries instead of veins that's why site of measurement affects PPG signal, and not only the site of measurement but the contact force between the site and the sensor, too.

1.13.2 The PPG Waveform

A typical PPG waveform is divided into two main parts. They are as follows;

- Alternating current (AC).
- Direct Current (DC).

The AC component shows the deviations in the frequency of pulsatile arterial blood with the heartbeat and it is superimposed on the DC signal. While at the other hand, the DC component depicts the slight variations with the respiration. It arises from the transmitted or reflected optical signal by the tissues. The following figure 1.13 shows the AC and DC components.

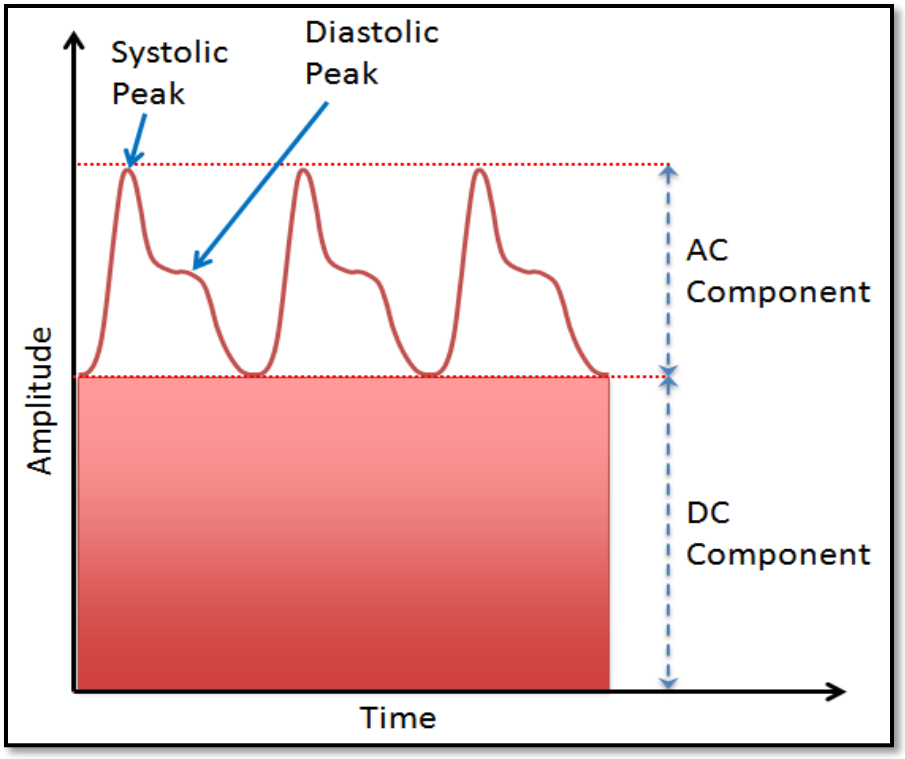

Figure 1.13 AC and DC components [28].

1.14 Hyperglycemia and Hypoglycemia

In case of insulin deficiency of insulin, glucose remains in the blood because it cannot enter into the cell. The body exhaust through urine and tries to get rid of extra sugar by drawing water from the cells. This type of situation is known as hyperglycemia. People suffering from hyperglycemia report frequent thirst and possess high sugar levels in urine. Whilst, hypoglycemia is a state in which a person's blood sugar is always low. Even lower than a normal range. Hence, cells depriving from glucose send signal to the brain to eat more food. In order to that, glucose may be readily available to body. So such type of patients are always starving. Body begins to convert fats and proteins into glucose to provide energy. This break down of fats leaves a chemical compound in the blood named Ketone, which is excreted in the urine (ketoacidosis).

1.15 Types of Hyperglycemia

There are mainly three types of diabetes and there details are as follows;

1.15.1 Type 1

Type 1diabetes is the most serious type of diabetes. People usually develop this type of diabetes in their childhood or adolescence. Type 1 diabetes is developed when there is a huge lack of insulin secretion in the body. This causes hyperglycemia in young teenager patients and has a marked tendency toward ketoacidosis [4, 29]. Every 10% out of 100 suffers from Type 1 diabetes.

1.15.2 Type 2

This is the most extensively found type and every 90% patients out of 100 suffers from Type 2 diabetes. This type of disease usually develops in adults and the people who are bit fat. This happens when body cannot fulfill the requirements of the insulin or the pancreas fail to produce as per body requirements [4, 29]. This type of diabetes can be cured by closely monitoring the diet and exercise. Patients of this type of diabetes inject insulin in their body with the help of an injection or a pump, whilst the other hand, type 2 diabetics can control it by a proper diet plan i.e. healthy eating, exercising, controlling obesity and taking oral medication.

1.15.3 Gestational

Third type of diabetes mellitus is gestational diabetes which affects females during their pregnancy. Females possessing this type of DM may undergo such high levels of glucose that their body fails to produce enough insulin. Perhaps, proper diet, medication and exercise yield better controlling of the said disease.

1.16 Acceptable Range of Glucose

The acceptable range of blood glucose is from 70 mg/dL (milligram of glucose in 100 milliliters of blood) to 110 mg/dL or 3.9 to 6.0 mM/L. But after consuming some meal the glucose concentration increases to a level as high as 140 mg/dL [30]. Blood glucose should come to a normal level two hours after eating, and in failing to do so will result in diabetic symptoms. Well some define the normal range as 60 to 180 mg/dL, too. In different literature, slight variation is observed that is hypoglycemia happens when the glucose level decreases more than 70 mg/dL, hyperglycemia befalls when the glucose level exceeds 180 mg/dL, and an individual is diagnosed with diabetes when the level exceeds 200 mg/dL after drinking a glucose enriched drink [31].

1.17 Reflectance and Transmittance Pulse Oximeters

There are two types of pulse oximeters, which use two different types of probes. On the basis of that, they are classified into two categories.

- Transmittance pulse oximetry probes.
- Reflectance pulse oximetry probes.

Both of these types are briefly described below.

1.17.1 Transmittance Pulse Oximetry Probes

Usually transmittance sensors are used in the majority of the commercially available pulse oximeters [20]. In this type of configuration, the fingertip is placed between the emitters and the receptor. Light is emitted by the LED source and passes through the fingertip and finally it reaches to the detector to find the oxygen saturation ratio, as described in figure 1.14.

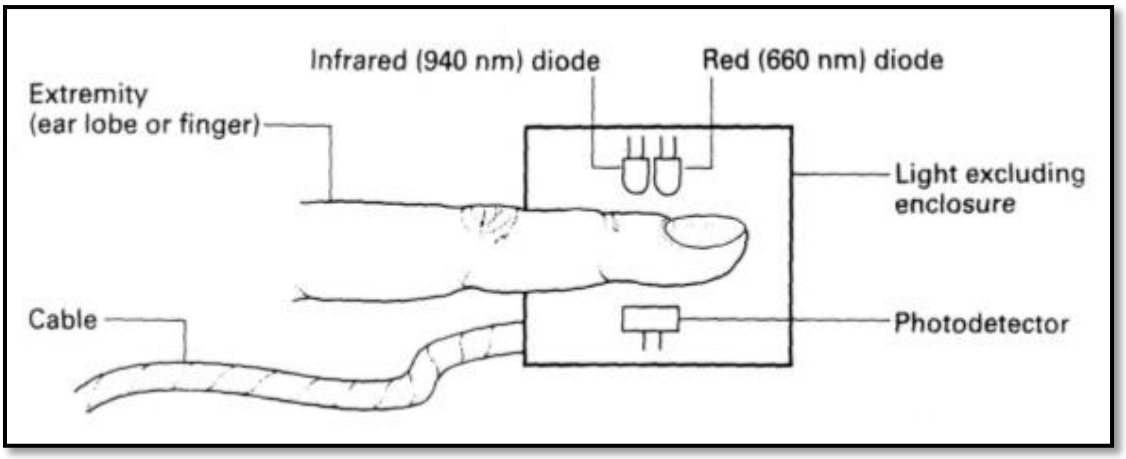

Figure 1.14 Transmittance pulse oximetry [32].

The probe includes two, red and infra-red, emitters. To let the light to pass through the tissue at any given point in time, the emitters are switched on and off alternately and the light reaching to the receptor is detected by the photodiode. At any given point in time, the intensity of the light get reduced by the arterial blood in the issue and it is used as to measure the heart rate because it varies with the arterial pulse [32]. The emitters are mounted at the opposite side of the photo detector in the transmittance oximetry because the photodiode detects the extreme light

diffused through tissue. The position of receptor matters a lot. It has to be somewhere near to the skin deprived of applying force on the tissue. Most of the time patient's finger, ear lobe or toe are the places to be inserted in the transmittance probe.

1.17.2 Reflectance Pulse Oximetry Probes

Transmittance pulse oximeters can only measure oxygen saturation from the peripheral part of the body such as ear, finger and toe etc. [33]. When it comes to measure the blood oxygen saturation virtually any part of the body, reflectance pulse oximeters are used. In reflectance pulse oximeters the light is transmitted in all directions from the emitters. A photodetector should sense all the light coming from the emitters in order to detect most of the back scattered light from skin. The output of the receptor is handled by the pulse oximeter in order to evaluate oxygen saturation level [33]. The figure 1.15 depicts the configuration of a reflectance pulse oximeter probe. The emitters and photo detector are placed on the same side adjacent to each other. In reflectance pulse oximetry, the most common surfaces for monitoring the oxygen saturation are the forehead and temple [34].

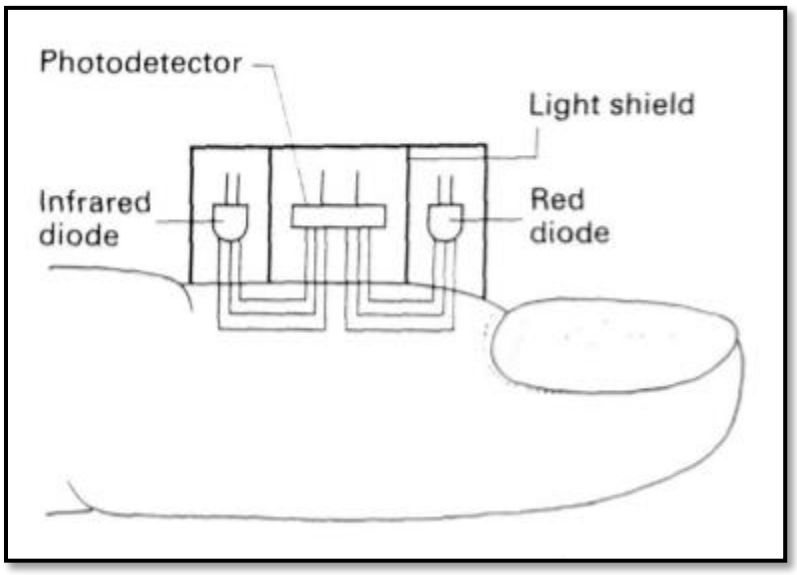

Figure 1.15 Reflectance pulse oximetry probe [32].

1.18 Advantages and Disadvantages of Transmittance and Reflectance Pulse Oximetry

The basic benefit of transmittance oximetry is the larger intensity of light usually sensed by the photodetector and therefore better signal to noise ratio (SNR) [33]. In transmittance pulse oximetry, increased light intensity is sensed by the receptor because the light diffused through the body and tissues is more than the reflected light. The quality of signal is better in case of transmittance oximetry if a robust light intensity is focused on a specific area. In comparison to the transmission through a relatively thick tissue volume, chances of the reflected light photons are much higher when the incident light intensity is reduced considerably. The drawback of the transmission pulse oximetry is that the instrument usage is confined to peripheral parts of the body such as ear lobe, toe and finger etc. or the foot and palms of infants. On the other hand, in reflectance pulse oximetry, the blood oxygen saturation level can be measured virtually from any part of the body. Referring to the AC component and the pulsatile nature of the arterial blood, the deviation in the photoplythsmographic signal relies on blood perfusion, bones, thickness of tissues and the intensity of light emanated from the light source.

1.19 Glucose Testing

Blood glucose tests are made to evaluate the blood glucose levels in the body to diagnose if the person is diabetic or not. Some cells in the brain and red blood cells are reliant on the blood glucose since it produces energy. Human brain needs certain amount of glucose concentration in the blood to functional normally. In the body, glucose concentration less than 30 mg/dL or greater than about 300 mg/dL produces unconsciousness [35].

There are three tests used to test for the disease

- The fasting blood sugar test (FBS)
- The 2-hour postprandial blood sugar test (2 hour PC)
- The random blood sugar test (RBS)

1.19.1 Fasting Blood Sugar Test (FBS)

The FBS test is the most commonly used test. In this test a patient's body response is tested, after fasting for at least 8 hours, to a large amount of sugar into the patient's body. A reading above

100 mg/dL is normal, while a reading above 125 mg/dL is diagnosed as diabetes. Readings in the middle show a potential for pre-diabetes.

1.19.2 2-Hour Postprandial Blood Sugar Test (2 hour PC)

This test is done after 2 hours a patient eats something. It is to determine how food affects the patient's body glucose level.

1.19.3 Random Blood Sugar Test (RBS)

The RBS test is done at random at any point in the day. A reading of 180 mg/dL or more along with other symptoms i.e. increased in thirst or unexpected weight loss etc. these symptoms indicate that the person is suffering from diabetes.

1.20 Home Glucose Tests

Home glucose tests are used by diabetics when they are away from a doctor's office or hospital. Typically, testing has to be done at least once a day. But depending on the severity of the patient, several times a day can be required. That kind of home testing is done with a device called glucose monitors or glucometers. Various types of glucometers are pictured in the figure 1.16.

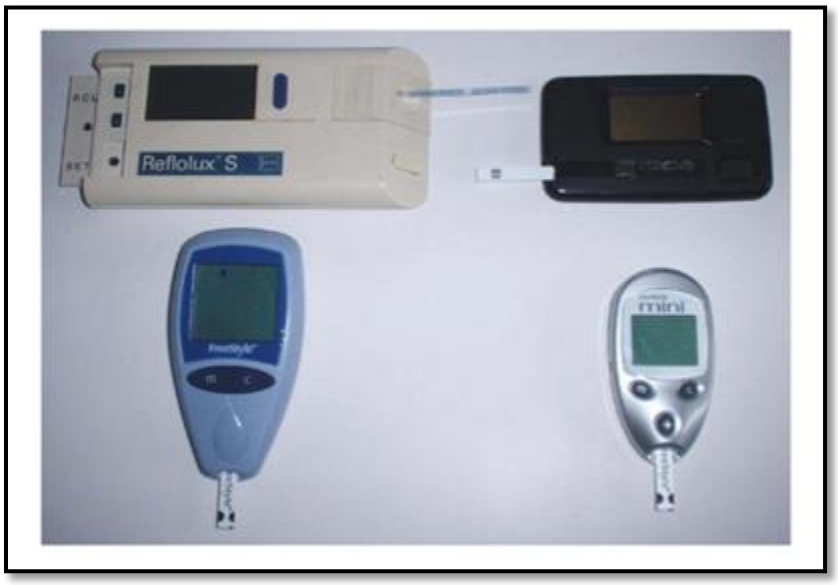

Figure 1.16 Different blood glucose meters [36].

1.21 Working of Glucometers

All commercially available glucometers are invasive and they require pricking or puncturing of a finger, palm, or forearm by using a lancet to collect few micro liters of blood. These glucometers undergo electrochemistry of tests strips to scrutinize the blood glucose. There are ten layers of spacers and chemicals, including glucose oxidase and microcrystalline potassium ferricyanide in each test strip of glucometer. The blood flows by capillary action into the test strip and the blood glucose reacts with the glucose oxidase to form glycolic acid, resulting to a reaction with the layer of ferricyanide to form ferro cyanide [37].

1.22 Accuracy of Glucometers

The measuring range of the commercially available glucometers is in between 0 mg/dL and 600 mg/dL. This range may vary meter to meter and the glucose results are not linear over total range. Moreover, these glucometers possess 6 to 7% error as well. That is why extremely low and high results are open for interpretation. It is required to confirm them either by taking various readings from different glucometers or by taking the repeated measurement from the same meter [38].

1.23 Accuracy in Different Oxygen Saturation Levels

All pulse oximeters undergo absorption spectrophotometry [20]. However, different manufacturers use different methods of obtaining and processing the data. Many factors could be the reason for the differences such as sampling frequency, LEDs, microprocessor programming and the look up table. It has been observed that accuracy of oxygen saturation is not the same at different levels.

Oxygen saturation can be categorized as follows:

- High saturation
- Normal saturation
- Low saturation

1.23.1 High Oxygen Saturation

The high range of a pulse oximeter is considered to be greater than 97.5%. The range of expected reading of a pulse oximeter is less than or equal to 100% and for this range the pulse oximeters are designed, which restricts positive errors and makes results better [20]. By the time when oxygen saturation level is more than 97%, the patients' conditions are encouraging, which does not require and emergency attention regarding oxygen saturation.

1.23.2 Normal Saturation

The normal range of a pulse oximeter reading is from 90 to 97.5% because pulse oximeter performs well in this range. The absolute mean error was found to be less than 1% in a study of the assessment of different pulse oximeters in this range [39]. Many other researchers also reported a reliable and accurate performance of pulse oximeters in this range.

1.23.3 Low Saturation

Although, there are major advancements in blood oxygen saturation monitoring but pulse oximeters fail to be used for measuring oxygen saturation level below 80%. The reason for this can be explained by absorption spectra and the wavelength used in pulse oximetry as shown in the figure 1.7. The absorption of hemoglobin at 660 nm is very high when the saturation is low, as compared to the high saturation level. Therefore, a slight error in peak wavelength may yield erroneous results.

1.24 Accuracy in Low Perfusion

The pulse oximeter readings are driven from the photoplethysmographic (PPG) signals and those signals illustrate the volumetric changes in the blood volume.

Deficiency of these pulsations would lead to the failure of the pulse oximeter. Reduction of the peripheral arterial pulsation caused by hypothermia, vasoconstriction, hypotension or cardiac arrest may affect the quality of the PPG waveform to be processed by the pulse oximeter [40, 41].

1.25 Accuracy in Motion

The performance of a pulse oximeter is badly affected by the movement of the patient. Including respiration. Because the PPG waveform can be overlapped by the frequency of these movements. The filter used in the pulse oximeters are of fixed cut-off frequency that is why it is difficult to filter these movements' artifacts [42].

Different approaches have been used to overcome this problem [43, 44, 45, and 46]. Motion artifacts i.e. trembling, exercise, or seizures are known by wrong heart rate or distorted PPG waveform. The R wave in the patient's electrocardiogram is also used to coordinate the optical assessments.

1.26 Hypoxia

Hypoxia is a disease which is defined as the lack of blood oxygen saturation level in the body tissues. Hypoxia is such a terrific disease that it can be the reason of the death of the cells but in fewer serious cases it depresses the mental activity and reduces the work capacity of the muscles [84].

Reasons of hypoxia can be many such as

- Insufficient conveyance and distribution of oxygen
- Insufficient tissue competences of using oxygen

1.27 Interim Conclusion

In this chapter, importance of pulse oximetry, heart rate and blood glucose monitoring was discussed. Their effects on human body are elaborated and it has been illustrated that how much it is important to continuously monitor the said parameters. Photoplythysmography (PPG) is explained because this is the basic technique followed for heart rate and SpO_2 calculation. The basic structure of a PPG signal is explained that how it depicts the absorption characteristics of the light absorbed in the body and how tissues and bones react differently to it. Diabetes mellitus is a chronic disease which requires frequent monitoring that is why glucose testing is also explained. In the last, accuracies of blood glucometers and pulse oximeters are discussed.

CHAPTER 2: LITERATURE REVIEW

Literature review is a key part of a research work. It is proven to be a beacon and leading us to achieve our set objectives. It makes us worthy to comprehend the previous work done by different enthusiasts. Also, it provides us a clear path to thoroughly analyze that work. This chapter focuses on the various research work carried out by different authors in the field of non-invasive pulse rate, blood glucose and blood oxygen saturation level measurement in arterial blood. In recent years, many major advancements have been done in this field to get better accuracy in results. In this chapter, multi research parameters different researchers are also discussed.

Rabinovitch et al. was the first person who made a device and proved that in vivo blood glucose measurement is possible, back in 1982. He measured the blood glucose levels by the optical rotation of the aqueous humor with scleral lens [55]. Likewise, Zeller et al. was the first person who detected the blood glucose level for an implantable artificial endocrine non-invasively by using the near infra-red spectroscopy (NIRS), back in 1989. He developed a sensor for a long time stability by using NIRS which responds directly to the glucose in the body. The investigation was carried out by using five different near and middle infra-red range. Only the 1040 cm^{-1} frequency gave the best measurement results for glucose and at this wavelength the signal was less affected by other substances like lipids, urea and proteins etc. [63].

Gelao et al. described a method to measure non-invasive and continuous blood glucose in the body based on dielectric spectroscopy [47]. The electrical circuit, schematic and the PCB designs were capable of working in a frequency range of 1 MHz to 160 MHz. The proposed design easily acquires the dependence of frequency for amplitude as well as phase of the dielectric constant and body glucose was estimated by measuring the change in the permittivity of the tissue.

Ashok et al. proposed a method based on Trans illuminated laser beam for valuation of non-invasive blood glucose. The author used an atomic gas laser which operates at 632.8 nm wavelength and also known as He-Ne laser. Since, monochromatic light source was required so single laser mode is being used. Using single mode laser eliminates the interference noise. By the implementation of this technique, it was possible to monitor the blood glucose level continuously and non-invasively for diabetic patients [48].

Abdallah et al. evaluated various methods for detecting blood glucose measurement, non-invasively. The proposed method was based on an optical multi sensor. Elastic and inelastic such as Raman scattering spectroscopy, fluorescence and infra-red spectroscopy were also discussed. The discussion was for development of a sloid device for continuous non-invasive glucose measurements at home. The sensor used in the technique measures the fluorescence and light scattering in the tissue through the finger, in and round the visible spectrum band of an optical window [49].

Burmeister et al. measured the blood glucose across human tongues by using the near infra-red spectroscopy. The author used an experimental protocol for collecting non-invasive human spectra. In this paper, author has used the first overtone transmission spectra and light is being passed through the tongue of five diabetic patients. Despite of the huge scattering, the correlations were not related to temporal variances because the protocol separates the glucose concentration and the time [50].

Wang et al. developed a prototype to measure non-invasive blood glucose working on the metabolic heat conformation (MHC). The system utilized three temperature sensors, two humidity sensors, an infra–red sensor and an optical measurement device. In the proposed prototype, the blood glucose level was assessed from the quantity of heat dissipation, local tissue blood flow rate and degree of blood oxygen saturation. Clinical trial was conducted and compared with the results with the commercial automated chemistry analyzer. Correlation depicts that correlation coefficient gets improved when heat dissipation is summed in from the evaporation of the skin. The achieved correlation coefficient was equal to 0.914. Although this result is closer but not adequate for clinical purpose [51].

Prof. Mrs. A. A. Shinde et al. discussed the application of occlusion spectroscopy. In this regard, a circuit is designed using infra-red sensors and FFT analysis is performed. Furthermore, the results generated from the spectral response were related to subject's health condition. Author used a NBM device by OrSense Ltd. to compare the results. The experimentation demonstrated the design issue and this information for identification of glucose concentration though it needs further analysis [52].

Amir et al. described the non-invasive glucose measurement using the same occlusion spectroscopy as described previously. In this method, author passed the light through the finger and light present on the other side of the finger was measured as photons. A clinical survey was conducted to quantify the in vivo analysis with the NBM device. Total 23 patients were examined for results validation. 12 of the patients were of type-1 and 11 of type-2 diabetes. Accuracy was evaluated by comparing the predicted results with the NBM data [53]. Clarke Error Grid (CEG) analysis is performed and the mean relative absolute difference was 17.2%. CEG analysis yields that 95.5% of the measurements lie in the clinically accepted zone A and B with 69.7% in zone A and 35.7% in zone B [53].

Unnikrishna et al. represented a system capable of measuring blood glucose using near infra-red spectroscopy. The proposed technique comprises of transmission and reception of near infra-red rays through fingertip. Blood flow is occluded in the fingertip before and after the near-infrared (NIR) light is passed through it. Glucose levels are estimated by measuring the received signal intensity changes and wirelessly transmitted to a remote PC [54].

Maruo et al. presented the near infra-red diffuse reflectance spectroscopy to determine the in situ non-invasive blood glucose. Light source and receptor were placed on the forearm skin surface of the patient by a distance of 0.65 mm. This kind of arrangement helped to reduce the noise caused by stratum corneum. Partial Least Square Regression (PLSR) analysis was done for 5 non diabetic and one type-1 diabetic patient. The value of coefficient of correleation was obtained as R = 0.934 and the Standard Error of Prediction (SEP) was 23.7 mg/dl. The Clarke Error Grid (CEG) analysis shows that 71.3% results lie in the clinically accepted zone A [56].

Heise et al. proposed the blood glucose measurement by the oral mucosa study by using non-invasive Attenuated Total Reflection (ATR) Fourier Transform (FT) infra-red spectroscopy. Oral mucosa has been suggested as an especially suited subject for drug delivery and in vivo monitoring of endogenous body metabolites. The ATR-FT method used to analyze the outer most epidermal layer of human oral mucosa. Fast Fourier transform analysis was done for lip and saliva. Frequent blood testing was done for patients to keep an eye on varying blood glucose concentration but the results were not satisfactory, hence not good for clinical usage [57].

Heinemann et al. presented the scattering coefficient analysis technique to measure blood glucose non-invasively. Experimental study involves 5 non-diabetic and 13 type 2 diabetic patients. Oral glucose tolerance test (OGTT) were performed and their glucose level were observed for every reading. Only 19 out of 26 measurements (73%) of patients with type 2 diabetes were in acceptable manner. The acquired results between the simultaneous measurements of the two sensor heads was acceptable in 13 out of 18 volunteers. The study showed that slow changes were observed for volunteers and they all were correlated and hence glucose changes can be observed by the obtained changes in the scattering coefficients [58].

Weiss et al. presented a novel sensor "Aprise™ (Glucon Inc., Boulder, CO)". It utilized the photoacoustic properties of blood for measuring glucose non-invasively. The intrusions of the perplexing features were evaded by unravelling the changes in the blood from those in the nearby tissue sections. This was done to create a consistent association in between the photoacoustic signal and the changes in glucose concentrations. 62 diabetic test specimens were analyzed and the study included 979 sets of reference and predicted values. The mean and the median values of the Relative Absolute Difference (RAD) were 19.9% and 13.2%, respectively. The Clarke Error Grid (CEG) analysis depicted that 66.5% of the test results lie in clinically accepted zone A. since, the proposed system failed to show satisfactory results for some hypoglycemia specimens that is why it was not recommended for clinical purpose [59].

Caduff et al. implemented impedance spectroscopy method and results were compared the classical glucometer. The results were pretty good and CEG analysis showed that 93% results fall in clinically accepted zones that is 56% and 37% in the A and B zones, respectively. Experimental study was carried out for adult diabetic test specimens who had a controlled diet. Local deviations, temperature and degree of hydration variations influenced the measurements [60].

Wang et al. discusses the method of measuring non-invasive blood glucose using near infra-red spectroscopy and double artificial neural network. Author used four different wavelengths of near infra-red spectrum i.e. 820 nm, 875 nm, 945 nm and 1050 nm. PPG signal analysis was carried out for every signal from each LED followed by wavelet transform algorithm to extract eigen values for every PPG signal. Double artificial neural network regression model improves the measurement accuracy. Experimental study carried out for 5 normal healthy test

specimens of age ranging from 23 to 26 years. Oral glucose tolerance tests (OGTT) were conducted. The acquired average percentile error was 16 mg/dL [61].

Lipson et al. presented a calibration model for non-invasive glucose monitoring via Raman spectroscopy. The factors considered for the model development were the distinguished estimation method, maintenance of the proposed device, the adequacy of the size of the data, and suitable exit standards to start the analytical value of the algorithm. A total of 30 test subjects were analyzed. CEG analysis shows that 92% pf the data lie in the clinically accepted zone A and B [62].

Yamakoshi et al. described a method based on spectrophotometric analysis stemming 100 transmittance spectra per second to determine optical spectra (900 to 1700 nm) of blood volume pulsations during the cardiac cycle. A partial least square (PLS) model was developed and data set had been established of 27 healthy volunteers. Total 603 data pairs were found and two-third of the data was used for the PLS calibration model and the remaining for the estimation. CEG analysis shows that 90.05% data pairs fell within region A and 9.95% lie in zone B [64].

Chen et al. presented the non-invasive glucose measurement by using the optical signal of pulsatile microcirculation (OSPM). Experimental research consists of 18 test subjects and a total of 179 data pairs. Results were validated with the finger-stick meter. The study underwent in much careful way to diminish any likely effect on microcirculation i.e. maintaining a steady pulse rate, avoiding any physical activity, and preserving a stable environment temperature 10 minutes before the reading and correct positioning of the finger. CEG analysis shows that 100% of the values lie in the clinically accepted zones A and B [65].

Gabriely et al. described the transcutaneous near-infrared spectroscopy system in vivo non-invasive blood glucose monitoring during euglycemia and hypoglycemia. Experimental studies involved 10 non-diabetic and 2 type-1 diabetic patients and a total of 27 studies. For every study, the test specimen's glucose was less than a normal hypoglycemic range. The glucose values obtained from standard method were used for results validation. CEG analysis showed that 97.7% of the readings fall in the clinically accepted zone A [66].

Ilana Harman-Boehm et al. described a method for non-invasive glucose assessment by using combination of independent techniques ultrasonic, electromagnetic, and thermal. Results

have been compiled and compared with Bayer glucometer and for diabetic and non-diabetic people the mean and median absolute relative differences (ARDs) were 29.9% and 19.9%, respectively. Clarke error grid analysis shows that 92% of the results lie in clinically acceptable zones A and B, with 50% in the zone A [67].

Later on, to get more accuracy they performed some more trails on 2 different days with targeted patients of type 1 and type 2 diabetes. For day 1, they were able to get ARDs means and median 22.4% and 15.9%, respectively. And for day 2 results the ARDs mean and median were 23.4% and 16.5%, respectively. According to Clarke error grid (CEG) analysis, 96% of the results lie in the clinically accepted zones A and B, with 60% in zone A [68].

Md Koushik Chowdhury et al. describes error grid analysis of reference and predicted glucose results of 10 adult volunteers (2 normal and 8 pre-diabetic) in fasting and random mode. Non-invasive measurement is done by using Amplitude Modulated Ultrasound and Infra-red (940 nm) sensor. Results have been compared to invasive glucometer "Accucheck" and Clarke and Parker error grid analysis is done. Both grids show the overall (Normal & Pre-diabetic patients) 85% results lie in clinically accepted zone A. Individually, for normal test specimens 87.50% and for pre-diabetic patient's 84.375% data points lie in zone A [69].

Back in 2001, there was a device named as "Glucowatch" which overruled the world for 5 years because it was the only FDAUS (Food and Drug Association United States) approved device to measure blood glucose level in humans but subsequently withdrawn in 2005 [67] due to uncertainties in the results. The device was operational on the working principle of reverse iontophoresis, involving direct contact with the skin using an iontophoresis current. Since, the device functioned on direct interaction with the ski that is why it was considered as minimal invasive in nature.

Shuming et al. described a nonlinear method of measuring the blood oxygen saturation rate (SpO_2) in wide range on the basis of spectrophotometry. He used the CAN bus communication and fast digital signal processing. The hardware consists of a microprocessor, equipped with the DSP chip TMS-320LF2407A of C-2000 series from TI as main CPU, which has a maximum frequency of 40 MHz, a 256 KB FLASH and a 2.5 KB RAM. The proposed method was capable

of solving the nonlinear problem in measuring the blood oxygen saturation in wide range. The accuracy of the designed pulse oximeter was 35% to 100% within ±3% accuracy. The pulse oximeter based on the method can be used for patients in clinic and the cosmonauts in the field of spaceflight [71].

Yichao et al. proposed a model for monitoring regional cerebral oxygen saturation (rSO_2). The study was based on the steady state spatially resolved spectroscopy (SRS), rSO_2 was obtained by the NIRS oximeter and the oximeter was calibrated by blood gas analyzer using the lipid tissue model. The obtained correlation was excellent, R = 0.99. The rSO_2 of 14 patients were monitored non-invasively during cardiopulmonary bypass (CPB) [72].

Barthelemy et al. described the method to measure the blood oxygen saturation level and in vivo analysis is being done. For proposed method, the apparatus consists of three laser diodes at wavelengths 660, 750 and 940 nm, respectively. Three optical fibers are used to traverse the light in a fully vascularized tissue region of test specimens. This was use to dismiss an opto-electronic sensor which was generating the overlapping signals of molecular absorption. Logic gates are used to switch the signals on all three channels and processed in a microprocessor to yield the blood oxygen saturation level and the carbon mono oxide fixation of the hemoglobin [73].

Ashoka et al. represented a novel model working on the photoelectric plethysmographic (PPG) principle, for non-invasive blood oxygen saturation measurement. The proposed method was great enough to get not influenced by the interfering parameters such as skin color and quantum of intervening tissue between the source and the detector, source and detector intensity etc. Preliminary results obtained on a prototype pulse oximeter, built and tested, demonstrate the practicality of the method [74].

Bagha et al. presented a low-cost and a miniaturized pulse oximeter to continuously measure patient's blood-oxygen saturation level (SpO2) and pulse rate. Author has used the photoplethysmographm (PPG) technique to measure the intensity of light as a voltage signal. Hardware involves the usage of two LEDs i.e. red and infra-red. Transmittance pulse oximetry technique is utilized. PPG signal analysis is being done to estimate SpO2 by comparing the

absorption characteristics of the two different colored light (red and infra-red). Software used for processing is LabView. Data is fetched to the system via DAQ card. The SpO_2 is calculated by computing the AC and DC components, and heart rate is calculated by the time domain peak detection algorithm in LabView signal processing module [75].

Liu et al. proposed a heart rate extraction method by using the fuzzy logic discriminator. This was done to discriminate the truth of each peak of the slope of the PPG signal based on weights. Author has used a smart algorithm to extract the pulse rate, and also to insert an interpolated peak near the time of a missing peak. Author applied 6 different PPG waveforms to test the credibility of the proposed system. The root mean square error values are evaluated, which comes out to be 5.15 beats per minute (BPM) [76].

Azmal et al. described the problem of measuring the SpO_2 level continuously. The proposed method was based on photoplethysmography (PPG) analysis. In this regard a prototype transducer is built to process the PPG signal. Basic amplification and filter circuitry is made and blood oxygen saturation levels are examined for different test patients [77].

Kiran et al. presented an algorithm, based on the wavelength decomposition technique to extract the respiratory activity and heart rate from the PPG signals. MATLAB software along with the MULTISIM tools are used. Obtained signal is compared with the standard respiratory sensor and it has been observed that the correlation was up to 90%. Results are sent to the physician's mobile phone for faster and better assessment of the patient from remote places [78].

CHAPTER 3: TECHNIQUES FOR NON-INVASIVE GLUCOSE MEASUREMENT

Non-invasive blood glucose measurement is not an easy thing. Since, an ideal non-invasive glucose monitoring system should possess few attributes for example it should be completely non-invasive in nature without making any sort of lesion in the human skin and should capable of measuring the accurate glucose results for conditions such as rapid blood glucose changes [85, 86]. That is the reason that development of a non-invasive glucose measuring device is very challenging and difficult task. Glucose can exist in any body fluid, besides blood, for example interstitial fluid, tears, vitreous fluid, urine and sweat etc. There are several techniques which undergo finding the glucose level by examining these body fluids. From the studies, it has been proven that glucose plasma values lag behind the glucose concentration in other body compartments [87-90]. This chapter focuses on the different non-invasive techniques to measure blood glucose level.

3.1 Non-invasive Methods Classification

The non-invasive (NI) techniques for glucose measurement, are classified into two groups. These are classified on the capability to track the intrinsic or extrinsic properties of glucose molecules. In body, glucose can interact with different chemical or physical methods. This interaction is either independent of the body compartment (the intrinsic property) or it can persuade tissue special local changes (the extrinsic property).

3.2 Classification on the Basis of Extrinsic Properties Calculation

Methods based on the extrinsic property calculation are the methods which are tissue compartment dependent. These methods track the changes developed at local level by glucose concentration and differences.

3.2.1 Light Scattering Coefficient

In few techniques, detection of the light scattering coefficient of a tissue compartment yields to estimate blood glucose level. Such technique is called as optical coherence tomography and temperature modulated localized reflectance. In heterogeneous media i.e. human skin, the

scattering of light relies on the balance between the refractive index of the medium and the molecules.

Changes in the refractive index between the glucose and the interstitial fluid lead to alter the tissue transparency which causes change of the scattering coefficient. Various physiological conditions can also affect it. Change in body temperature, shifts in the plasma or/and water between the intravascular and the interstitial compartments have a severe effect on scattering coefficient [91].

Due to these limitations, the devices working on this technique, face a serious problem, especially when the environmental constancy is not preserved because signal stability is essential for an accurate approximation [91, 92].

3.2.2 Ultrasound Technology

Ultrasound technique is the most commonly used technology in which ultrasonic waves are created by tissue absorption of pulsating laser light. This technique is also known as the photoacoustic spectroscopy [93]. In this method, fluid is excited by a laser pulse, which gets absorbed by a particular molecule existing in the fluid [94].

This light absorption results in microscopic localized heating in that particular medium which generates an ultra-pressure. There is a drawback of this technique that it limits the possible interference of water. This is because of the fact that water has a relatively poor photoacoustic response [95]. Whenever a laser light traverses through a denser media, its influence on the photoacoustic signal is not because of the absorption coefficient only but also the scattering coefficient. This is the major cause of the erroneous results obtained by this technique.

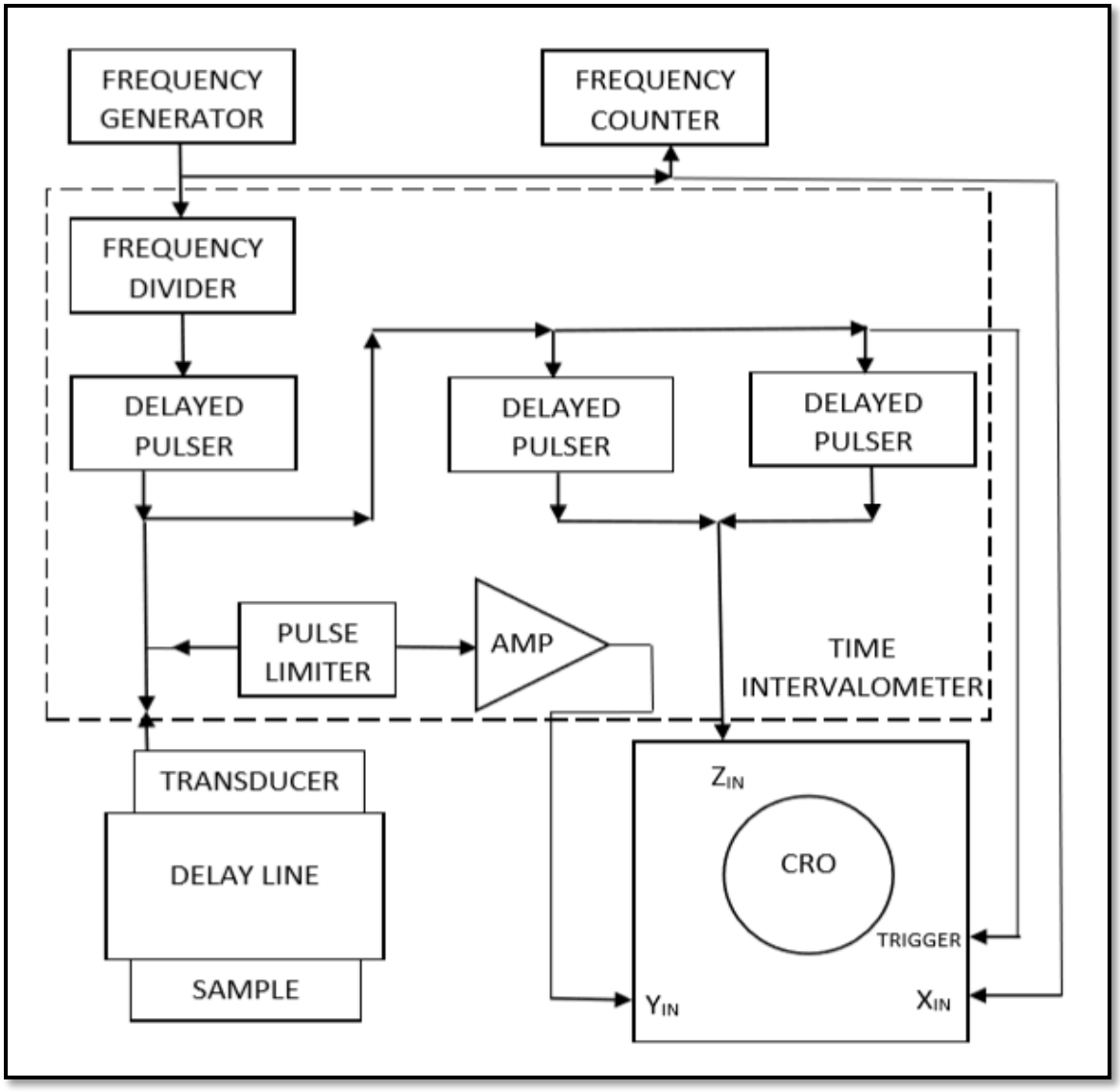

Figure 3.1 Block diagram showing the setup for ultrasound technology [116].

3.2.3 Impedance Spectroscopy

This technique refers to fact that the variations in blood glucose concentration result in decline in sodium concentration and an inclination in potassium level in the red blood cells. These changes generate a potential membrane which can be predicted by measuring the permittivity and conductivity of the cell membrane through the dielectric spectrum [96, 97]. This technique is also known as dielectric spectroscopy.

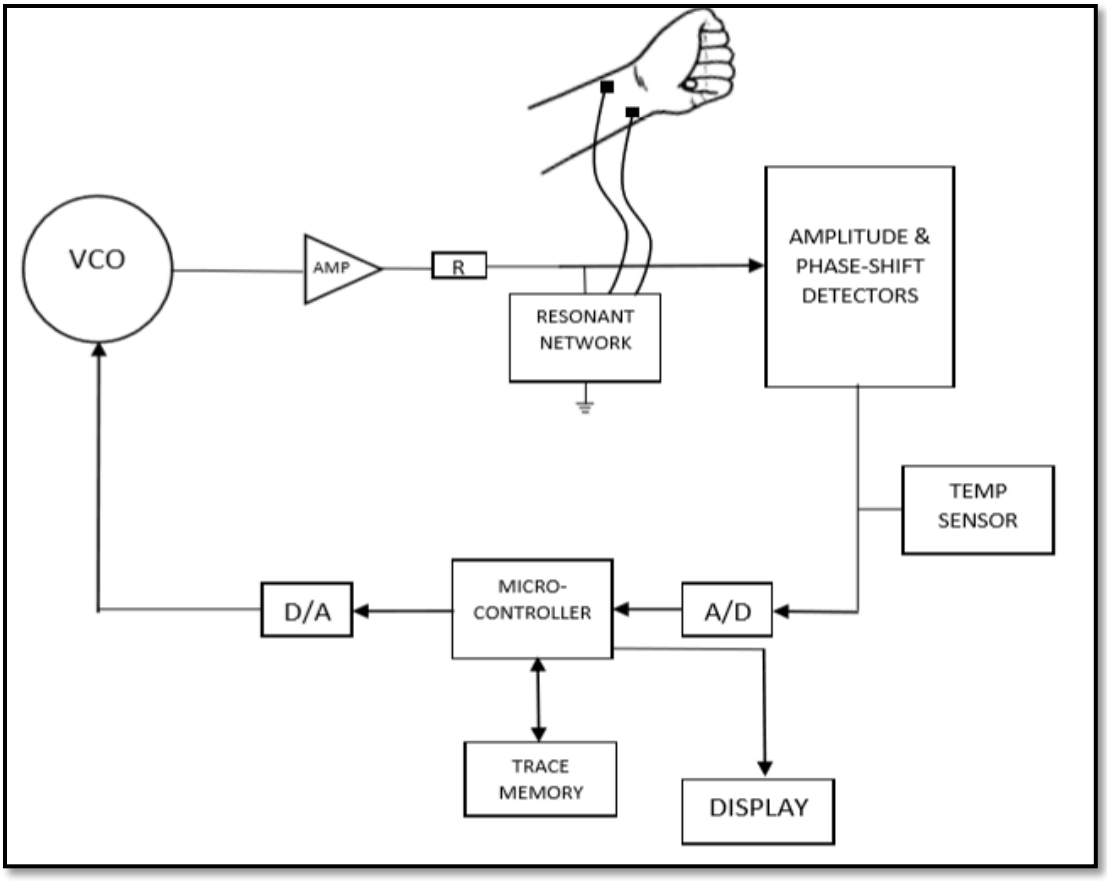

Figure 3.2 Block diagram showing the setup for impedance spectroscopy [115].

3.2.4 Electromagnetic Sensing

The working principle of this technique is the electromagnetic coupling of two inductors, used to detect the dielectric parameters of the blood. Variations in the glucose concentration have a specific voltage signal. Hence, glucose can affect the dielectric properties of a given solution [98, 99]. It is concluded from the experiments that the differences in the ambient or solution temperature have a robust influence on the dielectric properties. Whilst in case of the blood, there are other various solutes that affect the properties, too [100].

3.3 Classification on the Basis of Intrinsic Properties Calculation

Various methods based on intrinsic property are explained as follows.

3.3.1 Thermal Spectroscopy

In thermal spectroscopy technique, thermally modulated optical signals are used to measure the glucose level by the physiological and physical effects of blood glucose concentrations. Since, absorptive effect of glucose produces infrared radiation [90]. But this technique has its own limitation because differences in tissue temperature could change cutaneous vascular and refractive index responses [101].

3.3.2 Fluorescence Spectroscopy

This technique refers to the discovery of fluorescence when exciting a glucose solution by an ultraviolet laser light at 308 nm. However, the fluorescence phenomenon in humans, depends not only on the glucose concentration, but on skin pigmentations and epidermal thickness [102] and its accountability has been confirmed only in vitro experiments.

3.3.3 Raman Spectroscopy

In this type of spectroscopy, laser light is used to excite the molecules present in a given solution, as a results, emission of scattered light is formed by the rotations of those molecules. In a solution, the degree of vibration and rotation of a molecule relies on its concentration. Different wavelength ranges have been used for in-vitro analysis such as 200 to 1800 cm^{-1} and the Raman bands at 900 to 1200 cm^{-1} are considered as the best bands for glucose detection [103, 104].

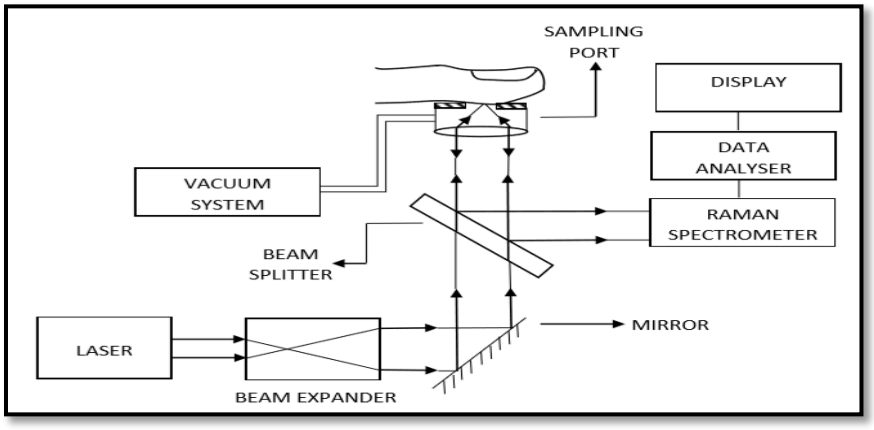

Figure 3.3 Block diagram showing the setup for Raman spectroscopy [114].

3.3.4 Mid-infrared Spectroscopy (MIR)

MIR spectroscopy is based on the detection of glucose level to specifically absorb light in the 2500-10000 nm spectrum [90]. The advantage of MIR is that the signals produced by glucose are sharp and have high absorption coefficient but allowed penetration of few micrometers is a significant limiting factor. In order to sort this issue, attenuated total reflection measurements are used. This method uses a beam of light guided through a crystal placed in contact with the skin, thus ensuring that the electromagnetic field created by the reflected light reaches the dermis. Dermis is the layers of the skin which contains the most of the glucose molecules.

3.3.5 Near-infrared Spectroscopy (NIR)

Near infrared spectroscopy (NIRS) is based on the transmission of a band of NIR light through a vascular area of the body (finger, ear, tongue, etc.), and the concentration of glucose in vivo is evaluated from the spectral information acquired at the reception. In some recent years, great importance has been located on systems working on NIRS principle [105, 106].

The basic idea of the systems working on NIRS is that specific information about glucose levels is represented in it [107, 108], since this region has several windows where hemoglobin, melanin and water absorption band intensities are low enough to allow light to penetrate into the tissue, enabling NI spectral measurements to be taken.

This helps to obtain glucose measurements in complex biological matrices such as synthetic biological mixtures by the combination of NIR spectroscopy techniques with calibration techniques based on multivariate analysis [109-112].

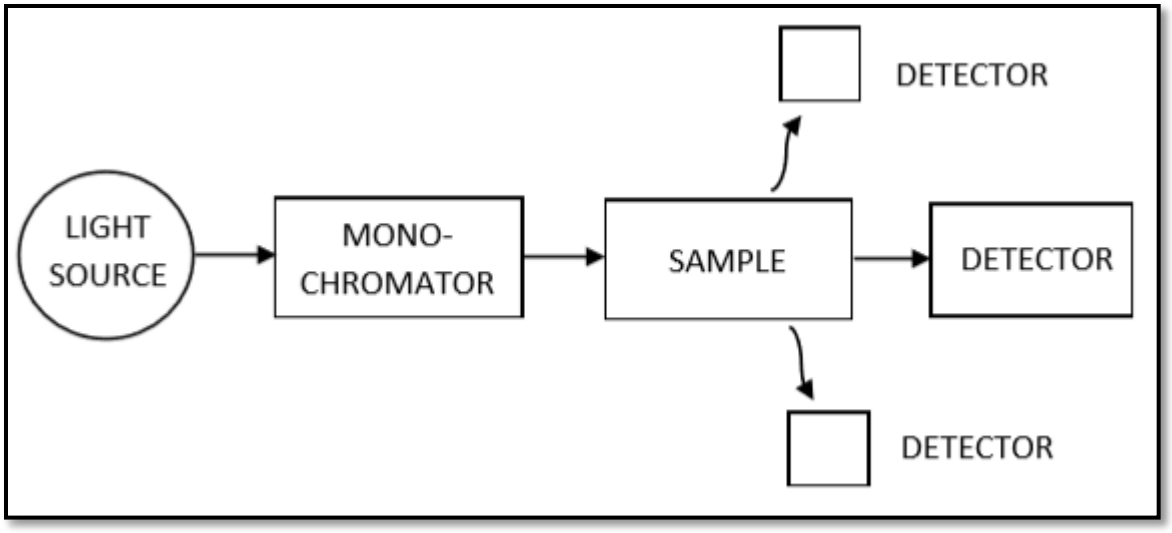

Figure 3.4 Block diagram showing the setup for near infra-red spectroscopy [113].

3.4 Interim Conclusion

In this chapter different non-invasive methods for measuring blood glucose level are described. NIRS detection is one of the most widely used technique which allows several wavelength windows such as high overtones, first overtones, to investigate the blood glucose level up to few millimeters of depth under the skin. In the following chapter, design and fabrication/development of the prototype capable of measuring pulse rate, oxygen saturation and blood glucose is being discussed. Acquired results, their comparison and achieved accuracy is presented in the second last chapter.

CHAPTER 4: DESIGN AND DEVELOPMENT OF THE PROTOTYPE

This chapter deals with the design and development of the non-invasive prototype, used to measure the pulse rate, oxygen saturation and blood glucose level. This chapter focuses on the block diagram, schematics for pulse rate and its wave form, blood oxygen saturation, and blood glucose level. Fingertip photoplythysmography (PPG) is performed to detect the oxygen saturation level in arterial blood. Since, accuracy plays a vital role to blood glucose measurements. Therefore, a specific protocol is used to reduce the error in the blood glucose results. The algorithm helps to overcome the error. Double regression model (DRM) is also implemented to attain an accuracy of 98.48%. Hence, all the materials and methodology is explained in this chapter.

4.1 Overview of the System

The figure 4.1 represents the overall block diagram of the measurement system. Amplified and filtered PPG signal is extracted from the hardware and further processed via micro controller Arduino UNO board. Model building involves the implementation of all the equations and performs all respective calculations.

Results are compared to the invasive chemistry analyzer AU-480 and it has been observed that some of the patients who are suffering from high glucose levels and they are on constant heavy dose of insulin, their readings depict more than 15% error. That error is overcome by performing the double regression analysis and the coefficient of determination is analyzed.

After the regression model building, it has been scrutinized that the value of R^2, the coefficient of determination gets improved to 0.9471. Furthermore, the correct results of pulse rate, oxygen saturation level and blood glucose are displayed on the laptop once the complete algorithm is executed.

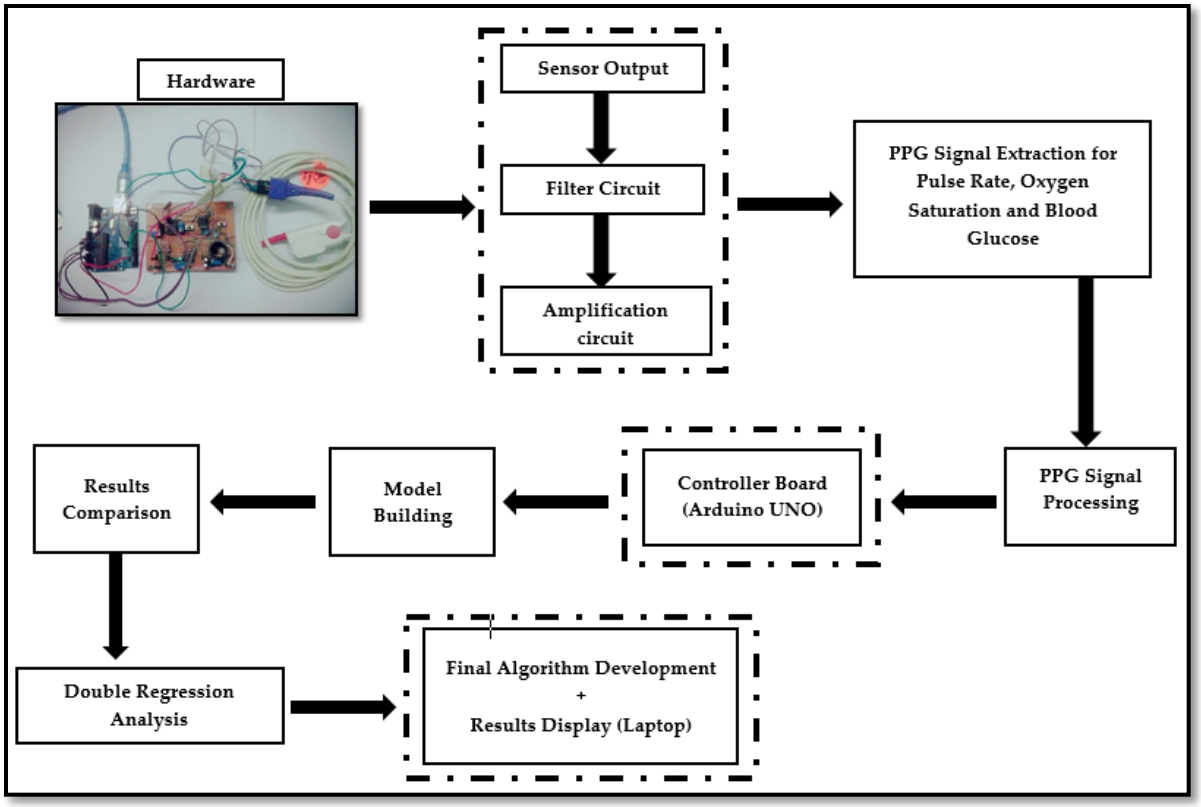

Figure 4.1 Block diagram of non-invasive pulse rate, oxygen saturation and glucose measurement system.

4.2 Blood Oxygen Saturation Detection and Measurement

For blood oxygen saturation detection, photoplythysmorgraphy (PPG) is done, which is extensively used in pulse oximetry. The pulse oximetry relies on the red and infra-red absorption spectrum physiognomies by comparing the light absorption difference of both lights in the blood. As far as pulse oximetry is concerned, there are two methods to find oxygen saturation ratio which are transmittance oximetry and reflectance oximetry. For proposed method, transmittance oximetry is used, in which light emitter and receptor both are mounted on the opposite sides. When light is emanated from the emitter, it passes through the fingertip tissues and reaches to the detector present at the other end. Since, oxygen is coined to hemoglobin in the arterial blood that is why photoplethysmography (PPG) signal analysis is necessary to notice the optical changes in the absorbed signal and its absorption characteristics are compared to make decisions about pulse rate (PR) and oxygen saturation level.

This follows Beer Lambert's law as shown in equation 4.1 [117]. This law relays the decrease of the incident light to any substance through which light is being traversed. Over here, $\boldsymbol{I_{out}}$ is the intensity of transmitted light via fingertip, $\boldsymbol{I_{in}}$ is the intensity of the incident light, $\in$ is the molar absorptivity, C is concentration level to be measured and L is optical path length. The Beer Lambert's law has some other names too such as the Beer' law, the Lambert-Beer law, or the Beer-Lambert-Bouguer law.

$$I_{out} = I_{in} e^{-\epsilon c l} \tag{4.1}$$

A fingertip pulse sensor probe, Nellcor DS 100A is being used, consisting of two LEDs having different wave lengths, 660 nm 940 nm. Figure 4.2 shows the Nellcor sensor probe. The fingertip PPG signals analysis gives the information about the DC and AC components. The DC component doesn't change with time and it shows light absorption via finger's skin, bone, muscles and, venous blood while the AC component gives information about the arterial blood and its pulsatile nature depicts that it changes with time, leading to the detection of heart beat.

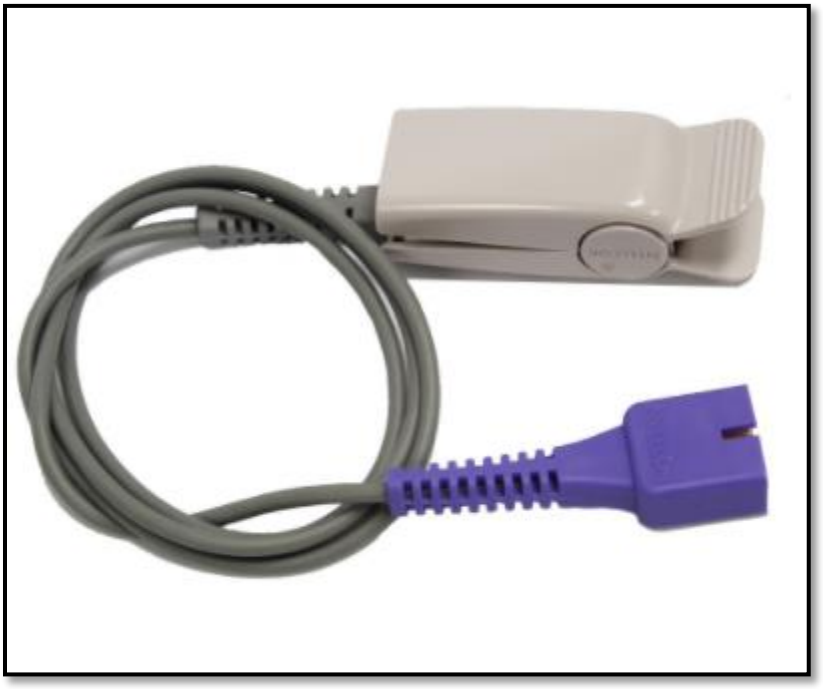

Figure 4.2 Nellcor DS 100A sensor

The figure 4.3 depicts the schematic design for blood oxygen saturation level measurement. The sensor's output is filtered with the help of a cascade passive low and high pass filter to remove base line and high frequency noise. The final filtered noise free output is fetched to Arduino

controller board. For amplification, LM 358 IC is used, which is an 8 pin, low power dual operational amplifier.

In hardware, Arduino's pins 7 and 8 are for the dual LEDs. The algorithm switches the red and infra-red lights with 50% duty cycle, manipulates the sensor's output data, analyzes the PPG signal's AC and DC components and implements the following equation 4.2 for calculating the blood oxygen saturation level.

$$\text{Oxygen Saturation} = \frac{AC\ Red / DC\ Red}{AC\ IR / DC\ IR} \tag{4.2}$$

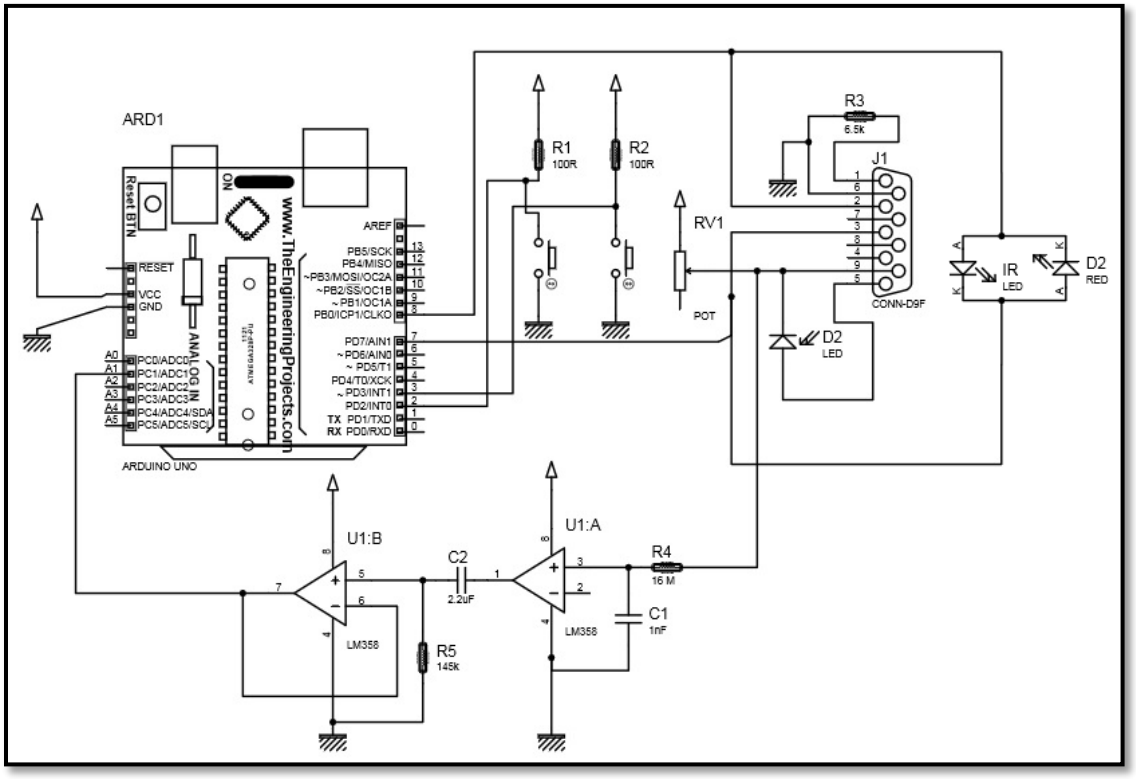

Figure 4.3 Schematic to find blood oxygen saturation level.

4.3 Data Acquisition Circuits for Pulse Rate

The acquisition of pulse rate is divided into two parts. At first, a circuit is designed to get the perfect noise free filtered pulse wave form, and then the heart rate circuit is designed to get the pulses in beat per minutes (BPM). Pulse rate waveform is obtained by using a basic signal conditioning circuit, consisting of cascade low and high pass filters as schematic depicts in figure 4.4. Low pass filter removes high frequency noise and power line intrusion. However, high pass

filter removes low frequency signal noise and it has a cut off frequency of 0.5 Hz. Small variations in the signal acquired by the photodetector are converted into square pulses to calculate heart rate which can be seen in figure 4.5. The reason of doing this is to get the precise pulse rate value from the PPG pulse wave form as shown in figure 4.6. The output of figures 4.4 and 4.5 goes to Arduino controller board and the algorithm developed by using the Arduino sketch software, interprets the sensor's output, records all pulses and, performs all the calculation to show the final pulse rate on the screen. Figures 4.3, 4.4 and 4.5 are made with the help of Proteus Design Suite software. The figure 4.7 shows the hardware for the measurement of the pulse wave form and pulse rate.

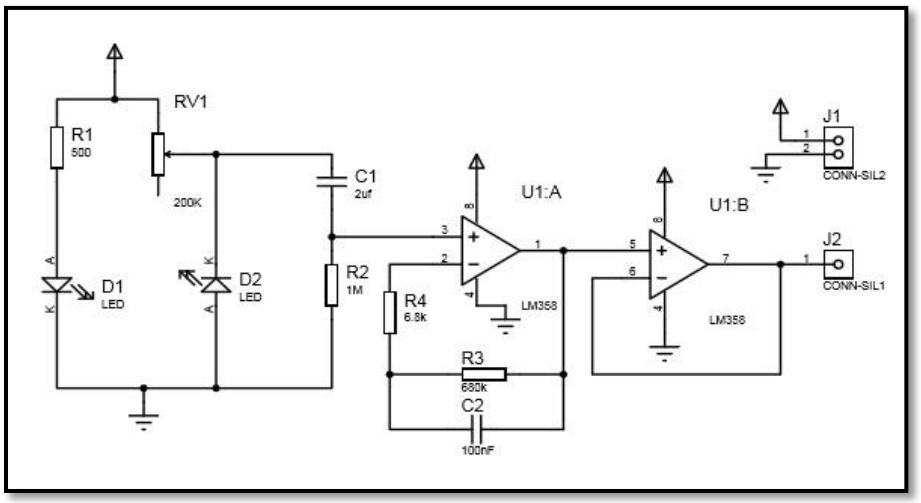

Figure 4.4 Schematic to get pulse waveform.

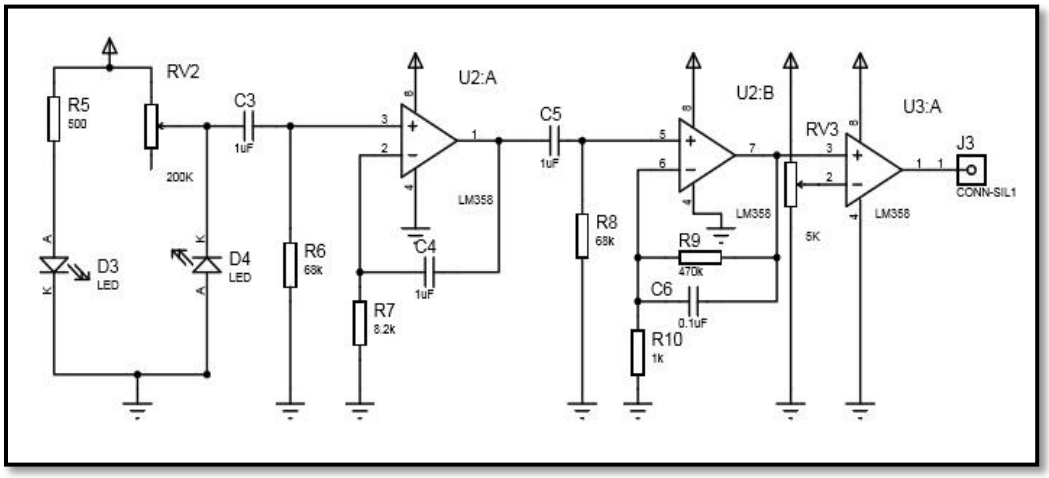

Figure 4.5 Schematic to calculate pulse rate.

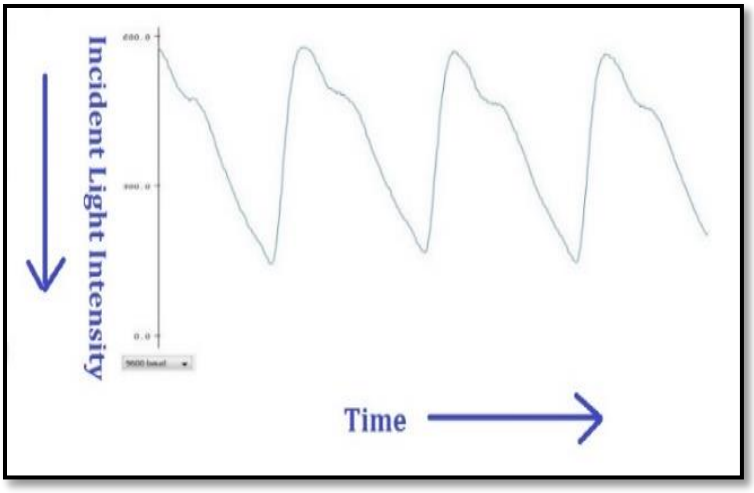

Figure 4.6 Noise free filtered PR wave.

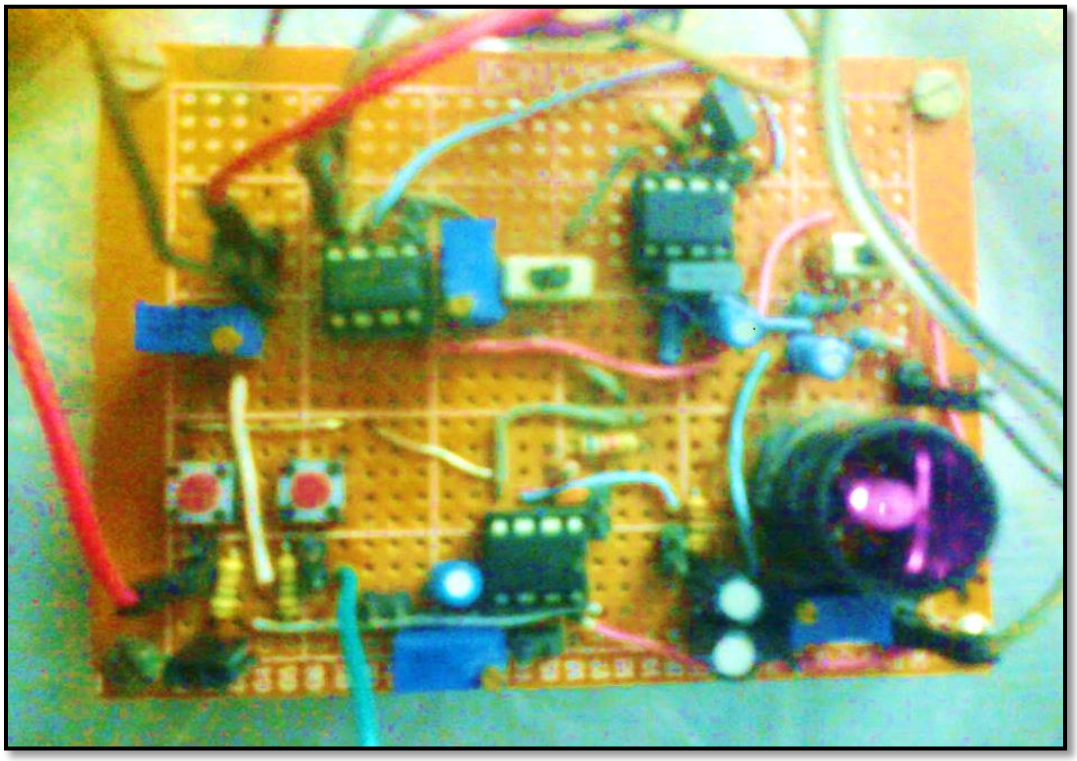

Figure 4.7 Hardware for the measurement of the pulse rate.

4.4 PPG Analysis and Analytical Modeling for Glucose

For glucose measurement, the basic working principle is the near infra-red spectroscopy (NIRS). NIRS is the most widely used technique in light spectroscopy beacsue it provides a wider range of wavenlength's windows to detect the blood glucose even under few milimeters depth of

the skin. The figure 4.8 (A) depicts the protocol to be followed for acquisition of the data for blood glucose measurement and 4.8 (B) describes the data processing and calculation algorithm. In figure 4.8 (A), first of all validation of sensor contact and correct placement of patient's finger inside the sensor is ensured because a slight mishap can yield to erroneous results. Patients were advised to remain static during the measurement and place their finger properly in the sensor. Especially, Female patients were instructed not to wear nail paint colors or hina (mehndi) during the clinical trials to avoid inaccurate readings.

Special sized rings are used to measure patient's finger thickness. The diameter of the ring yields the thickness of the fingertip. Since, light reaches to the receiver from the source in the sensor by passing through the fingertip. Hence, thickness of the finger is the ultimate measure of the path length. Sensor used for finding the glucose gives better signal output with high signal to noise ratio and the output of the sensor is filtered via paired low and high pass filter with cut off frequencies of 10Hz and 0.5Hz, respectively. Base line drift and unnecessary noise is removed from the acquired PPG signal.

The acquired data from hardware is fetched to Arduino controller board for further processing. In figure 4.8 (B), analog modeling of the signal is done whilst using Arduino sketch software, which involves the determination of intrinsic molecular credentials for example near infrared light absorption characteristics, molar extinction coefficients calculations, optical path length, transmitted and incident light intensity determination.

The fundamental working principle is based on Beer Lambert's law as already described in equation 4.1. The general form of the Beer's law to find blood glucose concentration can be seen in equation (4.6). Algorithm evaluates the transmittance, optical densities and absorbance as shown in equations (4.7), (4.8), (4.9) and (4.10); performs mathematical modeling and takes the mean of the optical density values which are taken at different times i.e. t, t+1 and so on as shown in equations (4.3) and (4.4). Equation (4.5) is used to find the difference of two densities.

Arduino's interrupts are used to get those intensities, simultaneously. Addition and subtraction of the mean value in the experimental values helps to overcome the error and after the results comparison, the final glucose values are displayed.

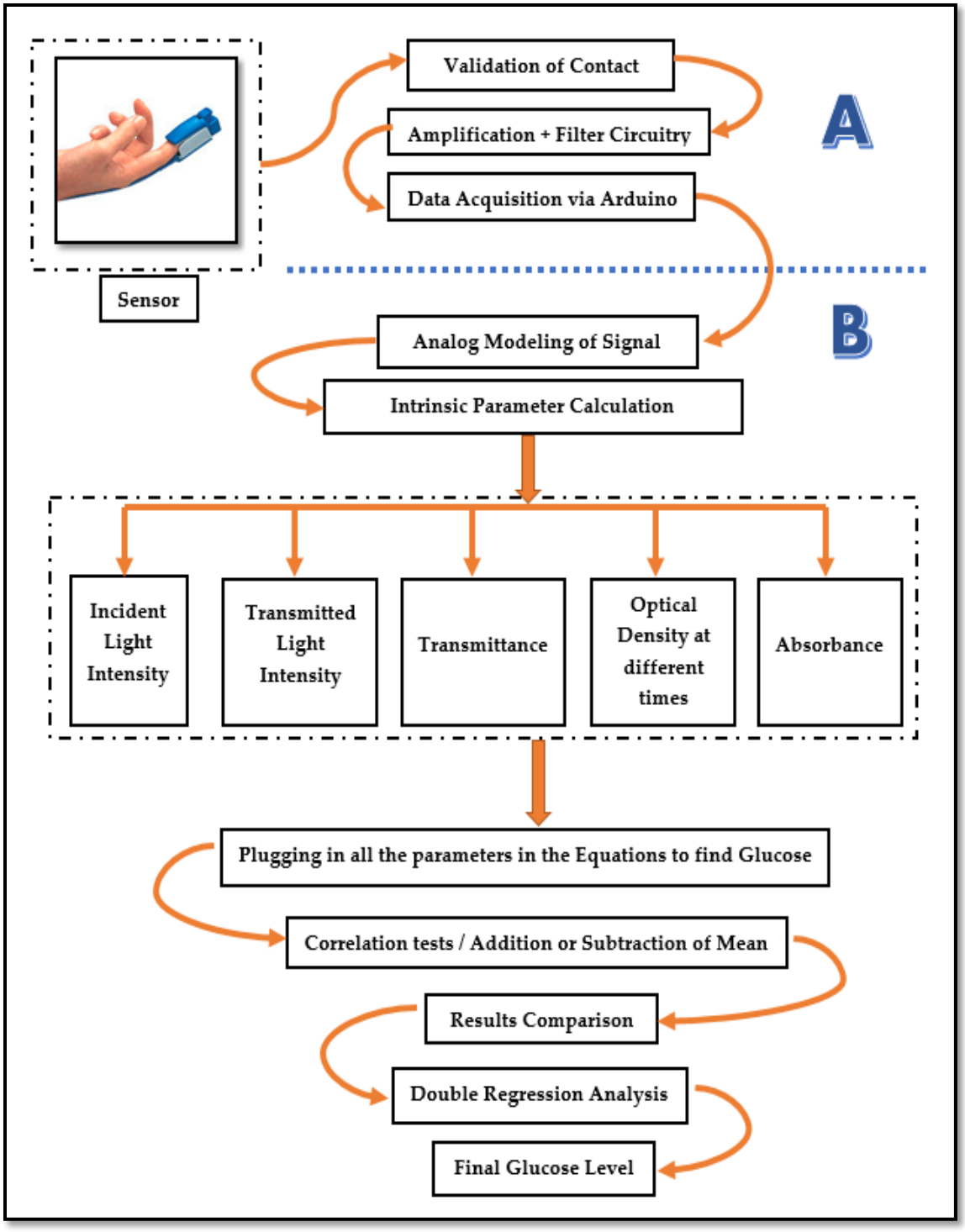

Figure 4.8 (A) Glucose measurement procedure. (B) Schematic of glucose evaluation protocol.

$$OD_{\lambda} = (\log \frac{I_o}{(I_{N\ (t_i)})}) \quad (4.3)$$

$$OD'_{\lambda} = (\log \frac{I_o}{(I_{N\ (t_i+1)})}) \quad (4.4)$$

$$\Delta OD_{\lambda} = \log(1 + (\frac{\Delta I_{\lambda(t_i)}}{I_{\lambda\ (t_i\ +\ 1)}})) \quad (4.5)$$

$$A\ [\ \{C_i\}, \lambda\ ] = \sum_i^n (\ C_i\ A_i\ (\ \lambda\ )) \quad (4.6)$$

$$A = a\ (\lambda)\ C\ L \quad (4.7)$$

$$A = \in C\ L \quad (4.8)$$

$$\%T = \frac{I_N}{I_0} * 100 \quad (4.9)$$

$$A = 2 - Log_{10}[\%T] \quad (4.10)$$

Over here; OD_{λ} = Optical density, ΔOD_{λ} = Difference in optical densities, A = absorbance, a (λ) = wavelength dependent coefficient, T = transmittance, I_N = transmitted light intensity, I_0 = incident light intensity, $A_i(\lambda)$ = optical density at wavelength λ of the ith component, C_i = Concentration of that component, A(λ) = Optical density of the mixture, ∈, C and L are already explained in Equation (1).

4.4.1 Double Regression Analysis

Results obtained from the above mentioned algorithm, show that there are 7 out of 132 readings which have error more than 15%. Those 7 results are of the patients suffering from hyperglycemia. Due to abnormal high glucose level, it was necessary to keep them in stable condition. That is why they were on constant high dose of insulin. Double regression analysis is being done to increase the sensitivity of the designed prototype and diminish the deviations. The regression model relates the relation between a reference and one or more predicted values. The equation (4.11) shows the regression equation, where β_o is the y-intercept, β_1 is the regression coefficient, Y_p is the predicted value and ε is the error term. For n number of terms, this expression can be transformed into matrix form as depicted in equation (4.12).

$$Y_p = \beta_o + \beta_1 x + \varepsilon \tag{4.11}$$

$$\begin{bmatrix} Y_1 \\ Y_2 \\ \vdots \\ Y_n \end{bmatrix} = \begin{bmatrix} 1 & X_1 \\ 1 & X_2 \\ \vdots & \vdots \\ 1 & X_n \end{bmatrix} \begin{bmatrix} \beta_o \\ \beta_1 \end{bmatrix} \tag{4.12}$$

The rest of the calculations are performed by coding in MATLAB software and all the coefficients values are acquired via software. The coefficient of determination R^2 is also evaluated to find the better fit of the model. Basically, it is the measure of how well a model can predict the data. Since, R^2 signifies the correlation in between the actual and estimated blood glucose levels so higher the value of R^2, better the model will be at predicating the data. Its value range is from 0 to 1. After the first regression model, the following equation (4.13) is used to evaluate the coefficient of determination.

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (y_i - y^{\bullet}_i)^2}{\sum_{i=1}^{n} (y_i - y^{\rightarrow}_i)^2} \tag{4.13}$$

Over here, $y^{\bullet}_i$ are the predicted values from the first regression model and $y^{\rightarrow}_i$ takes the mean of the values. The value of R^2 from the first regression model is sought out to be 0.8162. On the same lines, second regression model is evaluated, coefficients are found and the value of R^2 is acquired by using the equation (4.14).

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (y_i - y^{\sim}_i)^2}{\sum_{i=1}^{n} (y_i - y^{\rightarrow}_i)^2} \tag{4.14}$$

Over here, $y^{\sim}_i$ are the predicted values from the second regression model in which the y-intercept value is being used and rest all are explained already. The value of R^2 after second regression model is 0.9471 which clearly shows that model is being fit perfectly as it is closer to 1. The equation (4.15) represents the model equation generated by MATLAB code where Y_p is the predicted value.

$$Y_p = 1.7126x - 61.0644 \tag{4.15}$$

Accuracy plays a vital role in BG measurement. So far, all proposed non-invasive devices for glucose measurement suffer from inaccuracies due to signal to interference ratio (SIR), signal to noise ratio (SNR) and wavelengths used in the absorption spectra of near infra-red (NIR) band. Previously, much research has been done in the field of NI glucose measurement such as near infra-red, mid infra-red, optoacoustic and Raman spectrum detection etc. But all glucose monitors were not practical, precise, and/or economical. Hence, double regression model is being developed to lessen the deviations and upsurge the sensitivity of the prototype. Nevertheless, the proposed prototype is simple, reliable and yield the best results, as depicted in the following figure 4.9.

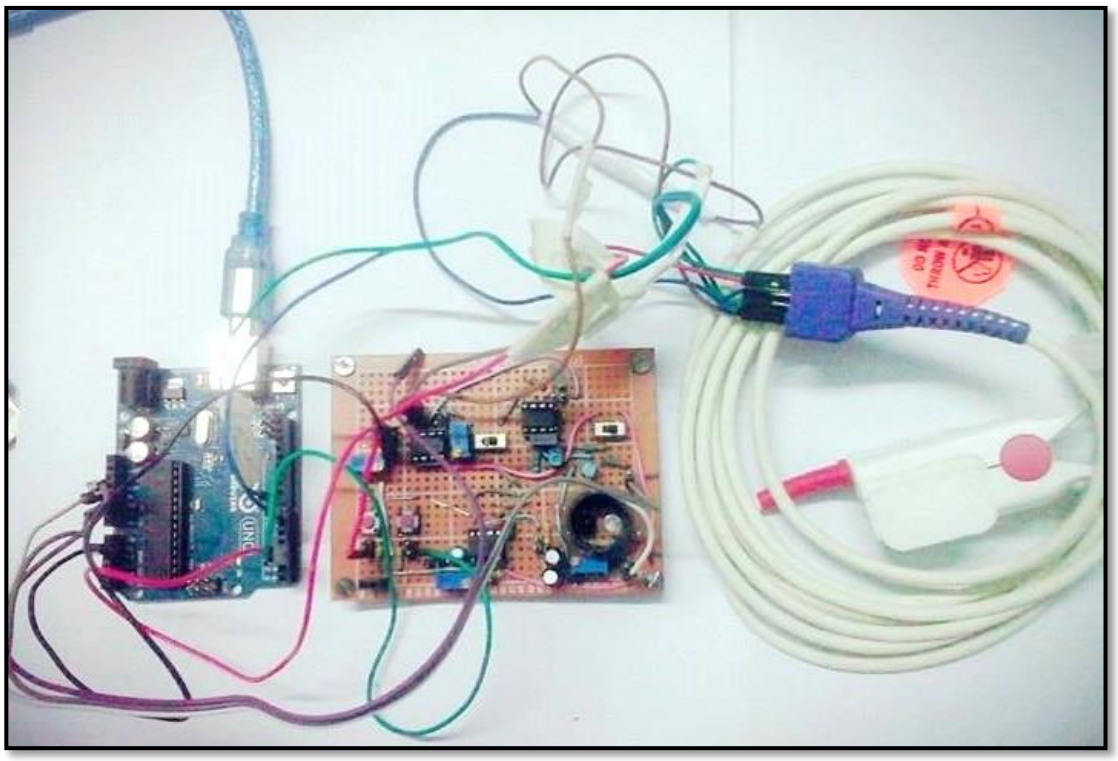

Figure 4.9 Final hardware diagram

CHAPTER 5: RESULTS AND DISCUSSION

This chapter deals with the results acquired by the prototype and the accuracy comparison of the results from the invasive commercially available glucometer i.e. “GlucoLab”. For blood oxygen saturation accuracy comparison, pulse oximeter is used, which is commercially available for clinical purpose. Furthermore, in order to validate the results, accuracy comparison is done with the invasive Beckman Coulter AU-480 chemistry analyzer, present in the Holy Family Hospital, Pakistan. Nevertheless, this chapter focuses on the case study of various test specimens, performed at the said hospital for in vivo analysis and results endorsement. International Standards Organization (ISO) accuracy criterion is tried to be followed by achieving 98.48% accuracy. Likewise, mean and median absolute relative differences (ARDs) are also get reduced to 8.25% and 7.94%, respectively. The coefficient of determination R^2, depicts the goodness of a fit that how close the predicted values are to reference glucose values. After the implementation of double regression model (DRM), the coefficient of determination R^2 gets improve to 0.9471.

5.1 Results for Pulse Rate and Blood Oxygen Saturation Level

Initially results have been acquired and compared to the commercially available fingertip pulse oximeter, can be seen in finger 5.1, which finds out the blood oxygen saturation level and pulse rate as per IEC60601-1-2 standards. Experimental study involves bunch of readings of various test specimens. Some of them are shown in Table 5-1. It shows 3 females (F) and 4 males (M) readings and their result comparison of PR in beats per minute (BPM) and SpO2 level with the commercially available pulse oximeter. The overall measurement accuracy of $\pm$ 3% for pulse rate and $\pm$ 1% for blood oxygen saturation level is being observed.

Figure 5.1 Commercially available pulse oximeter [118].

Table 5-1 Result Summary of Pulse rate and Oxygen Saturation.

Test Results for Blood Oxygen Saturation (SpO2) & Pulse Rate (PR)											
Test Specimens	Red Signal AC	Red Signal DC	IR Signal AC	IR Signal DC	Oxygen Saturation SpO2	Experimental Results		Commercial Pulse Oximeter		Percentile Error (%)	
						% SpO2	PR BPM	% SpO2	PR BPM	SpO2	PR
F	0.1326	2.8675	0.1430	2.9111	1.0026	98.82	96	99	98	1	2
M	0.1404	2.9604	0.1363	2.9095	1.0124	99.78	92	98	95	1	3
M	0.1411	2.9810	0.1323	2.8009	1.0021	98.77	76	99	79	1	3
M	0.1155	2.8765	0.1268	3.1296	0.9905	97.63	74	98	77	1	3
M	0.1406	2.9923	0.1410	3.0418	1.0134	99.89	80	99	83	0	3
F	0.1432	2.7822	0.1364	2.6538	1.0013	98.69	98	98	99	0	1
F	0.1406	2.9943	0.1405	3.0433	1.0131	99.86	93	99	96	0	3

5.2 Clarke Error Grid Analysis (CEGA)

Back in 1987, when for the very first time the Clarke Error Grid Analysis (CEGA) was established. CEGA is done to enumerate the clinical accuracy of the reference glucose results as compared to the non-invasive (NI) glucose results, obtained from NI prototype. It was the first time that CEGA seemed in the "Diabetes Care" in 1987 [119]. With the passage of time, the CEGA turn out to be putative as the "gold standards" for measuring the accuracy of different glucometers.

The CEG is classified into 5 different zones. Their detail is as follows as per International Standards Organization (ISO) standards;

- Zone A
  - Contains the results having error equal to or less than 15%.
- Zone B
  - Contains the results having error more than 15%.
- Zone C
  - Zone C are those results which require some unnecessary action.
- Zone D

- Zone D consists of those points which have hazardous failure to spot hypoglycemia or hyperglycemia.

- Zone E
  - Zone E involves those results which complicate the treatment of hypoglycemia for hyperglycemia and hyperglycemia for hypoglycemia.

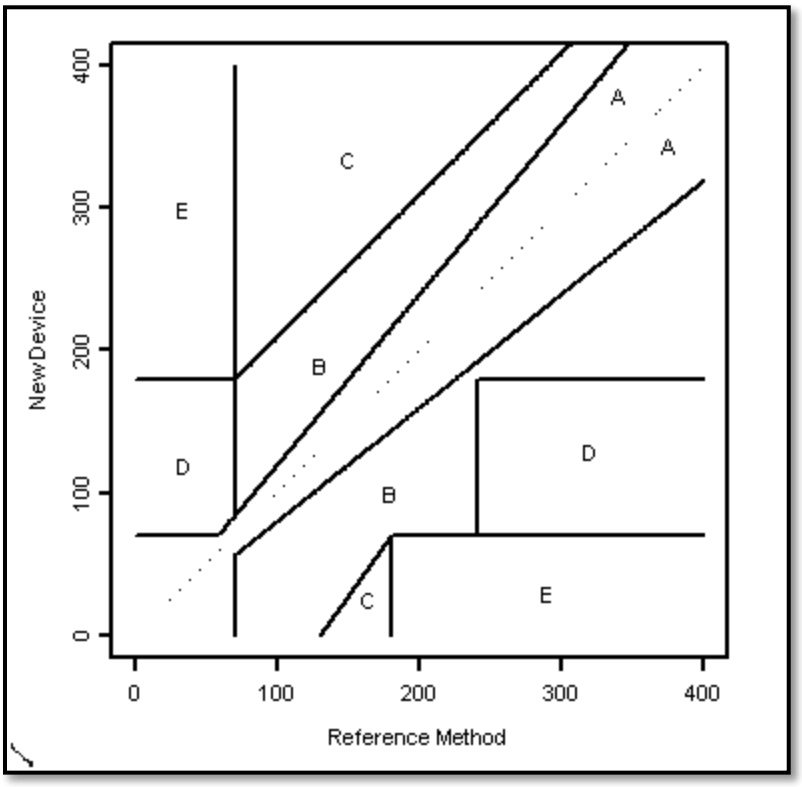

Figure 5.2 Basic structure of Clarke error grid [120].

5.3 ISO Accuracy Standards

The latest International Standards Organization (ISO) 2013 proposes a narrower accuracy range for high blood glucose levels for commercially available glucometers. According to that standard, results at or above 75 mg/dL must possess the measurement accuracy of $\pm$ 15%, previously it was $\pm$ 20%. For results below 75 mg/dL, the acceptable range is $\pm$ 15% which is same as per USFDA (United States Food and Drug Authority) standard [121]. Hence, obtained experimental results successfully follow the ISO as well as USFDA standards.

5.4 Clarke Error Grid Analysis for Glucometer Results

Initially results attained from NI prototype, are likened with the commercially available invasive glucose meter. The invasive glucometer consists of a lancet, needles, and some test strips. Every time a patient takes the reading, he/she has to insert a new needle into the lancet and prick their finger. A blood droplet is dropped on the test strip and inserted into the glucometer to give the glucose value. These test strips are also disposable and for one time use only. So this procedure is inconvenient and pain causing as well as expensive. For checking sugar level, 25 readings of different diabetic and non-diabetic test specimens have been taken in both fasting and random mode. The value of blood glucose of those test specimens is compared to commercially available invasive fingertip glucometer, named as “GlucoLab”.

5.4.1 Clarke Error Grid for Fasting Mode Results

The Clarke Error Grid (CEG) analysis is done by using MATLAB and graphically expressed as shown in figure 5.3, 5.4 and 5.5. The points lying in zone A are those which follows the International standards Organization (ISO) accuracy criteria and have maximum error not more than 15%. Likewise, points in zone B are those which have more than 15% error. For fasting mode results, the respective blood glucose level determining accuracy dependent values are further classified into different zones. For example zone A: 92.00%, zone B: 8.00%, zone C: 0.00%, zone D: 0.00% and zone E: 0.00%, respectively as shown in figure 5. Mean and median Absolute Relative Error (ARE) values for fasting mode are 6.97% and 4.76%, respectively.

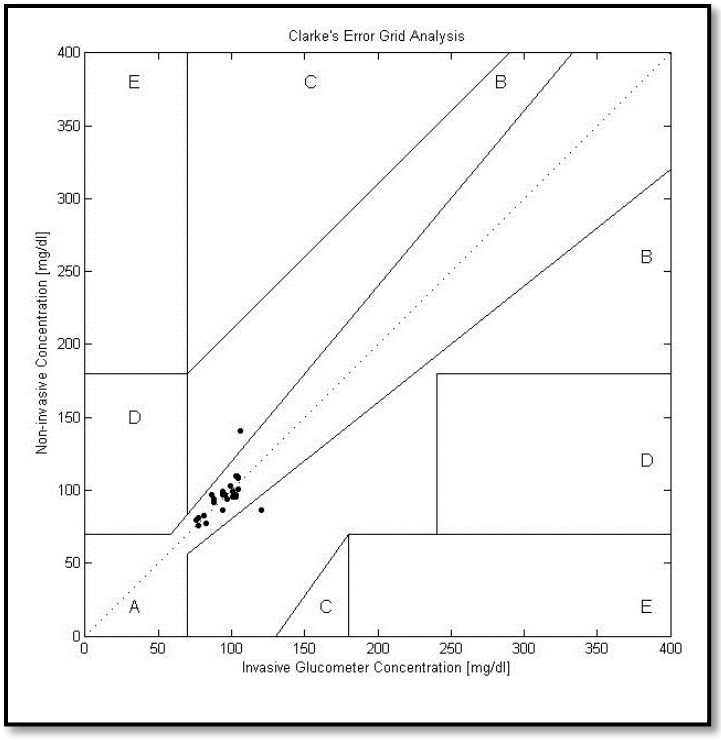

Figure 5.3 Clarke Error Grid (CEG) Analysis of the invasive glucometer (x-axis) and non-invasive blood glucose values (y-axis) of test specimens during the fasting mode.

5.4.2 Clarke Error Grid for Random Mode Results

For random test results, accuracy dependent values are further classified for example, zone A: 88.00%, zone B: 12.00%, zone C: 0.00%, zone D: 0.00% and zone E: 0.00%, respectively as shown in figure 6. For random mode, mean and median ARE values are 6.93% and 3.42%, respectively.

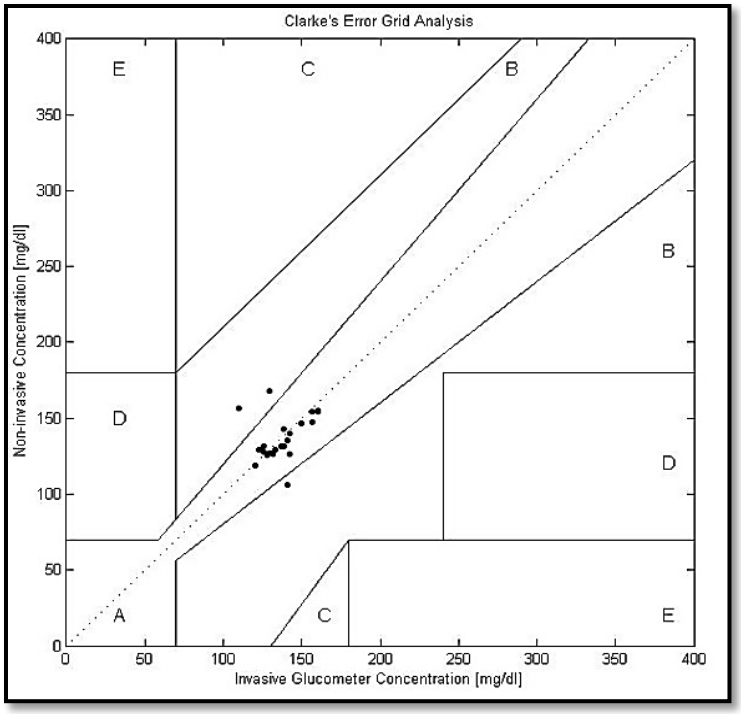

Figure 5.4 Clarke Error Grid (CEG) Analysis of the invasive glucometer (x-axis) and non-invasive blood glucose values (y-axis) of test specimens during the random mode.

5.4.3 Glucometer, LAB Spectrophotometer and Non-invasive Results Comparison

Since, commercially available invasive glucometers are not 100% accurate. They have 6% to 7% error. That is why extremely low and high results are open for interpretation. It is required to confirm them either by taking various readings from different glucometers or by taking the repeated measurement from the same meter [38]. That is why in order to validate the authenticity of the results, they are further compared with the chemistry analyzer AU-480 manufactured by Beckman Coulter.

5.4.4 Clarke Error Grid for Glucometer, LAB Spectrophotometer and Non-invasive System

The result comparison of non-invasive (NI) vs glucometer (GL) as well as NI vs LAB spectrophotometer is shown in table 5-2 also can be seen in figure 5.5. The green colored triangles show the NI vs LAB values and red colored dots are for NI vs Glucometer. The mean ARE percentage is better for NI vs LAB than NI vs GL which is 6.15% and 10.07%, respectively.

Table 5-2 Blood glucose comparison of Non-invasive, Glucometer and LAB spectrophotometer results.

Test Results for Blood Glucose Level										
Test Specimens	**Age (Years)**	**Glucometer (GL) Results**		**NI Experimental Results**		**Invasive LAB Results**		**NI vs GL ARE (%)**	**NI vs LAB ARE (%)**	**Remarks**
		mg/dL	**mmol/L**	**mg/dL**	**mmol/L**	**mg/dL**	**mmol/L**			
Male	24	87	4.83	76	4.22	75	4.16	12.64	1.33	Non diabetic
Male	27	103	5.72	94	5.22	87	4.83	8.74	8.05	Non diabetic
Male	3.5	92	5.11	81	4.50	77	4.27	11.96	5.19	Non diabetic
Male	55.5	204	11.32	174	9.66	193	10.71	14.71	9.84	Prediabetic (Type 2)
Male	21	86	4.77	84	4.67	79	4.38	2.33	6.33	Non diabetic

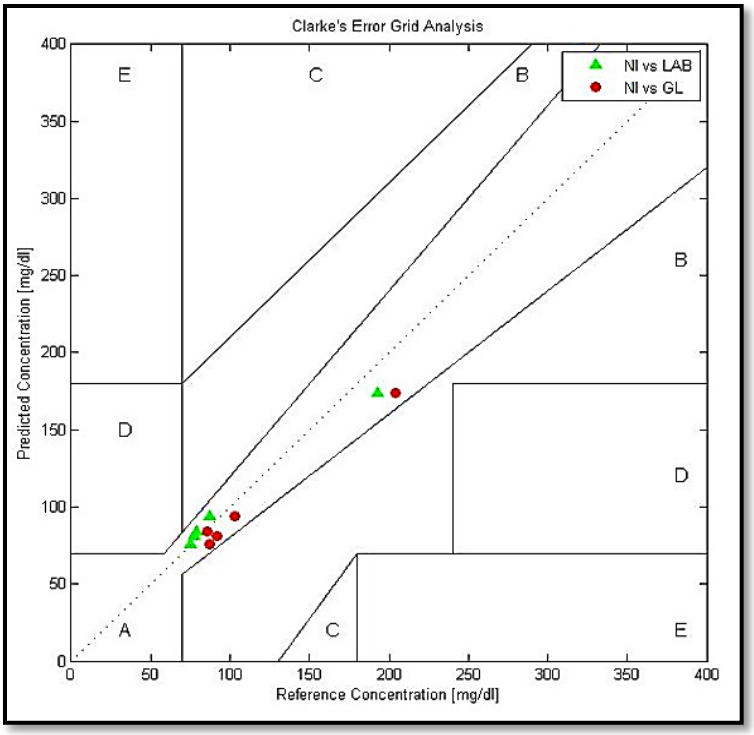

Figure 5.5 Clarke Error Grid (CEG) analysis of the invasive LAB & Glucometer (Reference) and non-invasive blood glucose values (Predicted) of test specimens during the random mode.

5.5 Clarke Error Grid Analysis for Beckman Coulter Results Comparison

5.5.1 Clinical Trials of Glucose

For blood glucose in-vivo analysis, a 3 day clinical trial is performed in the pathology LAB of Holy Family Hospital (HFH), Pakistan. Experimental study of all 3 days can be seen in the Table 5-3. A total of 132 test specimens were analyzed during the trial period, consisting of various diabetic and non-diabetic patients. Total 49, 57 and 26 test specimens were analyzed on day 1, 2 and 3, respectively. Out of which 14, 12 and 6 test specimens were analyzed in fasting mode on day 1, 2 and 3, respectively. And 35, 45 and 20 in random mode on day 1, 2 and 3, respectively. For diabetic test specimens, study involves total 13 type-2, 4 hypoglycemic and 4 gestational diabetic patients.

Table 5-3 Summary of experimental study subjects for clinical trials of glucose.

Days	Test Specimens	Fasting/ Random		Male/Female		Age (years) (min to max)	Diabetic	Non-diabetic
Day 1	49	Fasting	14	Male	3	30 to 40	Nil	3
				Female	11	18 to 37	3-Hypoglycemia	8
		Random	35	Male	7	23 to 74	1-Type 2	6
				Female	28	18 to 40	1-Type 2	27
Day 2	57	Fasting	12	Male	N/A	N/A	Nil	Nil
				Female	12	24 to 70	4-Type 2	8
		Random	45	Male	2	25 to 30	1-Hypoglycemia	1
				Female	43	21 to 65	4-Type 2	39
Day 3	26	Fasting	6	Male	2	57 to 69	2-Type 2	Nil
				Female	4	21 to 35	Nil	4
		Random	20	Male	1	10	Nil	1
				Female	19	21 to 38	4-Gestational 1-Type 2	14

5.5.2 Clarke Error Grid Analysis of ALL 3 Day Results

The Clarke Error Grid (CEG) analysis is done by using MATLAB software and graphically expressed as shown in figures 5.6, 5.7, 5.8, 5.9, 5.10 and 5.11. The figure 5.6 depicts the CEG of all 3 day results. The points lying in clinically accepted zone A are those which follows the International standards Organization (ISO) latest accuracy criteria and have maximum error equal or less than $\pm$ 15%. Likewise, points in zone B are those which have more than $\pm$ 15% error and so on. From CEG analysis, the respective blood glucose level determining accuracy dependent values are classified into different zones. For example zone A: 94.70%, zone B: 4.54%, zone C: 0.00%, zone D: 0.76% and zone E: 0.00%, respectively as shown in Figure 7. The mean Absolute Relative Differences (ARDs) value is 9.51% and median ARD is 8.05%.

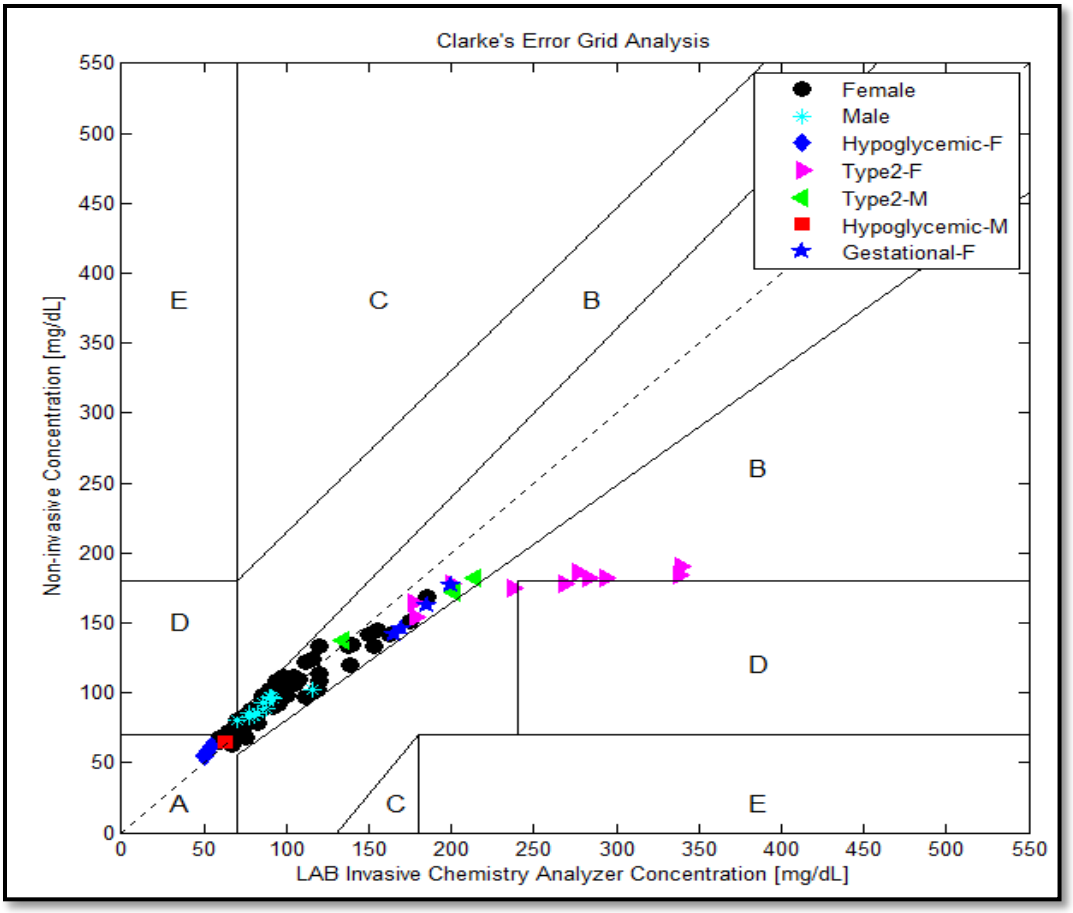

Figure 5.6 Clarke Error Grid (CEG) analysis of the LAB invasive chemistry analyzer Beckman Coulter AU-480 (x-axis) and non-invasive blood glucose concentration (y-axis) of test specimens of all 3 days experimental study.

5.5.3 Clarke Error Grid Analysis of ALL 3 Day Non-diabetic Results

The figure 5.7 shows the non-diabetic result values of all 3 day data. For non-diabetic patients, the blood glucose level determining accuracy dependent values are classified into CEG zones as; zone A: 100.00% and zone B, C, D and E = 0.00%, respectively as depicted in figure 8. The mean Absolute Relative Differences (ARDs) value of non-diabetic patients is 9.51% and median ARD value is 8.05%.

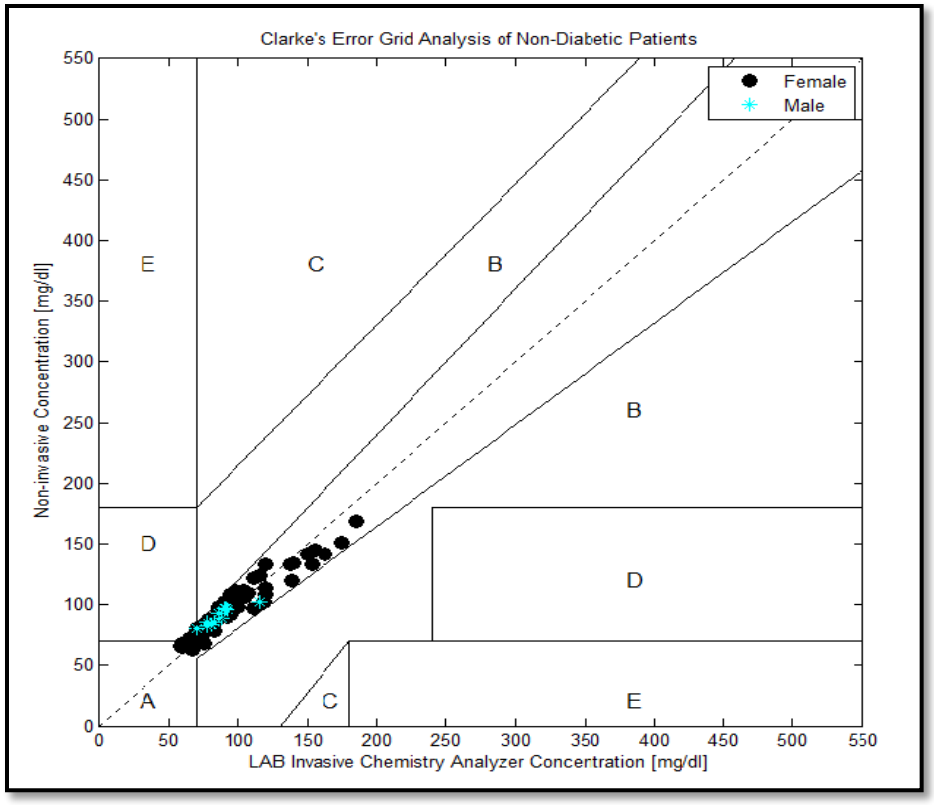

Figure 5.7 Clarke Error Grid (CEG) analysis of non-diabetic test specimens of all 3 days.

5.5.4 Clarke Error Grid Analysis of ALL 3 Day Diabetic Results

Figure 5.8 depicts the diabetic test results of all days and CEG zones for diabetic test specimens are classified as; zone A: 66.67%, zone B: 28.57%, zone C: 0.00%, zone D: 4.76% and zone E: 0.00%, respectively as shown in Figure 9. CEG analysis of diabetic patients involves 3 females suffering from hypoglycemia, 10 females of type-2 diabetes, 3 males of type 2, 1 male suffering from hypoglycemia and 4 females of gestational diabetes. The mean ARD value of diabetic test specimens is 19.53% and median ARD value is 13.94%.

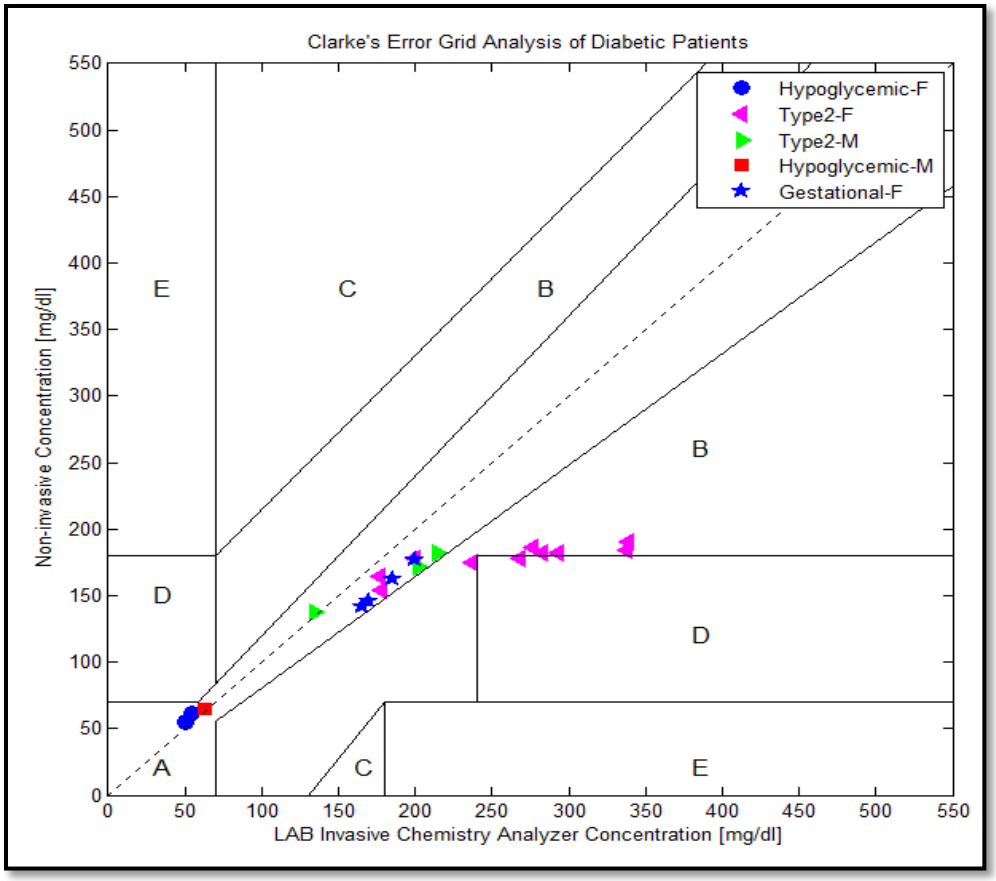

Figure 5.8 Clarke Error Grid (CEG) analysis of diabetic test specimens of all 3 days.

5.5.5 Clarke Error Grid Analysis of ALL 3 Day Diabetic and Non-diabetic Results in Fasting Mode

The figure 5.9 depicts the fasting mode readings of diabetic and non-diabetic test specimens of all 3 days. The blood glucose level determining accuracy dependent values are classified into CEG zones as; zone A: 90.63% and zone B: 6.25%, D: 3.13%, C and E = 0.00%, respectively as shown in figure 5.9.

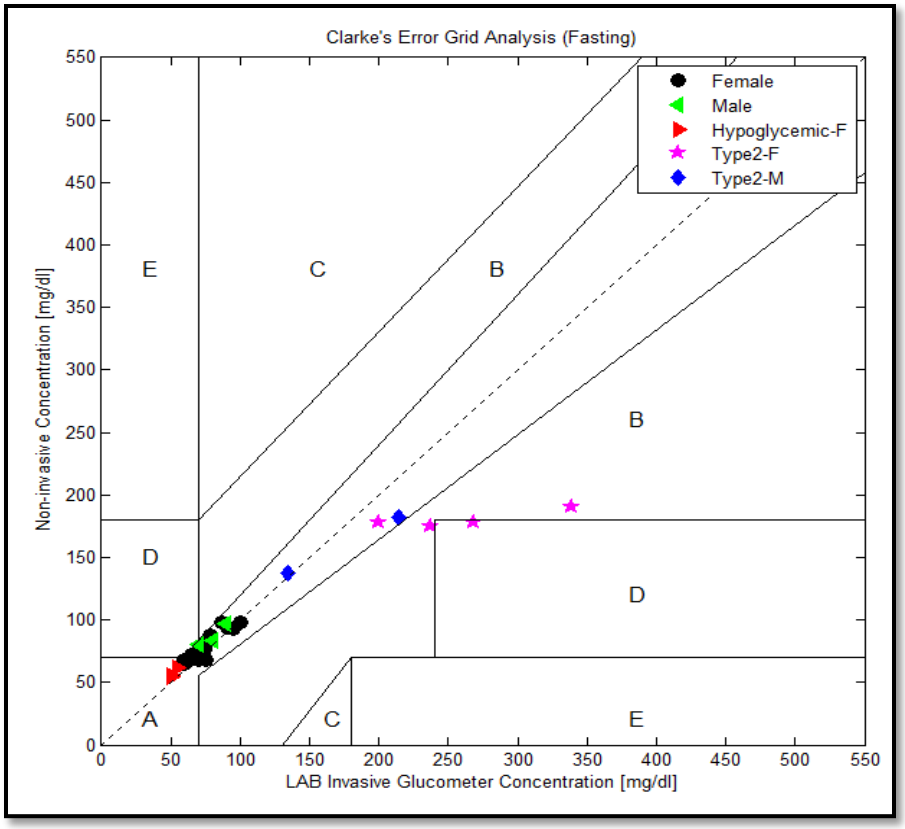

Figure 5.9 Clarke Error Grid (CEG) analysis of diabetic and non-diabetic test specimens of all 3 days in fasting mode.

5.5.6 Clarke Error Grid Analysis of ALL 3 Day Diabetic and Non-diabetic Results in Random Mode

CEG zones for random mode of diabetic and non-diabetic test specimens are classified as; zone A: 96.00%, zone B: 4.00%, zone C, D and E = 0.00%, respectively as depicted in figure 5.10. The mean ARD value of diabetic and non-diabetic test specimens in fasting mode is 11.20% and median ARD value is 9.67%, and the mean ARD value of random mode is 8.97% and median ARD value is 7.60%.

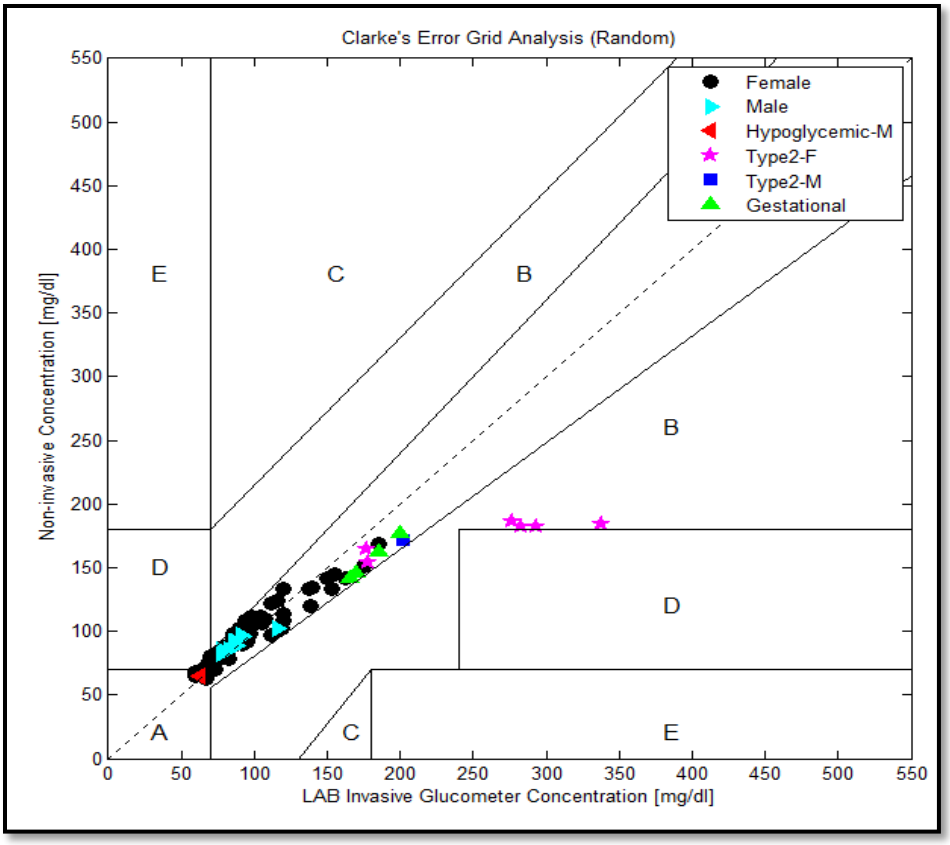

Figure 5.10 Clarke Error Grid (CEG) analysis of diabetic and non-diabetic test specimens of all 3 days in random mode.

5.6 Beckman Coulter AU-480

The invasive LAB chemistry analyzer installed in HFH is Beckman Coulter AU-480. It is a fully automated spectrophotometer, principally based on beer lambert's law. Blood glucose levels were analyzed through Endpoint-2 method at a primary source wavelength of 340 nm on this analyzer. First blood sample is centrifuged then serum is separated from blood via density separation method in centrifugal machine. Serum is then placed into chemistry analyzer AU-480.

A reagent is added in serum which reacts to it and develops a particular color. More the color, more will be the value of glucose in the serum. When visible light is passed on to this serum, a change in wavelength is being observed. Basically, instrument measures this change in wavelength. Instrument is further calibrated, for glucose reagent, with standard known valued calibration samples. Once reagent is calibrated, a standard curve is generated. Instrument saves

that curve for a limited period of time and any unknown glucose sample can be correlated to get the exact glucose value.

5.7 Double Regression Model Results

The figure 5.11 depicts the improved results by the implementation of double regression model. With respect to figure 5.6 which shows the CEG analysis of all the 3 day data. It can be seen clearly that there are 7 test results who have error more than 15% that is why they are out of the zone A.

These are some hyperglycemic diabetic patients' results who have extremely high glucose levels and are on heavy dose of insulin as depicted in figure 5.8. But after the application of double regression model (DRM), the accuracy has been improved and 98.48% of the test results lie in clinically accepted zone A.

Previously, it was 94.70%. Likewise, mean and median error ARDs are also get reduced to 8.25% and 7.94%, respectively. Previously, mean and median errors were 9.51% and 8.05%, respectively.

5.7.1 Improved Accuracy Analysis via Coefficient of Determination

The coefficient of determination R^2, depicts the goodness of a fit and it is evident from the figures 5.12 and 5.13 that it has been improved by the implementation of DRM. The figure 5.12 represents that the value of coefficient of determination, for diabetic patients of all days who have shown high glucose values and underwent more than 15% error, is 0.8162 and after the DRM implementation it gets improved to 0.9471, as shown in figure 5.13.

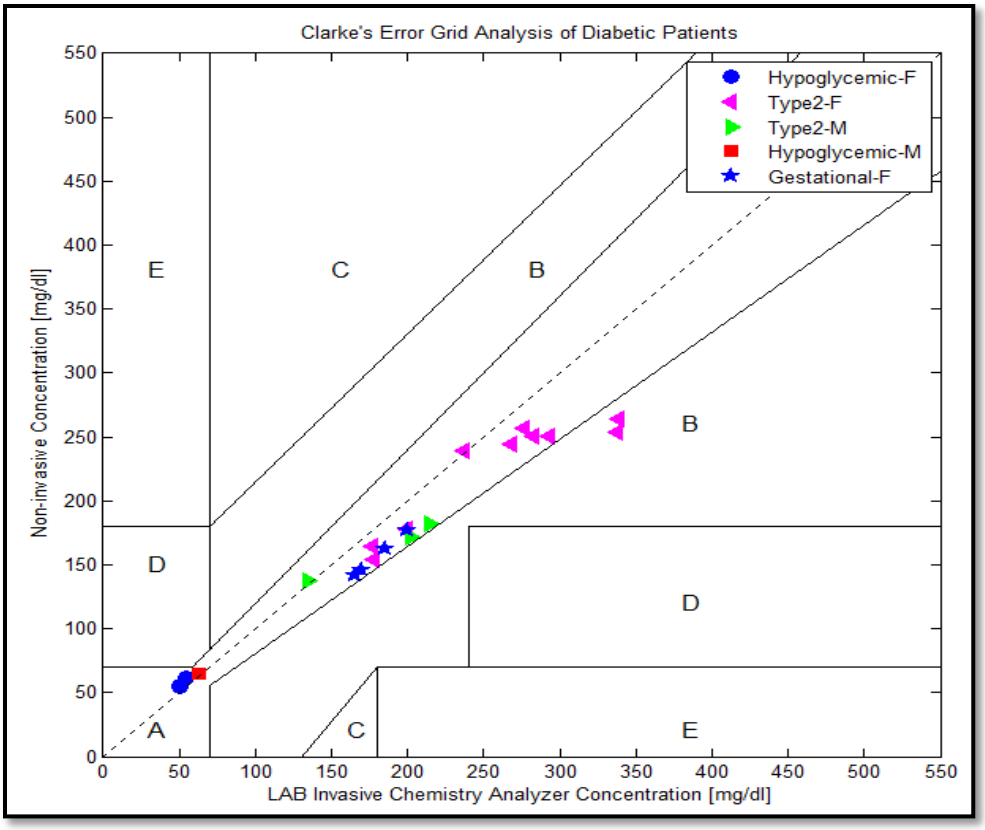

Figure 5.11 Clarke Error Grid (CEG) analysis of all 3 day diabetic test results after the implementation of double regression model.

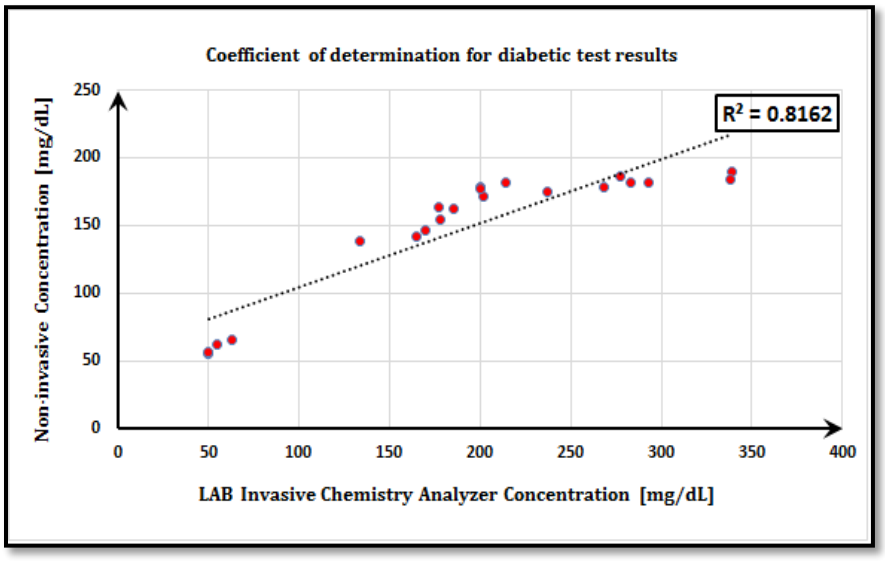

Figure 5.12 Coefficient of determination for diabetic test results without DRM implementation.

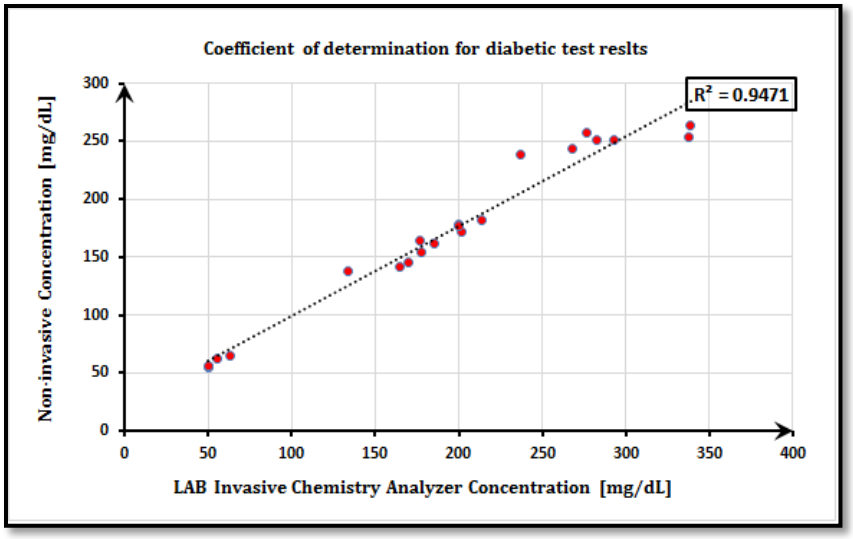

Figure 5.13 Coefficient of determination for diabetic test results with DRM implementation.

5.8 Discussion

This thesis research presents the design and development of a non-invasive system capable of measuring the pulse rate, blood glucose and oxygen saturation level in arterial blood. In the hustle and bustle of life, everyone wants to be healthy by keeping an eye on their body disorders and especially for those who are suffering from some chronic diseases like diabetes, heart and lung diseases. The conventional devices available in the market are invasive, pain causing, and require puncturing of skin every single time a person needs to take a glucose reading. Moreover, they are expensive because every time a new test strip and needle are required to check the glucose level. Diabetes is a cureless disease till the date. The only thing diabetic patients can do is to continuously monitor their blood glucose level and take insulin accordingly. That is why there was a need to propose a non-invasive approach to deal with all said issues.

Previously, much research has been done in the field of non-invasive glucose measurement by implementing various techniques. However, all previously invented glucometers are inaccurate because of comparatively weak absorption of the glucose in the near infra-red range, which leads to erroneous results. This happens because of overlying absorption from water, proteins, or other blood components, and especially from arterial blood and tissues in the optical path. Previously proposed glucose monitors were not proven to be practical, precise, clinically approved and/or economical. All of them were either for investigational purpose or market awareness.

The proposed prototype is based on NIRS which undergoes photoplethysmography (PPG) and double regression analysis. Near infra-red spectroscopy (NIRS) is most famous because it allows minimum penetration while measuring the blood glucose. NIRS helps to deal with the tissues having low absorption energy and permits glucose measurement up to few millimeters depth under the skin.

In order to validate the results, a 3 day clinical trial is conducted, to perform in vivo analysis, in HFH and a total of 132 diabetic and non-diabetic test specimens are analyzed. Non-invasive (NI) system's results are compared with the invasive Beckman Coulter AU-480 chemistry analyzer. The CEG analysis of all 3 day results yields the mean and median values of 9.51% and 8.05%, respectively. The CEG analysis proves that 94.70% of the readings lie in the clinically accepted zone A. However, there were some of the hyperglycemic results having more than 15% error and they were out of the clinically accepted zone A. In order to increase the accuracy, overcome the deviations and get more reliable results, the double regression analysis is performed. The analysis shows that 98.48% of the results fall in the clinically accepted zone A. Likewise, mean and median ARDs are also get reduced to 8.25% and 7.94%, respectively. The coefficient of determination R^2, depicts the goodness of a fit that how close the predicted values are to reference glucose values. After the implementation of DRM, the coefficient of determination R^2 get improves to 0.9471.

This thesis research also includes literature review which showers the light on the previously used NI techniques and resulting accuracies. People have used multiple and complexed techniques in order to achieve high accuracy but my motive was to get as maximum accuracy as possible by keeping the design simple and economical. And it has been observed that proposed NI system has achieved more accuracy then those previously proposed complexed NIRS designs. Nevertheless, this current prototype is not feasible for the clinical purpose because few of the test specimens who were on high dose of insulin have shown extremely high glucose levels. Thus in future, some other transduction method needs to be incorporated in the design to improve accuracy.

CHAPTER 6: CONCLUSION AND FUTURE WORK

Diabetes mellitus is a chronic disease which has no cure. The one thing diabetic patients do to keep themselves healthy is continuous glucose monitoring. All commercially available glucometers are invasive. Hence, it is pretty inconvenient to prink a finger 4 to 5 times a day to just to check the blood glucose. Since, necessity is the mother of invention so there has to be some solution to this problem.

A non-invasive glucose measuring system can ease the life of a diabetic person. Previously, much research has been done in the field of non-invasive glucose measurement but all suffer from inaccuracies because of overlying absorption from water, proteins, or other blood components, and especially from arterial blood and tissues in the optical path, which suppress the real glucose value and, resulting in erroneous results. That is why those glucose monitors were not proven to be practical, precise, and/or economically viable.

Therefore, the proposed design is simple, reliable and easy to use, which involves no pricking of finger, no puncturing of skin and no pain, eventually. Hence, the proposed system is promising to resolve patient's health issues whilst measuring pulse rate, oxygen saturation ratio and blood glucose non-invasively with maximum accuracy without making the design complex. International Standards Organization (ISO) accuracy criterion is tried to be followed whilst achieving 98.48% accuracy. Likewise, achieved mean and median absolute relative differences (ARDs) are 8.25% and 7.94%, respectively. Double regression model helped to improve the accuracy of the proposed prototype. The coefficient of determination R^2, which depicts the goodness of a fit, shows the value of 0.9471.

6.1Future Scope

In future, some other transduction method needs to be incorporated in the design to improve the accuracy.

REFERENCES

[1] Merrick, Edwin B., and Thomas J. Hayes. "Continuous, non-invasive measurements of arterial blood oxygen levels." *Hewlett-packard J.* 28.2 (1976): 2-9.

[2] Tremper, Kevin K. "Pulse oximetry." *Chest Journal.* 95.4 (1989): 713-715.

[3] Vijaylaskhmi Kamat. "Pulse oximetry." *Indian J. Anesthesiology*. 2002, 261–268.

[4] Hodavance, Michael S., Sarah L. Ralston, and István Pelczer. "Beyond blood sugar: the potential of NMR-based metabonomics for type 2 human diabetes, and the horse as a possible model." *Analytical and bioanalytical chemistry* 387.2 (2007): 533-537.

[5] Norman Endocrine Surgery Clinic, "Normal Regulation of Blood Glucose, "Norman Endocrine Surgery Clinic, 2002. [Online] 2006(November 5), Available: http://www.endocrineweb.com/insulin.html.

[6] Webster, John G., ed. *The Physiological Measurement Handbook*. CRC Press, 2014.

[7] Kyriacou, Panayiotis A. *Investigation of new electro-optical techniques for monitoring patients with compromised peripheral perfusion in anaesthesia.* Diss. School of Medicine and Dentistry, Queen Mary, University of London, London, 2001.

[8] Sulotto, F., et al. "Normal values of carboxyhemoglobinemia and methemoglobinemia in a sample of conscripts." *La Medicina del lavoro* 85.4 (1993): 289-298.

[9] Eisenkraft, James B. "Pulse oximeter desaturation due to methemoglobinemia." *The Journal of the American Society of Anesthesiologists* 68.2 (1988): 279-281.

[10] Friebel, Moritz, et al. "Influence of oxygen saturation on the optical scattering properties of human red blood cells in the spectral range 250to2000nm." *Journal of biomedical optics* 14.3 (2009): 034001-034001.

[11] http://www.lmtb.de/gb_bmo/optical_properties_blood_en.php#prettyPhoto/0/

[12] Hayashi, Yoshihito, Leonid Livshits, Andreas Caduff, and Yuri Feldman. "Dielectric spectroscopy study of specific glucose influence on human erythrocyte membranes." *Journal of Physics D: Applied Physics* 36, no. 4 (2003): 369.

[13] Livshits, L., et al. "Dielectric response of biconcave erythrocyte membranes to D-and L-glucose." *Journal of Physics D: Applied Physics* 40.1 (2006): 15.

[14] Beatty, P. C. W. "Design of pulse oximeters (medical science series)." (1998).

[15] http://www.goldbamboo.com/topic-t4272-a1 6Hemoglobin.html.

[16] Wukitsch, Michael W., et al. "Pulse oximetry: analysis of theory, technology, and practice." *Journal of Clinical Monitoring and Computing* 4.4 (1987): 290-301.

[17] Leahy, Martin J., Manus P. Henry, and David W. Clarke. "Sensor validation in biomedical applications." *Control Engineering Practice* 5.12 (1997): 1753-1758.

[18] Mannheimer, Paul D., et al. "Wavelength selection for low-saturation pulse oximetry." *IEEE Transactions on Biomedical Engineering* 44.3 (1997): 148-158.

[19] Khalil, Omar S. "Spectroscopic and clinical aspects of noninvasive glucose measurements." *clinical chemistry* 45.2 (1999): 165-177.

[20] Webster, John G., ed. *Electrical measurement, signal processing, and displays*. CRC Press, 2003.

[21] J. Webster, "Design of Pulse Oximeters," in *Medical Science Series*, W. J. G, Ed.: IOP Publishing Ltd, 1997.

[22] Shelley, Kirk H. "Photoplethysmography: beyond the calculation of arterial oxygen saturation and heart rate." *Anesthesia & Analgesia* 105.6 (2007): S31-S36.

[23] Nijboer, J. A., J. C. Dorlas, and H. F. Mahieu. "Photoelectric plethysmography-some fundamental aspects of the reflection and transmission methods." *Clinical Physics and Physiological Measurement* 2.3 (1981): 205.

[24] Roberts, V. C. "Photoplethysmography-fundamental aspects of the optical properties of blood in motion." *Transactions of the Institute of Measurement and Control* 4.2 (1982): 101-106.

[25] Challoner, A. V. J., and C. A. Ramsay. "A photoelectric plethysmograph for the measurement of cutaneous blood flow." *Physics in Medicine and Biology* 19.3 (1974): 317.

[26] Dorlas, J. C., and J. A. Nijboer. "PHOTO-ELECTRIC PLETHYSMOGRAPHY AS A MONITORING DEVICE IN ANAESTHESIA Application and Interpretation." *British journal of anaesthesia* 57.5 (1985): 524-530.

[27] Kyriacou, Panayiotis A. *Investigation of new electro-optical techniques for monitoring patients with compromised peripheral perfusion in anaesthesia*. Diss. School of Medicine and Dentistry, Queen Mary, University of London, London, 2001.

[28] https://hackaday.io/project/7177/logs

[29] Merriam-Webster's Medical Dictionary. Springfield, Massachusetts: MerriamWebster, Inc., 2006.

[30] Åsberg, Cristine, et al. "Fructose 1, 6-bisphosphatase deficiency: enzyme and mutation analysis performed on calcitriol-stimulated monocytes with a note on long-term prognosis." *Journal of inherited metabolic disease* 33.3 (2010): 113-121.

[31] Norman Endocrine Surgery Clinic, "Normal Regulation of Blood Glucose, "Norman Endocrine Surgery Clinic, 2002. [Online] 2006(November 5), Available: http://www.endocrineweb.com/insulin.html

[32] Moyle J.T.B. Pulse oxymetry. BMJ Publishing Group, London, 1994.

[33] Mendelson, Yitzhak, and Burt D. Ochs. "Noninvasive pulse oximetry utilizing skin reflectance photoplethysmography." *IEEE Transactions on Biomedical Engineering* 35.10 (1988): 798-805.

[34] Wood, Earl H., and J. E. Geraci. "Photoelectric determination of arterial oxygen saturation in man." *Journal of Laboratory and Clinical Medicine* 34.3 (1949): 387-401.

[35] Medline Plus Medical Encyclopedia, "Glucose Test," U.S. National Library of Medicine and National Institutes of Health. [Online] 2006(November 3), Available: http://www.nlm.nih.gov/medlineplus/ency/article/003482.html.

[36] Anonymous (2007, October 4, 2007). Glucose Meter. [Online]. 2007(October 4), Available: http://en.wikipedia.org/wiki/Glucose_meter.

[37] Sherman, Marie. "How do blood glucose meters work?." *Chem* 13 (2006): 5-6.

[38] Bergenstal, Richard M. "Evaluating the accuracy of modern glucose meters." *Insulin* 3.1 (2008): 5-14.

[39] Ralston, A. C., R. K. Webb, and W. B. Runciman. "Potential errors in pulse oximetry III: effects of interference, dyes, dyshaemoglobins and other pigments." *Anaesthesia* 46.4 (1991): 291-295.

[40] Shafique, Muhammad, Justin P. Phillips, and Panayiotis A. Kyriacou. "A novel non-invasive trans-reflectance photoplethysmographic probe for use in cases of low peripheral blood perfusion." *Engineering in Medicine and Biology Society, 2009. EMBC 2009. Annual International Conference of the IEEE. IEEE*, 2009.

[41] HeikkiPalve. "Reflection and transmission pulse oximetry during compromised peripheral perfusion." *Journal of Clinical Monitoring*, 8:12–15, 1992.

[42] Lee, Juwon, et al. "Design of filter to reject motion artifact of pulse oximetry." *Computer Standards & Interfaces* 26.3 (2004): 241-249.

[43] Visram, A. R., et al. "Use of two oximeters to investigate a method of movement artefact rejection using photoplethysmographic signals." *British journal of anaesthesia* 72.4 (1994): 388-392.

[44] Kim, Sang Hyun, Dong Wan Ryoo, and Changseok Bae. "Adaptive noise cancellation using accelerometers for the PPG signal from forehead." *Engineering in Medicine and Biology Society, 2007. EMBS 2007. 29th Annual International Conference of the IEEE. IEEE*, 2007.

[45] Hayes, Matthew J., and Peter R. Smith. "Artifact reduction in photoplethysmography." *Applied Optics* 37.31 (1998): 7437-7446.

[46] Foo, Jong Yong A., and Stephen J. Wilson. "A computational system to optimise noise rejection in photoplethysmography signals during motion or poor perfusion states." *Medical and Biological Engineering and Computing* 44.1-2 (2006): 140-145.

[79] Engber, William D., and James G. Gmeiner. "Palmar cutaneous branch of the ulnar nerve." *The Journal of hand surgery* 5.1 (1980): 26-29.

[80] Whiting, William Charles, and Ronald F. Zernicke. *Biomechanics of musculoskeletal injury*. Human Kinetics, 2008.

[81] http://www.becomehealthynow.com/article2/bodycardio/699/.

[82] Kassab, Ghassan S. "Biomechanics of the cardiovascular system: the aorta as an illustratory example." *Journal of the Royal Society Interface* 3.11 (2006): 719-740.

[83] McVeigh, Gary E., Alan J. Bank, and Jay N. Cohn. "Arterial compliance." *Cardiovascular medicine. Springer London*, 2007. 1811-1831.

[84] Dyson, Alex, et al. "Tissue oxygen monitoring in rodent models of shock." *American Journal of Physiology-Heart and Circulatory Physiology* 293.1 (2007): H526-H533.

[47] Gelao, Gennaro, et al. "Design of a dielectric spectroscopy sensor for continuous and non-invasive blood glucose monitoring." *International Journal of Advances in Engineering & Technology* 3.2 (2012): 55.

[48] Ashok, V., A. Nirmalkumar, and N. Jeyashanthi. "A novel method for blood glucose measurement by noninvasive technique using laser." *Evaluation* 376 (2010): 2624.

[49] Abdallah, O., et al. "Design of a compact multi-sensor system for non-invasive glucose monitoring using optical spectroscopy." *International Conference on Electronics. Biomedical Engineering and its Applications (ICEBEA).* 2012.

[50] Burmeister, Jason J., Mark A. Arnold, and Gary W. Small. "Noninvasive blood glucose measurements by near-infrared transmission spectroscopy across human tongues." *Diabetes technology & therapeutics* 2.1 (2000): 5-16.

[51] Tang, Fei, et al. "Non-invasive glucose measurement by use of metabolic heat conformation method." *Sensors* 8.5 (2008): 3335-3344.

[52] Shinde, A. A., and R. K. Prasad. "Non Invasive Blood Glucose Measurement using NIR technique based on occlusion spectroscopy." *International Journal of Engineering Science and Technology (IJEST)* 3.12 (2011).

[53] O. Amir, D. Weinstein, S. Zilberman, M. lka Less, D. Perl-Treves, H. Pri-mack, A. Weinstein, E. Gabis, B. Fikhte, and A. Karasik: “Continuous Noninvasive Glucose Monitoring Technology Based on "Occlusion Spectroscopy”, *Journal of Diabetes Science and Technology*, July 2007.

[54] Menon, KA Unnikrishna, Deepak Hemachandran, and T. K. Abhishek. "A survey on non-invasive blood glucose monitoring using NIR." In *Communications and Signal Processing (ICCSP), 2013 International Conference on*, pp. 1069-1072. IEEE, 2013.

[55] Rabinovitch, B., W. F. March, and Robert L. Adams. "Noninvasive glucose monitoring of the aqueous humor of the eye: Part I. Measurement of very small optical rotations." *Diabetes Care* 5, no. 3 (1982): 254-258.

[56] Maruo, Katsuhiko, Mitsuhiro Tsurugi, Mamoru Tamura, and Yukihiro Ozaki. "In vivo noninvasive measurement of blood glucose by near-infrared diffuse-reflectance spectroscopy." *Applied spectroscopy* 57, no. 10 (2003): 1236-1244.

[57] Heise, H. M., and R. Marbach. "Human oral mucosa studies with varying blood glucose concentration by non-invasive ATR-FT-IR-spectroscopy." *Cellular and molecular biology (Noisy-le-Grand, France)* 44, no. 6 (1998): 899-912.

[58] Heinemann, Lutz, Uwe Krämer, Hans-Martin Klötzer, Michael Hein, Dietmar Volz, Marcus Hermann, Tim Heise, and Klaus Rave. "Noninvasive glucose measurement by monitoring of scattering coefficient during oral glucose tolerance tests." *Diabetes technology & therapeutics* 2, no. 2 (2000): 211-220.

[59] Weiss, Ram, Yevgeny Yegorchikov, Alexander Shusterman, and Itamar Raz. "Noninvasive continuous glucose monitoring using photoacoustic technology—results from the first 62 subjects." *Diabetes technology & therapeutics* 9, no. 1 (2007): 68-74.

[60] Forst, T., A. Caduff, M. Talary, M. Weder, M. Brändle, P. Kann, F. Flacke, Ch Friedrich, and A. Pfützner. "Impact of environmental temperature on skin thickness and microvascular blood flow in subjects with and without diabetes." *Diabetes Technology & Therapeutics* 8, no. 1 (2006): 94-101.

[61] Guo, D. X., et al. "Noninvasive Blood Glucose Measurement Based on NIR Spectrums and Double ANN Analysis." *Journal of Biosciences and Medicines* 3, 06 (2015): 42.

[62] Lipson, Jan, Jeff Bernhardt, Ueyn Block, William R. Freeman, Rudy Hofmeister, Maya Hristakeva, Thomas Lenosky et al. "Requirements for calibration in noninvasive glucose monitoring by Raman spectroscopy." *Journal of diabetes science and technology* 3, no. 2 (2009): 233-241.

[63] Zeller, H., P. Novak, and R. Landgraf. "Blood glucose measurement by infrared spectroscopy." *The International journal of artificial organs* 12, no. 2 (1989): 129-135.

[64] Yamakoshi, Kenichi, and Y. Yamakoshi. "Pulse glucometry: a new approach for noninvasive blood glucose measurement using instantaneous differential near-infrared spectrophotometry." *Journal of Biomedical Optics* 11, no. 5 (2006): 054028-054028.

[65] Chen, Chung-Sen, et al. "Noninvasive blood glucose monitoring using the optical signal of pulsatile microcirculation: a pilot study in subjects with diabetes." *Journal of diabetes and its complications* 22.6 (2008): 371-376.

[66] Gabriely, Ilan, et al. "Transcutaneous glucose measurement using near-infrared spectroscopy during hypoglycemia." *Diabetes Care* 22.12 (1999): 2026-2032.

[67] Harman-Boehm, Ilana, et al. "Noninvasive glucose monitoring: a novel approach." *Journal of Diabetes Science and Technology*, 3(2), (2009): 253-260.

[68] Harman-Boehm, Ilana, et al. "Noninvasive glucose monitoring: increasing accuracy by combination of multi-technology and multi-sensors." *Journal of diabetes science and technology* 4.3 (2010): 583-595.

[69] Chowdhury, Md Koushik, et al. "Error Grid Analysis of Reference and Predicted Blood Glucose Level Values as Obtained from the Normal and Prediabetic Human Volunteers." *American Journal of Biomedical Engineering* 5.1 (2015): 6-14.

[70] Diabetes Research in Children Network (DirecNet) Study Group. "Accuracy of the glucowatch g2 biographer and the continuous glucose monitoring system during hypoglycemia. Experience of the diabetes research in children network (direcnet)." *Diabetes care* 27, no. 3 (2004): 722.

[71] Ye, Shuming, Feng Zhou, Hang Chen, Hong Yan, and Yan Li. "Non-Invasive Method and Experimental Study for Measurement of Oxygen Saturation Rate in Wide Range." In *Bioinformatics and Biomedical Engineering, 2008. ICBBE 2008. The 2nd International Conference on*, pp. 733-736. IEEE, 2008.

[72] Teng, Yichao, Haishu Ding, and Qingcheng Gong. "Non-invasive monitoring of human cerebral oxygen saturation by near infrared spectroscopy: instrumentation, calibration and application in cardiopulmonary bypass." In *Engineering in Medicine and Biology Society, 2005. IEEE-EMBS 2005. 27th Annual International Conference of the*, pp. 670-673. IEEE, 2006.

[73] Barthelemy, Jean-Claude, and Andre Geyssant. "Non-invasive method for the in vivo determination of the oxygen saturation rate of arterial blood, and device for carrying out the method." *U.S. Patent* 5,413,100, issued May 9, 1995.

[74] Reddy, K. Ashoka, Boby George, N. Madhu Mohan, and V. Jagadeesh Kumar. "A novel method for the measurement of oxygen saturation in arterial blood." In *Instrumentation and Measurement Technology Conference (I2MTC), 2011 IEEE*, pp. 1-5. IEEE, 2011.

[75] Bagha, Sangeeta, and Laxmi Shaw. "A real time analysis of PPG signal for measurement of SpO2 and pulse rate." *International journal of computer applications* 36.11 (2011): 45-50.

[76] Liu, Shing-Hong, Kang-Ming Chang, and Tsu-Hsun Fu. "Heart rate extraction from photoplethysmogram on fuzzy logic discriminator." *Engineering Applications of Artificial Intelligence* 23.6 (2010): 968-977.

[77] Azmal, Gazi Maruf, and Adel Al-Jumaily. "Continuous measurement of oxygen saturation level using photoplethysmography signal." *Biomedical and Pharmaceutical Engineering, 2006. ICBPE 2006. International Conference on. IEEE*, 2006.

[78] PS KB, Jatti A. Respiration and Heart Rate Monitoring from Photoplethysmograph Signal. 2015, 3(1), 8-16. *DOI:* 10.13189/asp.2015.030102.

[85] Heinemann, Lutz, and T. Koschinsky. "Continuous glucose monitoring: an overview of today's technologies and their clinical applications." *International journal of clinical practice. Supplement* 129 (2002): 75-79.

[86] Koschinsky, Theodor, Karsten Jungheim, and Lutz Heinemann. "Glucose sensors and the alternate site testing-like phenomenon: relationship between rapid blood glucose changes and glucose sensor signals." *Diabetes technology & therapeutics* 5.5 (2003): 829-842.

[87] Rebrin, Kerstin, et al. "Subcutaneous glucose predicts plasma glucose independent of insulin: implications for continuous monitoring." *American Journal of Physiology-Endocrinology and Metabolism* 277.3 (1999): E561-E571.

[88] Maruo, Katsuhiko, et al. "In vivo noninvasive measurement of blood glucose by near-infrared diffuse-reflectance spectroscopy." *Applied spectroscopy* 57.10 (2003): 1236-1244.

[89] Heise, H. M., and R. Marbach. "Human oral mucosa studies with varying blood glucose concentration by non-invasive ATR-FT-IR-spectroscopy." *Cellular and molecular biology (Noisy-le-Grand, France)* 44.6 (1998): 899-912.

[90] Khalil, Omar S. "Non-invasive glucose measurement technologies: an update from 1999 to the dawn of the new millennium." *Diabetes technology & therapeutics* 6.5 (2004): 660-697.

[91] Heinemann, Lutz, et al. "Noninvasive glucose measurement by monitoring of scattering coefficient during oral glucose tolerance tests." *Diabetes technology & therapeutics* 2.2 (2000): 211-220.

[92] Yeh, Shu-jen, Charles F. Hanna, and Omar S. Khalil. "Monitoring blood glucose changes in cutaneous tissue by temperature-modulated localized reflectance measurements." *Clinical Chemistry* 49.6 (2003): 924-934.

[93] Wickramasinghe, Y., Y. Yang, and S. A. Spencer. "Current problems and potential techniques in in vivo glucose monitoring." *Journal of fluorescence* 14.5 (2004): 513-520.

[94] Waynant, R. W., and V. M. Chenault. "Overview of Non-Invasive Optical Glucose Monitoring Techniques." *Food and Drug AdministrationOffice of Science Technology and Office of Device Evaluation* (1998).

[95] Shen, Yaochun, et al. "Measurement of the optical absorption coefficient of a liquid by use of a time-resolved photoacoustic technique." *Applied optics* 39.22 (2000): 4007-4012.

[96] Ermolina, I., Yu Polevaya, and Yu Feldman. "Analysis of dielectric spectra of eukaryotic cells by computer modeling." *European Biophysics Journal* 29.2 (2000): 141-145.

[97] Polevaya, Yulia, et al. "Time domain dielectric spectroscopy study of human cells: II. Normal and malignant white blood cells." *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1419.2 (1999): 257-271.

[98] Gourzi, Mohamed, et al. "Study of a new electromagnetic sensor for glycaemia measurement: in vitro results on blood pig." *Journal of medical engineering & technology* 27.6 (2003): 276-281.

[99] Gourzi, Mohamed, et al. "Non-invasive glycaemia blood measurements by electromagnetic sensor: study in static and dynamic blood circulation." *Journal of medical engineering & technology* 29.1 (2005): 22-26.

[100] Moran, Gerald R., et al. "A dielectric analysis of liquid and glassy solid glucose/water solutions." *Carbohydrate Research* 328.4 (2000): 573-584.

[101] Yeh, Shu-jen, Charles F. Hanna, and Omar S. Khalil. "Monitoring blood glucose changes in cutaneous tissue by temperature-modulated localized reflectance measurements." *Clinical Chemistry* 49.6 (2003): 924-934.

[102] Sandby-Møller, Jane, Thomas Poulsen, and Hans Christian Wulf. "Influence of epidermal thickness, pigmentation and redness on skin autofluorescence." *Photochemistry and photobiology* 77.6 (2003): 616-620.

[103] Hanlon, E. B., et al. "Prospects for in vivo Raman spectroscopy." *Physics in medicine and biology* 45.2 (2000): R1.

[104] Steffes, Paul G. "Laser-based measurement of glucose in the ocular aqueous humor: an efficacious portal for determination of serum glucose levels." *Diabetes technology & therapeutics* 1.2 (1999): 129-133.

[105] Khalil, Omar S. "Spectroscopic and clinical aspects of noninvasive glucose measurements." *Clinical Chemistry* 45.2 (1999): 165-177.

[106] Heise, H. M. "Non-invasive monitoring of metabolites using near infrared spectroscopy: state of the art." *Hormone and metabolic research* 28.10 (1996): 527-534.

[107] Chung, Hoeil, et al. "Simultaneous measurements of glucose, glutamine, ammonia, lactate, and glutamate in aqueous solutions by near-infrared spectroscopy." *Applied Spectroscopy* 50.2 (1996): 270-276.

[108] Riley, Mark R., et al. "Adaptive Calibration Scheme for Quantification of Nutrients and Byproducts in Insect Cell Bioreactors by Near-Infrared Spectroscopy." *Biotechnology progress* 14.3 (1998): 527-533.

[109] Arnold, Mark A., and Gary W. Small. "Determination of physiological levels of glucose in an aqueous matrix with digitally filtered Fourier transform near-infrared spectra." *Analytical Chemistry* 62.14 (1990): 1457-1464.

[110] Lu, Geng, et al. "Multivariate calibration models based on the direct analysis of near-infrared single-beam spectra." *Applied spectroscopy* 51.9 (1997): 1330-1339.

[111] McShane, Michael J., Gerard L. Coté, and Clifford Spiegelman. "Variable selection in multivariate calibration of a spectroscopic glucose sensor." *Applied Spectroscopy* 51.10 (1997): 1559-1564.

[112] Riley, Mark R., Mark A. Arnold, and David W. Murhammer. "Effect of sample complexity on quantification of analytes in aqueous samples by near-infrared spectroscopy." *Applied Spectroscopy* 54.2 (2000): 255-261.

[113] Ciurczak, Emil W., Kevin P. Bynum, and Howard Mark. "Near infrared blood glucose monitoring system." *U.S. Patent* No. 6,675,030. 6 Jan. 2004.

[114] Yang, Wei, and Shu Zhang. "Method and apparatus of non-invasive measurement of human/animal blood glucose and other metabolites." *U.S. Patent* No. 6,167,290. 26 Dec. 2000.

[115] Caduff, Andreas, Etienne Hirt, and Thomas W. Schrepfer. "Impedance spectroscopy based systems and methods." *U.S. Patent* No. 7,315,767. 1 Jan. 2008.

[116] Thomas, Graham H., Roger M. Watson, and J. Oakey Noell. "Method and apparatus for non-invasive monitoring of blood glucose." *U.S. Patent* No. 5,119,819. 9 Jun. 1992.

[117] Azmal, Gazi Maruf, and Adel Al-Jumaily. "Continuous measurement of oxygen saturation level using photoplethysmography signal." *Biomedical and Pharmaceutical Engineering, 2006. ICBPE 2006. International Conference on. IEEE*, 2006.

[118] https://www.amazon.com/Santamedical-Generation-SM-165-Fingertip-Saturation/dp/B00R59OTOC

[119] Clarke, William L., et al. "Evaluating clinical accuracy of systems for self-monitoring of blood glucose." *Diabetes care* 10.5 (1987): 622-628.

[120] https://en.wikipedia.org/wiki/Clarke_Error_Grid#/media/File:Clarkeerrorgrid.gif

[121] Wilson, G.S.; Zhang, Y. Introduction to the glucose sensing problem. In In Vivo Glucose Sensing; Cunningham, D.D., Stenken, J.A., Eds; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2009; *doi: 10.1002/9780470567319*. ch1.

Completion Certificate

It is certified that the thesis titled **"Non-invasive SPO2 & blood glucose measurement using near infrared spectroscopy (NIRS)"** submitted by registration no. NUST201362513MCEME35513F, NS Nazo Haroon of MS-78 Mechatronics Engineering is completed in all respects as per the requirements of MainOffice, NUST (Exam branch).

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