Non-Invasive Method To Detect The Changes Of Glucose Concentration In Whole Blood Using Photometric Technique

By

Shiny Amala Priya Rajan

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved November 2013 by the
Graduate Supervisory Committee:

Bruce Towe, Chair
Jitendran Muthuswamy
Jeffrey LaBelle

ARIZONA STATE UNIVERSITY

December 2013

ABSTRACT

A noninvasive optical method is developed to monitor rapid changes in blood glucose levels in diabetic patients. The system depends on an optical cell built with a LED that emits light of wavelength 535nm that is a peak absorbance of hemoglobin. As the glucose concentration in the blood decreases, its osmolarity also decreases and the RBCs swell and decrease the path length absorption coefficient. Decreasing absorption coefficient increases the transmission of light through the whole blood.

The system was tested with a constructed optical cell that held whole blood in a capillary tube. As expected the light transmitted to the photodiode increases with decreasing glucose concentration. The average response time of the system was between 30-40 seconds. The changes in size of the RBC cells in response to glucose concentration changes were confirmed using a cell counter and also visually under microscope.

This method does not allow measuring the glucose concentration with an absolute concentration calibration. It is directed towards development of a device to monitor the changes in glucose concentration as an aid to diabetic management. This method might be improvised for precision and resolution and be developed as a ring or an earring that patients can wear.

DEDICATION

This is dedicated to my parents, sister, relatives and all my friends who were always supportive. (Dad and mom, I did it!)

ACKNOWLEDGMENTS

I would like to thank Dr. Bruce Towe, committee chair for his impeccable support, guidance and patience in helping me complete this thesis. I also would like to thank Dr. Jeffrey LaBelle and Dr. Jitendran Muthuswamy for their support.

Without the help of Daniel Gulick I would not have found any components in the lab and worked with ease. I would like to thank Dan and other lab mates for their patience and assistance that they provided throughout my time in the lab.

I would also extend my gratitude to Mark Richards and Dmitry Derkach for their assistance with 3D printing, cell counter and their questions that molded my project work.

I am grateful to Laura Hawes for her academic advising and helping me throughout my defense process.

I would also thank my mom, sister, cousins and my roommates who have been a great source of support and encouragement for my work.

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CHAPTER 1

INTRODUCTION

Glucose is the primary source of energy for mammalian cells, controlled by glucagon hormone (increase blood sugar level) and insulin (decreases sugar level by uptake and conversion to energy). The normal plasma glucose level in human blood after fasting is <6.1 mmol/L or 110 mg/dL and without fasting is within the range of 6.1 mmol/L – 7.0 mmol/L [1]. The condition of increased glucose level beyond 7.0 mmol/L or 126 mg/dL in the blood due to insulin deficiency (Type I) or insulin resistance (Type II) is called Diabetes. The Type 1 diabetes is common among children of age 0-14 years but also affects other age group in smaller percentage [2]. It is a condition caused by deficiency of insulin due to cell-mediated autoimmune attack on pancreatic β-cells [3]. Type II diabetes or diabetes mellitus is common among the age group 40-60 years and has a strong genetic component associated with it. 90% of the diabetes cases fall under type II. It is a metabolic disorder that is characterized with hyperglycemia along with disturbance in the carbohydrate metabolism due to insulin resistance [4].

Other types of diabetes are gestational diabetes and Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG). Gestational occurs during pregnancy and has various physiological factors that cause the hyperglycemic symptoms [5]. It is diagnosed during prenatal screening. IGT occurs during the transition between normal and diabetic condition and are at high risk of Type II diabetes [6].

Need For Glucose Sensor

Any diabetes with hypoglycemia or hyperglycemia can lead to long term complications with time. The complications of diabetes vary from patient to patient and can affect various part of the body. Many diabetics are affected with cardiovascular disease, nephropathy, retinopathy, neural disorder and lower limb amputation.

Apart from the long term effects, diabetes also leads to ketoacidotic and hyperosmolar diabetic comas. Deficiency of insulin causes hyperglycemia and ketoacidosis. In the case of diabetic ketoacidosis (DKA), due to the decrease in insulin, there is a decrease in glucose use and increase in release of glucose precursors and free fatty acids by peripheral tissues [3]. It leads to hyperglycemia, osmotic dieresis, vomiting and difficulty in breathing [4]. Within few hours this can become a life threatening condition if proper medical attention is not provided . American Council on Science and Health has conducted a 6 year study that proved one third of the population in Maryland died with DKA that was undiagnosed. This is due to lack of awareness, no regular access to medical care, discomfort in using invasive method to detect glucose level and absence of continuous monitoring of glucose.

There has been lot of medical technologies developed over the years for diabetes management that includes not just glucose measurement in blood but also continuous monitoring of glucose to help in better medications and understanding. Thus being of greater importance continuous glucose monitors are being developed using various invasive and non invasive techniques.

Continuous Glucose Monitoring Sensor (CGMS)

Diabetes is one of the challenging health problems and has been estimated to increase to 21% of the US adult population in 2050 [7]. Diabetes is one of the most common chronic diseases and is projected to be 7th leading cause of death in 2030 by WHO. Along with the increasing percentage of diabetic patients, research on glucose sensor has also been developing over 50 years. International Diabetes Federation has reported in 2012 that 50% of the people with diabetes are left undiagnosed. These alerted the researchers for years to thrive in developing patient-friendly self monitoring blood glucose sensor (SMBG) and inexpensive sensors with high precision to be accessible to all population and be used without any discomfort.

Blood glucose meter provides a single snapshot of the present glucose level. This helps in monitoring glucose at a time point but not over a period of time to follow the trend of changes in glucose concentration in the blood in relation to the daily activities like food habits, physical activities and medication. Also the sudden changes of glucose level during sleep or normal activities cannot be monitored. Studies over years have proved that 55% of hypoglycemic events occur during sleep and are also asymptomatic due to altered counter regulation. Also SMBG are not preferred by most patients due to poor compliance and inconvenience and common patients of Type I diabetes is children who seldom needs to use the finger pick method.

CGMS helps in monitoring the trend of glucose level in the blood even during nocturnal sleep and gives deeper insight for appropriate medication for Type I diabetes patient, who are mostly unaware of the sudden changes of glucose level. Also help in providing timely medication during DKA and hypoglycemia which need continuous

monitoring. The data about the magnitude, direction, duration, frequency and the causes of fluctuation is not provided by the intermittent glucose sensors [8] that remain crucial for insulin therapy.

The need for self monitoring of glucose in diabetic patient has increased as research groups like Diabetic control and complication trial research group [9] proved tight control of blood glucose helps in slowing down the progression of micro vascular complications and long term complications related to retinopathy, nephropathy and neuropathy by 60%. CGMS can also be used in real time with patients who are critically ill and maintain a close looped control of glucose [10]. Thus CGMS is not only helpful in detecting hypoglycemia, it also helps in improving therapeutic management and reduce hypoglycemic occurrence [11].

The Development of close loop CGMS has been going on for two decades, where system measures glucose level at regular internal and administer insulin or glucagon as required. The first CGMS was developed and commercialized in 1977 by Miles Laboratory Inc., USA called Biostator which was able to maintain near-normal glucose homeostasis [12]. This was a bulky device that continuously drew venous blood from the patient and measures the glucose level.

Another successful product was CGMS, Medtronic MiniMed (USA) that was commercialized in 1999 and was able to store data of three days [13]. It had an electrochemical sensor, which was inserted into the subcutaneous tissue. This sensor was connected to a portable data processing unit by a lead and stored data every 5 min. The system has to be calibrated with SMBG at least four times a day.

There have been improvisations made on these technologies for better close loop control. But the sensitivity of the sensor has not reached 10% of the Clarke's error grid. These are being mainly designed for usage in ICU for critically ill patient. Hence outpatient monitoring is not possible. It has failed to display satisfactory results in reliability and accuracy along with an issue of time lag of 15 – 30 min. So far the systems developed are either invasive or minimally invasive, which can cause other risk factors and non compliance by patient.

Contribution of the thesis

The goal of the thesis is to develop a continuous glucose monitoring system that is completely non-invasive which patient can use outside hospitals for monitoring glucose level during normal day-to-day activities. It is based on photometric technique which uses LED of wavelength 535 nm and a photodiode sensitive to that wavelength. It is designed to be commercialized in future to be a worn as a finger-ring or ear-ring without causing discomfort during normal activities but monitoring every change in blood glucose level throughout the day. This system, though not capable of measuring accurate glucose concentration, its main purpose is to detect the sudden changes of glucose concentration in blood that can lead to hypoglycemia or hyperglycemia in diabetic patient and alarm them for medical attention or trigger insulin injection in case of hypoglycemia.

This can be an effective continuous monitoring system for Type 1 Diabetes patients and would be a great assistance for insulin therapeutic management. But this system is not limited to Type I diabetes patients, as Type II diabetic patients also need monitoring to help in studying the pattern of blood glucose level changes during their

daily activities. It can be a useful tool in developing a healthy lifestyle by maintaining the glucose level around the normal level and patient can be stress free from worrying about the sudden changes in glucose level, where monitoring or knowing the glucose level is not feasible.

This system can respond to the changes of glucose concentration in real-time, which gives the patient enough time to respond and get medical care. It is a simple device that can be made affordable to all patients and an easy to use technology. This work is focused on developing a technique for a product that is convenient and a patient can wear without developing other side effects or health hazards like skin irritation, allergic reaction or immune response. This system can also be used as a primary technology upon which sophisticated techniques can be adopted for high precision and improved features. Some of the features include alarm system, insulin injection and data storage.

CHAPTER 2

BACKGROUND

Why Non-Invasive technique?

There are three major types of glucose measuring systems: invasive, minimally invasive and non-invasive measuring systems. Most widely used and developed technique is the invasive needle-type technique or implanted sensors as it gives 1% accuracy and is dependable. The invasive system can be continuous or intermittent. The continuous monitoring comprises of a biosensor that is implanted on the patient and the data is continually collected externally. The intermittent method is the traditional finger prick method. In the ICU blood is drawn at regular interval and the glucose level is tested. There are also self monitoring systems available that can be used by patients at home by drawing a small amount of blood from the finger and dropping it on an electrochemical sensor. This is stuck into a small device with a monitor that displays the glucose concentration. These are accurate devices but does not allow for nocturnal monitoring which is essential for maintaining near normoglycaemia.

Similarly, the implantable sensors have the drawback of functional loss of detecting glucose [14]. Koschwanez et al [15] developed methods for testing the biocompatibility of these biosensors. The functional loss can be due to the histological changes like inflammatory reaction, fibrous encapsulation that takes place on the tissues around the implants. The fibrous encapsulation restricts the diffusion transportation of glucose to the sensor and thus reducing sensitivity [16-18]. The stability of glucose sensor was tested by Rebrin et al and reported instability in measurement and loss of sensitivity.

This was supported by similar results by other researchers [19-21]. Some of the risks of these implants are tissue injury and initiation of foreign body reactions due to long time interaction between the device and body. Apart from these factors, the glucose diffusion to the sensor is also affected by absorption of nonspecific protein from the tissue fluid [19-21]. Hence the challenge still remains for stability and sensitivity along with biocompatibility for these invasive techniques [14].

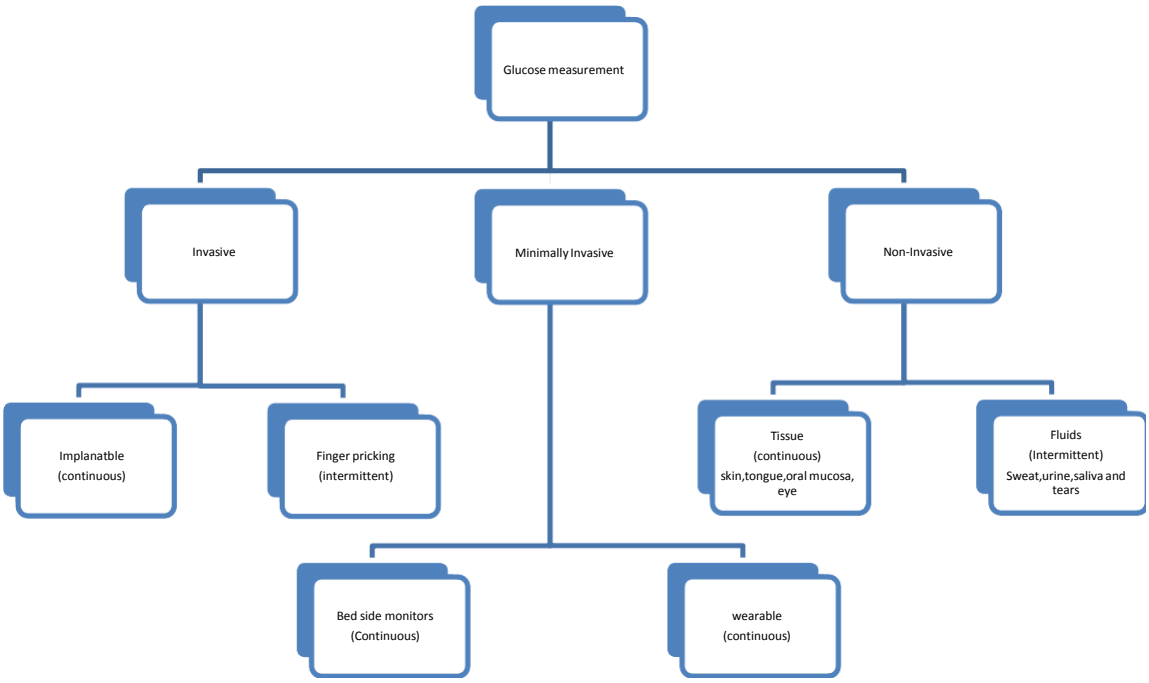

Figure 1. Overview of measuring techniques of blood glucose [22].

For the Minimally invasive technique, the glucose concentration is measured in the sub-cutis or interstitial fluid of skin. The sensor is placed in contact with the tissue or through iontophoresis the interstitial fluid is extracted to the sensor. The advantage of

this technique is accuracy and specificity [23]. But it has a latency period of 8-15 minutes which encompass the sensor reaction time, signal-processing delays, and ISF-to-plasma glucose equilibration time. There are also other risks like infection at the site of sensor installation on tissue, inflammatory reaction and skin irritation as the interstitial fluids are being dragged across the skin.

These risks are of great concern to the physician and the patient who use it. Hence the non-invasive sensor research has become a topic prime importance, influenced by the acceptance rate of the type-1 diabetes patients and advantages of reasonable biocompatibity and painless technique thus increasing the compliance by patients in glucose management.

Photometric Techniques

There are various non-invasive techniques to detect blood glucose. But widely researched technique is photometric or optical glucose sensor. Some of the methods developed are Impedance spectroscopy, ultrasound technology, thermal emission spectroscopy, ocular spectroscopy, electromagnetic sensing, fluid harvesting, thermal emission spectroscopy and photometric technique.

Depending on the wavelength used, different methods have been studied to measure the concentration of blood glucose. The NIR spectroscopy, mid IR spectroscopy, Optical coherence Tomography, Temperature modulated localized reflectance, Raman spectroscopy, polarization changes and fluorescence spectroscopy are the optical techniques studied so far to measure glucose concentration in blood. Basically, light beam is directed through the skin and the properties of the light emitted is measured.

There is change in the property of the incident light and reflected light due to direct interaction with glucose or the indirect effects induced by glucose.

The NIR (Near infrared) spectroscopy uses a light of wavelength 750-2500 nm that can penetrate up to 100mm depth [24]. The light is either transmitted through or reflected by the tissue containing glucose and the resulting light beam is altered by the changes in glucose concentration. The change in glucose concentration causes changes in the absorption and scattering coefficients that can be detected and correlated with the glucose concentration. Similar to this method is for mid IR expect the penetration is only few micrometers [25] hence only the scattered light is considered. A light of wavelength 2500-10,000 nm is used that responds better to glucose changes than NIR.

A low coherent light is used in the Optical coherence Tomography that is backscattered by the tissue which is combined with the reference light and the delay correlation is measured. As glucose concentration increases the refractive index also increases thus decreasing the mismatch of refractive index with the reference. This is similar to light scattering technique [26].

The tissue refractive index changes with temperature that is dependent on the glucose concentration. This theory is used as the basis for temperature modulated localized reflectance at 590nm and 935 nm to estimate glucose concentration.

The Raman spectroscopy is based on the theory that laser light causes oscillation of solutes that influence the scattering of light depending on the concentration [27, 28]. This helps in measuring glucose concentration with less overlapped spectra.

The polarization technique is based on the phenomenon that the polarized light rotates when passed through optically active solutes like glucose through an angle

proportional to the concentration of the solutes. It has the advantage of using visible light and ability to be miniaturized [29].

In the fluorescence technique, the glucose solution when excited at 308 nm emits fluorescence intensity at 380 nm that is dependent on glucose concentration [27].

Most of the photometric techniques mentioned above uses infrared or ultraviolet wavelength that might be harmful to the skin causing skin pigmentation, redness [30] and are not recommended or continuous exposure. The technique developed here is to choose a wavelength sensitive to glucose changes in the visible range thus being safe for continuous monitoring.

The technique developed in this thesis is based on the osmolarity property of the RBC in response to the glucose concentration changes in the blood.

Osmosis

Osmosis is a phenomenon in which the water molecules pass through a semi-permeable membrane along a concentration gradient. The water molecules diffuse from region of high water concentration to low water concentration. There is a semi-permeable membrane that separates two sugar solution of different concentration. In the figure, a solution with high water concentration and less sugar concentration is in the right side and a solution with low water concentration and high sugar concentration is in the left side. Hence the water molecule tends to move from left to right to achieve equilibrium. The net transfer of the water becomes zero when the back pressure 'P' equals to the relative osmotic pressure, until which the fluid level rises.

$P=\rho gh$

Where, *g* is gravity at the surface of overlaying material

ρ is density of liquid

h is height of liquid column or depth within a substance

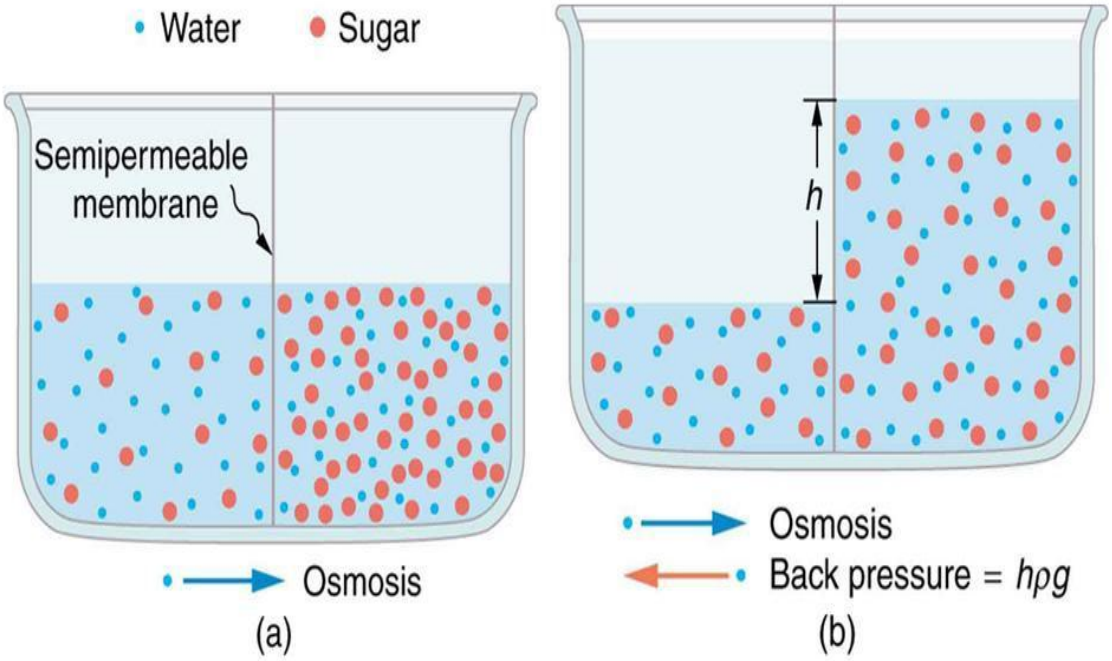

Figure 2. Demonstration of Principle of Osmosis [31]

Osmosis is an important phenomenon that takes place in cell to maintain equilibrium with their environment. It also helps in the transport of molecule in and out of the cell based on the concentration gradient.

CHAPTER 3

THEORY

Osmolarity of RBC

Osmosis is the phenomenon that takes place in the Red Blood Cell (RBC) with respect to change in the glucose concentration in the blood plasma. Osmolarity is one of the important properties of RBC that affects the optical property. As the glucose concentration increases in blood, the water molecules from the cells tend to diffuse outside, thus shirking the RBC cell. Similarly, as the concentration of blood glucose decreases, the water molecule tend to diffuse inside the RBC causing it to swell.

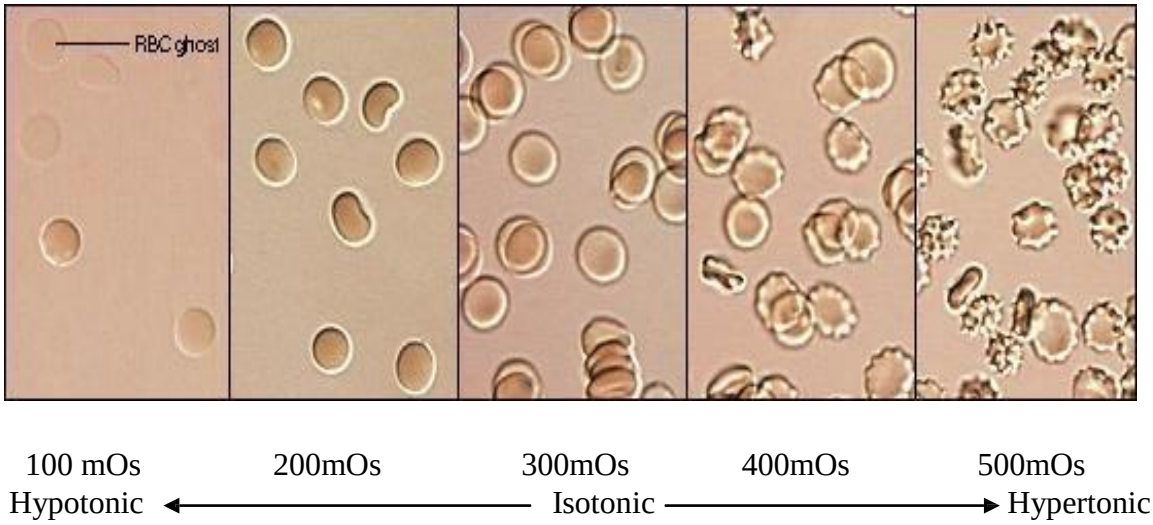

Figure 3. Change in size of the RBCs in different osmolarity solution [32].

The figure shows the changing size and shape of the RBC with varying osmolarity of blood serum. As the glucose concentration increases the RBCs shrink in size. The characteristic shape is called as spinocytes. But at 100mOs, there are lots of

ghost cells found as the cells swells to a spherical shape by the diffusing water inside and might rupture eventually.

The osmolarity variations not only change the shape and volume of the RBCs, but also the inner cell concentration [32]. The changes in RBC cause changes in optical property like refractive index, absorption, scattering and transmission of light.

As the osmolarity increases the absorption coefficient of RBC is also known to increase [33]. As the glucose concentration increases, the cell shrinks and the absorption coefficient increases. Thus more light is being absorbed and the transmitted light decreases. Similarly as the glucose concentration decreases, the cells expand and absorption coefficient decreases. This causes the RBCs be a poor absorbing medium and thus transmit more light. Hence the transmitted light increases with decreasing glucose concentration.

Apart from absorption coefficient, the scattering coefficient and refractive index also increases with increasing glucose concentration. The change in cell volume and cell shape has drastic influence on the optical properties. It has been predicted that for a 0.05% decrease in scattering coefficient, there is a 0.1%/mM change in glucose. In another test, 2.1% decrease in scattering coefficient was observed for an increase of 3.6mM of blood glucose concentration [34].

Considering the optical properties of the whole blood, it is similar to the changes as in single RBC because absorption takes place within the cells and the red blood cells are highly concentrated during flow condition [35].

Spectral Analysis of RBC, Hemoglobin, water and tissue at 540nm

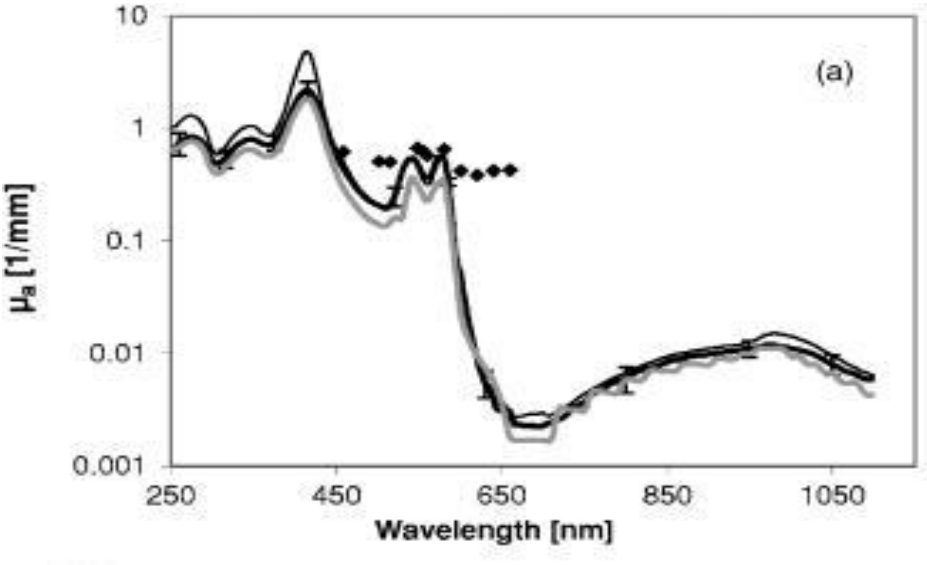

Figure 4. Absorption coefficient of flowing RBCs of a hemoglobin solution 0.27 g/dL compared to values calculated by Mie theory and to data from literature [35].

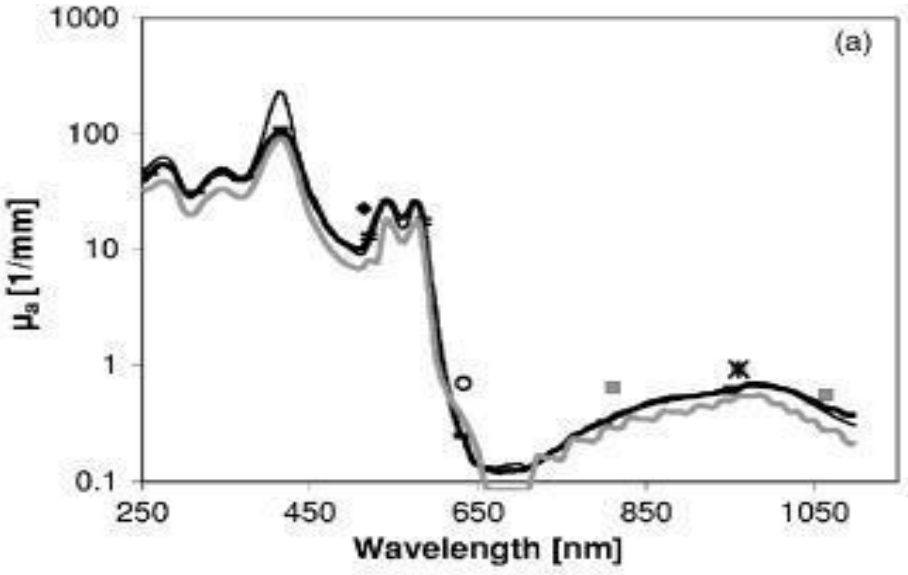

Figure 5. Absorption coefficient of flowing RBCs of a hemoglobin solution 12.9 g/dL compared to values calculated by Mie theory and to data from literature [35].

From the figure 4 and 5, it is clear that there is a maximum absorption peak at 540nm for oxygenated hemoglobin.

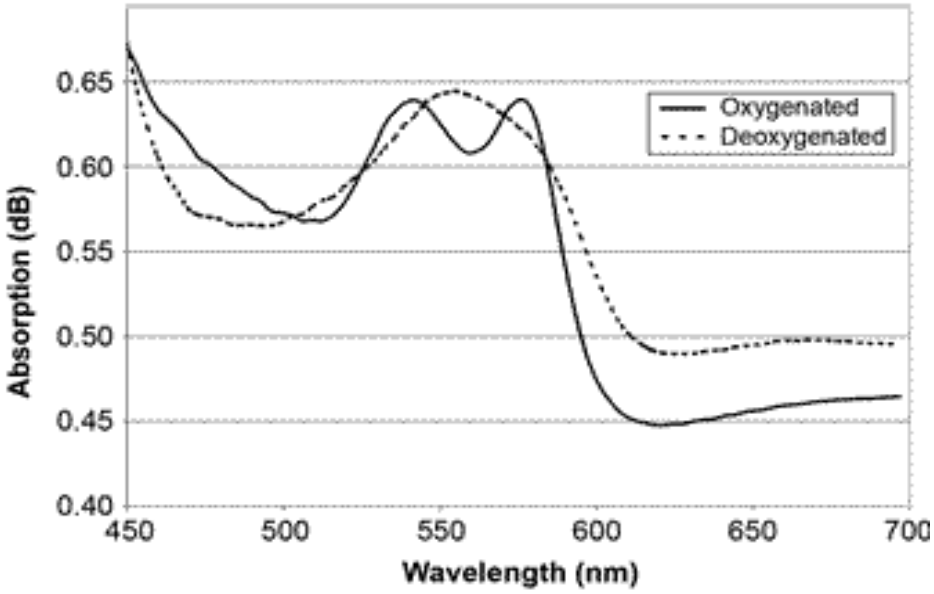

Figure 6. Spectral Absorption of oxygenated and deoxygenated hemoglobin [36].

From figure 6, we can see that the absorption of oxygenated and deoxygenated blood is same at 540 nm. This is called as isosbestic point. Isosbestic point is the wavelenght at which two different species have the same molar absorptivity or it is linearly related.

It can be infered from figure 7, that the absorption of water is minimal at 540 nm. This helps in the non interference of the water molecule in the absorption spectrum for RBC. But considering the spectral transmission of the tissue and skin, it is very low. Here is a challenge in transmiitting the light deep enough to penetrate through the tissue to monitor changes in the RBC absorption.

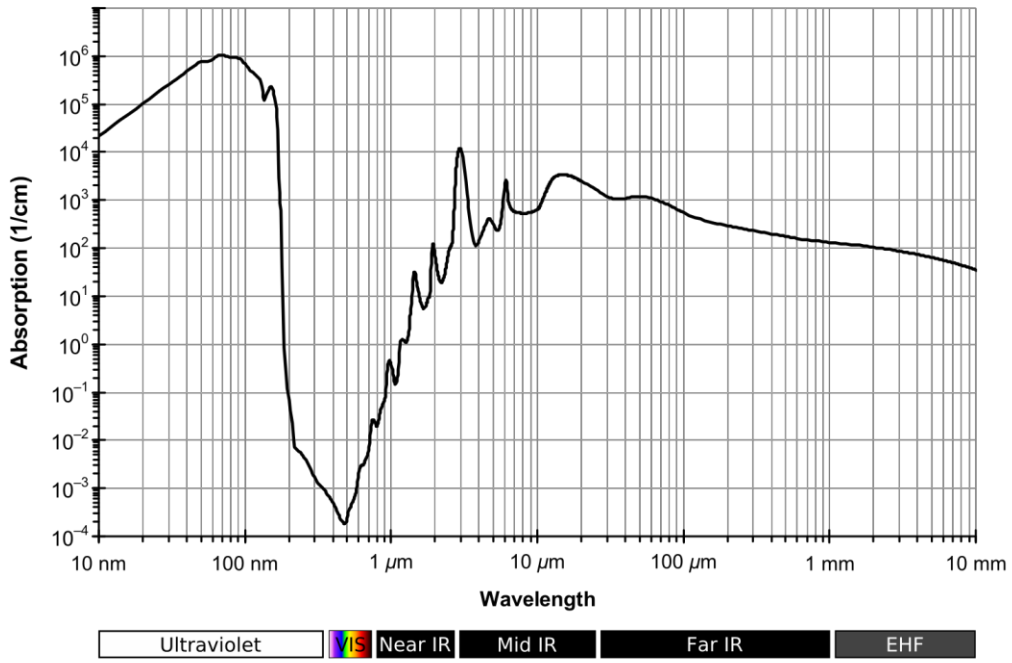

Figure 7. Spectral Absorption of water [37]

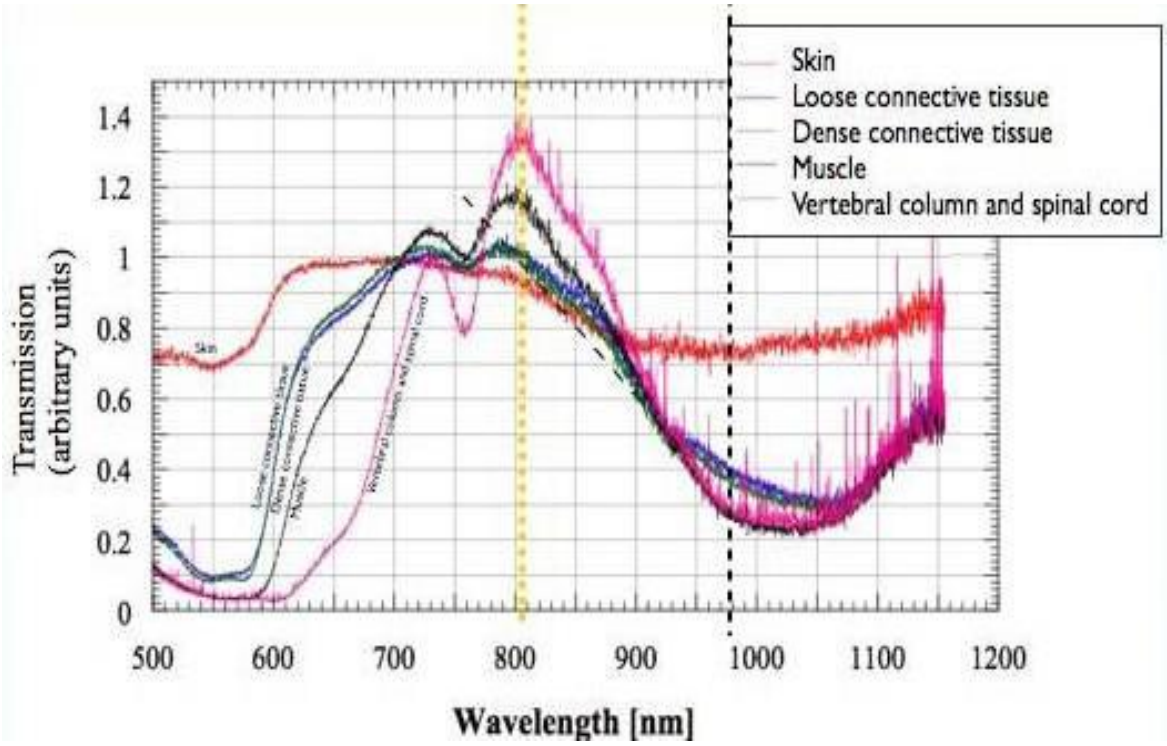

Figure 8. Spectral Transmission of tissue [38]

The tissue's optical property changes with the varying glucose concentration. The scattering coefficient of tissue depends on the mismatch between the extracellular fluid (ECF) and intracellular fluid (ICF). Increase in glucose concentration increases the refractive index of the ECF [34].

Changes in glucose concentration cause change in the optical properties of RBC,hemoglobin and tissues. These effects can be monitored in the visible range. These changes are tried to be monitored in 535 nm light in this thesis.

The experimental strategy of this research is to use a spectrophotometer to calibrate the performance of a miniature optical call that can accept small quantities of rodent blood. The cell illuminated volume is much smaller than the spectrophotometer, leading to relatively poor resolution in the spectrophotometer. The constructed optical cell is calibrated using a red dye prepared from red food color and distilled water. Then the dye, prepared in different concentration, is introduced into the capillary tube and absorbance readings at the 535nm wavelength are taken in the spectrophotometer. This reading is then used as reference for calibration of the optical cell in terms of absorbance.

CHAPTER 4

EXPERIMENTAL PROCEDURE

Blood sample collection.

The blood sample used in this research is taken from rodents. The blood is drawn from the saphenous vein. The back of the leg is shaved until the saphenous vein is visible. The vein is made to bulge out by compressing at the base of the leg and is punctured with a needle. A heparinized capillary tube is held against the droplet on the skin until sufficient volume is extracted through capillary action into the tube. The capillary tube is capped with clay. The sample is transported to the experimenting location within 10-20 minutes. It is stored in the refrigerator if needed to be used later.

RBC under microscope

Few drops of the blood sample collected are placed on a slide and diluted with sterile Phosphate Buffer Solution (PBS). The blood sample is also diluted with glucose solution of different concentration. It is smeared with another microslide by placing the slide at an angle and slide it across the blood sample to form a thin layer. A cover glass is placed over the blood sample to prevent it from drying. Then the smeared slide is placed under the microscope and viewed under a 50X objective lens. Once adjusted for focus, it is viewed in the camera attached to the lens and pictures of the view is snapped and stored using QCapture Pro software.

Different concentration of glucose was prepared from stock glucose of 19.5 mM concentration.

RBC in Cell Counter

Invitrogen Countess® Automated Cell Counter was used for this experiment. This machine has the advantage of comprehensive and reproducible data with convenient data archiving. It measures the cell count and viability accurately using trypan blue.

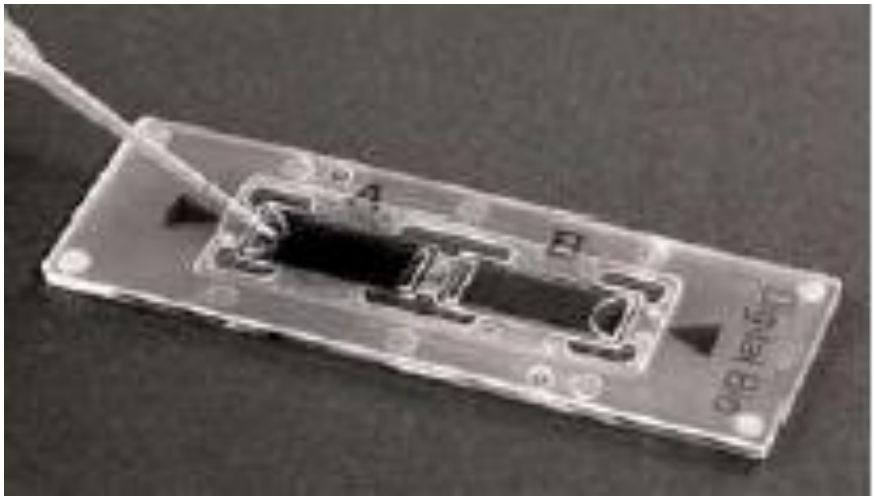

Figure 9. Invitrogen cell counting chamber slide with trypan blue

The blood collected in the heparinized capillary tube was diluted 10000 times. As the RBC was densely packed, it was difficult for the cell counter to count cells. Hence the sample was diluted with Phosphate Buffer Solution (PBS) to decrease the number of red blood cells to be within the counting range of the cell counter. Then glucose solution of concentration 2.5 mMol and 5 mMol was added to the diluted blood. Though initially PBS was used, due to the dilution, there were lot of ghost cells found. Hence the blood was later diluted with different molarity glucose solution itself.

10µL of diluted blood was then pipetted along with 10µL of trypan blue dye on to a petri dish and mixed well. Then 10 µL of the mixed solution was pipetted into the cell counting chamber slide. The cell counting machine counts the cell and its average diameter in 4 µL solution in the center of the chamber slide.

The same experiment was repeated for different glucose concentration and the diameter of the cells was noted.

Optical Cell

A device was designed to find the absorbance as a function of voltage. An LED was selected to emit at the wavelength sensitive to the RBC absorbance and suitable to measure the changes in glucose concentration based on the theory. Hence an LED emitting at dominant wavelength of 535nm was used. The LED is a 1 watt power with a maximum forward voltage of 4 Volts.

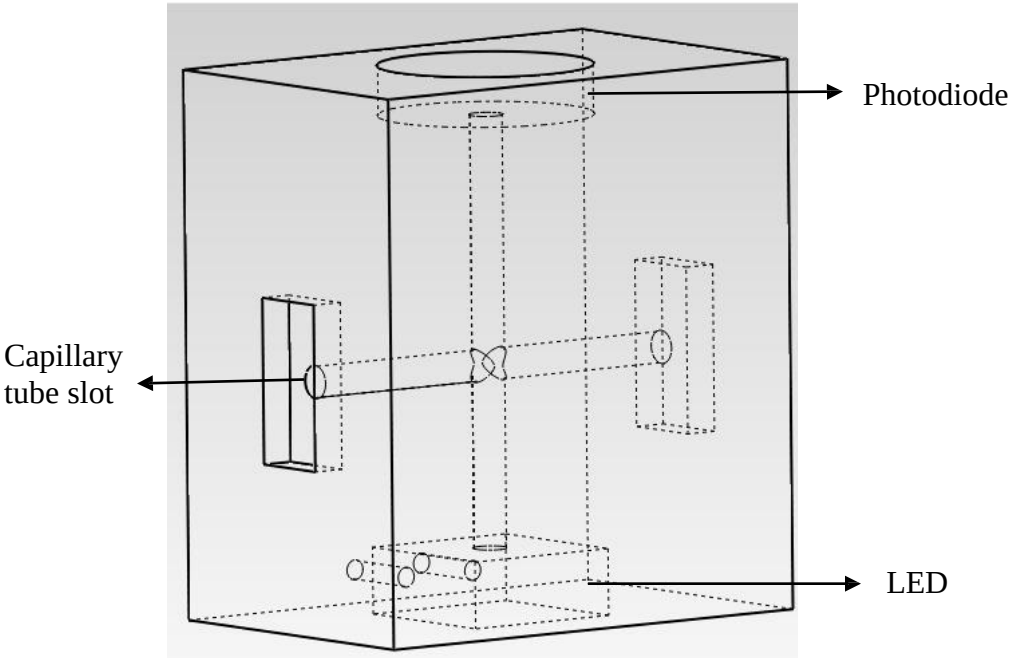

Figure 10. Optical Cell

A photodiode was used that is sensitive to green light. Function generator Tektronix AFG310 was used to produce a square wave at 40Hz and 200Hz with amplitude of 4V. LED was powered by Tektronix PS280 power supply. The output of

the diode was connected to single-ended signal input of the Lock-in amplifier SR530. Lock- in allows to detect the output of the photodiode that has frequency equal to the reference frequency of the Lock-in. The dynamic reserve of the Lock-in amplifier was set to Low as the voltage measured is in mV.

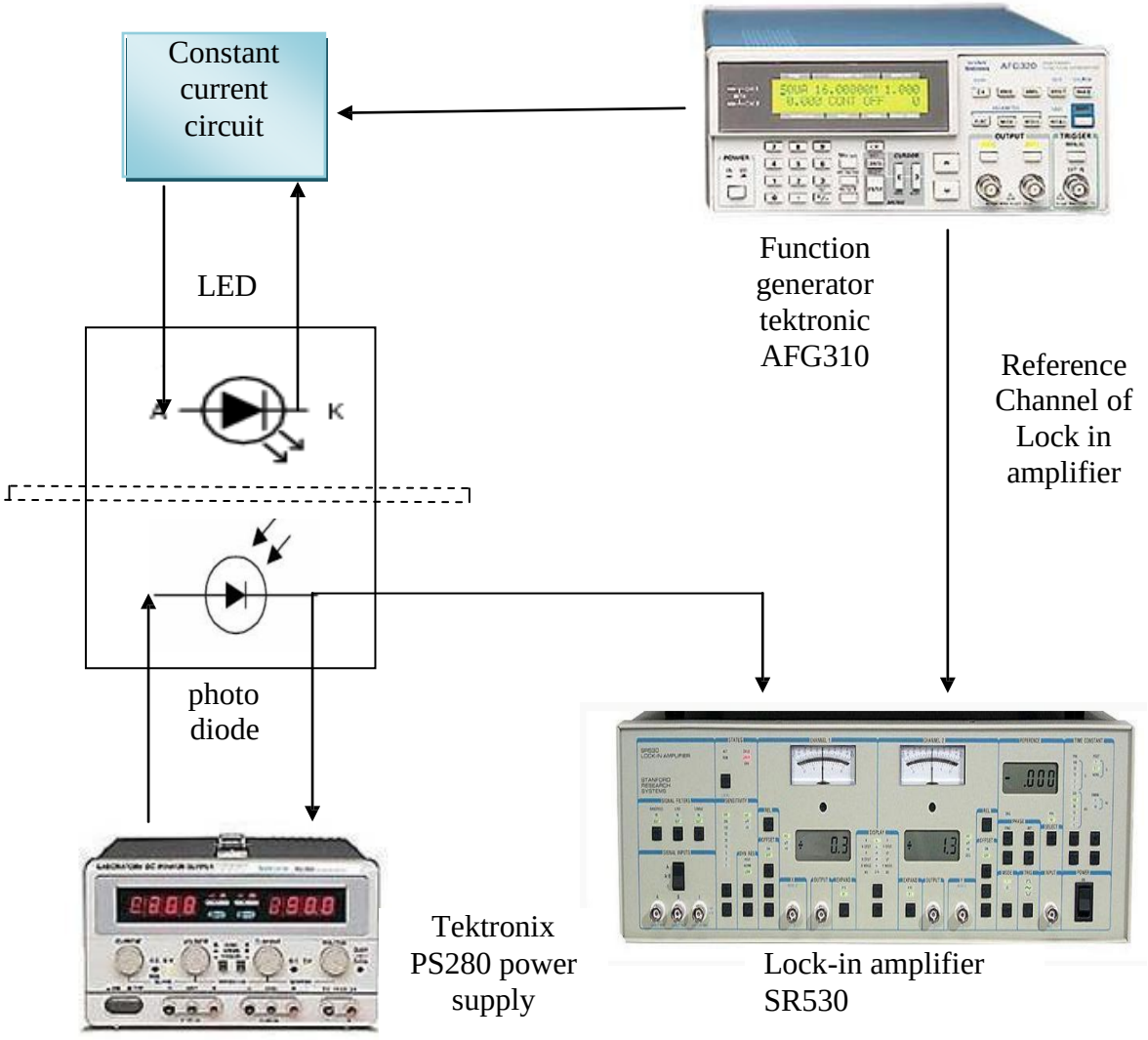

Figure 11. Experimental set up of the Optical cell

The function generator is connected to the constant current source circuit to power the LED and emit light at the set frequency. The function generator frequency is also connected to the reference of the lock-in amplifier. The LED and photo detector are encapsulated in a black holder of dimensions 1”x1”x0.5”, that has a slot to insert a capillary tube.

The system is waited for stability for 30-40 seconds. The capillary tube is inserted into the slot and the data from the Lock-in amplifier is noted for every 30 seconds. Glucose concentration of the blood is tested prior to the test and at the end of the experiment using TRUEtrack® blood glucose meter.

To increase the glucose concentration, a dialyzer fiber was introduced into the capillary tube with blood. The dialyzer fiber is inserted into a needle with a syringe filled with 10 mM glucose concentration. The syringe is operated with a syringe pump for constant flow of glucose through the dialyzer fiber.

CHAPTER 5

VERIFICATION AND OPTIMIZATION

The system has been verified for stability and repeatability of results. The optical cell is being calibrated against the spectrophotometer. Hence the absorption can be calculated from the voltage output reading from the lock-in amplifier based on the calibration curve.

Constant current Source

The LED used for the optical cell is a high efficient light source emitting at 535 nm. The optical cell system depends on the amount of light that is transmitted through the capillary tube on to the photo detector. As the output result depends on the light emitted, it is of prime importance for the LED to emit constant output, hence can detect the actual changes. If the LED output varies with time and temperature, the output readings are erroneous.

LED was tested for changes in output with time. The general theory is that, LED gets heated up with time. As the LED gets warmer, it draws more current and thus increasing the light output. In an open environment the LED is fairly constant because the heat is able to dissipate quickly into the environment. Figure 1 shows that the current drawn by the LED is steady.

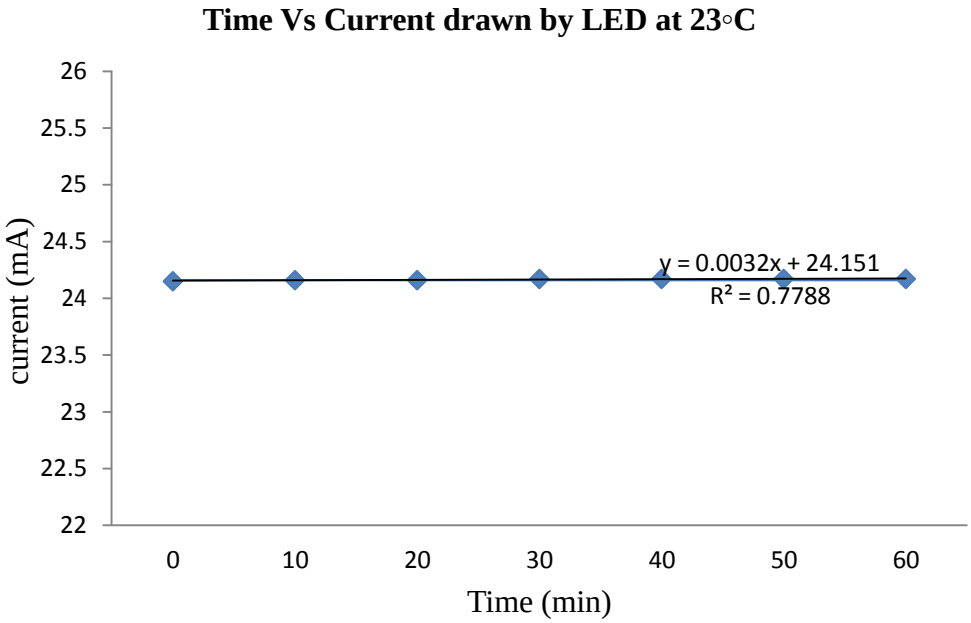

Figure 12. Graph representing the time Vs current drawn by the LED at room temperature.

Table 1. Tabulation of response of photodiode and current drawn by LED with time.

Time (min)	Temperature ◦C	Current drawn by LED (mA)±0.01	Photodiode Output (mV)±0.3
0	24.9	24.15	362.7
10	24.9	24.16	361.8
20	24.9	24.16	361.1
30	25	24.17	361.1
40	24.8	24.17	361.1
50	24.8	24.17	361.2
60	24.9	24.17	361.3

But in a closed or isolated environment and with a source of heating, it was observed that the LED was drawing more current. With increasing temperature, the current that was drawn by LED also increases. For every 1 ◦C, the current increases by 0.04 mA approximately. Even the small difference in the current is crucial as the changes to be observed are minimal and accuracy is important.

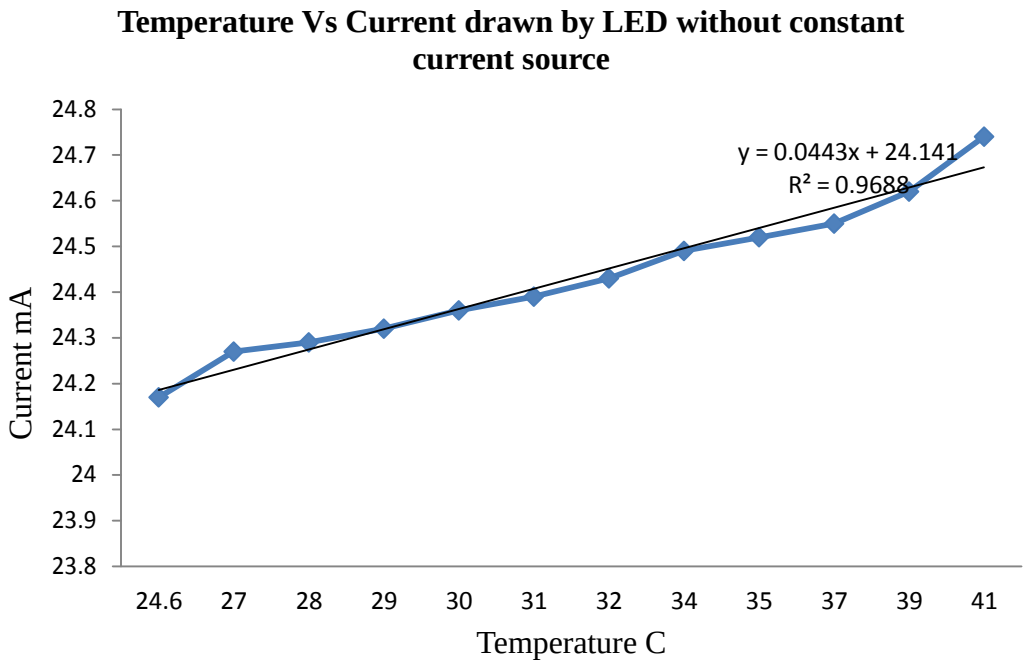

Figure 13. Graph representing the time Vs current drawn by the LED at varying temperature in an isolated environment without constant current source.

Table 2. Tabulation of response of photodiode and current drawn by LED at varying temperature in an isolated environment without constant current source.

Temperature ◦C	Current drawn by LED (mA)±0.01	Photodiode Output (mV)±0.3
24.6	24.17	367.7
27	24.27	370.5
28	24.29	371
29	24.32	371.6
30	24.36	372
31	24.39	372.8
32	24.43	374.2
34	24.49	374.7
35	24.52	375.8
37	24.55	376.1
39	24.62	377
41	24.74	377.5

To stabilize the optical cell, a constant current source is implemented. The circuit was adopted from the circuit design developed by Dan Goldwater. The system is observed to be 90% efficient and has a wide operating voltage from 3V to 30V. It is designed to maintain a constant current at any environmental condition that the LED is subjected to.

The circuit has a NFET and NPN transistor to control the current that drives the LED. The V_{DS} controls the voltage across the drain and source of NFET and draws current from the source. As the V_{DS} increases, the drain current I_D increases till it reaches the peak voltage V_P. I_D reaches the maximum. As the voltage increases the drain current also increases. This increases the resistance of the conductivity channel, meaning the depletion layer grows. With maximum current, V_{DS} is equal to V_P and V_{GS} is zero. But by increasing V_{GS}, the depletion layer increases narrowing the conducting channel with zero current.

The NFET IRFBG20 used in the circuit has the advantage of fast switching and easy driving requirements. It has wide operating range and low thermal resistance. The NPN transistor MJE3055T is a power switching circuit. The resistor R1 is used to protect the transistor as it can be easily damaged.

In this circuit the LED current is set by R2 value. The LED is driven by 5 mA current and the power dissipated by the resistor is approximately 2.5 mA.

This circuit is efficient than the LM317 voltage regulator which has only 80% efficiency in providing constant current to drive the LED. There is no gate current as JFET depends on the $V_{DS,}$ so less gain. It has high input impedance, hence high power transfer.

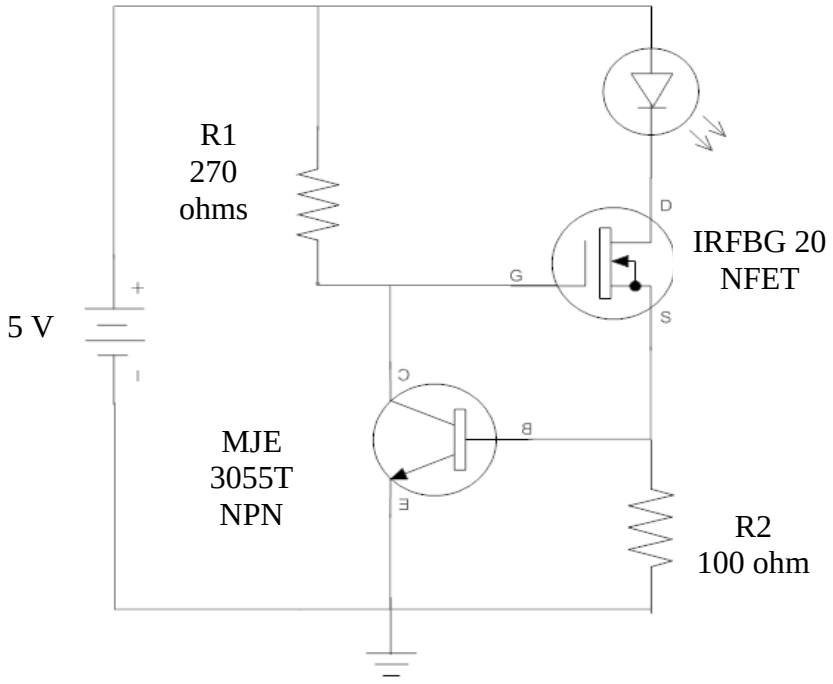

Figure 14. Circuit diagram of constant current source.

After implementing the constant current source, the performance of the system was stable over a wide range of temperature. In an isolated environment with increasing temperature, the current drawn by LED was more stable. For every 1◦C temperature increase, there was a change of 0.005mA.

The temperature of the LED was monitored using a RHXL3SD handheld thermometer hygrometer data logger from Omega which measures using thermocouple. It has an accuracy of ±0.8°C.

There are tradeoffs in selecting the current for LED. High current, in theory, improves the SNR, however causing heating of the LED and sample because it is in such close proximity. In this work 5V on the LED resulting in about 18.5 mA of current was preferred to minimize the heating effect.

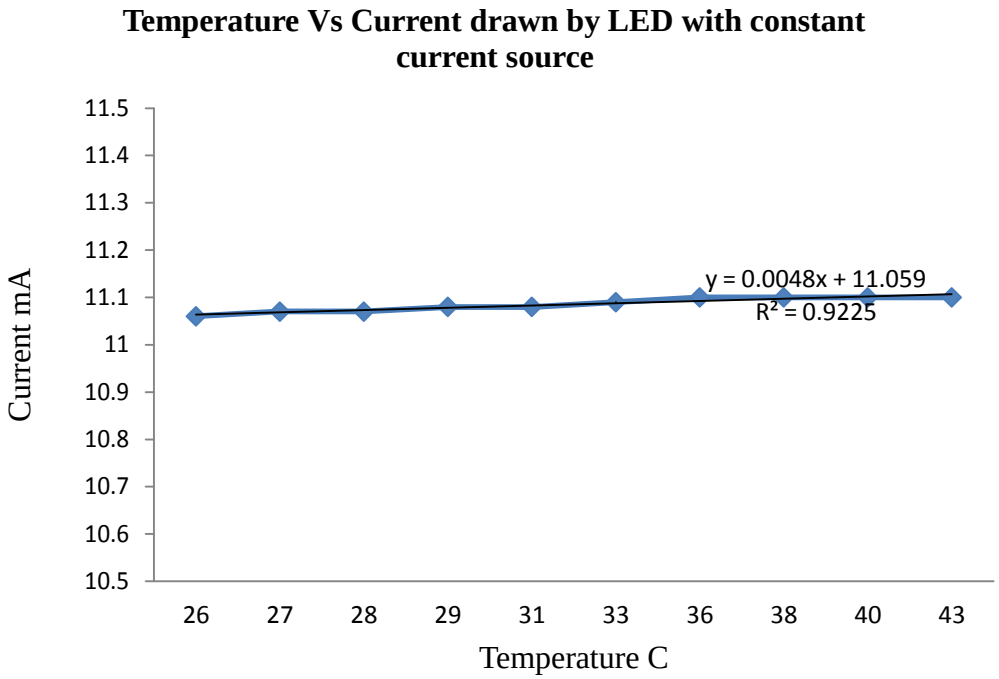

Figure 15. Graph representing the time Vs current drawn by the LED at varying temperature in an isolated environment with constant current source.

Table 3. Tabulation of response of photodiode and current drawn by LED at varying temperature in an isolated environment with constant current source.

Temperature ◦C	Current drawn by LED (mA)±0.01	Photodiode Output (mV) ± 0.3
26	11.06	345.2
27	11.07	346
28	11.07	346
29	11.08	347
31	11.08	347.1

33	11.09	347
36	11.1	348.2
38	11.1	348.3
40	11.1	349
43	11.1	349

Calibration Curve

The optical system was calibrated against the spectrophotometer using solutions that were prepared in varying concentration from red dye food color (Safeway Inc). For calibration in the spectrophotometer, a special cuvette was designed to hold a capillary tube as a capillary tube is used in the optical cell. This is because of the change in the path length. It is based on the Beer-Lambert law.

$A = \varepsilon lc$

Where A is the absorbance

ε is the molar absorptivity constant

l is the path length

c is the concentration

The equation shows that the absorbance depends on the path length also. As the path length increases, there is more solution in the path to travel by the light. More solution equals to more molecules that absorb the light. This makes the solution darker and increase the absorbance. As path length increases the absorbance increases. Also by increasing the concentration, the absorbance increases and appears darker.

To demonstrate this law, red solutions was prepared with different concentration with different volume of red food color and distilled water.

Table 4. Tabulation of spectrophotometer reading of absorption and transmission% in cuvette and capillary tube.

Volume of H20 in µL	volume of red dye in µL	Cuvette		Capillary Tube	
		Absorption	Transmission %	Absorption	Transmission %
2998	2	0.112	77.3	0.694	20.2
2996	4	0.746	17.8	0.797	16.4
2994	6	1.109	7.8	0.83	14.8
2992	8	2.164	0.7	0.982	10.4
2990	10	2.492	0.3	1.061	8.8
2988	12	2.61	0.2	1.215	6.1

From the table it is clear that there is a difference in absorption compared to the reading in the cuvette and capillary tube. Hence was designed a capillary tube holder for the spectrophotometer. The holder was 3D printed to the dimensions of a cuvette 0.49”x0.49”x1.71”. A hole was extruded along the length to fit in the capillary tube. A slot was then extruded for the light in the spectrophotometer to pass through. As the 3D printed material was white, the holder was spray painted with flat black paint. Any light that is reflected or scattered by the curvature of the tube can be absorbed by the black paint.

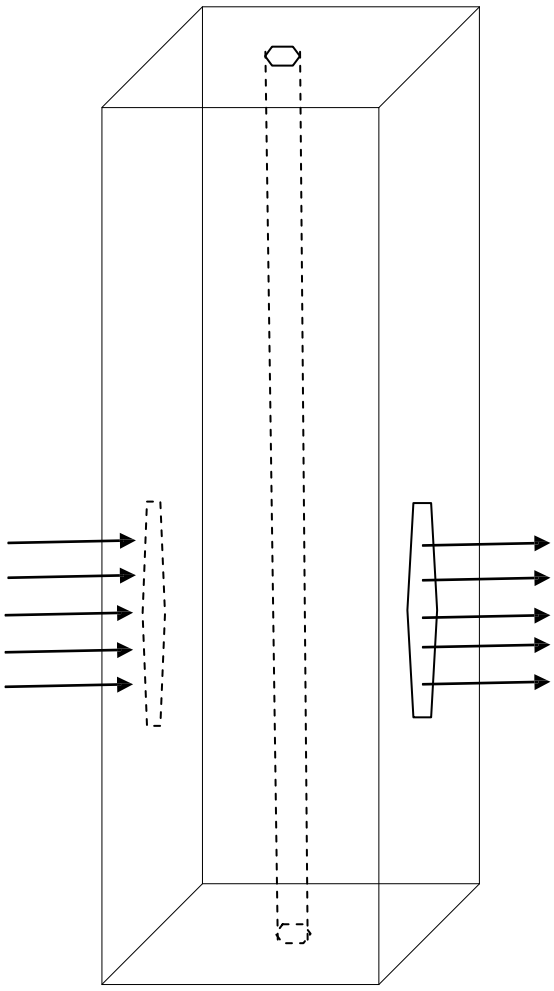

Figure 16. Capillary Tube holder for Spectrophotometer.

The absorbance of the solution was taken at 535nm. The spectrophotometer was calibrated for 535nm. The baseline absorption at535 nm was 0. Initially the absorption of the red dye was taken in a cuvette. Then the capillary tube holder was used. The absorption and transmission% was noted for 6 different samples.

The same capillary tube was used in the optical cell and the photodiode voltage from the lock-in amplifier was noted.

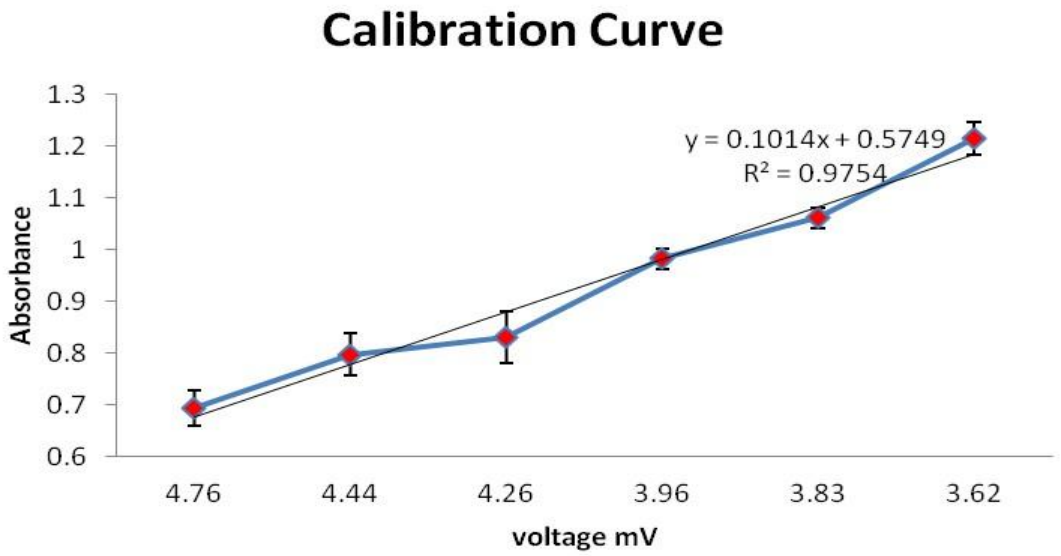

Figure 17. Calibration curve of the optical cell.

Table 5. Tabulation of the Absorption and transmission% of different concentration sample and their corresponding photodiode output.

Capillary tube		Photodiode Output mV			Average in mV
Absorption	Transmission %	Trial I	Trial II	Trial III	
0.694	20.2	4.72	4.79	4.76	4.76
0.797	16.4	4.42	4.42	4.49	4.44
0.83	14.8	4.25	4.31	4.21	4.26
0.982	10.4	3.94	3.96	3.98	3.96
1.061	8.8	3.85	3.81	3.83	3.83
1.215	6.1	3.61	3.65	3.59	3.62

CHAPTER 6

RESULTS

RBC under Microscope

The blood that is smeared on the glass slide is seen under the microscope and the images are captured using the QCapture Pro 6 software. The size of the RBC that was diluted in the PBS was around 5-5.8 µm. This is considered to be a control for comparing the change in size of RBC with the glucose concentration. The image was taken after 30 minutes so as the RBC to undergo the osmosis process.

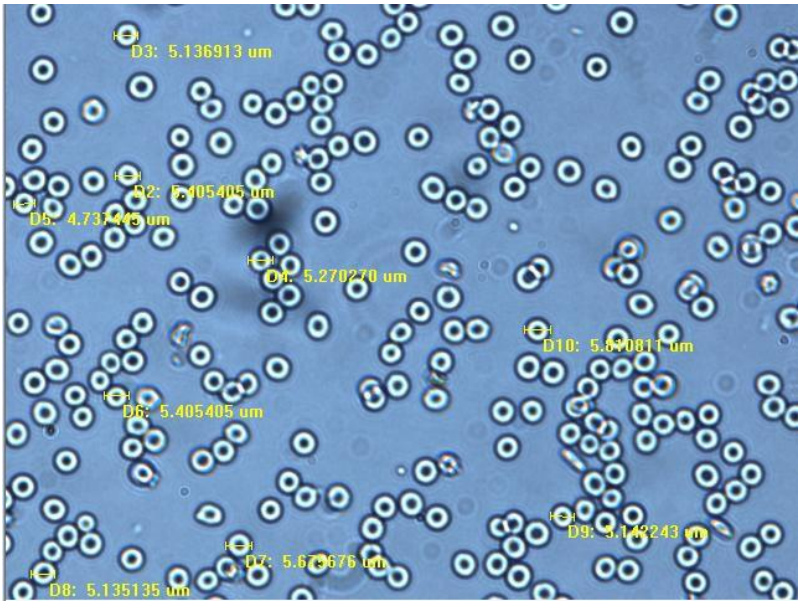

Figure 18. RBC diluted in PBS.

Another blood smear made by diluting the blood with 2.5 mM glucose concentration was seen under the microscope after 30 minutes. The image shows that the RBCs have increased in size to $\approx$ 6.5 µm.

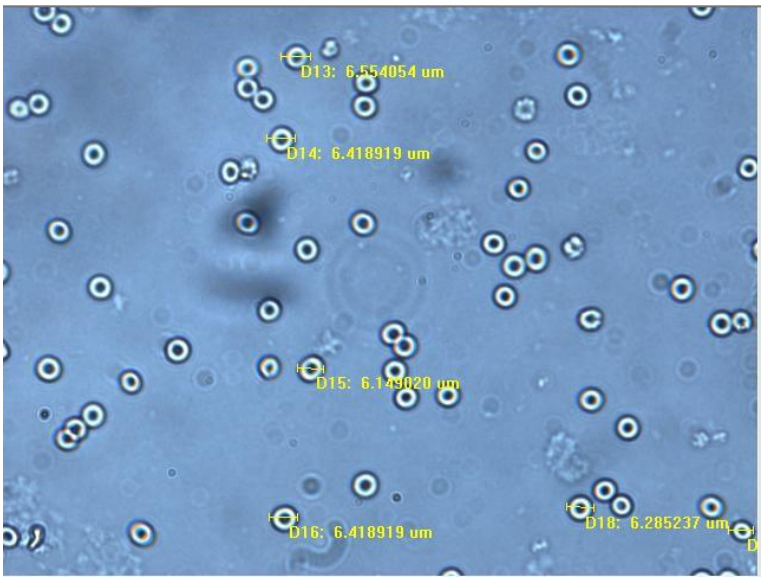

Figure 19. RBC diluted in 2.5 mM glucose concentration.

The blood smear diluted with 7.5 mM glucose concentration shows that the RBCs have shrunk in size < 4 μm.

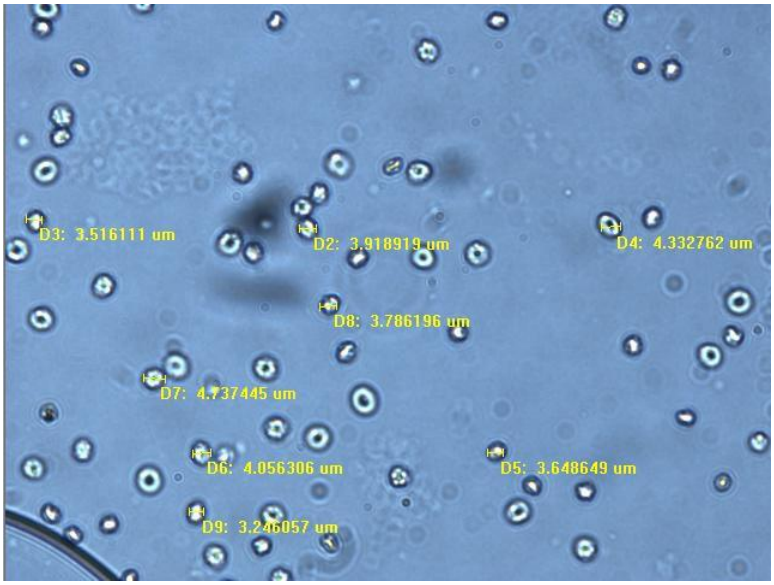

Figure 20. RBC diluted in 7.5 mM glucose concentration.

RBC in cell counter

The initial trial was conducted by diluting the blood by 10000 times in PBS and then adding different concentration of glucose to the diluted blood. This sample is then scanned in the cell counter.

Table 6. Cell counter reading of blood diluted in PBS.

Concentration	Average diameter of Viable cell in µm	
	Trial I	Trial II
PBS	6.4	6.1
2.5 mM	9.2	9.8
5 mM	5.8	5.5

Final trial was conducted by diluting the blood by 10000 timies in different glucose concentration like 2.5 mMol and 5 mMol.

Table 7. Cell counter reading of blood diluted in different glucose concentration.

Concentration	Average diameter of Viable cell in µm
PBS	7.6
2.5 mM	7.4
5 mM	7.8

Optical Cell

Tracking of opacity changes at 535nm in a capillary tube of whole rodent blood was performed. Glucose concentration changes in the unpreserved blood as a result of continuous metabolism of red blood cells. Initial blood glucose measurements were taken at the start of the experiment and at the end. This shows the changes in glucose concentration with time. Fresh rat blood in a hematocrit tube was introduced into the experimental test setup. Data was captured manually every thirty seconds and plotted using Excel.

The absorbance values as reflected photo detector output were continuously acquired from the output of the lock-in amplifier.

Figures 22-24 shows the results of these trials. The decrease in absorbance causing an increase in light transmittance can be seen on the plots over duration of an hour or till saturation is reached. The decrease was calculated as a slope of the line after stabilization.

The sample was examined visually at the end of the study and showed no evidence of coagulation if capped at the end with clay.

Figure 22 shows the absorbance test reading with 5 mA through the photodiode giving a relatively higher response than Figure 23 test where the current was 6 mA and hence the scale differences.

The output of the optical cell is the reading of the photodiode from the Lock-in amplifier.

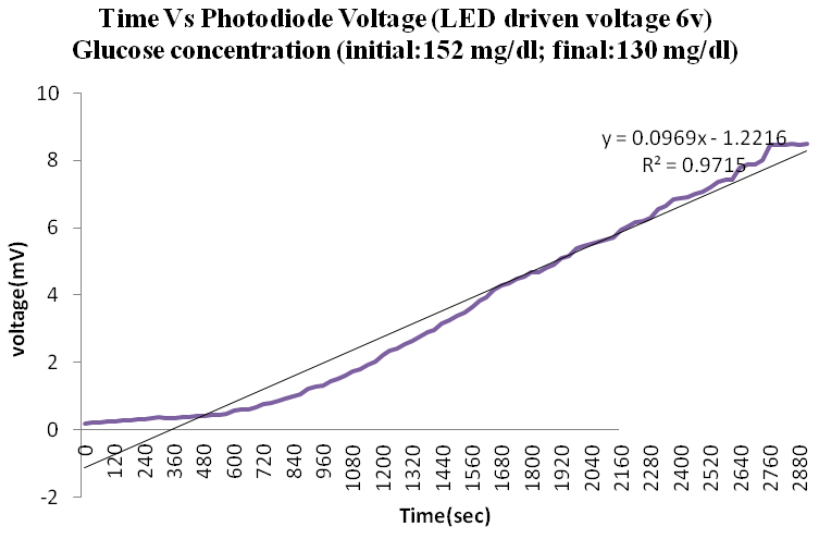

Figure 21. Plot of the photodiode output with LED driven at 6V

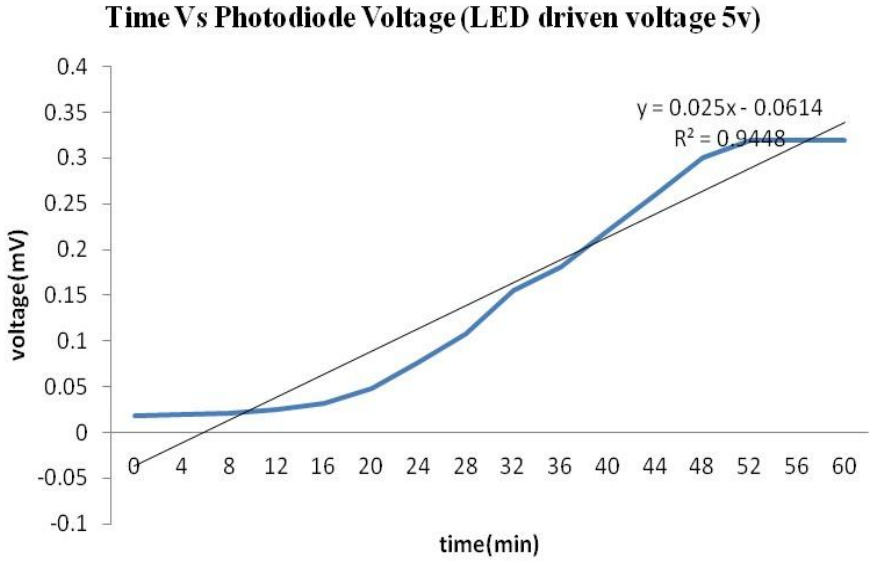

Figure 22. Plot of the photodiode output with LED driven at 5V; Trial I

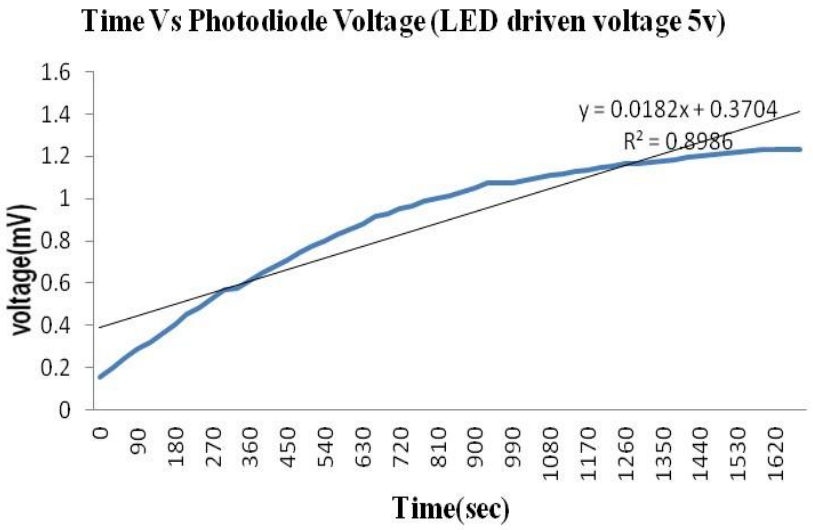

Figure 23. Plot of the photodiode output with LED driven at 5V; Trial II

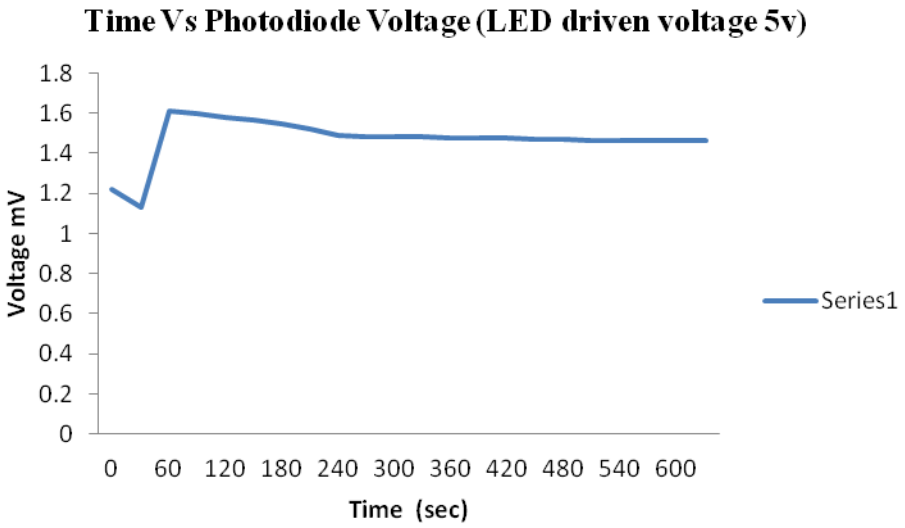

Figure 24. Plot of the photodiode output with blood sample delayed for 2 hours

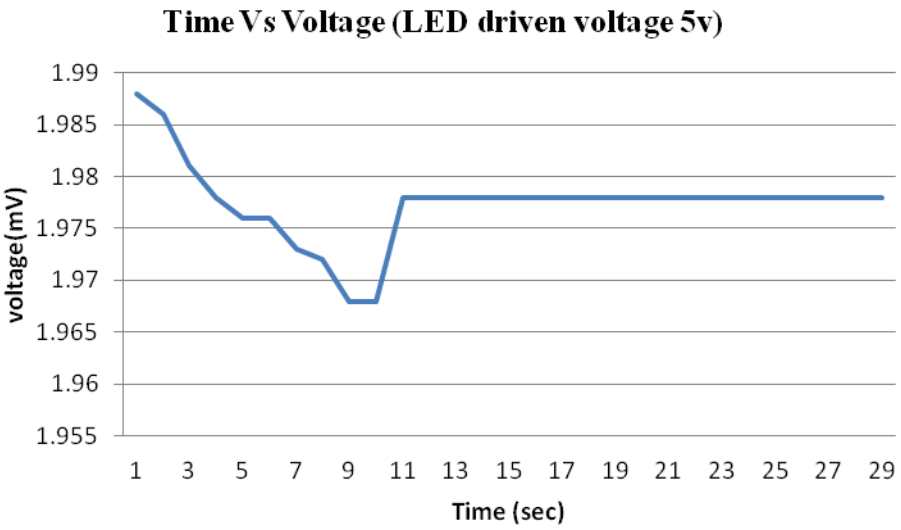

Figure 25. Plot of the photodiode output with blood sample with dialyzer fiber to increase glucose concentration.

CHAPTER 7

DISCUSSION

The results of this study support the hypothesis that optical absorbance changes at 535 nM can be used to as an indirect indicator of glucose concentration changes in whole blood.

The images under the microscope show that the RBCs change in diameter with the glucose concentration in the biological range. The change in their volume is correlated to changes in an absorbance cell. The control for the images is the blood diluted with PBS. In this slide, the diameter of the RBC varies between 5-5.8 µm. In the slide prepared by diluting the blood in 2.5mM glucose concentration, the cell tends to dilate as more water molecules enter the RBCs. The size of the RBCs increases up to 6.5µm. When the blood was diluted with 5 mM concentration of glucose, the cells started to shrink as the water molecule started to move out from the RBCs.

The cell counter proved the similar results. The average size of the cells where noted. Initially the blood sample was diluted 10000 times in PBS as the concentration of RBC was high and made it difficult for the cell counter to count. Then glucose solution was added to the diluted blood. Later the blood sample was diluted in solution of different glucose concentration itself. There were lots of dead cells found when test was not done within 30- 60 minutes of the blood extraction from rodent.

Initial data acquisition from the optical cells had challenges for stability and accuracy. LED emitted steady output by the use of constant current source. The frequency of the LED to emit light was initially set to 40Hz. Due to electronic noise

frequency close to the set frequency; there were fluctuations in the output. Hence the frequency was set to 200 Hz. Blood sample tested within 30 minutes of extraction showed better results. This was because the blood was fresh and has no coagulation. If the blood sample is left for more than 2 hours, it gets coagulated. Also if the heparinized tube is capped with the clay at one end, stayed un-coagulated till the end of the test. But it was difficult to take the glucose reading of the blood from the capillary tube. As expected the voltage increased with time with decreasing glucose concentration. In theory, the RBC uses glucose as a fuel and is an anaerobic metabolism. With time the glucose concentration decreases due to cell metabolism.

As the blood is drawn from the rodent, the cell still consumes the glucose in the sample, thus decreasing the concentration of the blood glucose. As the glucose concentration decreases, the cells dilate due to osmosis. This decreases the absorption coefficient, thus increasing the amount of light transmitted. Increasing transmission results in increased photodiode output. The changes in absorption is non linear. From the calibration curve, the changes in absorption can be calculated. Figure 25 shows the plot of the photodiode response to a blood sample that was tested after a 2 hour delay. Here, due to coagulation the change in absorption is not seen.

Attempts were made to increase the glucose concentration in the blood sample using the dialyzer fiber without increasing the volume of the sample. There were difficulties in getting the dialyzer fiber through the capillary tube with the blood sample. The results of the successfully inserted fiber through the capillary tube were not repeatable.

CHAPTER 8

CONCLUSION

The osmolarity phenomenon was tested in the RBC for the glucose concentration that is within the normal range of blood glucose. The images showed the changes in size of the RBC with different glucose concentration that are within the human blood glucose range. These changes were confirmed with the cell counter automatically too.

The optical cell that was built was able to detect the changes in absorption. But the optical cell can be made sophisticated with the use of heat sink and lens for the LED to reduce heating effect and focus the light on the capillary tube with less scattered light respectively. The photodiode can also be used with amplifier for increased signal output.

The set up for increasing the glucose concentration was not very successful. This method can be improvised and optimized to increase the blood concentration. The capillary tube holder used for calibration was used to see the absorption change in the blood sample too. But as the absorption was higher than the range of the spectrophotometer, it was not able to detect the changes. Hence a spectrophotometer that can detect higher range of absorption can be used to detect the absorption changes in the blood sample.

LED was placed on the ear lobe and the photodiode was able to pick up the light emitted through the tissues of the ear lobe. The calibration curve is used to find the absorption against the voltage value from the photodiode during experiment with the same parameters used during calibration.

It has been demonstrated that the changes in absorption due to changes in glucose concentration can be measured at 540 nm. Though the system is stable, repeatability of results depends on the consistent sample conditions like time between extraction of blood and the first data acquisition time, storage or transportation conditions, ambient temperature and light.

Though this system is designed only to track the changes in glucose concentration, optimizing absorption with the glucose concentration might help in detecting the exact concentration of glucose in the blood non-invasively.

Physiological conditions that might interfere in the readings were not considered in this stage of experiment. For later development, physiological conditions that might change the absorption of 540 nm wavelength should be considered and the system should be optimized for it.

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APPENDIX A

SYSTEM DEVELOPED FOR INCREASING THE GLUCOSE CONCENTRATION IN CAPILLARY TUBE THAT IS TO BE USED IN THE SPECTROPHOTOMETER.

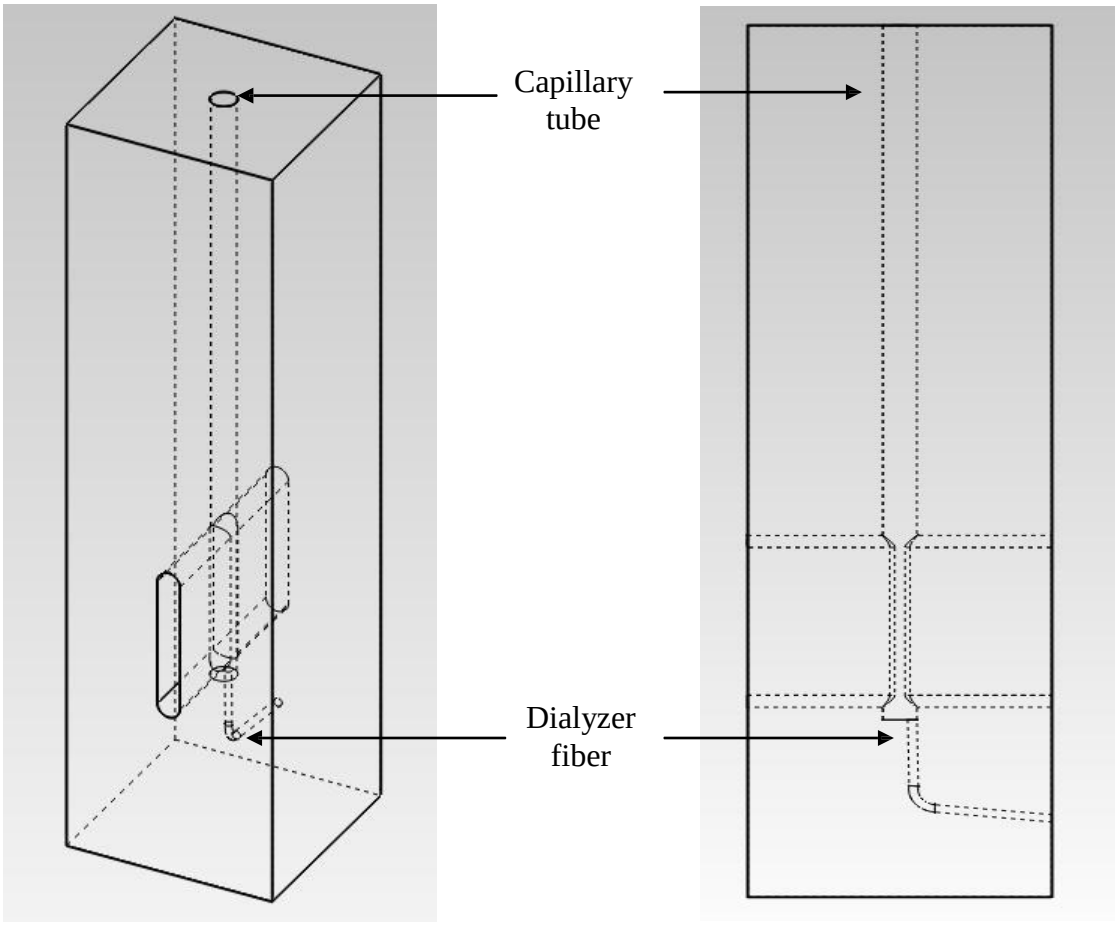

Figure 1. Capillary tube with dialyzer fiber holder for spectrophotometer reading.

This layout was 3D printed to be used in the spectrophotometer. A dialyzer fiber is introduced into the capillary tube and the end is taken out of the outlet port. The inlet of the dialyser fiber is inserted into a needle of a syringe with known glucose concentration solution. The syringe is operated by a syringe pump at a constant rate of 1mL/minute. The speed of the syringe pump can varied depending on the glucose concentration and need.

Initially the system was tested with a red dye to see if the diffusion works as expected. Color of the solution in the capillary tube seems to vary with time, proving the diffusion that takes place through the dialyzer fiber.

Though the system worked, it was not used as the spectrophotometer was not able to detect the absorption range. This can be useful if the spectrophotometer is able to detect the range.

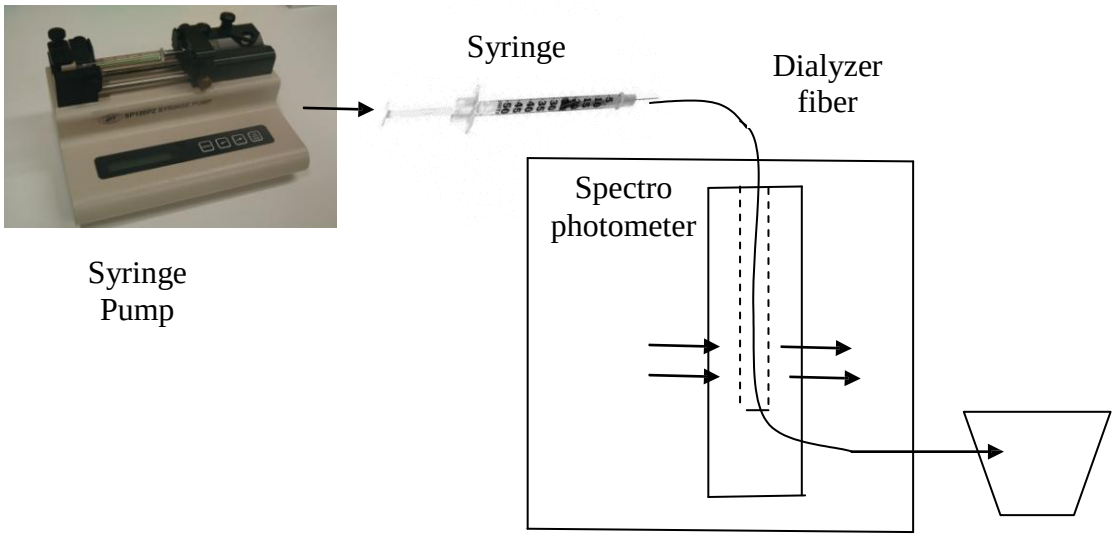

Figure 2. Experimental set up for increasing glucose concentration in capillary tube that is to be used in a spectrophotometer