Non- Invasive Method to Detect the Changes of Glucose Concentration in Whole Blood Using Photometric Technique

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Abstract— **A non-invasive 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, which is a peak absorbance of hemoglobin. As the glucose concentration in blood decreases, its osmolarity also decreases and the Red Blood Cells (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.**

I. Introduction

Diabetes is one of the most common chronic diseases and is projected to be 7th leading cause of death in 2030 by the World Health Organization. The International Diabetes Federation has reported in 2012 that 50% of the people with diabetes are left undiagnosed. There is a well recognized need for a patient-friendly self monitoring blood glucose sensor (SMBG) and inexpensive sensors to be accessible to all population and be used without any discomfort.

A blood glucose meter provides a single snapshot of the present glucose level. This helps in monitoring glucose at a time point but not a trend of changes in relation to the daily activities involving 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. SMBG are not preferred by most patients due to inconvenience. Type I diabetes is common in children who seldom wants to use the finger pick method.

Continuous glucose monitoring systems (CGMS) help in monitor the trend of glucose level in the blood even during nocturnal sleep and give deeper insight for appropriate medication. This can be important for Type I diabetes patient, who may be unaware of the sudden changes of glucose level. Also help in providing timely medication during diabetic ketoacidosis and hypoglycemia which need continuous monitoring. Information about the magnitude, direction, duration, frequency and the cause of fluctuation is not provided by intermittent glucose sensing [1] but this is crucial for insulin therapy.

Optical glucose sensors that use transillumination of tissue are generally based in infrared (IR) or near infrared absorbance techniques [2, 3]. Glucose has a strong absorbance in multiple regions of the infrared [3] and much effort has been directed towards making a truly effective noninvasive optical glucose monitor by this approach. There are unfortunately multiple problems with this technique including a lack of specificity, metabolic interferences, poor SNR, calibration problems, with the result that so far no commercially successful system has appeared on the market.

The goal of this research is to develop a continuous glucose monitoring system that is non-invasive and 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, compared to other optical glucose sensors using IR source . The goal is to be able to monitor relative or fast-acting changes in blood glucose level without necessarily a direct calibration. We conceive of a design as a finger-ring or ear-ring without causing discomfort during normal activities but monitoring every change in blood glucose level throughout the day. IR photometric techniques are harmful to the skin causing skin pigmentation, redness [4] and not recommended for continuous exposure. But we have chosen a wavelength sensitive to glucose changes and safe for continuous monitoring.

II. Background

Osmolarity is a measure of solute concentration in solution, while osmotic pressure is caused by concentration differences of a solute across a semipermeable membrane. It is well known that red blood cells shrink or swell in response to plasma osmolarity [5]. This swelling or shrinking is known to affect the optical properties of RBCs [6]. As the glucose concentration increases in blood, the water molecules from the cells tend to diffuse outside, thus shrinking the RBC cell. The characteristic shapes of these shrunken RBCs are called as spinocytes. Similarly, as the concentration of blood glucose decreases, water tends to diffuse inside the RBC causing it to swell.

Glucose concentration in the blood is known to be the major factor in determining blood osmolarity when other factors such as hydration and kidney function are within normal limits. Thus in normal health and homeostasis blood osmolarity is strongly correlated and an indirect indicator of blood glucose level. The osmolarity variations in blood not only change the shape and volume of the RBCs, but also the inner cell concentration [7].

This present work is based on the principle that changes

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in RBC cause changes in optical property of refractive index, absorption, scattering and transmission of light. As the osmolarity increases the absorption coefficient of RBC is also known to increase [8]. 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 [9].

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 [10].

The goal of this project is to investigate the relationship between the absorbance of whole blood and its glucose concentration but in a different optical spectrum than the usual infrared. We measure the variation in hemoglobin absorbance in a capillary tube as an indicator of the density of RBC's. We hypothesize that the concentration of hemoglobin in the optical path is affected by their size and water uptake. This is through hemoglobin concentration measurements that have a strong absorbance in the green region of the optical spectrum and an oxygen isosbestic point at about 540 nm.

III. Methods

An LED based optical absorbance cell designed for capillary tubes was made to determine the absorbance of fresh blood. It employs a wavelength sensitive to the RBC absorbance at 535nm. The LED is a 1 watt power with a maximum forward voltage of 4 Volts.

A photodiode is 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. To achieve the highest sensitivity the output of the diode was connected to single-ended signal input of the Lock-in amplifier SR530. The dynamic reserve of the Lock-in amplifier was set to Low as the voltage measured is in mV.

The circuit employs a constructed constant current source circuit to power the LED that was in turn controlled in frequency by the square wave. 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. This was taken to confirm that the glucose concentration changes during the experiment.

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.

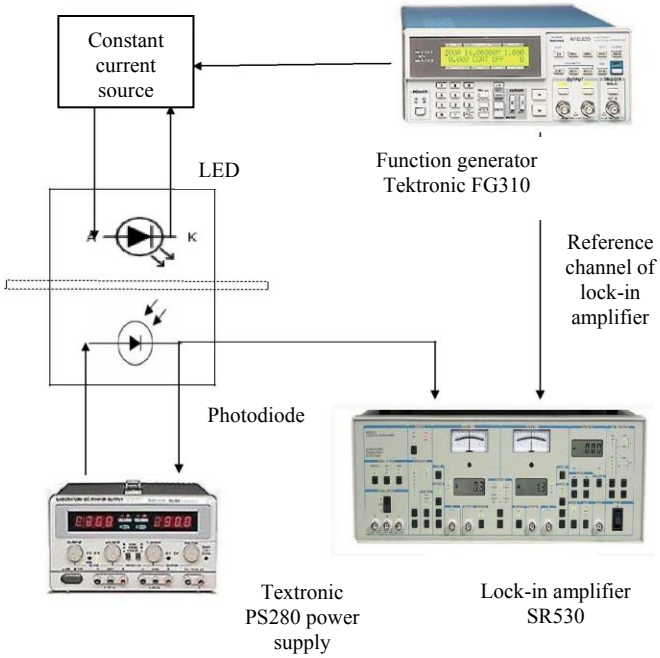

Fig 1. Experimental setup of the optical cell

IV. Verification and Optimization

The system is verified for stability and repeatability of results by calibrating it against the spectrophotometer. Hence the absorption can be calculated from the voltage output reading from the lock-in amplifier based on the calibration curve.

A. Constant current source

A constant current source is required to power LEDs when they are used in critical application since as the LED gets warmer it draws more current, thus increasing the light output. This is to stabilize its intensity since very small changes in absorbance are expected and no drift in the light source could be tolerated. The current drawn by LED for every 1 ◦C 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.

The circuit employed is observed to be 90% efficient and has a wide operating voltage from 3V to 30V. 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. In this circuit the LED current is set by R2 value. The LED is driven at 5 mA current and the power dissipated by the resistor is approximately 2.5 mA.

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.

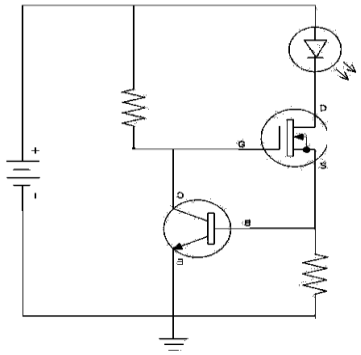

Fig 2. Circuit diagram of constant current source.

B. 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. 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.

The absorbance of the solution was taken at 535nm. 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.

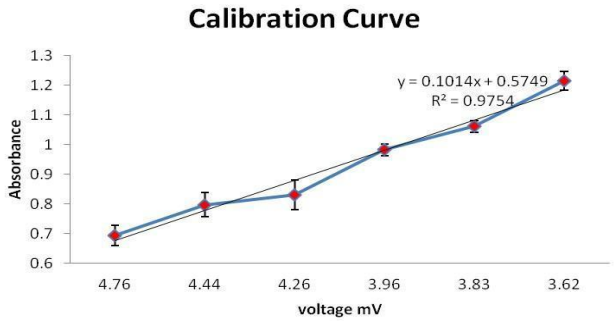

Fig 3. Calibration curve of the optical cell.

V. RESULTS

Fresh rat blood in a hematocrit tube was introduced into the experimental test setup and opacity changes at 535 nm were measured as a function of glucose concentration. Changes in blood glucose concentration occurred over time in the unpreserved blood as a result of continuous metabolism of red blood cells. Blood glucose measurements were taken at the start of the experiment and at the end to calibrate. Data was captured manually every thirty seconds and plotted using Excel.

Figures 4-6 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 sample was examined visually at the end of the study and showed no evidence of coagulation when capped at the end with clay.

Fig 4 shows the absorbance test reading with 5 mA through the photodiode giving a relatively higher response than Fig 5 test where the current was 6 mA and hence the scale differences. Both figures 4 and 5 show an easily detectable change in absorbance in fresh blood over time compared to figure 6 where the measurement was delayed for 2 hours.

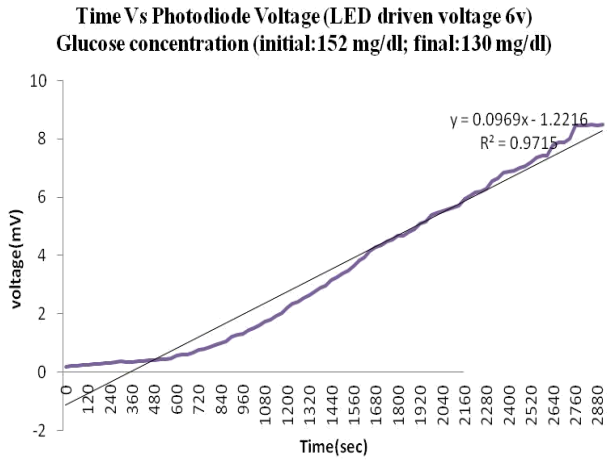

Fig 4. Absorbance decrease with time seen with fresh whole rat blood (LED driven at 6V).

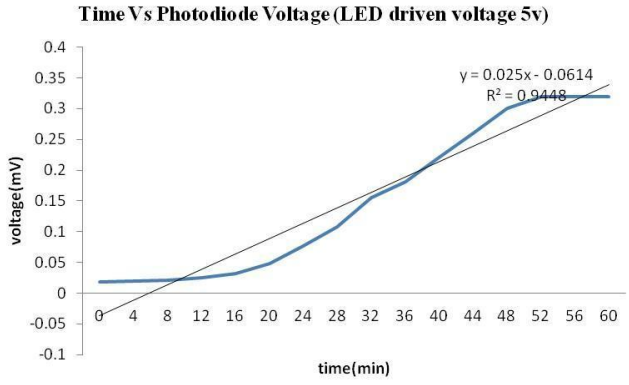

Fig 5. Another trial showing absorbance decrease with whole rat blood (LED driven at 5V).

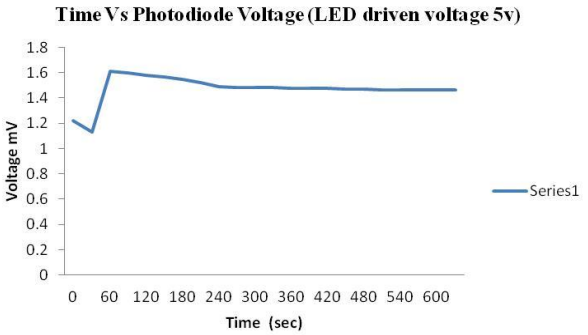

Fig 6. Absorbance over time for a blood sample aged for 2 hours as a control.

VI. 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.

Blood samples tested within 30 minutes of extraction were required to avoid coagulation. The capillary absorbance cell tests showed measureable changes in the opacity of the blood over time that was not seen with the control sample. The control sample was tested after a 2 hour delay at room temperature and it shows, presumably because of eventual cell death and coagulation, that no remarkable changes in absorption occur over time.

The results compared to calibration runs and colorimetric standards show that as the measured glucose concentration decreased in the sample tubes. We note that preservatives that stop cell metabolism are usually used within blood withdrawal tubes but were not in this case.

The nonlinearly of the plots with time would be both due to Beer's Law of absorbance which is an exponential function of concentration as well as possibly variations in the vitality of the cells as the sample ages. Clearly there would be changes in pH and conditions over time with cellular metabolism in the small contained volume.

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, and ambient temperature and light.

The measurement of small optical absorbance changes using LEDs requires the use constant current sources and careful attention to insuring that the environmental temperature is stable. The chopping frequency of the LED was initially set to 40Hz but proved unsuitable because of electronic noise frequencies in the ambient and so higher frequencies at 200 Hz were found to perform better.

The optical absorbance path in the capillary tube was quite high even though it was relatively short compared to a standard spectrophotometer cuvette. Whole blood in even a short-path special spectrophotometer cuvette at 535 nm in a relatively larger amount of rat blood was too opaque for the range of the spectrophotometer. Dilution of the whole blood sample to accommodate the spectrophotometer range was not possible because of the effects on osmolarity and dilution of the contained glucose. The capillary optical cell thus provided not only a way to measure the absorbance of small 10 microliter samples of rat blood but also served as a preliminary design to the realization of a transcutaneous system. Such a system we envision to transilluminate a capillary bed

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.

VII. Conclusion

We have designed and evaluated a system for tracking absorbance changes in small amounts of fresh blood from rats. This system allows rapid acquisition of blood from a rat and then tracking of the sample absorbance over time. Without the use of preservatives that stop cellular metabolism we find that the changes in blood absorbance track what would be expected from continued cellular glucose metabolism of RBCs. These changes in absorbance over time, along with comparative measurements using glucose meters, standards, and old blood are consistent with the hypothesis that they show variations due to osmolarity changes in blood correlated with its changing glucose concentration.

This method monitors only the change in glucose concentration. Hence for future work, this method might be improvised for precision and be developed as a device to monitor the changes in glucose concentration as an aid to diabetic management.

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