Optical Changes in Whole Blood vs. Glucose Concentration

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ABSTRACT

The effects of physiologically relevant glucose concentrations on the optical properties of whole blood were measured in-vitro. A concentration increase of +400 mg/dL caused a decrease in the scattering coefficient by 10% over all wavelengths studied. To determine potential mechanisms for the change in the scattering coefficient, we employed optical microscopy to quantify the change in erythrocyte geometry. A 15% change in cell thickness was observed following a glucose increase of +500 mg/dL.

Keywords: Blood Optical Properties, Erythrocyte Morphology, Glucose, Integrating Sphere

1. INTRODUCTION

Non-invasive measurement of glucose has been the elusive objective of significant research over the past several decades. Numerous optical methods that have been employed for non-invasive glucose detection are being investigated and can be grouped into five main categories (as proposed by Pullano et al.[1]): Raman spectroscopy, optical coherence tomography, photoacoustic spectroscopy, fluorescence, and NIR. However, the impact of glucose on the optical properties of blood has received little attention. Harkness et al.[2] have recently shown that changes in glucose concentration in whole human blood results in a change in scattering and absorption coefficients. In this prior study, steps in glucose concentration of +400 mg/dL resulted in few data points over the physiological range. Thus, one of our objectives here is to quantify the relationship between glucose concentration (in +100 mg/dL steps) and the optical properties of blood.

The mechanism behind the optical scattering changes following glucose addition is not completely clear. However, morphological changes in erythrocytes have been shown to affect scattering. Mauer et al.[3] have demonstrated numerically that the thermal undulations of erythrocytes should be detectable using dynamic light scattering. These undulations are typically on the order of 10-400 nm,[4] which is similar to the changes caused by glucose concentration. Borovoi et al.[5] employed a straight-ray approximation with a plane wave receiving a complex phase shift as it passed through the cell. They found cell dimensions to have a measurable effect on the scattered light. Streekstra et al.[6] employed an ektacytometer, which measured deformability and ellipticity of the erythrocytes. They demonstrated that cell morphology affects scattering properties. Sloot, Figdor, and Hoekstra used a modified Rayleigh-Gans-Debye scattering model. They showed both numerically[7] and experimentally[8] that forward scattering from osmotically shocked lymphocytes was dependent on their morphology. In short, these studies have shown that cell shape and dimension will affect the optical scattering of blood.

The goal of this study is twofold. First, we aim to quantify the effect of small glucose additions on the optical properties of whole blood. Secondly, we investigate the impact of additions on erythrocyte morphology.

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2. METHODS

Whole human blood was collected from healthy adult volunteers under BYU IRB #X-2021-135. The blood was collected in EDTA Vaccutainer Tubes. This blood was immediately used without refrigeration. All experiments were completed within 6 hours of draw. An isosmotic glucose solution (5400 mg/dL) was prepared using powdered d-glucose and water. This solution was used in both the integrating sphere experiments and the morphology experiments.

2.1 Integrating Sphere Experiments

2.1.1 Blood

The initial glucose concentration and hematocrit of the blood were collected before optical measurements were taken. The donated blood was separated into two measurement branches. One branch was used for glucose additions while the other branch was used as a control. In the first branch, glucose concentration was increased by 100 mg/dL every 45 min. At the same time, an identical volume of plasma from the same donor was added to the other branch of the experiment to act as a control for hematocrit. Figure 1 shows a top-down model of the experimental setup. Experiments started approximately 20 minutes after blood draw. The blood in each branch was stored in a temperature-controlled bath held at 37.5°C, and pumped out of the bath using a syringe pump. The blood was pumped back and forth with a shear rate of 255 s^{-1}. During this pumping, the blood passed through a custom-designed flow chamber held between the integrating spheres. The glass faces of the chamber were each 1 mm thick. The space between the glass (where the blood was flowing) was also 1 mm.

2.1.2 Optical setup

Light from a mercury arc lamp was focused onto a linear variable filter. This filter was attached to a kinematic mount controlled by a stepper-driver, which we used to select wavelength. After the linear variable filter, the light was recollimated back into a 10 mm beam. A beamsplitter divided the beam into the two measurement branches. Each branch was identical with an integrating sphere placed on each side of the blood flow chamber for that branch. Extended InGaAs photodiodes were used to measure the reflected and transmitted light. To reduce noise, the light beam was chopped at 2 kHz and our photodiode signal was passed through lock-in amplifiers (Zurich Instruments MFLI 500 kHz).

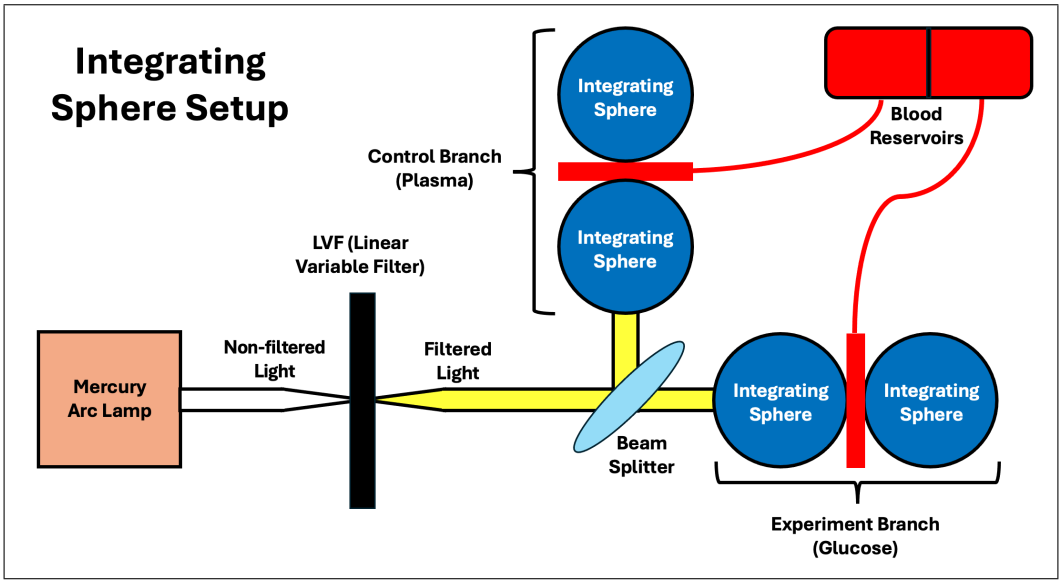


Figure 1. Optical setup for integrating sphere experiments. Light from a mercury arc lamp was focused onto an infrared linear variable filer (LVF). The filtered light was then split into both the glucose branch and the control branch. Integrating spheres captured the back-scattered and transmitted light for analysis.

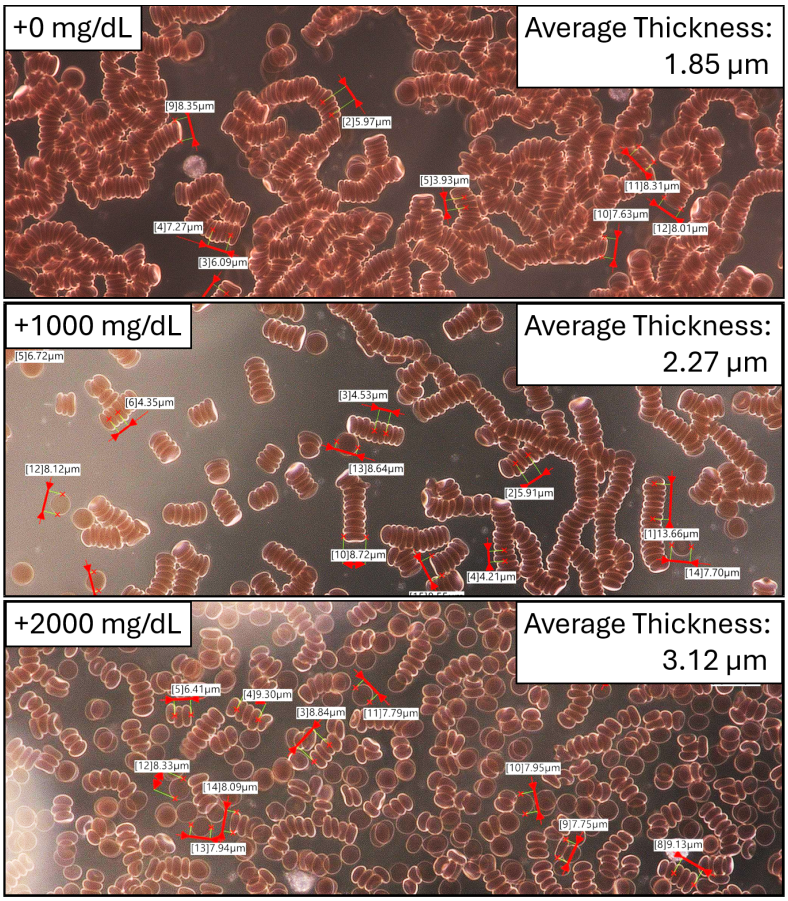


Figure 2. Erythrocytes as viewed by the Keyence microscope at approximately 1000x magnification. Rouleaux formations are clearly seen. The thickness difference between each sample is also visible, as is the reduction of Rouleaux stacking caused by the slightly deformed cells.

2.1.3 Data analysis

Before blood was added to the system, a calibration run was performed that measured total incident light. Then, after blood was added, the transmission and reflection data were collected for each branch as described in section 2.1.2. This data, along with the calibration data, was passed into Scott Prahl's Inverse Adding Doubling program.[9] This program extracted the reduced scattering coefficient (μ_s') and the absorption coefficient (μ_a) of the blood from each branch.

2.2 Erythrocyte Morphology Experiments

For erythrocyte morphology experiments, the glucose solution was added to 1 mL whole blood samples in plastic test tubes. The glucose solution volume added was calculated to increase glucose concentration by +500 mg/dL, +1000 mg/dL, +1500 mg/dL, and +2000 mg/dL. Another whole blood sample (with no glucose addition) from the same donor was set aside as a control. Following the glucose addition, each sample was gently mixed by slowly inverting the test tube. In order to minimize lysing and other disturbances of the blood, care was taken that the test tubes not be bumped or jostled significantly.

Samples were imaged using a Keyence VHX Optical Microscope for data collection. Images and measurements are seen in Figure 2, where a variety of diameter and thickness measurements are illustrated. Measurements were made using the two-point length measurement tool on the Keyence. For each blood sample, 32 cell diameter measurements and 32 cell thickness measurements were taken. Cells that appeared representative of those in the field of view were selected for measurement (i.e. if the cell appeared overly wide or narrow, it was not chosen).

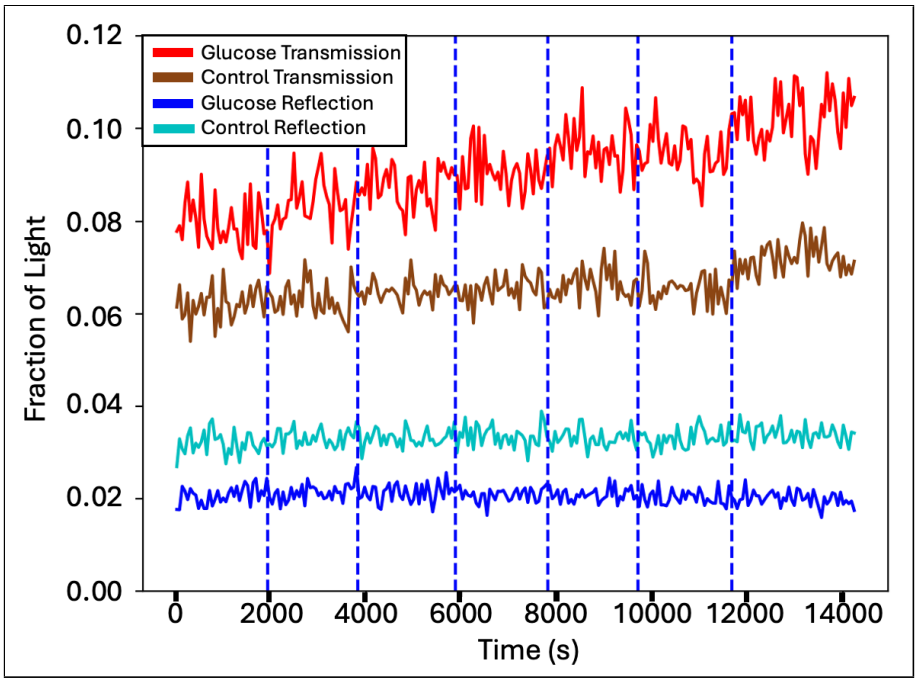


Figure 3. Fraction of light received in each of the 4 integrating spheres relative to calibration values. Dashed blue lines indicate glucose and plasma addtions to the blood.

Clicking on each end of the cell provided the diameter. This process was repeated for 32 cells, resulting in 32 diameter data points.

Thickness data was obtained using a similar pseudo-random selection method. Stagnant whole blood forms stacks of cells or "Rouleaux formations." An additional requirement for choosing these cells was that they be edge-on from the viewer's perspective. If a cell appeared at an angle (i.e. some of the concave face was visible), we did not measure that stack. Once an edge-on Rouleaux formation was identified, the two-point length measurement tool was used to find the length of the stack. Dividing by the number of cells in the stack gave an average cell thickness for each cell in that formation. Thus, each of the 32 thickness measurements included 1 to 7 cells.

3. RESULTS

3.1 Integrating Sphere Results

Raw integrating sphere data are seen in Figure 3. Across all wavelengths in our range, we saw an increase in transmission of about 2.5% with an overall increase in concentration of +400 mg/dL (see Figure 4). Over the same concentration change, the reduced scattering coefficient was observed to decrease approximately 10% over all wavelengths. Reflectance and absorption changes were not observed with the additions of glucose.

3.2 Morphology Results

The 32 data points for diameter measurements were averaged for each blood sample. The same was done with thickness. As seen in Figure 5, no statistically significant change in cell diameter was detected. On the other hand, average cell thickness increased 15% following a glucose concentration increase of +500 mg/dL. For the +1000 mg/dL and +2000 mg/dL samples, thickness increased 23% and 69% respectively.

4. DISCUSSION

4.1 Integrating Spheres

Our results show that the reduced scattering coefficient of blood is significantly affected by glucose concentration. The decreased scattering caused the increase in transmission, similar to what Harkness et al.[2] reported.

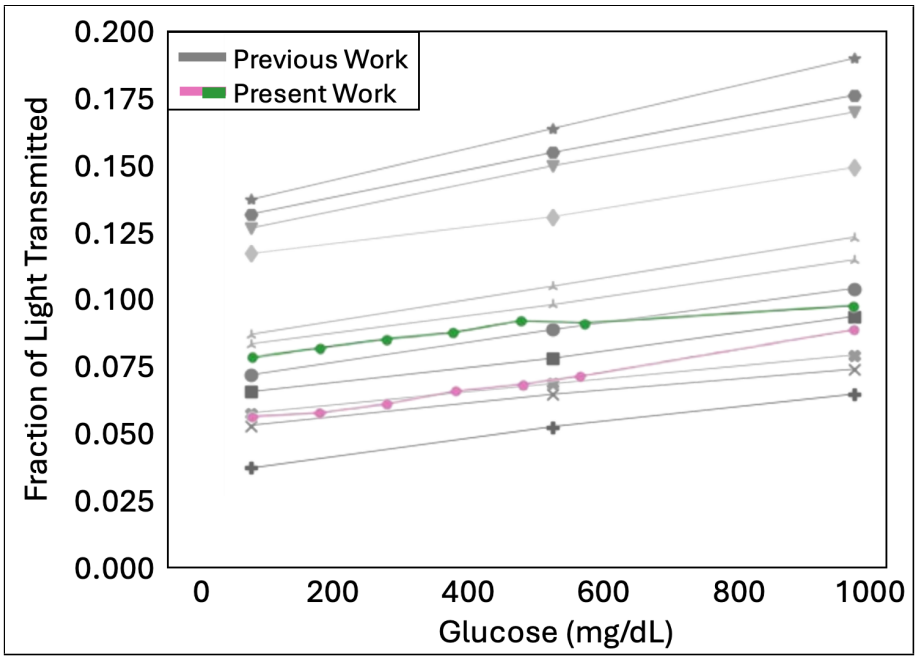


Figure 4. Transmission of Light at 1700 nm for various donations of blood.

4.2 Morphology

There are several general observations from our optical micrographs, as represented by Figure 2. First, any change in diameter was too small to be detected, which is unlikely to have a significant impact on the overall scattering. Second, we also see that increased glucose concentration yields fewer cells in each Rouleaux formation. While this reduced the number of cells in each thickness measurement, it did not significantly impact the ability to measure cell thickness (thickness uncertainty did not increase with glucose concentration). The substantial changes in morphology are likely the cause of this reduced Rouleaux stacking. The third observation is that changes in cell thickness vs. glucose concentration are clearly evident.

While we observed that increased glucose concentration resulted in reduced scattering and also increased cell thickness, a precise mechanism is required to connect these phenomena. Optical modeling is likely required to determine whether the observed morphological changes are sufficient to explain the observed scattering changes.

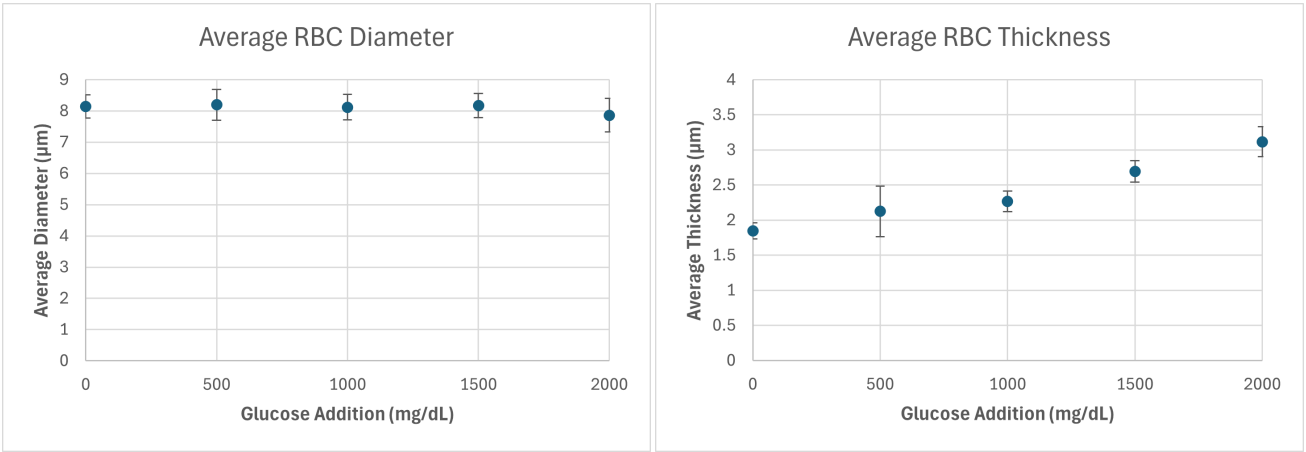


Figure 5. Average diameter and thickness dimensions of erythrocytes in varying glucose additions. 0 mg/dL refers to whole, unaltered blood. Error bars represent standard deviations.

5. CONCLUSION

Understanding the relationship between glucose concentration and the optical properties of whole blood could be crucial for future technologies, and may guide future glucose detection techniques. In this work, we have quantified the scattering effects of physiologically relevant glucose additions to whole human blood. The scattering coefficient was found to drop by approximately 10% after a glucose increase of +400 mg/dL. The morphological response of the erythrocytes was also quantified. Following a glucose addition of +500 mg/dL, erythrocyte thickness increased 15%. Further work is needed to elucidate a more precise relationship between glucose and the scattering coefficient. Optical modeling may also clarify the relationship between erythrocyte morphology and scattering.

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