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EXPERIMENTAL / LABORATORY STUDIES

Polarized Mueller Matrix Analytical Model for Glucose Measurement in Vitro

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Abstract: In this study we present a noninvasive and nonradioactive glucose in vitro measurement technique using the Mueller polarization analytical model. The glucose solution and aqueous humor phantoms are illuminated with a He-Ne (632.8 nm) laser. This in vitro study provides a base and reference data for in vivo eye glucose monitoring. The 16 Mueller polarization matrix element images give information about eye absorption and scattering, and changes in polarization patterns to measure glucose concentration. The interior part of the eye (aqueous humor) contains a birefringent scattering medium for glucose measurement. The Mueller matrix polarimetric technique for simultaneous extraction of rotating linear and circular polarized light is discussed theoretically and experimentally implemented. This method requires calculation of the Mueller matrix for different orientations of polarized light through turbid samples. The results are in good agreement with those in the literature.

Key Words: Polarization, glucose sensor, birefringence, depolarization, aqueous humor, Mueller matrix polarimeter.

Introduction

Optical glucose monitoring is routinely used for diabetes mellitus. Diabetes mellitus is a leading cause of death. The primary defect in diabetes mellitus is a lack of insulin production (1,2). Insulin is a hormone that regulates the transport of glucose from the blood stream into fat and muscle tissue, where glucose is utilized as an energy source. The insulin deficiency in diabetes leads to hyperglycemia and the inability to utilize glucose as a source of energy (3,4). Insulin therapy is commonly prescribed in the treatment of diabetes mellitus (5,6). Diabetic patients regulate their insulin dosage by monitoring blood glucose levels. Long-term hyperglycemic states have been shown to accelerate the evolution of the complications associated with diabetes. Therefore, with tight control of blood glucose concentration the complications associated with diabetes mellitus can be minimized (7,8). Unfortunately, many patients do not monitor their blood glucose concentrations at recommended frequencies. Lack of compliance in blood glucose monitoring is partly due to the inconvenience, pain and tediousness of current home monitoring techniques. Patients who fail to maintain euglycemia suffer higher rates of morbidity and mortality caused by the associated complications of diabetes mellitus (9). Due to the increased risk of infection whenever the blood-skin barrier is broken, a noninvasive blood glucose monitor would be ideal (10-12). A sensitive polarimeter was designed to measure glucose concentrations within the aqueous humor of the eye. The proposed glucose sensor is noninvasive and produces no medical waste. Finally, the polarimeter does not require a blood sample, thus reducing the risk of infection. The non invasive detection of blood glucose levels in humans is an ambitious goal for managing diabetes which can lead to severe complications over time. These can include blindness, renal and cardiovascular diseases, and peripheral neuropathy associated with the limbs (3).

The proposed model is primarily based upon the measurement of the glucose concentration in the aqueous

humor using polarimetry (14,15). Test sites being explored include the eye, fingertips, cuticle, finger web, forearm and ear lobe. However, many hurdles remain before these products reach the commercial marketplace. Glucose concentration in the aqueous humor closely mimics glucose levels in the blood (16,17). Polarimetric techniques use the property of glucose as an optically active analyte, which rotates the plane of polarization of incident linearly polarized light. The first documented use of polarized light to determine sugar concentration dates back to the late 1800s, when it was used for monitoring industrial sugar production processes (18,19). However, it is only in the last 2 decades that the use of polarized light has been applied to the physiological measurement of glucose. In 1982, March et al. were the first to propose the use of polarimetry to indirectly estimate blood glucose levels via the aqueous humor of the eye (20,21). They found that in order to measure millidegree sensitive rotations due to glucose at physiological levels a very sensitive and stable polarimeter is required, and in the past decade considerable work has been done on the development of such a polarimeter (22,23). Coté et al. reported on the potential of millidegree sensitivity by utilizing a true phase technique (24). This work was later followed by Cameron et al., who reported on a Faraday-based polarimeter using a digital closed-loop feedback technique with submillidegree sensitivity (25). Since then, different polarimetric variations have been illustrated by several groups to measure glucose concentration.

In general, photons propagating in turbid media have their incident direction, phase, and polarization randomized by multiple scattering. The Mueller matrix polarimetric technique provides a simple analytical model for a noninvasive glucose sensor. It is an experimental polarization birefringent measurement system (26).

Background

Theory

A beam of light is composed of electromagnetic waves oscillating perpendicularly to the direction of light propagation. The polarizers and retarder rotate the polarization plane of light as it propagates through the sample. The plane of polarization may be rotated either clockwise or counter-clockwise. The equation that relates optical rotation to a medium specific rotation is given by the equation (26)

$$[\alpha]^T \lambda,pH = \frac{\alpha}{LC} \quad (1)$$

where α is the specific rotation of an optically active compound in degrees in a specific temperature, T, and PH of the system, λ is wavelength, L is the optical path length in dm, and C is the sample concentration in grams of mass per milliliter of solution. The relationship in terms of wavelength for specific rotation is given by Drude's equation (27).

$$[\alpha]^T \lambda,pH = \frac{k_0}{\lambda^2 - \lambda_0^2} \quad (2)$$

Equation 2 is an approximation of Drude's equation and is valid only outside the absorption region for the molecule of interest. The optical instrument used to measure rotation due to an optically active sample in a polarimeteric system has the main components of a polarizer, quarter wave plate, sample cell, a second polarizer known as the analyzer, a detector and a CCD camera [Figure 1]. As the beam passes through the sample, the plane of polarization will rotate according to the concentration of the sample and the path length of the container. If an optically active sample is introduced into the system, the intensity of transmitted light will be proportional to the amount of rotation in polarization due to the sample (28).

If the optical properties of a substance are same in all directions regardless of its orientation, the substance is said to be isotropic. In many crystalline structures and some organic substances the optical properties are not the same in all directions and they have more than one index of refraction; these materials are known as anisotropic. Birefringence is a property of anisotropic substances in which 2 orthogonally oriented different refractive indices of light exist, the ordinary refractive index (along the slow axis) and the extraordinary refractive index (along the fast axis). This difference in the speed of propagation between the x and y polarized components induces a phase difference, depending on the magnitude of the components and the relative phase retardance. We can see different states varying from linear to circular light (29).

For modeling the polarization effects of various optical components we represented the Mueller matrices. Using Mueller calculus, an optical element that acts on a

light beam is represented by multiplication of the incident light Stokes vector by the Mueller matrix for that optical element, and is given as (30)

$$[S_{out}] = \lfloor M_{system} \rfloor [S_{in}] \quad (3)$$

where $[M_{system}]$ is the Mueller matrix representing the entire experimental optical system given as

$$[M_{system}] = [QW][A_M][M][QW][P_M] \quad (4)$$

and the output Stokes vector can be calculated by the relation in Equation 4, where the Mueller matrix for the polarizer [P], turbid medium [M], analyzer [A] and quarter wave plate [QW] at the horizontal fast axis and Stokes input vector $[S_{in}]$ are given as (31)

$$[P_M] = \frac{1}{2}\begin{bmatrix} 1 & C_{2i} & S_{2i} & 0 \\ C_{2i} & C_{2i}^2 & \frac{S_{4i}}{2} & 0 \\ S_{2i} & \frac{S_{4i}}{2} & S_{2i}^2 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}, [A_M] = \frac{1}{2}\begin{bmatrix} 1 & C_{2o} & S_{2o} & 0 \\ C_{2o} & C_{2o}^2 & \frac{S_{4o}}{2} & 0 \\ S_{2o} & \frac{S_{4o}}{2} & S_{2o}^2 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$[QW] = \frac{1}{2}\begin{bmatrix} 1 & -1 & 0 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}, [M] = \frac{1}{2}\begin{bmatrix} m_{11} & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{bmatrix} \quad (5)$$

$$\text{and } S_{in} = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix}$$

where $C_{2i} = \cos(2\theta_i)$, $S_{2i} = \sin(2\theta_i)$, $S_{4i} = \sin(4\theta_i)$, $C_{2o} = \cos(2\theta_o)$, $S_{2o} = \sin(2\theta_o)$, $S_{4o} = \sin(4\theta_o)$. All the Mueller matrix elements can be determined experimentally [see figure 1]. It can be shown that 49 intensity measurements with various orientations of polarizers and analyzers are necessary to obtain the 16 elements of the Mueller matrix. Table 1 lists the necessary measurements for each matrix element. Once all 16 elements of the matrix are obtained, the medium is completely described in terms of its optical characteristics (32).

Experimental Setup

The block diagram of the glucose sensing system developed for this project is shown in Figure 1. A He-Ne laser beam 632.8 nm in wavelength was passed through the sample. The sample was prepared with 5 g of D-glucose in a total volume of 200 ml of deionized water, and aqueous humor solution was analyzed for composition as indicated in Table 2. Once the observed mV/mg was determined for each individual substrate and wavelength, the mathematical model was developed for the mixtures of solutions. To test the validity of this model, a series of experiments was executed. In these experiments, solution combinations involving glucose, ascorbic acid and albumin were analyzed for polarized light within the system. In the first series of combinations, each glucose concentration was combined with deionized water. In the second series, ascorbic acid concentrations were combined with albumin at the physiological aqueous humor concentration. The laser beam is polarized by polarizers and the retarder present in the optical train. Polarimetric measurement for each set is taken from a 1 cm diameter cell. The polarizing optics transform the modulated polarization vector into intensity modulation according to Malus' law. The intensity is detected by a silicon photo diode, which outputs a voltage proportional to the detected light intensity, and images are taken by a charge coupled device (CCD) camera.

Table 1. Calculation of the 16-image Mueller matrix. The notation is as follows: the first term represents the input polarization state, while the second term represents the output polarization state. The states are defined as H for horizontal, V for vertical, P for +45°, M for -45°, R for right circular, and L for left circular.

M_{11} = HH+HV+VH+VV	M_{12} = HH+HV-VH-VV	M_{13} = PH+PV-MH-MV	M_{14} = RH+RV-LH-LV
M_{21} = HH-HV+VH-VV	M_{22} = HH-HV-VH+VV	M_{23} = PH-PV-MH+MV	M_{24} = RH-RV-LH+LV
M_{31} = HP-+HM+VP-VM	M_{32} = HP-HM-VP+VM	M_{33} = PP-PM-MP+MM	M_{34} = RP-RM-LP+LM
M_{41} = HR-HL+VR-VL	M_{42} = HR-HL-VR+VL	M_{43} = PR-PL-MR+ML	M_{44} = RR-RL-LR+LL

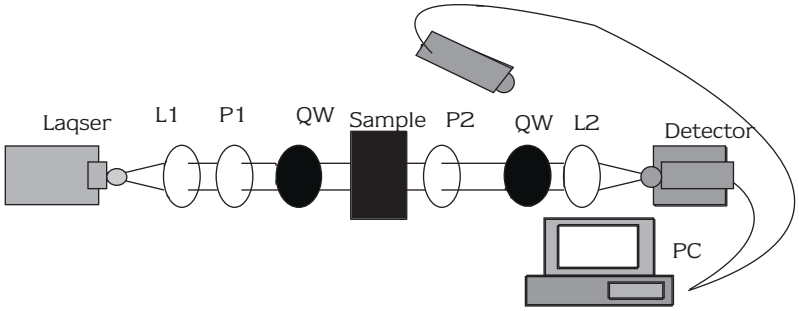

Figure 1. Experimental setup for measurements of transmitted Mueller matrix elements. A He-Ne laser beam with an output power of 5 mW at a wavelength of 632.8 nm is used as the light source. The laser light is focused on polarizer P1 to obtain linearly polarized light. The circularly polarized light is generated by inserting a quarter mica retardation plate behind the linear polarizer. The output polarized light is focused on the polystyrene sphere suspension turbid medium by lens L1 (f = 15 cm) and again passed through the linear polarizer and quarter wave plate and recorded on a photodiode detector and CCD camera, controlled and operated with Lab software.

Table 2. Constituents of the aqueous humor solution.

Constituent	Concentration (mmol/l)	Constituent	Concentration (mmol/l)
Glucose	51	Phosphorus	04
Protein	150	HCO_3	20
Urea Nitrogen	12	Sodium	140
Magnesium	1.5	Potassium	3.5
Calcium	04	Chloride	100

Results and Discussion

A total of 8 experiments were conducted with a 632.8 nm He-Ne laser using a hyperglycemic concentration range for both the glucose-doped water and aqueous humor media. The solution concentrations are illuminated to determine the model. Figure 2 shows the results of the measured glucose concentrations in water solution. The experimental results of the mixture of glucose with ascorbic acid are shown in Figure 3. The rotational accuracy needed to perform these experiments can be calculated through the experimental setup shown in Figure 1 using the specific rotation, path length and concentration. Therefore, for our system, the minimal rotational accuracy needed for normal blood glucose concentrations is α = 0.5 * 100 * 44.8/100 = 22.4 m deg. The predicted glucose concentration was evaluated for actual concentrations without a posterior retarder and polarizers in the optical train [see Figure 4]. The

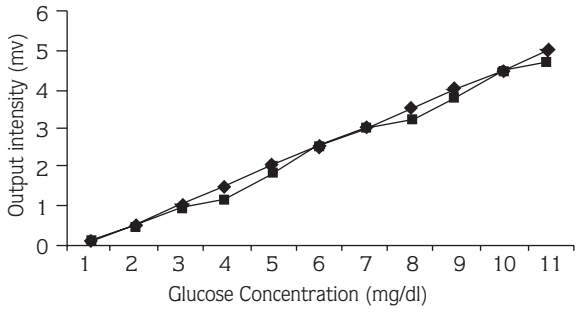

Figure 2. The output laser intensity versus glucose concentration for water glucose solution.

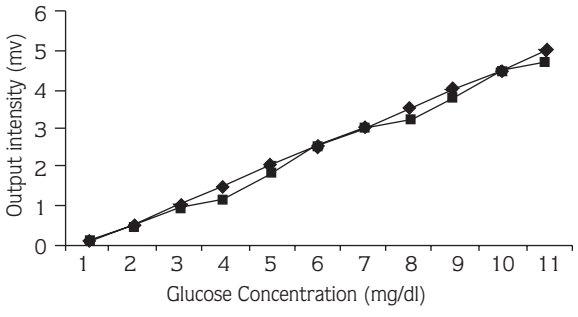

Figure 3. The output laser intensity versus glucose concentration for aqueous humor solution.

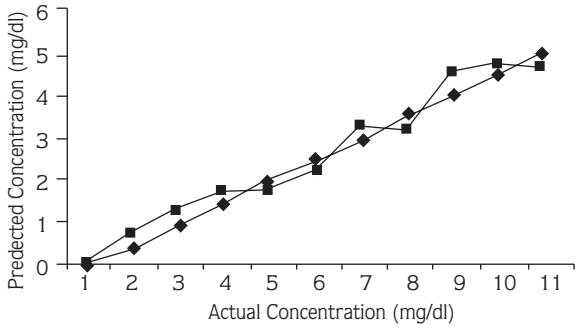

Figure 4. Predicted versus actual glucose concentration for the water mixed and glucose-doped aqueous humor experiment.

average glucose concentration in this model is 4.5 mmol/l and less than 10 mmol/l, which means the accuracy is less then 0.5, comparable to the study by Goetz (33). Figure 5 shows the output intensity in volts for the given solution concentration. The decrease in the intensity of aqueous humor solution is due to its birefringent behavior. In this measurement we used only linear light (as our laser source was linear polarized). In water dissolved glucose solutions the depolarization and intensity decrease varies gradually with changes in glucose concentration. This process was repeated several times for the same composition.

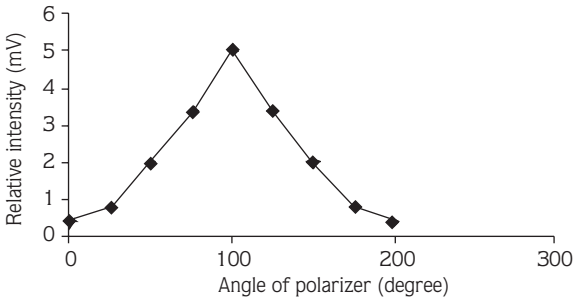

Figure 5. The output intensity of the sample at a linear polarizer.

The Mueller matrix 2D images [see Figure 6] of the glucose concentration taken for aqueous humor solution by polarimetric setup and the resultant matrix of Figure 7 provide comprehensive details about the sample. The M_{11} element of the matrix shows the direct intensity pattern and the characteristic of the illuminating light. The other matrix is the results of linear and circular polarization output. These images and the matrix were acquired through 49 orientations, and the measurements

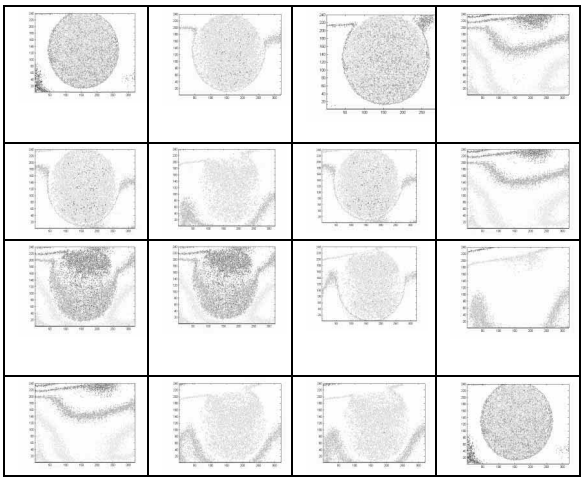

Figure 6. The 2 dimensional images of 16 Mueller matrix transmitted intensity elements for aqueous humor.

1.102	0.501	-.254	0.059
0.582	0.651	0.831	0.061
0.385	0.324	0.519	0.321
0.0653	0.325	-0.136	0.739

Figure 7. Mueller matrix data for a transmitted polarized laser beam from aqueous humor glucose solution.

of the polarized optics are given in Table 1. Mueller matrces M_{22}, M_{33} and M_{44} are unitary and represent the horizontal, vertical, ±45°, L-circular and R-circular polarization. Only looking at the diagonal matrix of the Mueller train one can estimate the material concentration. These images, along with the matrix for normal glucose concentration, can be taken as a model for a polarimetric glucose sensor, and can be compared to other samples.

The results of this investigation with a polarimetric system for glucose concentrations from ascorbic acid and albumin were measured. The effect of ascorbic acid and albumin alone at physiological concentrations was minimal compared to that of hyperglycemic glucose in the aqueous humor of the eye. Furthermore, since ascorbic acid, albumin and other confounders in the aqueous humor are contrarotatory, the effect of their combination at physiological concentrations was even less. It can be assumed that material in the sample will also possess a unique optical rotation spectrum. No complete study of the optical rotation of the medium found in the aqueous humor is readily available, and one therefore needs to be

performed. Based on the work presented here and using the new equipment, the optical rotation of molecules in the aqueous humor will be established.

In general, scattering and absorption that are wavelength-dependent could cause both intensity variation and depolarization of the light. As the conformation of the protein changes, the optical properties, including specific rotation, scattering and absorption, also change. If the albumin was undergoing any type of conformational change, then the voltage out of the polarimeter would also fluctuate. Furthermore, naturally occurring small fluid movements in the test cell would also affect the position of the protein molecules in the solution. These changes could cause the system to drift since the conformational variations and fluid movements of the solution occur as random events throughout the solution. Although a nonlinear analysis might be appropriate for the in vitro system that may be affected by conformational changes, these changes are not expected to occur in vivo due to the association constants of the molecules naturally occurring in the aqueous humor. Therefore, the analysis for this study focused on the use of a polarized model, which should be more appropriate for the final system. Moreover, with more optimized equipment the polarization analysis could likely be developed for a glucose sensor. Lasers demonstrated an increase in the rotation observed when ascorbic acid was added to the glucose concentrations and a decrease in the observed rotation when albumin was added to the glucose concentrations.

Conclusion

The results demonstrate the ability of the polarimeter to accurately measure glucose concentrations in both water and aqueous humor media. This research represents an important step toward the development of a noninvasive glucose sensor that may eventually be capable of detecting glucose levels in the aqueous humor of the eye. The polarimeter is the simplest method for a glucose sensing system and its development for in vivo measurement provides an easy to use home-based glucose sensor. The Mueller matrix provides a fingerprint of the solution and detected analysis for glucose aqueous humor solution.

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