Investigation of glucose-hemoglobin interaction by optical coherence tomography

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ABSTRACT

In this study, the refractive index of glucose-hemoglobin solutions at different glucose concentrations was measured. Measurements were performed using Abbe refractometer at 589 nm and OCT system at 1300 nm. The different amount of glucose was added to hemoglobin solution. Theoretical values of refractive index of the glucose-hemoglobin solutions were calculated in assumption that hemoglobin and glucose molecules do not interact. The difference between the measured and calculated values of refractive index can be connected with glucose binding to hemoglobin. It is shown that the refractive index measurements can be applied to the evaluation of glycated hemoglobin amount.

Keywords: glycated hemoglobin, refractive index, Abbe refractometer, optical coherence tomography, glucose

1. INTRODUCTION

Measurement of glycated hemoglobin is widely used for routine blood glucose control in patients with diabetes. It is known that glucose combines with hemoglobin via a slow irreversible non-enzymatic reaction; the rate of the reaction is determined by the glucose concentration in plasma of blood. The amount of glycated hemoglobin does not depend on changes of glucose level in blood during a day. Glycated hemoglobin is used as an index of mean glycemia and as a measure of risk for the development of diabetes complications. Concentrations of the other blood-based glycated proteins (e.g., glycated plasma proteins, "fructosamine") also reflect mean glycemia, but over a much shorter time than glycated hemoglobin (15-30 days and 60-120 days, respectively). Glycated hemoglobin is a useful marker for long-term glucose control in diabetic patients and assessing the quality of diabetes care, therefore, the search of quick and high sensitive methods for measurement of glycated hemoglobin portion in blood is important.

Glycated hemoglobins comprise HbA1 and other hemoglobin-glucose adducts, since HbA1 is made up of HbA1a, HbA1b and HbA1c. HbA1c is the major component of HbA1, about 80% of HbA1. There are many different glycated hemoglobin assay methods in current use.[1] Most methods quantify hemoglobin A1c, defined as hemoglobin A with glucose attached to the NH_2-terminus valine of one or both beta chains. The methods can be classified into two groups based on assay principle. The first group includes methods that quantify glycated hemoglobin based on charge differences between glycated and nonglycated components (e.g. cation-exchange chromatography and agar gel electrophoresis). The second group includes methods that separate components based on structural differences between glycated and nonglycated components (e.g. boronate affinity chromatography and immunoassay). Nevertheless, the obtained results can be erroneously increased or decreased depending on the particular hemoglobinopathy and assay method. Alternative non-hemoglobin-based methods for assessing long-term glycemic control may be useful in this case. In particular, optical methods may be suggested for estimating the level of free glucose and glycated hemoglobin in blood, since glucose and glycation of hemoglobin have influence the optical properties of blood and erythrocytes.[2-5] This study is focused on the assessment of refractive index of glucose-hemoglobin solutions at different glucose concentration using Abbe refractometer at 589 nm and OCT system at 1300 nm.

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2. MATERIALS AND METHODS

Theoretically, in depth scale of OCT images are determined by the optical path length Δz_{opt} between two points along the depth direction, which is proportional to the group refractive index n_g and geometrical path length Δz in a homogeneous medium:

$$\Delta z_{opt} = n_g \Delta z \,. \tag{1}$$

There is a small difference between phase and group refractive indices for many tissues, and the mean refractive index is the geometrical mean of the phase and group indices $n = (n_g n)^{1/2}$, i.e.

$$\Delta z_{opt} = n \Delta z \,, \tag{2}$$

whence

$$n \cong \frac{\Delta z_{opt}}{\Delta z} \,. \tag{3}$$

In our experiments Δz was equal to thickness of empty tube (De) on OCT image and Δz_{opt}=Ds (thickness of tube or dish with a sample). So, the refractive index of solution was calculated as ratio Ds/De. Geometrical thickness of the tube is 1 mm.

Two groups of glucose-hemoglobin solutions were investigated:
(1) Solutions with hemoglobin concentration of 35 g/l in all samples and glucose concentration changed from 0 to 1000 mg/dl (with a step 100 mg/dl). Time of incubation was up to 11 days.
(2) Mixtures of two simple solutions: first – aqueous 40%-glucose solution, second – aqueous solution of hemoglobin with concentration of 120 g/l.

Scheme of the OCT system was presented in Fig. 1. The superluminescent diode (λ = 1300 nm) was used as the light source. Spectral bandwidth of the superluminescent diode was 26 nm. Operation of OCT scanning was automated and controlled using PC.

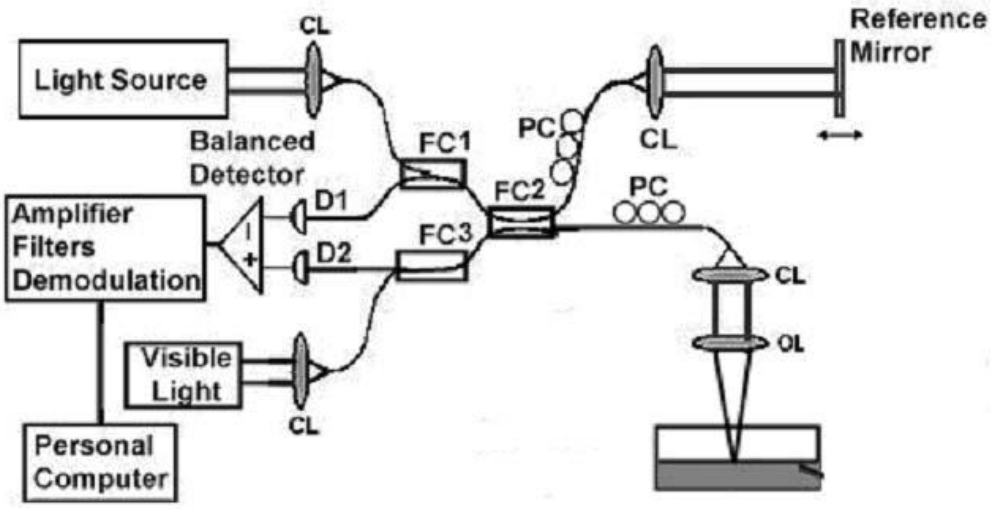

Fig. 1. Scheme of the OCT system

The axial resolution in OCT is determined only by the coherence length of the light source. The coherence length is inversely proportional to the width of the power spectrum. Spectral bandwidth of the superluminescent diode used in our experiments is 26 nm, hence the coherence length is about 30 μm. If the thickness of cuvette is 1 mm, the reproducibility of the refractive index evaluation from OCT measurements can be provided as $\delta n \approx 0.03\text{-}0.003$.

The light-emitting diode with the wavelength 660 nm was used in Abbe refractometer as a light source. Quasi-monochromatic red light irradiation provides more contrast visibility of the dark/lightened field border of Abbe refractometer, and therefore more precise and stable measurements of refractive index. Measured values of refractive index correspond to refractive index at 589 nm because of Amici prism action.

The refractive index of two non-interacting solutions can be estimated from the equation: [6]

$$n_{calc} = n_{Hb}V_{Hb} + n_{gl}V_{gl}\,, \tag{4}$$

where V_{Hb}, V_{gl} is the volume fractions of hemoglobin and glucose solutions, respectively.
The refractive index of glucose solution n_{gl} can be calculated from the equation: [7]

$$n_{gl} = n_{H_2O} + 0.1515 \cdot C_{gl}\,, \tag{5}$$

where C_{gl} is glucose concentration, g/ml.

3. RESULTS AND DISCUSSION

Figures 2, 3 and Table 1 represent experimental and theoretical values of refractive index of glucose-hemoglobin solutions.

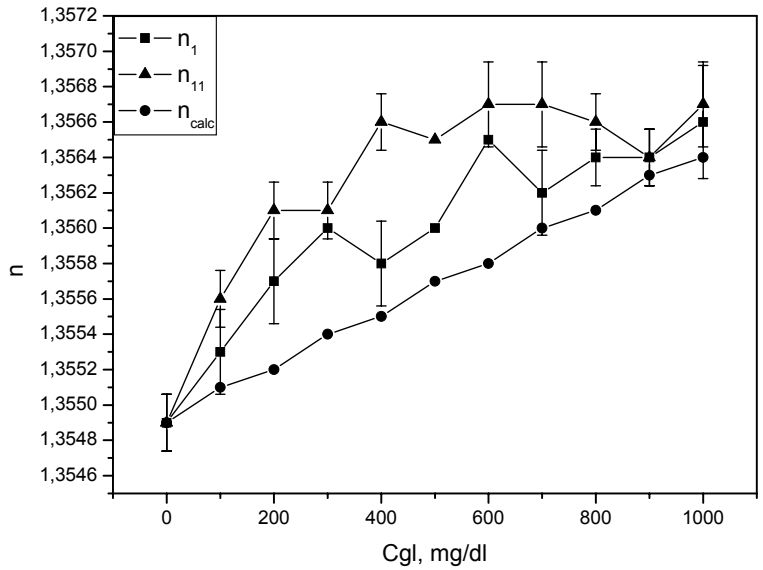

Fig. 2. The experimental (n_1, n_{11}) and calculated values (n_{calc}) of refractive index of glucose-hemoglobin solutions (group 1). The incubation time for n_1 is 1 day, for n_{11} – 11 days. The hemoglobin concentration is 35 g/l. Measurements were performed using Abbe refractometer.

Table 1. The refractive index of mixtures of hemoglobin and glucose solutions (group 2).

Hb/Glucose, % (vol/vol)	Refractive index										
100/0											1.388
90/10										1.394	
80/20									1.395		
70/30								1.391			
60/40							1.393				
50/50						1.398					
40/60					1.393						
30/70				1.391							
20/80			1.391								
10/90		1.395									
0/100	1.393										

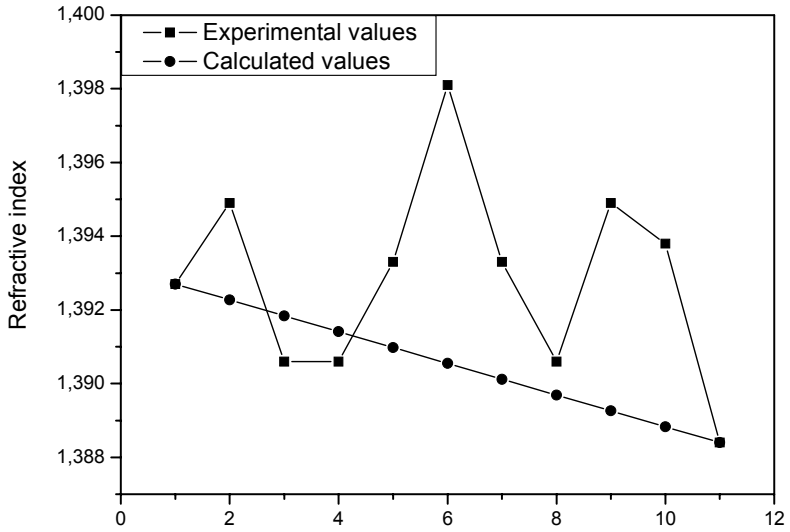

Fig. 3. The refractive index of glucose-hemoglobin solutions (group 2). 1 – 100% glucose solution; 2 – 10% hemoglobin solution + 90% glucose solution; 3 – 20% hemoglobin solution + 80% glucose solution; 4 – 30% hemoglobin solution + 70% glucose solution; 5 – 40% hemoglobin solution + 60% glucose solution; 6 – 50% hemoglobin solution + 50% glucose solution; 7 – 60% hemoglobin solution + 40% glucose solution; 8 – 70% hemoglobin solution + 30% glucose solution; 9 – 80% hemoglobin solution + 20% glucose solution; 10 – 90% hemoglobin solution + 10% glucose solution; 11 – 100% hemoglobin solution.

As it can be seen from Figs. 2, 3 and Table 1, there is a difference between the measured and calculated values of refractive index. The difference may be explained by assumption of chemical interaction between glucose and hemoglobin molecules and formation of glycated hemoglobin. The experimental data presented at Figs. 2, 3 may be explained by the classical theory of light dispersion in condense matter:[8]

$$n = 1 + \alpha N \frac{q^2}{m}, \tag{6}$$

where α is the wavelength dependent coefficient,
N is the number of molecules,
m is the molecule's mass,
q is the molecule's charge.

From Eq. (6) the refractive index of hemoglobin solution at the addition of different glucose concentrations depends on molecular weight and charge. At the increase of glucose concentration the increase of R-group charge of hemoglobin is higher than the increase of molecular weight at glucose binding, $\Delta(q^2) > \Delta m$, and the refractive index of solution increases. The saturation of refractive index n_{11} at high glucose concentrations (Fig. 2) may be connected with the charge saturation and increase of m, so refractive index saturates with the increasing of glucose level in the sample. The similar results were obtained by OCT measurements [2], where high concentrations of glucose (more than 400 mg/dl) in glucose-hemoglobin solutions caused the saturation and even decrease of refractive index values. The experiments (group 1) were performed under conditions of constant pH (about 7.9) and temperature (23.5 ± 0.3 ^{0}C), so the excess of experimental data over the calculated values can be explained due to glucose binding to hemoglobin.

Glycated proteins are formed of the slow non-enzymatic reaction between glucose and amino groups of proteins (Maillard reaction). Normal adult hemoglobin consists of four chains of amino acids (2α2β). There are different forms of hemoglobin (A0, A1, F, C, S). HbA1 consists of HbA1a, HbA1b and HbA1c. HbA1c is the major component of HbA1, about 80% of HbA1. HbA1c is formed by a non-enzymatic irreversible binding of aldehyde group of glucose with the amino-terminal valine of the β-chain of hemoglobin. This process comprises a sequence of non-enzymatic reactions (Fig. 4). The first is the rapid but reversible formation of an aldimine (or Schiff base). The next is the considerably slower formation of a stable ketoamine via a process known as the Amadori rearrangement. The ketoamine accumulates over the life of the erythrocyte and forms the main part of the glycated hemoglobin. The rate of formation of glycated hemoglobin is proportional to the free blood glucose concentration.

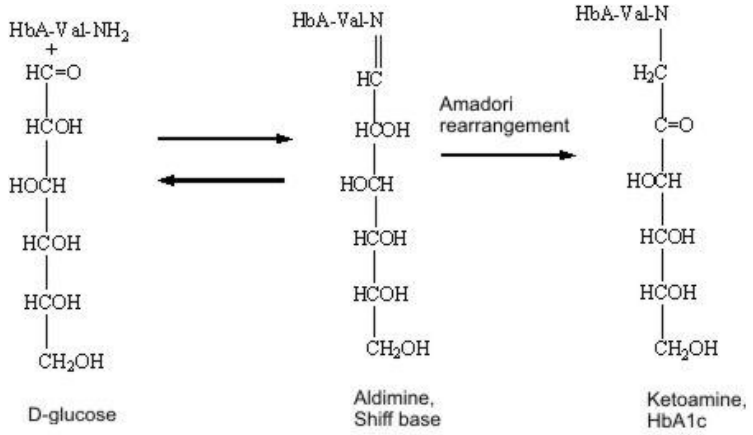

Fig. 4. Nonenzymatic formation of HbA1c from hemoglobin and glucose (L. Maillard)

Glycation of hemoglobin can occur at different points in the chains. Total glycated hemoglobin includes all hemoglobin that has reacted with glucose. In our experiments the excess of the measured values of refractive index over calculated data is due not only to HbA1c, but to all hemoglobin-glucose adducts which may appear as a result of glycation (e.g., glucose-lysine adducts and glucose α-chain NH_2-terminal valine adducts).

It is known that in a normal state less than 7% of Hb is glycated and this value rises at the increasing of free glucose concentration.[9] Rolhfing et al.[10] estimated the relationship between concentration of glucose in plasma of blood and amount of HbA1c. HbA1c measurements and corresponding values of blood glucose were obtained from 1441 patients

with type 1 of diabetes (Diabetes Control and Complications Trial data). It was found that each 1% increase of HbA1c amount corresponds to a 35-mg/dl (2 mmol/l) increase of mean plasma glucose concentration (Fig. 5).

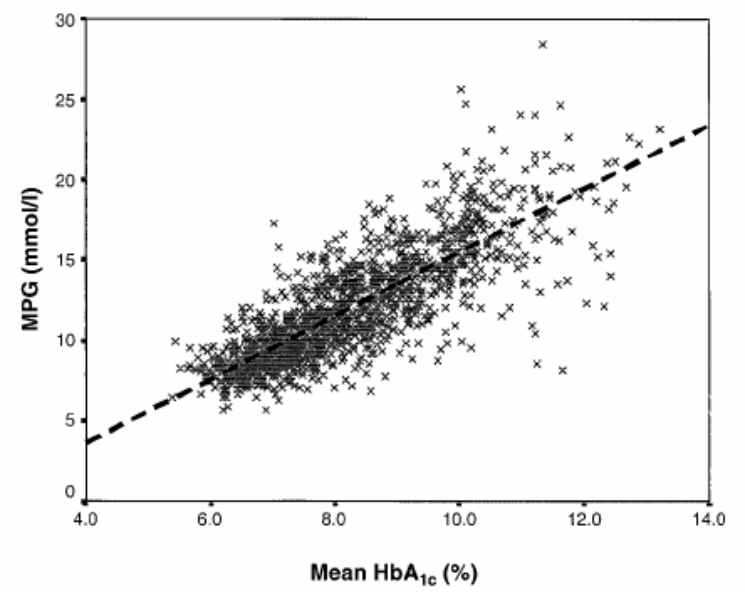

Fig. 5. Correlation between HbA1c level and mean plasma glucose concentration (MPG) [10]

There are some factors which may influence refractive index of hemoglobin solution and blood. In this study the measurements were performed considering 100% hemoglobin oxygenation, but oxygen saturation influence refractive index of hemoglobin rather strongly. Faber et al.[11] presented experimental values for the refractive index of oxygenated and deoxygenated hemoglobin. OCT-measurements showed that the absolute difference between refractive index of oxygenated and deoxygenated hemoglobin is 0.004 at 800 nm (1.392±0.001 for completely oxygenated hemoglobin and 1.388±0.002 for deoxygenated hemoglobin). Furthermore, Maksimova et al.[12] reported that high concentrations of glucose increase the hemoglobin affinity to oxygen. For example, 15-20 mM increase of glucose concentration in blood produces increase of hemoglobin affinity to oxygen up to 200%. The influence of pH on refractive index of intact erythrocytes taken from healthy and diabetic patients was investigated by Mazarevica et al.[4] It was shown that for intact erythrocytes the refractive index decreases from 1.65 to 1.55 with the increase of pH value from 2 to 7.4, and increases from 1.55 to 1.65 with the increase of pH from 7.4 to 13. At physiological pH = 7.4 refractive index values were 1.55 at norm and 1.65 for diabetics.

4. CONCLUSIONS

This study shows that the refractive index measurements can be potentially applied to the estimating of the total glycated hemoglobin amount. Changes of refractive index of glucose-hemoglobin solutions caused by glycation of hemoglobin may be observed using refractive index measurements. The difference between the measured and calculated values of refractive index may be explained by assumption of chemical interaction between glucose and hemoglobin molecules and formation of glycated hemoglobin that takes place. The method based on Abbe refractometer is inapplicable for refractive index measurements of blood due to strong blood scattering and absorption. The advantage of using OCT is the possibility of refractive index measurements of highly scattering media, such as whole blood.

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