In vitro correlation between reduced scattering coefficient and hemoglobin concentration of human blood determined by near-infrared spectroscopy

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ABSTRACT

We study the correlation between μ_s' and THC obtained *in vitro*, in a highly scattering medium containing human blood. We used a frequency domain near infrared spectrometer (modulation frequency: 110 MHz, wavelengths: 758 and 830 nm) to measure in real time (acquisition time: 0.64 s) μ_s' and THC. We used Liposyn suspension and red blood cells in saline buffer solution. After a couple of minutes of baseline acquisition, several consecutive increments of 3-5 ml blood were added to the solution yielding THC=15-100 µM and μ_a=0.03-0.3 1/cm. At the last amount of blood added, increments of glucose in the range of 0.5-20 g/L were added. For each step of blood and glucose added, data were acquired for a couple of minutes. This was repeated 6 times. Average of data was calculated for both μ_s' and THC for each of the red blood cells and glucose increments added. We found a high correlation between μ_s' and THC (0.018xTHC+4.51, R^2=0.98 at 758 nm and 0.012xTHC+4.86, R^2=0.97 at 830 nm). We studied the effect of glucose on μ_s' and we found a high correlation between the glucose added to the suspension and the decrease in μ_s' for the case of high glucose concentrations. The slope of this correlation is –0.011 at both wavelengths and the correlation factors were R^2=0.96 at 830 nm and R^2=0.91 at 758 nm (case shown). The effect of glucose was less significant at 830 nm than at 758 nm in general. This work is a proof of principle for detection of μ_s' changes with glucose. This approach also establishes limits for glucose detection in physiological conditions.

Keywords: Frequency-domain spectroscopy, near infrared, light scattering, blood, hemoglobin concentration.

1. INTRODUCTION

1.1. Diabetes Mellitus Disease

Diabetes mellitus is a disorder caused by decreased production of insulin, or by decreased ability to use insulin. Insulin is a hormone produced by the pancreas that is necessary to facilitate the blood sugar (glucose) to go from the blood to the inside of the cells to be used. The excess sugar remains in the blood and is then removed by the kidneys.

Diabetes occurs in several forms. The most two common types are type I, or insulin-dependent diabetes mellitus (IDDM); type II, or non-insulin-dependent diabetes mellitus (NIDDM). Diabetes mellitus affects almost 16 million people in the US. Blood sugar testing or self-monitoring of blood glucose is very important for people affected by this disease, and has to be done on a regular basis, several times a day. Despite the fact that this disease is very common, self-testing is still a problem being done invasively by a several painful finger lancing a day.

Thus, the continuous determination of blood glucose non-invasively is of interest for a more accurate and comfortable glucose monitoring that will allow diabetic patients to check and control their metabolism.

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1.2. Different Techniques for Glucose Monitoring

Several techniques have been proposed and tested, some which are minimally invasive and same which are non-invasive. The minimally invasive techniques are[1-4]:

- *implantable subcutaneous glucose sensors* (enzyme electrodes and fluorescence based)
- *subcutaneous interstitial fluid sampling by microdialysis or open-tissue microperfusion* (microdialysis and reverse iontophoresis)
- *transdermal glucose monitoring*

These minimally invasive techniques are based on the analysis of the interstitial fluid, which involved insertion of certain elements into the tissue. This gives rise to problems such as lack of biocompatibility or drifts in the signals at long periods of time, which implies loss of sensitivity and stability.

Recently, new non-invasive techniques are developed, such as optical glucose sensors. There are many approaches for the optical glucose sensors, and some of them are briefly described below:[2-3]

- *mid or near infrared spectroscopy* (involves measurements of absorption, diffuse-reflectance or frequency-domain reflectance)
- *light scattering*
- *photo-acoustic spectroscopy* (involves measurement of the photoacoustic signal and thermal properties)
- *optical activity and polarimetry* (involves measurement of change in the rotated angle of the polarization with glucose)
- *Raman scattering* (involves measurement of Raman shift, O-H and C-H fundamental vibrations with change in glucose concentration)

It is well known that near infrared light penetrates deep into the tissue allowing for glucose monitoring non-invasively. Light propagation through tissues can be expressed by optical parameters such as absorption coefficient (μ_a), reduced scattering coefficient (μ_s'), which is dependent on the density, diameter of the scatter, and the ratio between refractive index of the scatters and of the medium (interstitial fluid). By changing the glucose concentration in tissue there will be a change in the μ_a of the tissue through changes in absorption corresponding to water displacement or changes in the intrinsic absorption due to temperature or hydration changes. The glucose absorption is low in the near infrared region and is much smaller than that of water. The glucose absorption is high at longer wavelengths. Changes in glucose concentration will affect the intensity of the scattered light.

Kohl *et al*.[5] theoretically calculated the influence of the glucose concentration upon optical properties such as absorption, scattering, refractive index of a multi-scattering artificial medium, in the near infrared domain. They found that the effect is very small and this might be a challenge for *in vivo* clinical studies. It was reported[5-6,8] decreasing μ_s' values with increasing concentrations of glucose, in the range of 0.05-6%/mM.

Many non-invasive studies involved *in vitro* evaluation of glucose effect in samples such as whole blood[7,8], plasma[7,8], aqueous solutions[6,8], serum-like matrices or tissue-simulating phantoms[5]. Several studies using different non-invasive techniques were performed also *in vivo*, on non-diabetic subjects or subjects affected by diabetes. Data were collected from different locations of the body such as oral mucosa[6], blood[7], human lip[9], fingertip[10] or skin surface[2,8,11].

All the attempts to develop non-invasive techniques to measure glucose, which were briefly described, showed some correlation between blood glucose and optical signal even though none of the results provided a clear correlation, or sensitive enough for physiological range of glucose in blood. Nevertheless, from theoretical considerations and in vitro experiments of the effect of glucose on the scattering coefficient indicate that this is a predominant factor for glucose related variations[1-4].

In our present study, we used near infrared frequency-domain technique for in vitro determination of the effect of glucose on the correlation between reduced scattering coefficient and total hemoglobin concentration. We have previously reported a correlation between increase in reduced scattering coefficient and increase in total hemoglobin concentration[12]. The novelty of the present work resides in our results for the effect of glucose on the correlation coefficient between the reduced scattering coefficient and total hemoglobin concentration. Since it is possible to change the relative amount of total hemoglobin concentration in tissues non-invasively, for example, using venous occlusion protocol, we investigate this correlation to assess the sensitivity of this kind of approach.

2. EXPERIMENTAL AND THEORETICAL METHOD

2.1. Near Infrared Frequency-Domain Technique

Near infrared (NIR) frequency-domain spectroscopy permits one to determine the absorption and scattering coefficients of a strongly scattering medium such as tissue. Numerous studies used the near infrared tissue spectroscopy method for determination of the absorption and reduced scattering coefficients in order to determine different physiologic states of the tissues.

Near infrared frequency-domain techniques are based on sinusoidally modulated intensity of the light source and on a phase detection system to measure the amplitude of the intensity oscillations (AC component), the average intensity (DC component) and the phase (Φ) of the detected light.[14,15] Measuring these quantities (AC, DC and Φ) at different source-detector separations, the optical coefficients (absorption, μ_a, and reduced scattering, μ_s') are determined (for a semi-infinite geometry using either DC and phase or AC and phase)[13]. We used a near infrared frequency-domain oximeter (Model 96208, ISS, Inc., Champaign, IL) with modulation frequency of 110 MHz, operating at two wavelengths (4 laser diodes at 758 nm and 4 laser diodes at 830 nm). The measurements were performed in real time (acquisition time: 0.64 s).

The equations for μ_a and μ_s' have been previously derived from the slopes (S) of the functions: $f(r, DC, \mu_a, \mu_s')$ versus r and $h(r, \Phi, \mu_a, \mu_s')$ versus r.[15] Using an iterative process, the unknown optical coefficients are found.

$$\mu_a = -\frac{\omega}{2\upsilon}\frac{S_{DC}}{S_\Phi}\left(\frac{S_\Phi^2}{S_{DC}^2}+1\right)^{-1/2} \tag{1}$$

$$\mu_s' = \frac{S_{DC}^2}{3\mu_a} - \mu_a \tag{2}$$

The two wavelengths (758 nm and 830 nm) are necessary to determine the deoxy-hemoglobin ([Hb]) and the oxy-hemoglobin concentrations ([HbO_2]).[16]

$$[HbO_2] = \frac{\mu_a^*(\lambda_1)\cdot\varepsilon_{Hb}(\lambda_2) - \mu_a^*(\lambda_2)\cdot\varepsilon_{Hb}(\lambda_1)}{\varepsilon_{HbO_2}(\lambda_1)\cdot\varepsilon_{Hb}(\lambda_2) - \varepsilon_{HbO_2}(\lambda_2)\cdot\varepsilon_{Hb}(\lambda_1)} \tag{3}$$

$$[Hb] = \frac{\mu_a^*(\lambda_2)\cdot\varepsilon_{HbO_2}(\lambda_1) - \mu_a^*(\lambda_1)\cdot\varepsilon_{HbO_2}(\lambda_2)}{\varepsilon_{HbO_2}(\lambda_1)\cdot\varepsilon_{Hb}(\lambda_2) - \varepsilon_{HbO_2}(\lambda_2)\cdot\varepsilon_{Hb}(\lambda_1)} \tag{4}$$

where $\mu_a^*(\lambda) = \mu_a(\lambda) - 0.7\mu_a^{H_2O}(\lambda)$ indicates the water-corrected absorption coefficient, while $\varepsilon_{Hb}(\lambda)$ and $\varepsilon_{HbO_2}(\lambda)$ are the molar extinction coefficients of deoxy- and oxy- hemoglobin, respectively. Total hemoglobin concentration ([THC]) is the sum of the deoxy-hemoglobin and the oxy-hemoglobin concentrations.

$$[THC] = [Hb] + [HbO_2] \tag{5}$$

The optical probe was placed on the surface of the suspension, in contact with the suspension. The experimental set-up is shown in Fig. 1.

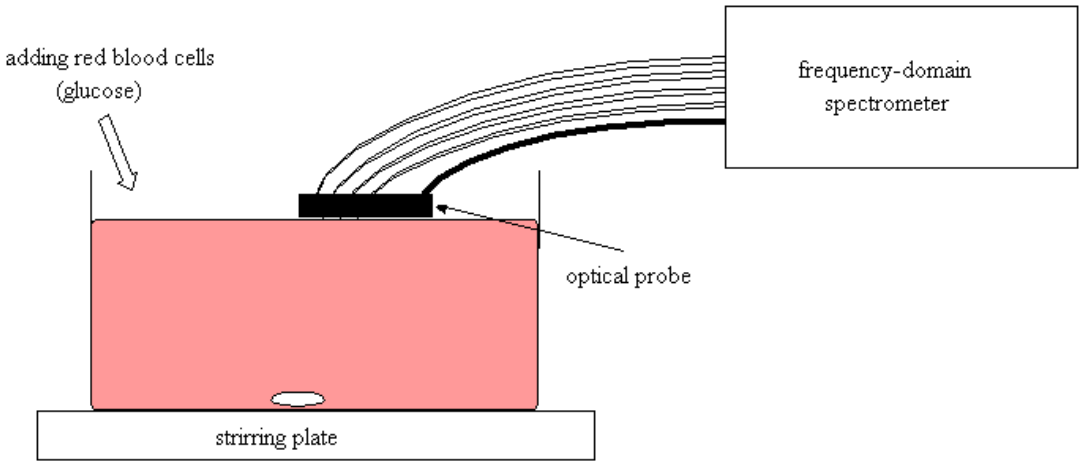

Fig. 1. Experimental set-up.

2.2. Experimental Procedures

2.2.1. Sample preparation

Following institutional review board approval, we measured turbid media of intralipid and human red blood cells. A specialized collection laboratory provided the red blood cells units. The red blood cells units that were used were expired units with a concentration of hemoglobin of 20 mg/dl. The turbid suspension (1 L) was made out of Liposyn 20% (30 mL) in a buffer solution with normal physiologic pH-value of 7.4. The buffer solution was an aqueous solution of sodium phosphate dibasic (13 g/L) and hydrochloric acid. To prevent red blood cells from lyses 90% NaCl was added to the solution. The concentration of 0.5 % solid content of Liposyn 20% was chosen to yield a reduced scattering coefficient of about 5 1/cm in the near-infrared region (typical value for in vivo reduced scattering coefficient of skeletal muscle). The suspension was placed in a cylindrical container with a total volume of 2 L. Several consecutive increments of 3-5 ml red blood cells were added to the solution yielding a hemoglobin concentration in the range of 15-100 μM. The blood concentration was chosen to yield absorption coefficients in the range of 0.03-0.3 1/cm. The measurements were done in 100% hemoglobin saturation conditions. The suspension was gently stirred (5cmx1cm stirrer) to prevent the red blood cells and the Liposyn particles from settling down. A light heat was also applied to the suspension during the measurements in order to keep normal physiological temperature of the red blood cells. We repeated the same measurements with red blood cells from 3 different units. At the last quantity of red blood cells added, increments of glucose in the range of 0.5-20 g/L were added to the suspension.

2.2.2. Determination of optical coefficients of the solution

Experimental data were continuous acquired using the near infrared frequency-domain spectrometer shown in Fig. 1. The suspension was continuously stirred gently and lightly heated. A baseline value was acquired for the Liposyn suspension for few minutes. Consecutive increments of 3-5 ml red blood cells were added and the data were continuously taken. For each amount of the red blood cells that were added few minutes of data were measured. At the last quantity of red blood cells added, increments of glucose in the range of 0.5-20 g/L were added to the suspension. For each of the glucose increments that were added few minutes of data were acquired.

The AC, DC, and Φ data were sent from the instrument to a computer, which calculates the optical coefficients (μ_a and μ_s') according to the equations (1) and (2). Total hemoglobin concentration in the suspension was also calculated.

We analyzed the correlation between the reduced scattering coefficient and the total hemoglobin concentration in the suspension. We showed previously (data not shown here) that there is a linear relation between the reduced scattering coefficient and the total hemoglobin concentration from data acquired *in vivo* on human skeletal muscle during venous occlusion protocol. We calculated an average of the few minutes of data acquisition for both reduced scattering coefficient and total hemoglobin concentration for each of the 10-12 increments of 3-5 ml of red blood cells that were added consecutively to the Liposyn suspension.

We studied the effect of the glucose on the relationship between the reduced scattering coefficient and the total hemoglobin concentration in the suspension. It is known[3,5,6] that glucose changes the index of refraction of the suspension, thus changing the reduced scattering coefficient. We calculated an average of the reduced scattering coefficient and the average of the total hemoglobin concentration for each of the 4-5 glucose increments in the range of 0.5-20 g/L that were added at a specific amount of red blood cells in the suspension. The average was done for each quantity of glucose for a period of few minutes of continuous data acquisition. The amount of red blood cells in the suspension was kept constant for the addition of glucose.

For error evaluation, we considered the contribution due to the measurement noise effecting the determination of the μ_s' and THC calculated as the standard deviation of the values for each period of acquisition time.

3. RESULTS AND DISCUSSION

First, we determined the *in vitro* correlation between reduced scattering coefficient and the total hemoglobin concentration measured by the near infrared spectrometer, during the consecutive addition of scatters, red blood cells. An example of the correlation of the μ_s' and THC is presented in Fig. 2. The first points for each wavelength are the values for Liposyn suspension.

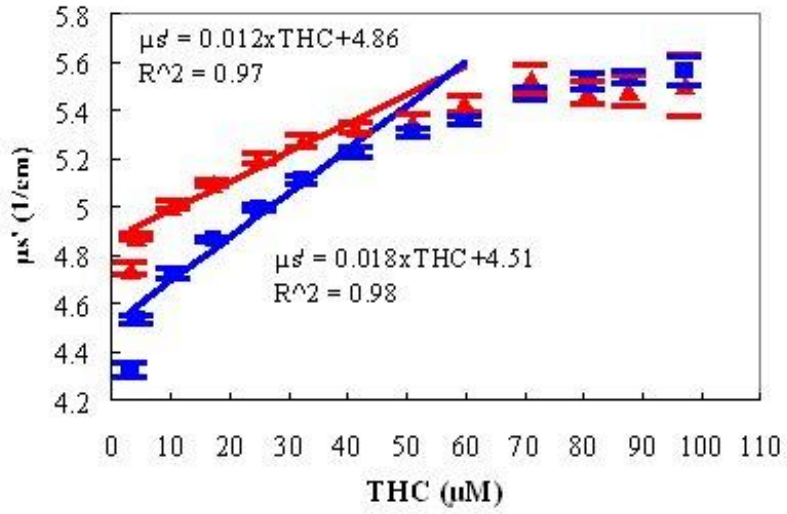

Fig.2. Correlation between μ_s' and THC at 758 nm (square) and at 830 nm (triangle).
The lines represent the results of the linear regressions at each wavelength.

This correlation was calculated up to a THC of 55 μM. We found a high correlation between the reduced scattering coefficient and the total hemoglobin concentration, similar at both wavelengths (758 and 830 nm). The regression equations are at 830 nm: 0.012xTHC+4.86 with R^2=0.97, and at 758 nm: 0.018xTHC+4.51 with R^2=0.98.

The correlation was verified 6 times. The slope values (ranging from 0.01 to 0.02) and the correlation factors (in the range 0.91-0.99) for all 6 cases. These values correspond to our previous correlation (0.02xTHC-0.02, with R^2=0.96) obtained *in vivo* on 3 healthy subjects[10].

For large amounts of red blood cells added to the suspension (total hemoglobin concentration above 55 μM) we observed no linear correlation. We noted a plateau of the relationship μ_s' versus THC that might be due to approaching of the limiting value when the intrinsic scattering of the red blood cells matches the intrinsic scattering of the Liposyn particles.

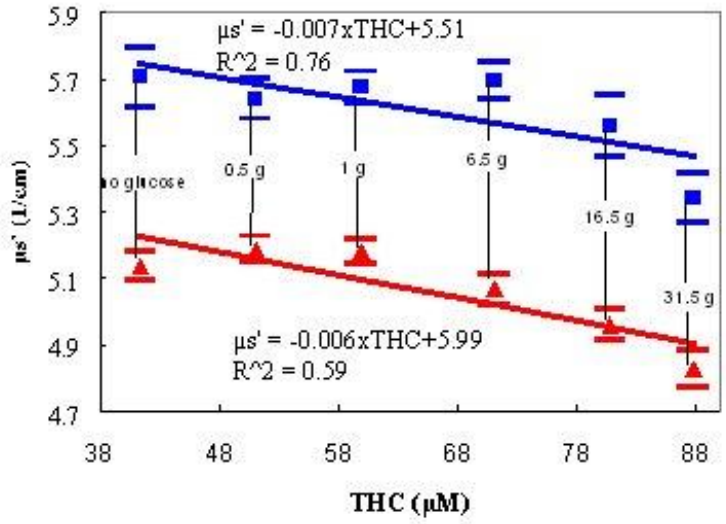

Fig.3. Reduced scattering coefficient versus total hemoglobin concentration measured by NIRS at different concentration of glucose at 758 nm (square) and at 830 nm (triangle). The lines represent the results of the linear regressions.

Figure 3 represents the effect of different glucose concentrations on the relationship of the reduced scattering coefficient and the total hemoglobin concentration at both wavelengths (758 and 830 nm). The amount of hemoglobin in the suspension was 40 μM. At this concentration we added different amounts of glucose in the suspension. The regression equations are at 830 nm: -0.006xTHC+5.99 with R^2=0.59, and at 758 nm: -0.007xTHC+5.51 with R^2=0.76.

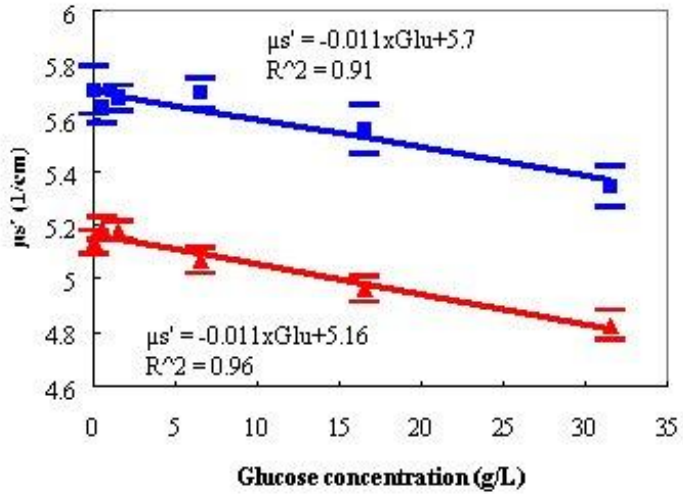

Fig.4. The effect of glucose on the reduced scattering coefficient at THC=55 μM, at 758 nm (square) and at 830 nm (triangle). The lines represent the results of the linear regressions.

Figure 4 represents effect of glucose upon reduced scattering coefficient considering that the amount of hemoglobin in the suspension is constant at 55 μM. Figure 4 shows a decrease in the μs' with the increase of the glucose concentration. We found a high correlation between the amount of glucose added to the suspension and the decrease in the reduced scattering coefficient. The slope of this correlation is –0.011 at both wavelengths and the correlation factor is R^2=0.96 at 830 nm and R^2=0.91 at 758 nm.

Similar results on the effect of glucose were reproduced in 3 other samples. In general, the effect of glucose is less significant at 830 nm than at 758 nm.

4. CONCLUSION

We found that the reduced scattering coefficient decreases with the increase of the glucose concentration of the suspension of Liposyn and red blood cells. The decrease in the reduced scattering coefficient with respect to the glucose concentration was 0.01 (cm^{-1}) / (g/L). We found a high correlation (R^2=0.91-0.96) between the glucose concentration and the reduced scattering coefficient. In most of the cases, this correlation was significant at 758 nm and less significant at 830 nm. Also, this correlation was found at high concentrations of glucose.

This is a proof of principle that the near infrared frequency-domain spectroscopy can detect changes in the scattering coefficient with glucose due to changes in the total hemoglobin concentration. The changes are on the order of 1% per g/L of glucose. For this approach to work, we need to measure changes in total hemoglobin concentration and reduced scattering coefficient with a relative precision of better than one percent. Note that using this approach we need to improve on the relative values and not on the absolute values like in previous approaches.

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