

Influence of glucose concentration on light scattering in tissue-simulating phantoms

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The presence of glucose dissolved in an aqueous solution increases the refractive index of the solution and therefore has an influence on the scattering properties of any particles suspended within it. We present experimental data on the effect of glucose concentration on the scattering coefficient of a suspension of spherical polystyrene particles. The experimental results are in good agreement with Mie theory. The effect of glucose on light transport in highly scattering, tissue-simulating phantoms is demonstrated both experimentally and theoretically by application of diffusion theory. The possible application of this effect for noninvasive glucose monitoring of diabetic patients is discussed.

A significant percentage of the population (approximately 7% in the United States¹) is diabetic, and a considerable fraction of diabetic people measure their blood glucose concentration several times a day. The results from a recently completed study² show that the long-term effects of diabetes can be significantly reduced by frequent monitoring and tight control of blood glucose levels. This is particularly true for type I diabetic patients, who depend on exogenous insulin. Blood glucose monitoring is most commonly done by pricking the fingertip with a needle and placing a drop of blood into a test strip that undergoes a color change dependent on the blood plasma glucose concentration (typically 4–7 mM for healthy persons). The development of a noninvasive glucose system for diabetic persons has been the goal of many investigators for several years. Techniques suggested to date have included implants based on electrochemical sensors³ and noninvasive spectroscopic techniques based on chemometrics.^{4,5}

Here we follow a proposed noninvasive optical method⁶ that relies on the refractive-index change caused by dissolving glucose in an aqueous medium.⁷ The change in refractive index in body fluids will in turn cause a change in the overall scattering coefficient of the body tissues that could be detected by various optical measurements. The magnitude of the effect caused by changes in the scattering coefficient on optical measurements can be predicted by diffusion theory,⁸ which can describe the transport of light in highly scattering media.

Monitoring glucose concentration by the scattering coefficient (μ_s) rather than by the absorption coefficient (μ_a) has several potential advantages. Tissue μ_s is broadly wavelength independent, and hence its effect is evident at near-infrared wavelengths in addition to midinfrared and far-infrared wavelengths, where glucose has significant absorption bands but light penetration into tissue is poor. Additionally,

optical sources and detectors in the 650–1050-nm wavelength region have relatively high output intensities and low noise-equivalent powers, respectively, compared with those at other wavelengths.

In this Letter we present theoretical and experimental evidence of the glucose-dependent scattering effect with suspensions of polystyrene microspheres in both single- and multiple-scattering samples. The aim is to estimate the magnitude of the effect and hence discuss its potential application for continuous noninvasive monitoring of blood glucose.

The single scattering coefficient of a cuvette containing a dilute aqueous suspension of microspheres was measured as a function of wavelength with the attenuation of a highly collimated light beam. Details of the equipment, consisting of a white-light source in combination with a CCD spectrophotometer, have been given elsewhere.⁹ The apparatus directly measures the total attenuation coefficient μ_t , which is the sum of μ_s and μ_a and can be calculated according to

$$\mu_t(\lambda) = \mu_a(\lambda) + \mu_s(\lambda) = 1/z \ln[I_0(\lambda)/I(\lambda)], \quad (1)$$

where z is the physical path length of the cuvette and I_0 and I are the transmitted light intensities of the reference solution (normally pure water) and of the solution under investigation, respectively. One then calculates μ_s by subtracting changes in μ_a from the measured μ_t . Spectra of the total attenuation coefficient μ_t of a suspension of polystyrene microspheres (Seradyn, Indianapolis, Ind.) with a diameter $d = 1.27 \pm 0.09 \mu\text{m}$ at a concentration of $c_s = 0.0019\%$ vol./vol. have been determined for different glucose concentrations c_g . The spectra of the fractional change of the scattering coefficient caused by glucose $\delta_g \mu_s(c_g) = 2[\mu_s(c_g) - \mu_s(c_g = 0 \text{ mM})] / [\mu_s(c_g) + \mu_s(c_g = 0 \text{ mM})]$ have been derived after subtraction of the small change in absorption

coefficient and are shown in Fig. 1 for glucose concentrations of 85 and 144 mM.

The theoretical scattering properties of the microspheres, i.e., μ_s and the angular distribution and its g value, can be calculated from Mie theory for a diameter d , the wavelength λ of the light, and the refractive indices of both the spheres n_s and the medium n_m . Here the algorithm given by Bohren and Huffman¹⁰ was applied. To include the wavelength dependence of the refractive indices, we extrapolated data from the literature for the refractive index of water and polystyrene at visible wavelengths into the near-infrared region, using a Cauchy fit.⁷ The refractive-index increment of an aqueous glucose solution Δn_m for visible wavelengths is $\Delta n_m = 2.5 \times 10^{-5}/\text{mM}$ glucose,⁷ and this relationship was assumed to be correct over the whole wavelength region under investigation.

In Fig. 1 the experimental data of $\delta_g \mu_s$ can be compared with predictions from Mie theory. The accurate interpretation of the experimental spectra for wavelengths $\lambda > 900$ nm is aggravated by a high noise level caused by the low responsivity of the CCD spectrophotometer at these wavelengths. Nevertheless, the overall agreement between experimental and theoretical $\delta_g \mu_s$ is good. $\delta_g \mu_s$ was found to be a linear function of glucose concentration over the range used. The magnitude of $\delta_g \mu_s$ increases with wavelength and has values of $-0.012\%/ \text{mM}$ at 700 nm and $-0.016\%/ \text{mM}$ at 955 nm.

The angular dependence of the scattered light intensity, i.e., the phase function, has been measured with a goniometer⁹ for the same batch of polystyrene particles. Two glucose concentrations, 0 and 1700 mM, were used to produce a measurable effect. The change of the phase function with glucose concentration was found to be in agreement with Mie theory. This permitted the use of calculated values for the mean cosine of the phase function, i.e., the g value, in further experiments. For $d = 1.27 \mu\text{m}$ and $\lambda = 700$ nm the g value is $g(c_g = 0 \text{ mM}) = 0.9282$ with a change in g of $\Delta g = 8.07 \times 10^{-6}/\text{mM}$ glucose.

Following the investigation of the influence of glucose concentration on the single-scattering properties, we performed experiments to measure the transmitted intensity and its dependence on glucose concentration in highly scattering, tissue-simulating phantoms. We used a sample of polystyrene microspheres¹¹ with a broad but specified size distribution between 4 and 7 μm . A Mie theory calculation of the average μ_s of the sample as a function of wavelength agreed with our own single-scattering measurements. For multiple-scattering media, the reduced scattering coefficient [$\mu'_s = \mu_s(1 - g)$] characterizes light transport within a medium. Here a microsphere concentration of 0.97% vol./vol. suspended in water was used that gave a μ'_s of 0.825 mm^{-1} at 700 nm and 0.870 mm^{-1} at 1000 nm. The calculated fractional change $\delta_g \mu'_s$ per millimolar change of c_g was -0.011% at 700 nm, and its magnitude increased monotonically with wavelength to -0.020% at 1000 nm. The absorption coefficient of the tissue phantom was purely that of water and the effect of added glucose. The suspension was

placed in a rectangular cuvette (60 mm \times 80 mm \times 20 mm) to simulate a 20-mm-thick slab of tissue. Light transmission across the sample was measured with the same CCD spectrophotometer used for the single-scattering experiments but connected by optical fiber bundles. Light-transmission spectra were measured as a function of glucose concentration. Figure 2 (dotted curve) shows the ratio of two such transmission spectra at concentrations of 200 and 0 mM. The general features of the spectrum closely match those of water absorption. Diffusion theory was used to compare the predicted contribution of the glucose-dependent changes in scattering and absorption coefficients ($\delta_g \mu'_s$ and $\delta \mu_a$) with the experimental data by the known μ'_s and μ_a of the phantom. The transmitted intensity of a point source in slab geometry was calculated with formulas given by Arridge *et al.*⁸ The different refractive indices at the boundaries among the

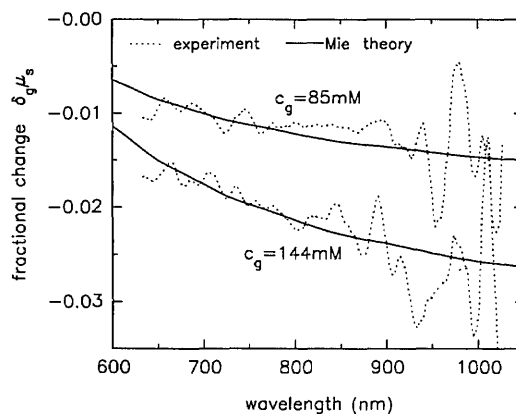


Fig. 1. Fractional change of the scattering coefficient $\delta_g \mu'_s(c_g) = 2[\mu'_s(c_g) - \mu'_s(c_g = 0 \text{ mM})]/[\mu'_s(c_g) + \mu'_s(c_g = 0 \text{ mM})]$ of an aqueous suspension ($c_g = 0.0019\%$ vol./vol.) of polystyrene spherical particles (diameter 1.27 μm) for glucose concentrations of 85 and 144 mM. The solid curves depict $\delta_g \mu'_s$ calculated from Mie theory for a change in the refractive index of the solution corresponding to the glucose concentrations used.

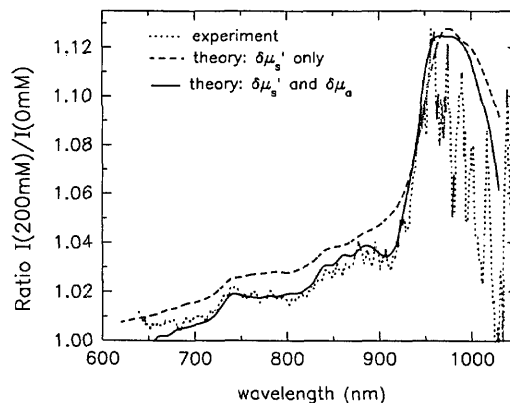


Fig. 2. Ratio of the transmitted intensities for zero glucose concentration and $c_g = 200$ mM through an aqueous suspension of polystyrene spherical particles ($4 \mu\text{m} < d < 7 \mu\text{m}$, $c_s = 0.968\%$ vol./vol.) of 20-mm path length. The experimental data (dotted curve) are compared with diffusion theory that takes into account the change $\delta_g \mu'_s$ (dashed curve) and the changes of both the scattering and the absorption coefficients (solid curve).

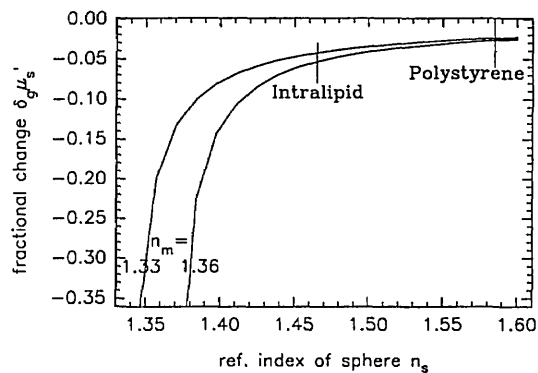


Fig. 3. Fractional change $\delta_g \mu'_s$ as function of the refractive index of the sphere n_s calculated from Mie theory for spherical particles of diameter $d = 1 \mu\text{m}$ and $\lambda = 700 \text{ nm}$ and a change in the refractive index of the medium of $\Delta n_m = 0.0025$ corresponding to $c_g = 100 \text{ mM}$ glucose concentration. n_m was assumed to be either 1.33 or 1.36.

solution, the cuvette, and air resulted in mismatched boundary conditions that we accounted for by additionally incorporating the method of extrapolated boundaries.¹² The diffusion theory results are also shown in Fig. 2, where the solid curve shows the combined effect of glucose-dependent changes $\delta_g \mu'_s$ and $\delta \mu_a$, whereas the dashed curve shows the effect of $\delta_g \mu'_s$ alone. Most of the observed intensity increase can be explained by $\delta_g \mu'_s$ alone. Its effect on the light intensity spectrum is to reflect the μ_a of the medium. Had another absorber, e.g., hemoglobin, been present in the phantom, the intensity increase would have been larger and would also have reflected its absorption spectrum. The increase in glucose concentration to 200 mM reduces the concentration of water and hence its absorption coefficient by 2.2%.⁷ Incorporating the effect of the change in absorption caused by adding glucose results in the solid curve of Fig. 2, which now very closely matches the experimental data.

The changes of the scattering properties with glucose concentration discussed above are small. To be clinically acceptable any instrumentation would need to be able to detect a change in blood glucose of approximately 1 mM. Such instrumentation would have to be very precise to detect the magnitude of $\delta_g \mu'_s$ described above and additionally be insensitive to much larger absorption coefficient changes resulting from hemoglobin. To estimate the likely $\delta_g \mu'_s$ in tissue, one must take into account different refractive indices. One distinct possibility is that the scattering change in tissue may be much greater than that found with polystyrene microspheres. $\delta_g \mu'_s$ has been calculated for spherical particles of diameter 1 μm , $\lambda = 700 \text{ nm}$, and $\Delta n_m = 0.0025$, corresponding to $c_g = 100 \text{ mM}$. Figure 3 shows $\delta_g \mu'_s$ versus the refractive index of the sphere n_s and a refractive index for the medium of $n_m = 1.33$ or $n_m = 1.36$. For polystyrene ($n_s \approx 1.58$) the change is approximately $\delta_g \mu'_s = -0.02\% \text{ mM}^{-1}$. Any decrease of n_s increases the magnitude of $\delta_g \mu'_s$. This effect has been confirmed experimentally for Intralipid (a soybean oil suspension, $n_s = 1.465$). This finding makes it difficult to predict the likely change in μ'_s caused by glucose in bi-

ological tissues. First, tissue scattering is caused by a variety of substances and organelles (membranes, mitochondria, nucleus, etc.) that all have different refractive indices varying between values near that of water (extracellular fluid, $n \approx 1.335$; intracellular fluid, $n \approx 1.354$) and that of protein ($n \approx 1.50$).¹³ For a sphere of the refractive index of intracellular fluid surrounded by extracellular fluid a change of $\delta_g \mu'_s \approx -0.3\%/\text{mM}$ can be predicted. Second, the effect of blood glucose concentration and its distribution at the cellular level is a complex issue. To clarify these topics further investigation is required. An instrument suitable for measuring glucose concentration based on scattering coefficient would need to separate μ_a and μ'_s with negligible interference. Preliminary results suggest that an intensity-modulated spectrophotometer is capable of detecting changes in μ'_s correlated with blood glucose in human muscle.¹⁴ Such instrumentation is likely to require *in vivo* calibration against an invasive blood glucose measurement, as the absolute tissue μ'_s is dependent on such additional factors as cell density.

There are many problems to overcome. Factors that might change tissue μ'_s are variations in temperature, red-blood-cell concentration, electrolyte levels, and movements of intracellular/extracellular water. All these factors are potentially the most difficult to separate.

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