MONTE CARLO MODELING STUDIES OF THE EFFECT OF PHYSIOLOGICAL FACTORS AND OTHER ANALYTES ON THE DETERMINATION OF GLUCOSE CONCENTRATION *IN VIVO* BY NEAR INFRARED OPTICAL ABSORPTION AND SCATTERING MEASUREMENTS

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ABSTRACT

Determining the concentration of glucose *in vivo* by near-infrared spectroscopy is complicated by the effects of optical changes caused by fluctuations in temperature, tissue water content, and the concentration of other analytes. Mie theory and Monte Carlo computer simulation of light transport in optically absorbing and scattering media were used to investigate the magnitude of the changes in diffuse reflectance and transmittance from changes in glucose. Similarly, the possible interference in the glucose measurement from the other tissue parameters has been assessed and found to be significant. © *1997 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(97)00603-5]

Keywords tissue optics; noninvasive glucose measurements; Monte Carlo modeling.

1 INTRODUCTION

The present method by which many diabetics control their blood glucose levels is by a finger prick several times a day to obtain a drop of blood, in which the glucose is then determined by an analytical chemical reaction. This invasive procedure limits the frequency of monitoring and so may give inadequate control of the long-term complications of the disease. Thus, a continuous, noninvasive method for monitoring body glucose levels would be of great advantage.

Many different techniques have been proposed for this purpose, such as implanted electrochemical sensors or optical methods.[1–3] In particular, some possible approaches are based on the effect of glucose on light transport in tissue. Until recently, most attention has been focused on absorption spectroscopy, exploiting the fact that glucose has an identifiable spectral signature in the near-infrared (IR). However, the absorption changes are very small at the glucose concentrations of interest, so that challenging problems remain in extracting accurate quantitative information from the tissue absorption spectra. More recently, it has been reported[4] that glucose also changes the optical scattering properties of tissues, either the scattering coefficient, μ_s, and/or the scattering anisotropy, g. The scattering of light in tissue is due to the differences in refractive index between the intra/extracellular fluid and the cellular components (e.g., cell membranes, nuclei, mitochondria). Since the refractive index of glucose solution is higher than that of water,[5] the major compartment of normal tissue fluid, increases in glucose concentration reduce the refractive index mismatches in the tissue and so reduce μ_s and increase g. These changes can be detected by measuring, for example, the diffusely reflected or diffusely transmitted light signal from tissue. Typically, the changes in these param-

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eters, at least in simplified model systems, are on the order of −0.02% change in μ_s and + 0.0007% change in g per millimolar change in glucose concentration, resulting in a −0.03% change in the transport scattering coefficient, $\mu_s' = \mu_s(1 - g)$.

A potentially important factor in applying these optical spectroscopy techniques is the interference in glucose detection due to fluctuations of other factors, such as tissue water content, temperature, and the concentrations of other analytes. In this work, the changes in light transport caused by varying glucose concentration were modeled using Monte Carlo simulation,[6] in which the absorption coefficient, μ_a, and transport scattering coefficient, μ_s', were varied. The dependence of μ_s' on physiological parameters was estimated using Mie theory[7] with varying refractive index mismatches between the cellular and extracellular components.

2 MATERIALS AND METHODS

2.1 EFFECT OF GLUCOSE ON LIGHT TRANSPORT

As a model, we have used a suspension of spherical scattering particles in an aqueous glucose solution. The glucose concentration was set in the range 0 to 30 mM, which covers normal physiological levels and levels for hyperglycemia (1.5 to 30 mM). The effect of glucose on the optical properties was considered by two different mechanisms. First, the molarity of the fluid compartment (here water) was reduced by glucose, due to volume displacement. Second, the intrinsic absorption and refractive index of the glucose in solution was added to those of molarity-reduced water.

A micropipet was used to measure the volume of water solutions before and after adding known concentrations of pure glucose. The coefficient of water volume displaced by aqueous glucose was then determined and found to be 0.0112 (± 0.0001)% mM^{-1} for glucose concentrations from 200 to 900 mM, which is consistent with previously published results[5] of 0.0111% mM^{-1}. The intrinsic absorption coefficient of glucose in water has been measured by Kohl et al.[8] and is 1 to 2 orders of magnitude smaller than that of water in the near IR (700 to 1100 nm). Hence, at physiological concentrations, the difference in absorption between aqueous glucose solution and pure water is mainly determined by the water displacement factor.

Several groups have reported experimental measurements of the effects of glucose on optical properties and light transport in phantoms.[4,8,9] To date, the glucose concentrations used experimentally have typically been much higher than physiological levels, due to the small changes in optical signals caused by variations in tissue glucose concentration. In order to examine the influence of other factors on the glucose signals at physiological levels and to be independent of particular instrumenta-

tion, we have chosen to use Monte Carlo simulations to calculate the diffusion reflectance (R) and transmittance (T) signals from tissue-like media.

In simulating tissue, a suspension of spherical polystyrene particles (1 μm diameter) was chosen as the scatterer at a concentration of 1% (v/v). Mie theory was used to calculate μ_s and g at the wavelengths of interest.[7,8] For this calculation, the refractive indices of aqueous glucose solution and of the scattering particles are required. An approximate value for the latter has been published.[8,10] (In principle, it would have been preferable to do the calculations for lipoprotein suspensions, such as Intralipid, which have been used widely to simulate tissue optical properties,[11] since the particle refractive index of 1.4 to 1.5 is closer to that of cellular structures. However, the irregular shape and size of the scattering particles make calculation of the optical properties much less accurate than for the polystyrene microspheres.) The near-IR refractive index of water has been reported by Schiebener et al.[12] We measured the increase of the refractive index of aqueous glucose solution as a function of glucose concentration integrated over the visible range with a refractometer: the value of 2.52 ($\pm$0.02)$\times 10^{-5}$ mM^{-1} is close to the published value[5] of 2.5×10^{-5} mM^{-1}.

The simulations were performed for an infinite-slab geometry with 5 mm physical thickness and a collimated incident beam. The total diffuse reflectance and transmittance were calculated at 800 and 960 nm, where the absorption differences between water and glucose are maximum and minimum, respectively.[13] The corresponding refractive indices of the polystyrene spheres were calculated as 1.5788 and 1.5742, respectively. The calculated optical properties (at 20 °C) are listed in Table 1.

2.2 EFFECT OF OTHER TISSUE PARAMETERS

2.2.1 Water Content
Over 90% of tissue fluids, including plasma and extra/intracellular fluid, are water, which has strong absorption bands from near- to mid-IR. Around 900 nm, water is the major absorber in tissue. Thus, the influence on light transport of small fluctuations in the water content of tissue, which can occur for many reasons, may cause errors in measuring the glucose level by absorption and scattering spectroscopy.

The changes in R and T were determined for total water variations in the range of − 1.5% to 1.5%. Over this small range, the change of glucose concentration caused by the altered water content can be ignored. This means that there are no significant changes in the absorption and refractive index of the tissue fluid, so that μ_a and g remain the same. However, μ_s decreases linearly with increasing water content due to dilution of the scatterers.

Table 1 Optical properties of phantom as a function of glucose concentration.

Glucose level (mM)	Refractive index n	Scattering coefficient μ_s (cm^{-1})	Absorption coefficient μ_a (cm^{-1})	Anisotropy g	Transport coefficient μ_s' (cm^{-1})
			960 nm		
0	1.32634	182.58	0.4240	0.87171	23.86
10	1.32659	182.23	0.4235	0.87177	23.79
20	1.32684	181.89	0.4231	0.87184	23.73
30	1.32709	181.54	0.4226	0.87191	23.67
			800 nm		
0	1.32923	256.50	0.02	0.89973	25.74
10	1.32948	256.05	0.01998	0.89980	25.68
20	1.32973	255.60	0.01996	0.89986	25.62
30	1.32998	255.16	0.01994	0.89993	25.55

2.2.2 Temperature

The above calculations were for a temperature of 20°C. However, the refractive index and absorption of water depend on temperature,[8,9,12] so tissue temperature fluctuations may interfere with the glucose signal. The change of water absorption on temperature at 960 nm was found to be $\approx +0.5\%$ per degree Celsius.[8,14] The modeling was therefore performed at 960 nm, over the temperature range 20 to 30°C.

2.2.3 Macromolecules

As indicated earlier, the effect of glucose on the optical properties of tissue is caused mainly by the displacement of water by dissolved glucose. This means that the effect of glucose on light transport may not be to produce a positive spectral "signature," for example an increase in absorption, per se. Proteins occupy most of the volume in tissue fluid except for water, so that fluctuations in protein concentration will also affect the water displacement factor, thereby changing the absorption and refractive index of tissue fluid. Hence, we also examined possible interference due to tissue proteins.

The average protein concentration[15] in tissue fluid is 185 g/liter. One of the major protein molecules in human tissue fluid is albumin. The same methods that were used to measure displacement of water by glucose were used to measure the displacement of water by albumin in water; we found a mean value of 0.0646($\pm$0.0009)% (g/liter)$^{-1}$. In order to calculate the influence of albumin concentration on scattering, the resulting increase of refractive index was also measured using a refractometer, giving a mean value of 1.6($\pm$0.02) $\times 10^{-4}$ (g/liter)$^{-1}$. Simplifying the tissue model, we assumed that these two parameters have the same values for all proteins. The optical absorption

of aqueous albumin solution was measured in a spectrophotometer, which showed that the albumin contribution is negligible compared with the water absorption.
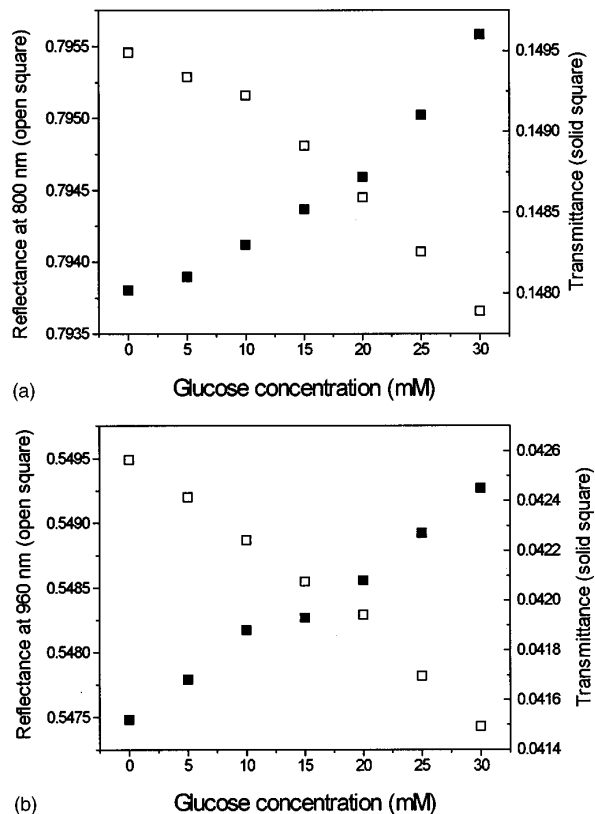

(a)

(b)

Fig. 1 Effect of glucose concentration on total reflectance (□) and transmittance (■) at (a) 800 nm and (b) 960 nm.
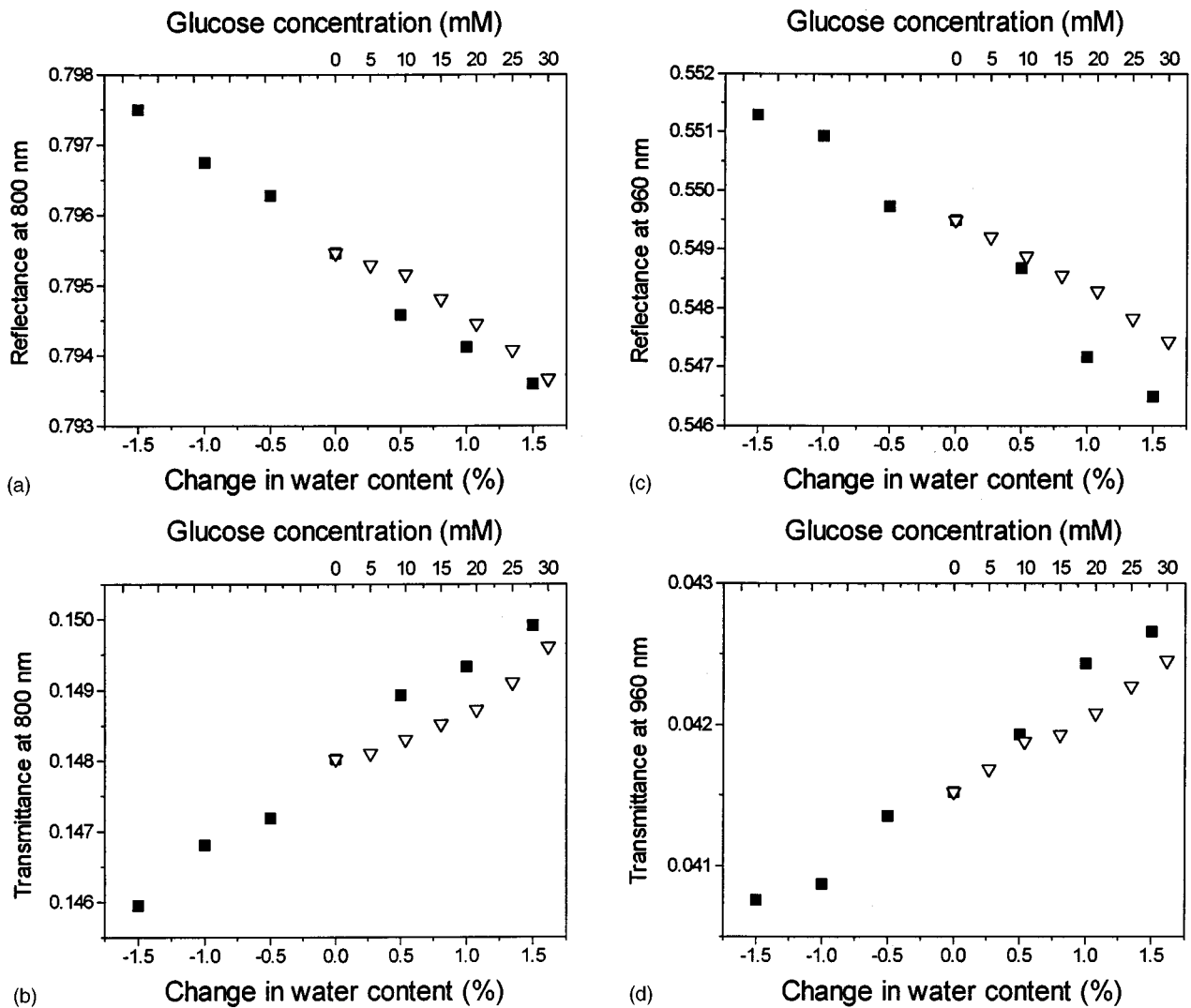
Fig. 2 Influence of water content on total reflectance and transmittance at (a,b) 800 nm and (c,d) 960 nm. ■, water content and ▽, glucose concentration.

3 RESULTS

The calculated reflectance and transmittance values for the tissue-simulating phantoms are shown in Figure 1. As can be seen, the transmittance in this scattering-dominated medium increases with glucose concentration at both wavelengths, due to the decrease of μ_s'. By contrast, the reflectance decreases with glucose concentration. In principle, such changes in R and/or T may be used to monitor the glucose level. However, as shown in Figure 1, the effect is very small at physiological levels. For example, the changes in transmittance at 800 and 960 nm are only $\approx +0.08$ and $+ 0.03\%/\mathrm{m}M$, respectively. The corresponding changes in reflectance are ≈ -0.01 and $- 0.006\%/\mathrm{m}M$.

The effects of fluctuation of water content on the reflectance and transmittance were calculated and are shown in Figure 2, where the glucose signals are included for comparison. As can be seen, the interference from fluctuations in water content is very strong. The interference in R and T from a change in tissue temperature is shown in Figure 3, again compared with the effect of glucose. The changes in reflectance and transmittance induced by protein and glucose are shown together in Figure 4, where the possible interference from protein is clear.

4 DISCUSSION AND CONCLUSIONS

The Monte Carlo results show that variations of glucose concentration in tissue-like absorbing and scattering media do change the light transport properties. However, the change is very small, typically less than 0.1% $\mathrm{m}M^{-1}$ for measurable noninvasive *in vivo* signals such as diffuse reflectance or transmittance for tissue thicknesses of less than 1

Glucose concentration (mM)
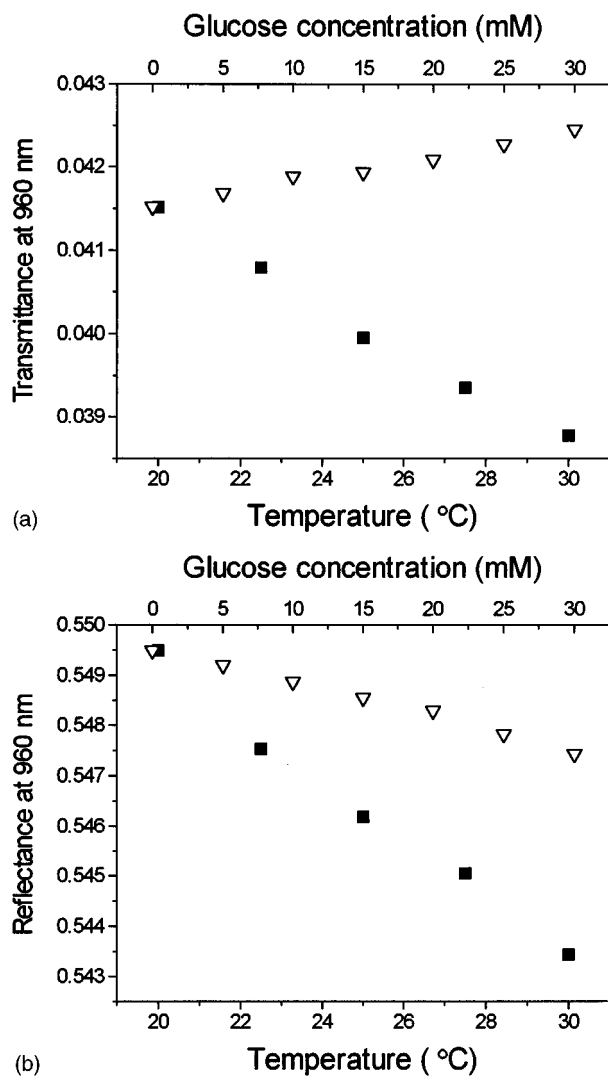

(a)

Glucose concentration (mM)

(b)

Fig. 3 The effect of temperature on total reflectance and transmittance. ■, temperature and ▽, glucose concentration.

cm. Accurate and reliable detection of such small effects requires technologies with extremely high sensitivity and stability.

Table 2 summarizes the changes in the various physiological factors that result in changes in diffuse reflectance and transmittance approximately equivalent to those caused by a + 1 mM change in glucose concentration.

Minor fluctuations in water content in tissue only affect the scattering coefficient, not the absorption or scattering anisotropy. If the contributions to the light transport from absorption and scattering can be separated to some extent, then the interference from the fluctuation of water content could be significantly reduced. In order to illustrate this, we have calculated the absorption coefficient and reduced scattering coefficient from the simulated reflectance and transmittance by inversely solving the diffusion model.[16] The results are shown in Figure

5. The scattering coefficient decreases with increasing water content, whereas the absorption coefficient appears relatively insensitive to it.

Interference from other analytes, such as protein molecules as modeled here, may cause an intrinsic error in glucose quantification *in vivo*. However, in the absence of overt clinical conditions such as anemia or starvation, the fluctuations in tissue protein are usually much less than those of water content.

From the simulation results it is clear that the tissue temperature must be controlled or at least monitored precisely in order to account for the dependence of the light propagation on the temperature-dependent changes in optical properties. The extent to which the temperature varies throughout the volume of tissue sampled during *in vivo* measurements is an additional potential source of error.

Some important limitations of the present study should be noted. First, as mentioned earlier, these results were obtained for a suspension of polystyrene microspheres of refractive index 1.57 to 1.58 in the near-IR, whereas in real tissue the refractive indices of natural scatterers such as cell membranes, nuclei, and mitochondria are more in the range of 1.4 to 1.5. This is much closer to that of the intra/extracellular fluid, which itself may be higher than the refractive index of water due to other solutes. Thus, changes in the fluid refractive index should produce correspondingly larger changes in the optical scattering. For example, using a refractive index of 1.45 for the microspheres, the sensitivity of the transmittance and reflectance to glucose concentration increase by factors of 3.7 and 1.8, respectively. However, this does not alter the degree of interference due to temperature or macromolecule concentration, since these also change the scattering due to the refractive index mismatch. Thus, the values in Table 2 for these two factors are independent of the assumed refractive index.

On the other hand, the interference due to the water content should be less in real tissue by the same factors of 3.7 and 1.8. This reduced interference is probably advantageous since the percentage change in tissue water content over time may be significantly higher than the ± 1.5% range used in the modeling, for example, because of edema associated with premenstrual syndrome or to dehydration. Very recently, Jagemann et al.[17] reported an *in vivo* study of glucose measurements that showed a significant relationship between the predicted blood glucose values using non-invasive NIR spectroscopy and the reference values obtained by standard invasive methods. This positive result is consistent with the prediction that the glucose-dependent signals in real tissue are likely to be greater than those simulated here.

Second, light scattering in tissue is most likely due to a complex combination of both intra- and extracellular refractive index mismatches. Hence, alterations in glucose, water content, and macromo-
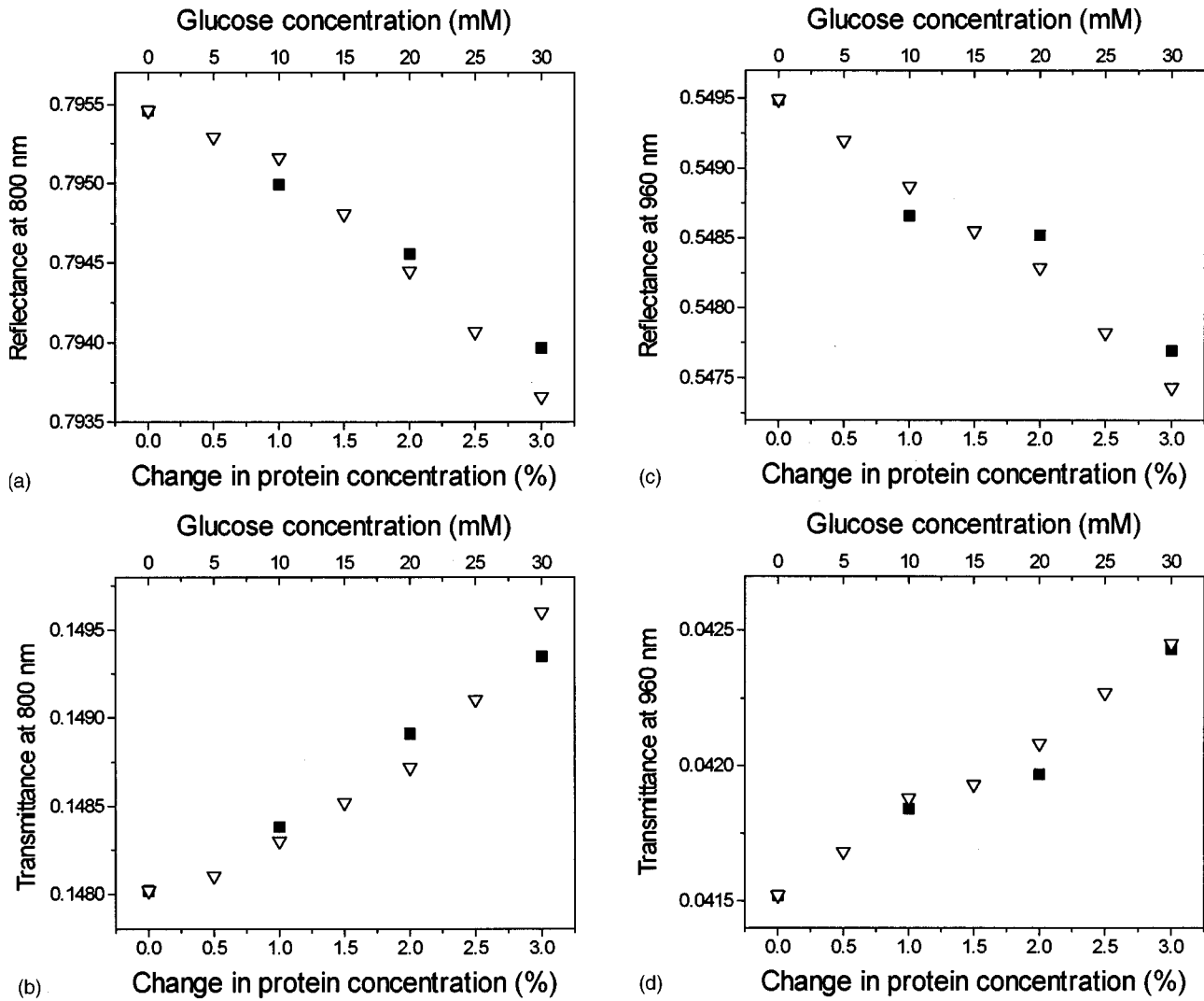
Fig. 4 The influence of protein concentration on total reflectance and transmittance at (a,b) 800 nm and (c,d) 960 nm. ■, albumin concentration and ▽, glucose concentration.

lecular concentrations are not necessarily equal in both compartments, and this has not been included in the modeling. It should also be noted that the normal finger-prick technique used in diabetic self-monitoring measures the glucose content in blood. With *in vivo* optical spectroscopy, given the small blood volume in the fraction of skin and subdermal connective tissues sampled noninvasively the measurement is primarily of interstitial glucose.

Third, we have not included the effect of variations in other tissue chromophores, the absorption coefficients used (Table 2) being for water only. At these wavelengths the absorption of hemoglobin (oxy- or deoxy-), in particular, may be significant: e.g., for 5% tissue blood content and 40% hematocrit, the absorption coefficient of blood at the isobestic point around 800 nm is $\sim 0.25 \, \mathrm{cm}^{-1}$. Thus fluctuations in blood content could certainly cause changes in the transmittance and reflectance that

are significant relative to the glucose-dependent changes.

However, in some measurement geometries (e.g., through the finger), the blood content and variations thereof can be substantially reduced by applying gentle pressure. This was shown by Jagemann et al.[17] to markedly reduce the variance in principal component analysis of diffuse reflectance spectra

Table 2 Optical equivalence of physiological factors.

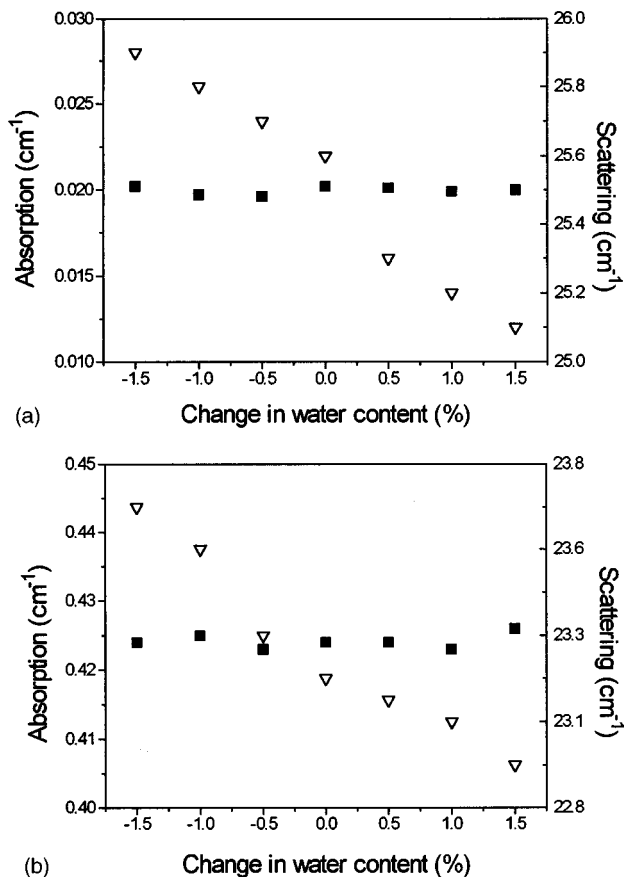| Physiological factor	Change ≡ +1 mM change in glucose
Water content	+0.2%
Temperature	−0.1 °C
Protein concentration	+0.1%

Fig. 5 Calculated absorption and reduced scattering coefficients at (a) 800 nm and (b) 960 nm. ■, absorption coefficient and ▽, reduced scattering coefficient.

from human subjects used to measure glucose concentration *in vivo*. Under these conditions, the observations made in this paper should remain essentially valid. Variations in other tissue solutes (e.g., glycogen and glycolipids) that might alter the tissue scattering and/or absorption spectra, have also not been considered here.

As a result of these limitations, the predictions for the dependence of the optical signals on non-glucose factors may not represent the true numerical values for real tissue. In addition, the degree to which these interfering factors mitigate against accurate *in vivo* optical measurements of glucose will depend also on the extent, precision, and accuracy of the spectral information obtained and on the sophistication and power of the subsequent spectral analysis. Nevertheless, the predicted effects are large enough to warrant close attention in the future development and clinical application of optically based glucose measurements.

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