Near-Infrared Studies of Glucose and Sucrose in Aqueous Solutions: Water Displacement Effect and Red Shift in Water Absorption from Water-Solute Interaction

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We used near infrared spectroscopy to obtain concentration dependent glucose absorption spectra in aqueous solutions in the near-infrared range (3800–7500 cm⁻¹). Here we introduce a new method to obtain reliable glucose absorption bands from aqueous glucose solutions without measuring the water displacement coefficients of glucose separately. Additionally, we were able to extract the water displacement coefficients of glucose, and this may offer a new general method using spectroscopy techniques applicable to other water-soluble materials. We also observed red shifts in the absorption bands of water in the hydration shell around solute molecules, which comes from the contribution of the interacting water molecules around the glucose molecules in solutions. The intensity of the red shift gets larger as the concentration increases, which indicates that as the concentration increases more water molecules are involved in the interaction. However, the red shift in frequency does not seem to depend significantly on the concentration. We also performed the same measurements and analysis with sucrose instead of glucose as solute and compared.

Index Headings: Near infrared spectroscopy; Water displacement coefficient; Glucose solution; Sucrose solution.

INTRODUCTION

The possibility of providing a direct, non-invasive approach to measuring glucose concentrations in blood has inspired studies of the applications of infrared spectroscopy for analyte detection in various solutions. It has been shown that glucose has many distinct infrared (IR) absorption features in the far-infrared (FIR),1–3 mid-infrared (MIR),4–8 and near-infrared (NIR)9–15 regions. However, water, the main component of blood, also displays strong IR absorption features in these regions, with increased absorption as you move further toward FIR. Water absorption modes in a wide spectral range can be found in the literature.16

In studies dealing with MIR analyte detection in blood,4,5 the experiments were performed on dry samples or using second derivative spectra to obtain concentration dependence.7 This causes large inaccuracies due to an increase in noise because of derivation. Water IR absorption in the MIR region as well as the FIR is overwhelming,17 rendering non-invasive glucose detection at physiological concentrations extremely difficult due to high water content in blood. In recent work,18 glucose absorption bands were extracted by using an independently measured water displacement coefficient of glucose. The water displacement coefficient is a measure of the second order effect of the presence of glucose on the spectrum of water. Because at physiologically relevant concentrations of glucose in blood, the water bands are orders of magnitude stronger than the glucose bands, incorrect treatment, a major source of error, can result.

In this study we introduce a new method to obtain reliable glucose absorption bands without measuring the water displacement coefficient separately. Because there is a strong water absorption peak at 5200 cm⁻¹ and negligible or almost no glucose absorption at this frequency, we take advantage of this strong and isolated water absorption peak to remove water absorption from the measured transmission of aqueous glucose solutions. By adjusting the effective thicknesses of water in a liquid cell to match the amplitude of the water peak for six different glucose solutions in the same liquid cell we are able to remove water absorption bands accurately and to extract an accurate concentration dependent glucose absorption coefficient in solution. Additionally, we are able to obtain a water displacement coefficient of glucose by using the concentration dependent effective thickness of water in the cell. We also observed red shifts in the absorption bands of water in the solution. Similarly there are red shifts from interacting water in macroscopic air–water and oil–water interfaces and in the hydration cell around nonpolar hydrocarbon solute groups.18,19 Water structure enhancement within hydration shells was reported.20,21 This indicates that water molecules around solute molecules are not free; they are interacting with solute molecules. Our analysis shows that the number of interacting water molecules seems to increase as the concentration of solute increases. We also applied the same method to another water soluble material, sucrose, which has a higher molecular weight than glucose. We compared the results of sucrose with those of glucose.

EXPERIMENTS

We prepared six different aqueous glucose and six sucrose solutions: the concentrations were 1.00, 2.00, 4.00, 6.00, 8.00 and 10.00 g/dL. All solutions were prepared using anhydrous D-(+)-glucose (C₆H₁₂O₆) purchased from Sigma-Aldrich (USA), sucrose (C₁₂H₂₂O₁₁) purchased from Junsei Chemical (Japan), and grade–3 deionized water. Aqueous samples were placed in a 250 ± 10 μm path-length liquid cell composed of glass. The cell was made using epoxy glue to attach 155 μm thick pieces of microscope cover glass (Sargent-Welch, USA) to a 1mm thick microscope slide (VWR Scientific, USA) forming a rectangular chamber (≈0.250 × 11.2 × 17.5 mm³). By covering this with another microscope slide we created a 250 ± 10 μm thick liquid cell appropriate for aqueous sample measurements. Reproducibility and stability of the measurement system were tested before proceeding with the study. We...
also prepared a pure amorphous glucose pellet and a sucrose pellet melting the D-glucose and sucrose powders, respectively. We measured those pellets to obtain the absorption coefficients of pure glucose and sucrose.

A commercial Fourier transform infrared (FT-IR) spectrometer, Bruker Vertex 80v was used for collecting near infrared spectra. The optical setup consists of a 75 W tungsten lamp as a light source, a CaF2 beam splitter, and a room temperature deuterated triglycine sulfate (DTGS) detector. We measured transmittance spectra, $T(x)$, of samples on a sample holder with a 5.0 mm diameter circular aperture and a resolution of 5 cm$^{-1}$ over a range of 3800–7500 cm$^{-1}$. An empty glass cell was used as the reference for all transmittance measurements except for the amorphous glucose and sucrose pellets. To get transmittances of the two pellets an empty hole was used as the reference. For all transmittance measurements of liquid samples we used the same liquid cell. All transmittance spectra were taken at room temperature (23 °C). Absorption coefficient spectra, $\alpha(\omega)$, were calculated from measured transmittances.

**MEASURED DATA AND ANALYSIS**

We measured transmittance spectra of the six different glucose and six sucrose solutions and pure water in the cell as well as pure amorphous glucose and sucrose pellets. The absorption coefficient can be extracted from a measured transmittance spectrum by using the well-known Beer–Lambert formula:

$$\alpha(\omega, C) = -\frac{\ln T(\omega, C)}{d}$$  \hspace{1cm} (1)

where $\alpha(\omega)$ is the absorption coefficient, $T(\omega)$ is the measured transmittance, $C$ is the concentration of the solution and $d$ is the thickness of the sample. Figure 1 shows raw absorption coefficients for the six glucose solutions, pure water, and a pure amorphous glucose pellet. For the solution samples (water and solutions) we used the same thickness $d_0 \approx 252 \mu$m because we used the same liquid cell. We also show a water absorption peak at 5200 cm$^{-1}$, which is a Lorentzian function. Because glucose absorption in the solution is very weak compared with the water absorption at these concentrations, we cannot see large differences among solution spectra. In these solution spectra, we observe three strong water absorption peaks in a spectral range between 3800 and 7500 cm$^{-1}$, which are near 4000, 5200, and 6900 cm$^{-1}$. There are two physiologically relevant windows in the water absorption through this measured spectral range; one between 4000 cm$^{-1}$ and 5200 cm$^{-1}$ is the combination region where four distinct glucose peaks are visible and the other between 5200 cm$^{-1}$ and 6900 cm$^{-1}$ is the first overtone region, which shows two broad glucose peaks that are not as distinct or strongly absorbing as those in the combination region.

To obtain the absolute magnitude of glucose absorption from a glucose solution, we initially subtracted the measured water absorption coefficient from that of each solution. As a first approximation, we assumed that the thicknesses of water are the same. Then we were able to formulate the subtraction procedure as follows:

$$\alpha_{sol}(\omega, C) - \alpha_w(d_0, \omega) = -\frac{\ln[T_{sol}(\omega, C)] - \ln[T_w(\omega)]}{d_0}$$  \hspace{1cm} (2)

where $d_0$ is the thickness of our liquid cell. $\alpha_{sol}(\omega)$ and $\alpha_w(d_0, \omega)$ are respectively the absorption coefficients of a solution and pure water calculated using the cell thickness, $d_0 = 252 \mu$m. $T_{sol}(\omega)$ and $T_w(\omega)$ are the measured transmittance spectra of the solution and pure water, respectively. Figure 2a shows spectra resulting from this analysis procedure. There is only one clearly

![Fig. 1. Absorption coefficients of pure amorphous glucose (dotted dark blue line), pure water (dash-dotted orange line), and six glucose aqueous solutions (from top to bottom; from low to high concentrations). We also show a water peak at 5200 cm$^{-1}$. Insets (A) and (B) show expanded views near 5150 cm$^{-1}$ and 4750 cm$^{-1}$ respectively.](image-url)
visible glucose peak at 4700 cm$^{-1}$, offset from the actual peak position seen in Fig. 1 of 4740 cm$^{-1}$. Also, at 5200 cm$^{-1}$, there is a sharp downward peak, with the larger peak for the higher concentrated solution. This concentration-dependent downward peak appears in the difference spectra because we did not consider the water displacement effect due to the glucose presence in the solution. When glucose is dissolved in water the volume of the solution changes because each glucose molecule takes up a finite space. We have to take into account this (water displacement) effect to subtract an appropriate water spectrum from the solution spectra.

Our approach for solving the downward peak problem in the difference spectra shown in Fig. 2a is as follows. There is no or very weak absorption of glucose around 5200 cm$^{-1}$, where water has a very strong absorption peak. This means that at that frequency absorption values of all solutions including pure water should be the same if we use a proper thickness of water for each solution. We performed the following procedure to remove an appropriate water absorption from the total absorption of each solution: by adjusting the thickness of pure water so that its absorption value at 5200 cm$^{-1}$ is the same as the absorption value of each solution, we can then subtract a proper water spectrum from each solution spectrum to obtain pure glucose absorption in each solution. We call the proper thickness of water for each solution the effective thickness of water. The procedure can be formulated as follows:

Fig. 2. (a) Glucose absorption bands in solutions obtained at six different concentrations by subtracting the water spectrum from those of the glucose solutions. Water displacement coefficient of glucose has not been considered on these spectra (see text). (b) Glucose absorption coefficients in six different solutions. Water displacement effects have been taken into account for the subtracting water absorption procedure (see text). The green dotted line is fitted to the feature near 5200 cm$^{-1}$ with both peak and dip from a model calculation (see Fig. 3b and corresponding text). The black dashed line in the lower frame is the pure glucose absorption spectra with its intensity reduced by a factor of 22. In the inset we display an expanded view to better show spectral features in the first overtone region.
where $d_{\text{eff}}(C)$ is the effective thickness of pure water for each concentration and $\alpha_{w, \text{def}}(\omega)$ is the absorption coefficient of water calculated using the effective thickness $d_{\text{eff}}(C)$, which is dependent on the concentration. By performing the procedure, the concentration dependent negative peak, caused by subtracting too much water absorption, is removed, although not completely. We will discuss this remaining downward peak in the following paragraphs. This uncovers new absorption peaks due to glucose that agree with the pure glucose absorption shown in Fig. 1, the dotted dark blue curve. The resulting absorption spectra due to glucose in six solutions are shown in Fig. 2b. In contrast to the single peak evidence in Fig. 2a at 4700 cm$^{-1}$, three others are obvious in the combination

\[
\alpha_{\text{sol}}(\omega, C) - \alpha_{w, \text{def}}(\omega) = -\frac{\ln[T_{\text{sol}}(\omega, C)]}{d_0} - \left[ -\frac{\ln[T_{w}(\omega)]}{d_{\text{eff}}(C)} \right]
\]  (3)

Fig. 3. (a) Reference peak with its center at 5200 cm$^{-1}$ and width 400 cm$^{-1}$. Two horizontal shifted peaks by negative 5 cm$^{-1}$ (dashed dotted red curve) and positive 5 cm$^{-1}$ (dashed blue curve), respectively. In the inset: Expanded graphs near the peak region show the shifts more clearly. (b) Resulting differences subtracted the reference peak at 5200 cm$^{-1}$ from the shifted peaks by seven different amounts (see in the text). (c) Resulting differences subtracted the reference peak at 5200 cm$^{-1}$ from the peaks at 5205 cm$^{-1}$ with four different amplitudes (see in the text). (d) (e) Resulting differences subtracted the reference peak at 5200 cm$^{-1}$ from the peaks at 5205 cm$^{-1}$ with six different widths (see in the text).
region and two in the first overtone region. Also, the peak centered at 4700 cm\(^{-1}\) in Fig. 2a has undergone a shift to the actual peak position of glucose at 4740 cm\(^{-1}\). Glucose absorption bands in the first overtone region were also uncovered (see the inset of Fig. 2b), displaying absorption at 5700 and 6360 cm\(^{-1}\). The relative intensities of the glucose peaks are revealed as well. The concentration dependent peak heights of the four peaks in the combination region are displayed in Fig. 5a. They show an almost linear dependence on concentration.

In the inset of Fig. 2b the peaks centered at 5700 and 6360 cm\(^{-1}\) have some visible discrepancies showing variation from the expected concentration dependence. In the first overtone region it seems that the 4 g/dL solution (green curve) has a larger absorption value than 6 g/dL (blue curve). However, looking at the 6 g/dL peak, it has a better-defined shape. Even though the 4 g/dL solution has a higher absorption value, the 6 g/dL solution has a more well-defined absorption compared with its average height in the first overtone region indicating a stronger real absorption. The same can be said for the 2 g/dL absorption (red curve) due to glucose, which appears to have a lower absorption than the 1 g/dL solution (black curve) in this region. Even though the area under the curves suggests that some weaker solutions have stronger absorption, the shape of the absorption peaks gives additional information about the concentration and a more accurate depiction of the concentration dependence in the first overtone region. These results can be attributed to the broader absorption peak of glucose in this region.\(^{14}\) Due to a broader or less well-defined peak, it is more difficult to detect proper concentration levels through aqueous media. A sharper or narrower peak provides a greater chance to see the concentration dependence of the absorption peak at that frequency as it is a better defined peak.

As we pointed out previously in Fig. 2b we still have an extra feature near 5200 cm\(^{-1}\), which has both a peak (on the lower frequency side) and a dip (on the higher frequency side). To understand this feature we simulate it with Lorentzian (reference) peaks. We found that there are three ways to

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**Fig. 4.** Comparison of the pure glucose absorption and the extracted glucose absorption \(\alpha_{glc}(\omega, C) - \alpha_{w,def}(\omega)\) from 10g/dL solution. Reduced intensity of the pure glucose absorption by a factor of 22. The water spectrum is shown for comparison with its intensity reduced by a factor of 40.

**Fig. 5.** (a) The concentration dependent peak height of four absorption modes of glucose in the combination region. (b) The concentration dependent height and depth of the new feature in Fig. 2b. (c) Concentration dependent effective thickness of the cell extracted from the water subtraction procedure (see in the text). A strong linear relationship is shown between the effective thickness of water and the glucose concentration.
produce such a feature. We show results of the three ways in Fig. 3b, c, and d, respectively. In Fig. 3a we show the reference Lorentzian peak along with three peaks where the horizontal axis shifted by ±5 cm⁻¹ and one peak shifted by −100 cm⁻¹. The Lorentzian peak can be described as follows:

\[
L_{\text{peak}}(\omega) = \frac{A}{\pi}\frac{\Gamma/2}{(\omega - \omega_{\text{shift}})^2 + (\Gamma/2)^2}
\]  

(4)

where \( A \) is area under the peak, \( \omega_0 \) is the center frequency of the peak, and \( \Gamma \) is the width of the peak, which is the full width at half maximum (FWHM). In Fig. 3b we show the difference between each shifted peak with various shifting amounts (\( \omega_{\text{shift}} = -100, -5, -4, -3, -2, -1, +5 \) cm⁻¹) with a fixed width (\( \Gamma = 400 \) cm⁻¹) and a fixed amplitude (\( A = 200 \) cm⁻²) and the reference peak; \( L_{\text{shift}}(\omega) - L_{\text{ref}}(\omega) = (A/\pi)\{(\Gamma/2)^{-1}/(\omega - \omega_{\text{shift}} - \omega_0)^2 + (\Gamma/2)^2\} - 1/[(\omega - \omega_0)^2 + (\Gamma/2)^2] \}. As we can see in the figure the more shifting produces the larger and better defined the difference spectra. We note that the difference between the peaks and dip positions do not change very much up to the −100 cm⁻¹ shift because of a large width (400 cm⁻¹) of the peak; 243 cm⁻¹ for −100 case and 230 cm⁻¹ for −5 case. But more shifting causes the larger interval between the peak and the dip. The frequency at the zero crossing is shifted by half the frequency shift amount; the difference for −100 cm⁻¹ case (the zero crossing frequency) is 1150 cm⁻¹. In Fig. 3c we show the difference between each peak at 5195 cm⁻¹ (i.e., \( \omega_{\text{shift}} = 5 \) cm⁻¹) with various amplitudes (\( A = 50, 100, 150 \) and 200 cm⁻²) and the reference peak; \( L_{\text{amplitude}}(\omega) - L_{\text{ref}}(\omega) = (A/\pi)((\Gamma/2)^{-1}/[(\omega - \omega_{\text{shift}} - \omega_0)^2 + (\Gamma/2)^2] - 1/[(\omega - \omega_0)^2 + (\Gamma/2)^2]) \}. Here we also change the amplitude of the reference peak according to each peak amplitude. The results are shown in the figure; the more intense peaks give the larger differences. We note that the peak and dip positions are not at all amplitude dependent. In Fig. 3d we show the difference between each broadened peak at 5195 cm⁻¹ (i.e., \( \omega_{\text{shift}} = 5 \) cm⁻¹) with various widths (\( \Gamma' = 400, 450, 500, 550, 600, \) and 650 cm⁻¹) and the reference peak; \( L_{\text{width}}(\omega) - L_{\text{ref}}(\omega) = (A/\pi)((\Gamma/2)^{-1}/[(\omega - \omega_{\text{shift}} - \omega_0)^2 + (\Gamma/2)^2] - 1/[(\omega - \omega_0)^2 + (\Gamma/2)^2]) \}. We can see in the figure the sharpest peak gives the largest and most-defined difference. We also note that the peak (dip) position is red (blue) shifted as the width increased.

From observation of these three cases we concluded that the extra feature in Fig. 2b can be attributed to the second case, i.e., amplitude changes with concentration. The intensity of the sharp absorption band edge due to the interacting water around 5200 cm⁻¹ is getting larger as the amount of glucose increases, which is reasonable because more water molecules get involved in the interaction with the glucose molecules as the concentration increases. We do not expect a concentration dependent change in the frequency of the interacting water as long as we keep a relatively low glucose concentration in the solution.

We fit the observed peak and dip feature near 5200 cm⁻¹ in Fig. 2b by using the model of our second case. In Fig. 1 we show the reference water peak at 5210 cm⁻¹ (green dashed line): Here we only considered the sharp absorption edge part of the water absorption band near 5200 cm⁻¹. In Fig. 2b we show an example fit (green dashed line) to the peak and dip feature near 5200cm in the 10 g/dL spectrum. We note that the amplitude (or area) of the reference peak is 3300 cm⁻² and its width is 170 cm⁻¹. From the fit we found that the amount of red shift is quite small, 2 cm⁻¹. However, the resolution of the frequency shifting depends on the width of the reference peak considered. In our case the width (170 cm⁻¹) is quite large compared with the shift (2 cm⁻¹) so it is not very resolvable. Still what we can tell clearly is that the water absorption peak undergoes a red shift. Here one may wonder why the instrumental resolution used to collect the spectra is 5 cm⁻¹ whereas the observed shift in the water absorption band is around 2 cm⁻¹. However, we measured the resulting feature from the shift, which is much broader (about the width of the reference peak) than the instrumental resolution shown in the figure. We also expected to observe red shifts from other water peaks. To show this we displayed the absorption of pure glucose and the extracted glucose absorption spectrum, \( \omega_{\text{shift}}(\omega) - \omega_{\text{sol}}(\omega) \) for \( C = 10g/dL \) in the same panel as shown in Fig. 4. The figure clearly shows signatures of red shifts for other water peaks. The signatures, which are strong dips, appear near sharp edges for water absorption namely, 3800 cm⁻¹, 5200 cm⁻¹, and 7000 cm⁻¹. So the red shifts seem to occur for all water absorption peaks. It is clear that the height of the peak or depth of the dip of the feature can be a measure of the intensity of the interaction (see Fig. 3c). The resulting concentration dependent intensities of the red shift, height, and depth are displayed in Fig. 5b. As we expected, the extracted intensity is roughly proportional to the glucose concentration. The deviation from the linearity may come from the uncertainty in the water subtraction procedure.

From the appropriate water subtraction procedure, which we described previously, we can obtain the effective thickness of water for each glucose solution. Figure 5c displays the extracted effective thickness of water as a function of the glucose concentration. It shows a strong linear relationship between the effective thickness and the concentration from 1 g/dL through 10 g/dL. The water displacement coefficient is defined by the molar concentration change of water caused by the dissolution of a unit molar concentration of the solute. The molar concentration is defined by the number of moles per a liter of solvent (in our case, water). More practically, the water displacement coefficient is the number of water molecules that are replaced by a solute molecule in the solution. By using these definitions we can write down the effective thickness of water in the cell as a function of glucose concentration.

\[
d_{\text{eff}}(C) = d_0 \left[ 1 + \frac{C' \cdot w_{\text{dis}}}{1 + C' \cdot w_{\text{dis}}} \right]
\]

\[
C' = \frac{M_{\text{water}}}{100 \cdot M_{\text{solute}}} \cdot C
\]

where \( C \) is the concentration in g/dL, \( d_{\text{eff}}(C) \) is the concentration dependent effective thickness of water, \( M_{\text{water}} \) is the molecular weight of water, \( M_{\text{solute}} \) is the molecular weight of solute, \( d_0 \) is the real thickness of the cell (in our case, 252 μm) and \( w_{\text{dis}} \) is the water displacement coefficient. We see that \( C' \) is a small quantity; 0.01 for 10 g/dL glucose solution and 0.0053 for 10 g/dL sucrose solution.; these are the maximum values for glucose and sucrose solutions. The water displacement coefficient is roughly a single digit value. So \( C' \cdot w_{\text{dis}} \) is small and we can rewrite Eq. 5 approximately as follows:

\[
d_{\text{eff}}(C) \cong d_0 \left[ 1 + C' \cdot w_{\text{dis}} \cdot (1 - C' \cdot w_{\text{dis}}) \right]
\]
The water displacement coefficient of glucose shows a strong temperature dependence; 5.051 at 21 °C23 and 6.245 at 37 °C14 and our extracted water displacement coefficients of the solution. If the total volume of the solution consists of glucose alone the absorption coefficient of the solution sample would be identical to that of pure glucose. Because the absorption intensity of glucose is proportional to the effective thickness (or amount) of glucose alone in solution the intensity ratio is the same as the effective thickness ratio as in the following equation,

$$\frac{A_{\text{solute pure}}}{A_{\text{solute sol}}} = \frac{C/M_{\text{solute}} \cdot w_{\text{dis}} \cdot N_A}{[100/M_{\text{water}} + C/M_{\text{solute}} \cdot w_{\text{dis}}] \cdot N_A}$$

(8)

where $A_{\text{solute pure}}$ and $A_{\text{solute sol}}$ are the peak heights of pure glucose and glucose in solution, respectively, C is the concentration in g/dL and $N_A$ is Avogadro’s number. When we solve for the water displacement coefficient, $w_{\text{dis}}$, we get the following equation,

$$w_{\text{dis}} = \frac{A_{\text{solute sol}} \cdot 100 \cdot M_{\text{solute}}}{A_{\text{solute pure}} - A_{\text{solute sol}}} \cdot C \cdot M_{\text{water}}$$

(9)

This equation means that for a given concentration, if we know the water displacement of solute and its absolute absorption coefficient we can easily estimate the absorption coefficient of solute alone in solution. In other words, if we can measure the absorption coefficient of solute alone in solution for a given concentration we are able to obtain the water displacement coefficient of the solute. For example, we consider 10 g/dL glucose solution and the absorption peak at 4000 cm$^{-1}$. Then by using Eq. 9 $w_{\text{dis}} = (3.52 \times 100 \times 180)/[(72.5–3.52) \times 10 \times 18] \approx 5.10$. Even though the value is slightly smaller than the previously extracted value (5.91) it is consistent with the previous one. The concentration dependent water displacement of glucose is shown in Fig. 6 along with the concentration dependent water coefficient of sucrose. It seems to be independent of concentration even though there are some noisy data points in the low concentration region due to the uncertainty in the water subtraction procedure.

The water displacement coefficient of glucose shows a strong temperature dependence; 5.051 at 21 °C23 and 6.245 at 37 °C14 and our extracted water displacement coefficients of...
glucose seem to be consistent with other studies; at least our value is in between those two values obtained at two lower and higher temperatures. The temperature dependence in the water displacement coefficient probably comes mostly from thermally induced morphology of glucose molecules in the water. More systematic studies should be performed on temperature dependent water displacement of glucose. We note that, at very high concentrations, the linear trend cannot hold because of the interactions between the nearest glucose molecules, which is an indirect repulsive interaction in water, will become stronger as the concentration increases.

We performed the same experiment and data analysis with a different solute, sucrose (C\textsubscript{12}H\textsubscript{22}O\textsubscript{11}), which has a larger molecular weight than glucose. The measured data and analysis results are displayed in Figs. 7, 8, and 9. Although there are some detailed qualitative differences, the overall qualitative concentration dependent trends are very similar to those of glucose. There are four sucrose absorption peaks in the combination region and two peaks in the first overtone region as for glucose. It is interesting to note that the relative intensities between the peaks are different. As we can see in the pure sucrose absorption spectrum, the peak at 4390 cm\textsuperscript{-1} is relatively large. The recovered concentration dependent peak height of sucrose absorption modes in the combination region and two peaks in the first overtone region as for glucose. It is interesting to note that the relative intensities between the peaks are different. As we can see in the pure sucrose absorption spectrum, the peak at 4390 cm\textsuperscript{-1} is relatively large. The recovered concentration dependent peak height of sucrose absorption modes in the combination region is displayed in Fig. 9a. This shows a linear dependence on the concentration as expected. An additional feature other than sucrose absorption peaks shows up in Fig. 8b and in Fig. 2b. We show an example fit to the feature for 10 g/dL only for the absorption edge region. This fitting shows that the red shift is very small, around 2 cm\textsuperscript{-1}. The concentration dependent height and depth of the feature are shown in Fig. 9b. This indicates that water molecules around a sucrose molecule are not free; the absorption peaks due to the water will be shifted to lower frequencies, i.e., red shifts, due to water structure enhancement within the hydration shells around solute molecules.\textsuperscript{20,21}

We also display the concentration dependent effective thickness, \(d_{\text{eff}}(C)\), of water in the liquid cell in Fig. 9c. We fit the data to a straight line; \(d_{\text{eff}}(C) = 252.3 \pm 0.4 + 1.427 \pm 0.065 \times C\) (in \(\mu\text{m}\)). By using Eq. 7, and the extracted slope of the line and \(d_0\) from the fit we can get the water displacement coefficient of sucrose, 10.76 \(\pm\) 0.46 at 23 °C. The water displacement coefficient tells us that a sucrose molecule can take up space equivalent to 10.76 water molecules in the solution. We also used the other method (see Eq. 9) to obtain the water displacement coefficient of sucrose. For example, we consider the 10g/dL sucrose solution and the absorption peak at 4000 cm\textsuperscript{-1}. We found, using Eq. 9, that \(w_{\text{dis}} = (3.57 \times 100 \times 342) / [(72.0 – 3.57) \times 10 \times 18] \approx 9.35\). Even though the value is slightly smaller than the previously extracted value (10.76) it is quite consistent with the previous one. The concentration dependent water displacement of sucrose is shown in Fig. 6. In low concentration regions the data show more noise and seem to deviate from the concentration independent trend shown at high concentration. This is probably due to the uncertainty in the water subtraction procedure in the low concentration region. The molecular weight of sucrose (\(M_{\text{sucrose}} = 342\)) is almost twice of glucose (\(M_{\text{glucose}} = 180\)) i.e., in solution a sucrose molecule takes up almost twice the space of a glucose molecule. This may indicate that sucrose molecules in solution have elongated shapes instead of global ones.
CONCLUSIONS

It is clear from our work as well as previous studies that water displacement is an important quantity that must be considered for a proper study of glucose concentration in aqueous media and blood. Our new method is fundamentally different from the study of Amerov, Chen, and Arnold,\textsuperscript{14} who realized the problem introduced by water displacement and compensated for it by using an independently measured water displacement coefficient based on density measurements. We introduce a simple new spectroscopic method in which we remove water absorption bands accurately from measured glucose aqueous solutions without independent measurement of water displacement by the glucose. Using the linear relationship of concentration found for the effective thickness of water in the liquid cell we could properly manipulate the spectra to remove water absorption and obtain reliable concentration dependent glucose absorption bands. In the spectra obtained from the subtraction procedure we observed signatures of interaction between water and solute molecules in the solution. The red shift of the water absorption near 5200 cm\textsuperscript{-1} is around 2 cm\textsuperscript{-1}, which is very small but clearly observable in the spectra. As we mentioned previously, the amount of shifting is not easily resolvable as the width of water absorption is quite broad compared with the red shift. Additionally, we were able to extract the water displacement coefficient of glucose, which is consistent with values reported in literature.\textsuperscript{14,23} These results may help to non-invasively monitor the glucose level in the human body.

Fig. 8. (a) Sucrose absorption bands in solutions obtained at six different concentrations by subtracting water spectrum from those of the sucrose solutions. Water displacement coefficient of sucrose has not been considered on these spectra (see text). (b) Sucrose absorption coefficients in six different solutions. Water displacement effects have been accounted for the subtracting procedure (see in the text). The black dashed line in the lower frame is the pure glucose absorption spectra with its intensity reduced by a factor of 20. Inset: An expanded view to better show spectral features in the first overtone region.
This method has several advantages: By removing the need to measure the water displacement coefficient independently, a reliable water displacement coefficient from our concentration dependent spectrum can be estimated, making it a self-contained method that can be applied to other solutions. In our study, we applied the same method to sucrose aqueous solution and got a reasonable water displacement coefficient for sucrose. The method of water displacement extraction described in this paper can serve as a new general method for sucrose. The method of water displacement extraction described in this paper can serve as a new general method for sucrose. The method of water displacement extraction described in this paper can serve as a new general method for sucrose. The method of water displacement extraction described in this paper can serve as a new general method for sucrose. The method of water displacement extraction described in this paper can serve as a new general method for sucrose.

ACKNOWLEDGMENTS

We thank T. Timusk and R. Peters for useful discussions. This work has been supported by the special fund of the Department of Physics at Pusan National University, Busan, Republic of Korea. The National Research Foundation of Korea also supported this work in the form of a grant funded by the Korean Government (NRF-2010-371-B00008).

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