

Molar Absorptivities of Glucose and Other Biological Molecules in Aqueous Solutions over the First Overtone and Combination Regions of the Near-Infrared Spectrum

AIRAT K. AMEROV, JUN CHEN, and MARK A. ARNOLD*

Department of Chemistry and Optical Science and Technology Center, University of Iowa, Iowa

Molar absorptivities are measured for water, glucose, alanine, ascorbate, lactate, triacetin, and urea in the near-infrared spectral region at 37 °C. Values are based on the Beer–Lambert law and cover the first overtone (1550–1850 nm; 6450–5400 cm⁻¹) and combination (2000–2500 nm; 4000–5000 cm⁻¹) spectral windows through aqueous media. Accurate calculations demand accounting for the impact of water displacement upon dissolution of solute. In this regard, water displacement coefficients are measured and reported for each solute. First overtone absorptivities range from 2 to 7 × 10⁻⁵ mM⁻¹mm⁻¹ for all solutes except urea, for which absorptivity values are below 0.5 × 10⁻⁵ mM⁻¹mm⁻¹ across this spectral range. Molar absorptivities over the combination spectral region range from 0.8 to 3.2 × 10⁻⁴ mM⁻¹mm⁻¹, which is a factor of four to five greater than the first overtone absorptivities. Accuracy of the measured values is assessed by comparing calculated or modeled spectra with spectra measured from standard solutions. This comparison reveals accurately modeled spectra in terms of magnitude and position of solute absorption bands. Both actual and modeled spectra from glucose solutions reveal positive and negative absorbance values depending on the measurement wavelength. It is shown that the net absorbance of light is controlled by the magnitude of the absorptivity of glucose compared to the product of the absorptivity of water and the water displacement coefficient for glucose.

Index Headings: Molar absorptivity; Water displacement; Near-infrared spectroscopy.

INTRODUCTION

The ability to model near-infrared spectra is essential for the advancement of noninvasive analytical measurements with near-infrared spectroscopy. Such models are particularly useful in developing analytical methods for measurements in complex samples of biological and clinical origin. Accurate spectral modeling provides an efficient means to establish the instrumental specifications required to achieve a targeted selectivity and limit of detection. Spectral models can also be used to examine the impact of critical experimental parameters, such as scattering, temperature, and interferences from chemical components in the sample matrix. Accurate values for molar absorptivities are required before spectral modeling is possible.

Water displacement is a critical factor that must be included in models for near-infrared spectra of aqueous samples. The relatively strong absorption properties of water throughout the near-infrared region of the spectrum^{1–6} cause significant changes in absorbance spectra upon dissolution of solutes. Basically, solute molecules

displace a specific molar volume of solvent, which results in fewer water molecules within the optical path of the spectroscopic measurement. Negative absorbance values can be created depending on the relative magnitude of molar absorptivities for solute and solvent. For this reason, near-infrared absorbance spectra reported for aqueous solutions include regions of both positive and negative absorbance.^{7,8}

The principal objective of this work is to determine molar absorptivities for a selected group of solutes of biological significance. In addition, these absorptivities are used to model actual near-infrared spectra and the corresponding accuracy is determined. Absorptivities are independently measured over the first overtone (1550–1850 nm; 6450–5400 cm⁻¹) and combination (2000–2500 nm; 5000–4000 cm⁻¹) regions of the near-infrared spectrum. These spectral regions correspond to the first overtone of C–H stretching vibrations and the combination of stretching and bending vibrations for C–H, N–H, and O–H bonds, respectively. These spectral ranges represent potential regions for noninvasive analytical measurements in biological samples.^{9–11} Molar absorptivities are reported for water, glucose, lactate, urea, triacetin, alanine, and ascorbate.

EXPERIMENTAL METHODS

Instrumentation and Apparatus. Spectra were collected with a Nexus 670 Fourier transform spectrometer (Thermo Nicolet, Madison, WI). This spectrometer was equipped with a standard 20-watt tungsten filament lamp, calcium fluoride beam splitter, and cryogenically cooled indium antimonide (InSb) detector. Multi-layer optical filters were used to isolate separately the first overtone and combination spectral regions. For both spectral regions, the filters were obtained from Barr & Associates (Westford, MA). First overtone spectra were collected with a 1550–1850 nm (6450–5400 cm⁻¹) bandpass H-band interference filter. Combination spectra were collected with a 2000–2500 nm (5000–4000 cm⁻¹) bandpass K-band interference filter.

Density measurements were carried out with a DMA 5000 density meter (Anton Paar GmbH). This instrument rigorously maintained solution temperatures at 37.0 ± 0.1 °C. Adjustments were applied for atmospheric pressure and relative humidity in the laboratory at the time of these measurements.

Chemicals and Reagents. Reagent-grade preparations of glucose, lactate, ascorbate, alanine, urea, and triacetin were purchased from common suppliers. Standard solutions were prepared by dissolving appropriate amounts of

Received 4 March 2004; accepted 8 June 2004.

* Author to whom correspondence should be sent. E-mail: mark-arnold@uiowa.edu.

dried solid in purified water, except for triacetin, which was used neat. Distilled, deionized water was prepared by passing house-distilled water through a Milli-Q three-stage water purification unit. This preparation generated type I, reagent-grade water with a resistance greater than 18 MΩ.

Methods and Procedures. Single-beam spectra were collected as 256 coadded, 16k double-sided interferograms. Single-beam spectra were obtained by using the spectral processing functions from the Nicolet OMNIC operating system. Interferograms were apodized with a common triangular apodization function prior to Fourier processing. One level of zero filling was used. Final spectra had a point spacing of 0.96 cm⁻¹ and the average root mean square (RMS) noise on 100% lines was 10.8 and 14.2 μAU (micro-absorbance units) at the peak transmission wavelengths for the first overtone and combination spectra, respectively.

Spectra were collected with 3- and 1.5-mm-thick samples for first overtone and combination spectra, respectively. For each spectral range, samples were placed in a Wilmad model 118-3 water-jacketed sample cell (Buena, NJ) equipped with a pair of 1-mm-thick sapphire windows. Teflon spacers were used to set the sample thickness and a VWR model 1140 circulation water bath (VWR Scientific, Chicago, IL) was used to maintain a sample temperature of 37.0 ± 0.1 °C. A copper-constantan thermocouple (Omega, Inc., Stamford, CT) was placed directly in the sample solution and the sample temperature was monitored with an Omega 670 digital meter.

Molar absorptivities were obtained from simple solutions composed of only the solute of interest dissolved in an aqueous buffer. The corresponding absorbance spectra were computed by ratioing single-beam spectra for the sample solution to a reference single-beam spectrum of air. Dry air was an excellent reference material for such measurements because of its transparency throughout the near-infrared spectrum, stable composition, insensitivity to temperature, and availability. When collecting near-infrared spectra from air and aqueous solutions, care was necessary to ensure that the detector response was linear with respect to radiant power for both the reference and sample measurements. In our case, the incident light was attenuated with standard neutral density filters to avoid detector saturation and nonlinear effects when collecting the air reference spectra. Reference spectra were collected in triplicate immediately before collecting sample spectra for each solute.

RESULTS AND DISCUSSION

Absorptivity Calculations. Molar absorptivities originate from the Beer–Lambert relationship and correspond to the wavelength dependent absorption strength for a molecule normalized for concentration. The Beer–Lambert law is

$$A = \sum_{i=1}^n \varepsilon_i c_i b \quad (1)$$

where A is a sample absorbance, ε_i and c_i are molar absorptivities and concentrations for each of the n components in the sample, respectively, and b is the optical path

length. In the absence of molecular interactions between solutes, the total absorbance for a sample is simply the summation of the individual absorbance values for the composite solutes.

Typically, the solvent is non-absorbing and the impact of solvent is easily compensated by using a reference spectrum of blank solvent. This approach is not sufficient for near-infrared spectra of aqueous solutions, where solvent absorption is significant. As described above, the dissolution of solute displaces a fraction of the absorbing water molecules from the optical path. This water displacement effect can be taken into account by summing absorbance for each solute and subtracting the loss of absorbance associated with the displacement of water by each solute. The following expression provides the net absorbance when a solvent blank is used as the reference material and water is the solvent:

$$\begin{aligned} A_{\text{sample}} &= \sum A_{\text{solute}} - \sum A_{\text{solvent displacement}} \\ &= \sum_{i=1}^n \varepsilon_i c_i b - \sum_{i=1}^n \varepsilon_w f_w^i c_i b \end{aligned} \quad (2)$$

where ε_w is the molar absorptivity of water and f_w^i is the water displacement coefficient for solute i . This water displacement coefficient corresponds to the molar concentration change of water caused by the dissolution of a unit molar concentration of the solute.

Equation 2 is not a general expression because it only considers the displacement of solvent by the individual solute molecules. Technically, the dissolution of any solute will also displace molecules of co-solutes from the optical path. An additional term can be added to Eq. 2 in order to account for the loss of absorbance due to co-solute displacement. At milli-molar concentrations, however, the effects of co-solute displacement are negligible and, to a first approximation, can be ignored.

For absorbance measurements of a single solute relative to air, Eq. 2 can be rewritten as:

$$A_{\text{sample}} = \varepsilon_w c_w b + \varepsilon_s c_s b - \varepsilon_w f_w^s c_s b \quad (3)$$

where the first term corresponds to the absorbance of water relative to air, the second term is the absorbance of solute relative to air, and the third term accounts for water displacement by the sole solute. Rearrangement of Eq. 3 gives an expression for calculating the molar absorptivity of the solute, as shown in Eq. 4:

$$\begin{aligned} \varepsilon_s &= \frac{A_{\text{sample}} - \varepsilon_w c_w b + \varepsilon_w f_w^s c_s b}{c_s b} \\ &= \frac{A_{\text{sample}} - A_{\text{buffer}} + \varepsilon_w f_w^s c_s b}{c_s b} \end{aligned} \quad (4)$$

where A_{sample} and A_{buffer} are absolute absorbance values (i.e., measured relative to air) for the solute containing sample and the blank aqueous buffer solution. A phosphate buffer is used in this work. The phosphate buffer salts are transparent throughout the near-infrared spectrum, thereby eliminating complications associated with the absorption properties of the buffer salts.

Absorbance values were corrected for the dispersion of water^{12–14} and sapphire¹⁵ by accounting for the loss of reflected light at the air–window and solution–window

TABLE I. Water displacement coefficients and regression results for individual solutes.

| Solute | Glucose | Alanine | Ascorbate | Lactate | Urea | Triacetin |
|------------------|---------|---------|-----------|----------|----------|-----------|
| $(f_w^s)^a$ | 6.245 | 3.23 | 5.32 | 3.163 | 2.483 | 10.013 |
| $(SE-\beta_1)^b$ | 0.0035 | 0.015 | 0.011 | 0.0037 | 0.0023 | 0.018 |
| $(SER)^c$ | 0.148 | 0.478 | 0.771 | 0.120 | 0.161 | 0.239 |
| $(\beta_0)^d$ | 0.039 | 0.548 | -0.330 | 0.043 | -0.089 | -0.205 |
| $(SE-\beta_0)^e$ | 0.107 | 0.446 | 0.523 | 0.108 | 0.105 | 0.192 |
| $(R^2)^f$ | 0.99999 | 0.99993 | 0.999994 | 0.999998 | 0.999997 | 0.999996 |

^a Slope of regression line and water displacement coefficient.

^b Standard error of the slope and, hence, of the water displacement coefficient.

^c Standard error of the regression, (mM).

^d y-intercept, (mM).

^e Standard error of the y-intercept, (mM).

^f Square of the regression correlation coefficient.

interfaces. The relevant radiant powers are obtained by the following expressions:

$$P_a = P_0(1 - r_{aw})^4 \quad (5)$$

$$P_b = P_0(1 - r_{aw})^2(1 - r_{bw})^2 10^{-A_{\text{buffer}}} \quad (6)$$

$$P_s = P_0(1 - r_{aw})^2(1 - r_{sw})^2 10^{-A_{\text{solution}}} \quad (7)$$

where P_a , P_b , and P_s correspond to radiant powers after passing through the sample cell filled with air, buffer, and sample, respectively, and r_{aw} , r_{bw} , and r_{sw} correspond to the reflectance coefficients at the air/window, buffer/window, and sample/window interfaces, respectively. These coefficients are obtained from the Fresnel equations under the assumption of normal incidence of the propagating light and are based on the refractive index of each medium according to the following expressions:

$$r_{aw} = \frac{(\eta_{\text{win}} - \eta_a)^2}{(\eta_{\text{win}} + \eta_a)^2}, \quad r_{bw} = \frac{(\eta_{\text{win}} - \eta_b)^2}{(\eta_{\text{win}} + \eta_b)^2},$$

$$r_{sw} = \frac{(\eta_{\text{win}} - \eta_s)^2}{(\eta_{\text{win}} + \eta_s)^2} \quad (8)$$

where η_{win} , η_b , and η_s correspond to the refractive index for the window material, buffer solution, and sample solution, respectively. Refractive indices were computed for each wavelength by using the Malitson fit for sapphire¹⁵ and the Cauchy and Sellmeier dispersion relationships for water.¹²⁻¹⁴ In addition, the impact of glucose concentration on the refractive index was investigated for the glucose containing solutions.¹⁶⁻¹⁸

Accordingly, the measured absorbance for the buffer and sample (A_b and A_s , respectively) are given by the following expressions, where A_{buffer} and A_{sample} are the intrinsic absorbance values for these two solutions:

$$A_b = \log\left(\frac{P_1}{P_{2b}}\right) = \log\left[\frac{(1 - r_{aw})^2}{(1 - r_{bw})^2} 10^{A_{\text{buffer}}}\right]$$

$$= \log K_b + A_{\text{buffer}} \quad (9)$$

$$A_s = \log\left(\frac{P_1}{P_{2s}}\right) = \log\left[\frac{(1 - r_{aw})^2}{(1 - r_{sw})^2} 10^{A_{\text{sample}}}\right]$$

$$= \log K_s + A_{\text{sample}} \quad (10)$$

with

$$K_b = \frac{(1 - r_{aw})^2}{(1 - r_{bw})^2}, \quad K_s = \frac{(1 - r_{aw})^2}{(1 - r_{sw})^2}$$

and K_b and K_s are coefficients that describe the reflectance of light at the air/window/solution interfaces for the buffer and sample solutions, respectively.

Final equations for buffer and solute absorptivities are:

$$\epsilon_b = \frac{A_b - \log K_b}{c_w b} \quad (11)$$

$$\epsilon_s = \frac{(A_s - \log K_s) - (A_b - \log K_b) + \epsilon_w c_s f_w^s b}{c_s b}$$

$$= \frac{A_s - A_b + \epsilon_w c_s f_w^s b + 2 \log\left(\frac{1 - r_{sw}}{1 - r_{bw}}\right)}{c_s b} \quad (12)$$

Water Displacement Coefficients. Water displacement coefficients can be obtained from accurate density measurements of purified solvent and solutions composed of different solute concentrations.¹⁹ The solution density was measured for several concentrations of solute and the corresponding water displacement was calculated as the difference between the density of each solution and the sum of densities for the blank solvent and the solute concentration. For a narrow concentration range (i.e., 100 and 500 mM), the dependence of water displacement on solute concentration is linear. Slope of the corresponding linear plot gives the water displacement coefficient. Results of linear regression analysis are tabulated in Table I for each solute. Listed information includes the slope or water displacement coefficient (f_w^s), uncertainty of the slope ($SE-\beta_1$), standard error of the regression (SER), y-intercept (β_0), uncertainty of the y-intercept ($SE-\beta_0$), and correlation coefficient (R^2) between concentration of displaced water and concentration of the solute. The order of water displacement coefficients is triacetin > glucose > ascorbate > alanine \approx lactate > urea. Relative uncertainties in these measured values range from 0.05% for glucose to 0.46% for alanine, which provides high confidence for the accuracy of these values in subsequent calculations.

Absorptivity Measurements. Molar absorptivities were computed from Eqs. 11 and 12 from near-infrared spectra collected for a series of solutions. Absorptivity values for water and triacetin were obtained from a set of triplicate spectra collected from neat solutions. Values for glucose, lactate, urea, alanine, and ascorbate were obtained from a series of spectra corresponding to different

solute concentrations. For each solute, triplicate spectra were collected for solute concentrations of 100, 200, 300, 400, and 500 mM. Although these concentrations are much higher than the normal physiological range, they were used to provide high signal-to-noise ratios for the absorptivity calculations.

Results are presented in Tables II and III for the first overtone and combination spectral regions, respectively. Reported values for water and triacetin correspond to the mean (\pm standard deviation) for the triplicate spectra. Values for the other solutes correspond to the overall mean and pooled standard deviation for the five sets of triplicate spectra. The values are reported here with a point spacing of approximately 40 cm^{-1} resolution or 20 nm for combination spectra and 10 nm for first overtone spectra. Tabulated elsewhere is a full listing of computed molar absorptivities with a point spacing of approximately 1 cm^{-1} .²⁰

Molar absorptivity spectra are presented in Figs. 1A and 1B for water over the first overtone and combination spectral regions, respectively. These absorptivity values were calculated by using Eq. 11 with a water concentration of $55.138 \pm 0.001\text{ M}$, which was determined from density measurements at $37\text{ }^\circ\text{C}$. This water concentration is consistent with the value of 55.135 M reported by the National Institute of Standards and Technology at this temperature.²¹

Molar absorptivity spectra are plotted in Fig. 2 for glucose, lactate, urea, ascorbate, alanine, and triacetin. As shown in tables and figures, uncertainties in the molar absorptivity values increase at the wavelength extremes for both the first overtone (Fig. 2A) and combination (Fig. 2B) spectral regions. The strong absorption of water at the wavelength extremes significantly reduces the measurement signal-to-noise ratio,²² thereby increasing measurement uncertainty.

As indicated in Eq. 12, the need to correct for reflective losses is based on the magnitude of $\log K_b$ and $\log K_s$ relative to the measured absorbance values for the buffer (A_b) and solute solution (A_s), respectively. Our analysis indicates that $\log K_b$ values are approximately 7% and 4% of the measured buffer absorbance values in the combination and first overtone spectral ranges, respectively, thereby necessitating this correction. On the other hand, the value of $\log K_s$ depends on the refractive index of the sample solution, which depends on the solute concentration. Glucose has a particularly high impact on the refractive index of the solution and this effect can be estimated by the following equation:

$$\eta = 1.325 + 2.73 \times 10^{-5}[C_g] \quad (13)$$

where η is the refractive index for the solution and $[C_g]$ is the milli-molar concentration of glucose.¹⁸ At 400 mM glucose, $\log K_s$ is approximately 1% of absorbance measurements over the first overtone spectrum and less than 1% over the combination spectral range. Such values for glucose indicate that this correction term is negligible throughout.

As expected, molar absorptivity values are similar in magnitude for each of the tested solutes. Over the first overtone spectral range, absorptivity values are generally in the range of 2 to $7 \times 10^{-5}\text{ mM}^{-1}\text{mm}^{-1}$. The notable exception is urea, for which the absorptivity spectrum is

below $0.5 \times 10^{-5}\text{ mM}^{-1}\text{mm}^{-1}$ and is essentially flat over the entire first overtone spectral range. This finding is consistent with a lack of C–H bonds within the urea molecule. The expected N–H urea stretching overtone band should be centered around 1470 nm,^{23,24} which is masked by the strong water absorbance band centered at approximately 1450 nm.

Likewise, absorptivities over the combination spectral range are similar in magnitude for each solute. The highest absorptivity is displayed by triacetin. The characteristic three carbohydrate absorption bands for glucose are located at 2123, 2272, and 2325 nm (4710, 4400, and 4300 cm^{-1} , respectively). Overall, C–H combinations can be identified from approximately 2250 to 2400 nm, N–H combinations from 2150 to 2250 nm, and O–H combinations around 2100 nm. In addition, combination absorptivities are larger compared to first overtone absorptivities. Over the combination spectral range, absorptivities range from 0.8 to $3.2 \times 10^{-4}\text{ mM}^{-1}\text{mm}^{-1}$, which is a factor of four to five greater than first overtone absorptivities.

Absorptivity Accuracy. The absorptivity values reported here for water (Figs. 1A and 1B) compare favorably to those reported by others.^{1–6} For example, Bayly et al.¹ used a double-beam spectrometer to measure water absorptivities over a wide range of infrared wavelengths. We estimate from Fig. 1 in the Bayly paper that the molar absorptivity of water is $7.0 \times 10^{-6}\text{ mM}^{-1}\text{mm}^{-1}$ at both 1566 and 1800 nm. The corresponding values reported here are $7.075 (\pm 0.008) \times 10^{-6}$ and $7.078 (\pm 0.006) \times 10^{-6}\text{ mM}^{-1}\text{mm}^{-1}$, respectively. Similarly, molar absorptivities computed from the imaginary part of the refractive index of water values reported by Kou and Chylek² and by Hale and Query³ are within 10% of the values listed here. Values reported here are within 2.5% of those published by Bertie and Lan⁴ for the combination spectra and 7.8% for the first overtone spectra. These previous measurements were made at temperatures other than $37.0\text{ }^\circ\text{C}$, which likely explains the observed differences. Jensen, Bak, and Andersson-Engels⁵ report molar absorptivities for water at $37\text{ }^\circ\text{C}$ and our values are within 2.0% and 4.4% of these over the first overtone and combination ranges, respectively.

Accuracy of solute absorptivity values for the solutes is judged by comparing calculated spectra generated from these absorptivities to real spectra collected experimentally. Two sets of comparison spectra are presented. The first is a series of spectra corresponding to glucose dissolved in water. The second is a spectrum from a mixture of glucose, urea, and lactate.

Figure 3A shows a series of absorbance spectra collected for solutions composed of 0, 100, 300, and 500 mM glucose dissolved in water. These spectra span the first overtone spectral region and correspond to absorbance spectra relative to air. By using air as the reference material, this absorption spectrum is dominated by water and absorption features associated with glucose are not apparent. The glucose absorption spectrum is readily apparent, however, by subtracting the water spectrum from the glucose spectra (data not shown). The most interesting feature in this figure is the effect of glucose concentration on the measured absorbance. At wavelengths between 1520 and 1804 nm, the measured absorbance

TABLE II. Molar absorptivities over the first overtone spectral range at 37 °C. The absorptivity values and standard deviations are presented in $10^{-5} \text{ mM}^{-1}\text{mm}^{-1}$ units.

| Wavenumber cm^{-1} | Wavelength nm | Alanine | Ascorbate | Glucose | Lactate | Triacetin | Urea |
|--------------------------------|------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 5404.6 | 1850.3 | 1.37 ± 0.42 | 4.13 ± 0.53 | 2.73 ± 0.10 | 1.35 ± 0.30 | 1.30 ± 0.02 | 1.43 ± 0.39 |
| 5440.3 | 1838.1 | 2.37 ± 0.15 | 4.73 ± 0.22 | 3.55 ± 0.06 | 1.71 ± 0.12 | 1.23 ± 0.02 | 1.52 ± 0.18 |
| 5479.8 | 1824.9 | 2.65 ± 0.06 | 5.00 ± 0.10 | 3.78 ± 0.04 | 1.76 ± 0.06 | 1.27 ± 0.02 | 1.69 ± 0.08 |
| 5520.3 | 1811.5 | 2.98 ± 0.06 | 5.25 ± 0.06 | 4.18 ± 0.03 | 1.96 ± 0.03 | 1.29 ± 0.02 | 1.53 ± 0.06 |
| 5559.9 | 1798.6 | 3.22 ± 0.05 | 5.45 ± 0.05 | 4.63 ± 0.04 | 2.09 ± 0.03 | 1.38 ± 0.01 | 1.06 ± 0.07 |
| 5600.4 | 1785.6 | 3.58 ± 0.04 | 5.57 ± 0.04 | 5.03 ± 0.04 | 2.34 ± 0.03 | 1.58 ± 0.01 | 0.70 ± 0.07 |
| 5639.9 | 1773.1 | 3.89 ± 0.04 | 5.65 ± 0.04 | 5.18 ± 0.04 | 2.69 ± 0.03 | 2.23 ± 0.01 | 0.57 ± 0.07 |
| 5680.4 | 1760.4 | 4.26 ± 0.04 | 5.90 ± 0.04 | 5.15 ± 0.05 | 3.08 ± 0.03 | 2.60 ± 0.01 | 0.52 ± 0.07 |
| 5719.9 | 1748.3 | 4.77 ± 0.04 | 6.23 ± 0.04 | 5.06 ± 0.05 | 3.05 ± 0.03 | 2.60 ± 0.01 | 0.50 ± 0.07 |
| 5760.4 | 1736.0 | 4.81 ± 0.04 | 6.08 ± 0.04 | 4.95 ± 0.05 | 3.13 ± 0.03 | 3.60 ± 0.01 | 0.51 ± 0.07 |
| 5800.0 | 1724.1 | 4.68 ± 0.04 | 5.37 ± 0.04 | 4.76 ± 0.05 | 3.11 ± 0.03 | 5.60 ± 0.01 | 0.54 ± 0.07 |
| 5840.5 | 1712.2 | 4.96 ± 0.04 | 5.00 ± 0.05 | 4.61 ± 0.06 | 2.75 ± 0.03 | 5.28 ± 0.01 | 0.55 ± 0.07 |
| 5880.0 | 1700.7 | 4.91 ± 0.04 | 4.89 ± 0.05 | 4.58 ± 0.06 | 2.64 ± 0.04 | 3.16 ± 0.01 | 0.55 ± 0.07 |
| 5920.5 | 1689.0 | 4.74 ± 0.05 | 4.62 ± 0.05 | 4.63 ± 0.06 | 3.22 ± 0.04 | 2.92 ± 0.01 | 0.55 ± 0.07 |
| 5960.0 | 1677.8 | 4.37 ± 0.05 | 4.36 ± 0.05 | 4.46 ± 0.06 | 3.00 ± 0.04 | 4.96 ± 0.01 | 0.55 ± 0.07 |
| 5999.6 | 1666.8 | 4.57 ± 0.05 | 4.31 ± 0.05 | 4.56 ± 0.06 | 2.33 ± 0.04 | 3.38 ± 0.01 | 0.57 ± 0.07 |
| 6040.1 | 1655.6 | 4.38 ± 0.05 | 4.34 ± 0.05 | 4.78 ± 0.06 | 2.08 ± 0.04 | 1.64 ± 0.01 | 0.58 ± 0.08 |
| 6079.6 | 1644.8 | 4.32 ± 0.05 | 4.42 ± 0.05 | 5.06 ± 0.07 | 2.05 ± 0.04 | 0.99 ± 0.01 | 0.59 ± 0.08 |
| 6120.1 | 1634.0 | 4.44 ± 0.05 | 4.54 ± 0.05 | 5.40 ± 0.07 | 2.09 ± 0.04 | 0.68 ± 0.01 | 0.58 ± 0.08 |
| 6159.6 | 1623.5 | 4.59 ± 0.05 | 4.67 ± 0.05 | 5.75 ± 0.07 | 2.17 ± 0.04 | 0.46 ± 0.01 | 0.53 ± 0.08 |
| 6200.1 | 1612.9 | 4.75 ± 0.05 | 4.80 ± 0.05 | 6.11 ± 0.07 | 2.26 ± 0.05 | 0.34 ± 0.01 | 0.49 ± 0.08 |
| 6239.7 | 1602.7 | 4.89 ± 0.05 | 4.91 ± 0.05 | 6.44 ± 0.07 | 2.32 ± 0.05 | 0.28 ± 0.01 | 0.45 ± 0.07 |
| 6280.2 | 1592.3 | 5.00 ± 0.06 | 5.01 ± 0.05 | 6.74 ± 0.07 | 2.40 ± 0.05 | 0.24 ± 0.01 | 0.44 ± 0.07 |
| 6320.7 | 1582.1 | 5.09 ± 0.06 | 5.07 ± 0.05 | 6.99 ± 0.07 | 2.48 ± 0.06 | 0.22 ± 0.01 | 0.48 ± 0.08 |
| 6360.2 | 1572.3 | 5.11 ± 0.06 | 5.07 ± 0.05 | 7.17 ± 0.07 | 2.53 ± 0.06 | 0.21 ± 0.01 | 0.55 ± 0.08 |
| 6399.7 | 1562.6 | 5.01 ± 0.06 | 4.98 ± 0.05 | 7.25 ± 0.07 | 2.54 ± 0.07 | 0.22 ± 0.01 | 0.66 ± 0.08 |
| 6440.2 | 1552.7 | 4.79 ± 0.06 | 4.76 ± 0.05 | 7.23 ± 0.07 | 2.47 ± 0.08 | 0.23 ± 0.01 | 0.84 ± 0.08 |
| 6479.8 | 1543.3 | 4.50 ± 0.07 | 4.49 ± 0.05 | 7.19 ± 0.07 | 2.34 ± 0.09 | 0.24 ± 0.01 | 0.97 ± 0.09 |
| 6520.3 | 1533.7 | 4.22 ± 0.06 | 4.24 ± 0.04 | 7.33 ± 0.07 | 2.25 ± 0.10 | 0.28 ± 0.01 | 1.04 ± 0.08 |
| 6557.9 | 1524.9 | 4.05 ± 0.11 | 4.05 ± 0.08 | 7.51 ± 0.06 | 2.28 ± 0.18 | 0.32 ± 0.01 | 1.22 ± 0.11 |

TABLE III. Molar absorptivities over the combinational spectral range at 37 °C. The absorptivity values and standard deviations are presented in $10^{-5} \text{ mM}^{-1} \text{ mm}^{-1}$ units.

| cm^{-1} | nm | Alanine | Ascorbate | Glucose | Lactate | Triacetin | Urea |
|------------------|--------|-------------|------------|--------------|--------------|---------------|--------------|
| 3999.7 | 2500.2 | 66.2 ± 8.8 | 68.3 ± 15 | 66.3 ± 20.1 | 60.19 ± 12.1 | 17.09 ± 0.04 | 140 ± 7.17 |
| 4040.2 | 2475.1 | 73.2 ± 7.4 | 80.9 ± 14 | 82.5 ± 22.4 | 81.72 ± 11.3 | 22.76 ± 0.03 | 176.2 ± 10.1 |
| 4079.7 | 2451.1 | 40.9 ± 4.3 | 54.4 ± 1.8 | 42.1 ± 3.2 | 46.74 ± 4.3 | 17.44 ± 0.03 | 83.84 ± 5.99 |
| 4120.2 | 2427.0 | 10.6 ± 0.6 | 32.6 ± 0.6 | 25.05 ± 0.7 | 21.08 ± 0.43 | 13.58 ± 0.02 | 30.16 ± 0.81 |
| 4159.8 | 2404.0 | 3.9 ± 0.3 | 24.3 ± 0.2 | 19.24 ± 0.2 | 12.79 ± 0.1 | 16.85 ± 0.00 | 17.93 ± 0.11 |
| 4200.3 | 2380.8 | 3.9 ± 0.2 | 19.3 ± 0.1 | 15.74 ± 0.1 | 8.717 ± 0.08 | 20.26 ± 0.00 | 13.34 ± 0.16 |
| 4239.8 | 2358.6 | 5.5 ± 0.1 | 16.6 ± 0.1 | 15.48 ± 0.1 | 7.64 ± 0.04 | 25.8 ± 0.00 | 11.21 ± 0.13 |
| 4280.3 | 2336.3 | 7.3 ± 0.1 | 14.4 ± 0.1 | 15.57 ± 0.08 | 7.17 ± 0.04 | 23.58 ± 0.01 | 9.855 ± 0.08 |
| 4319.8 | 2314.9 | 10.1 ± 0.1 | 12.9 ± 0.1 | 13.77 ± 0.07 | 7.895 ± 0.03 | 22.67 ± 0.01 | 9.086 ± 0.07 |
| 4360.3 | 2293.4 | 13.5 ± 0.1 | 12.1 ± 0.1 | 11.30 ± 0.06 | 10.22 ± 0.04 | 24.09 ± 0.00 | 9.227 ± 0.05 |
| 4399.9 | 2272.8 | 11.2 ± 0.1 | 13.9 ± 0.1 | 12.86 ± 0.06 | 7.574 ± 0.04 | 25.79 ± 0.01 | 9.959 ± 0.04 |
| 4440.4 | 2252.1 | 12.57 ± 0.1 | 11.7 ± 0.1 | 10.33 ± 0.06 | 7.83 ± 0.04 | 31.93 ± 0.03 | 11.17 ± 0.04 |
| 4479.9 | 2232.2 | 14.3 ± 0.1 | 11.2 ± 0.1 | 9.83 ± 0.05 | 5.607 ± 0.04 | 12.8 ± 0.01 | 13.32 ± 0.04 |
| 4520.4 | 2212.2 | 12.38 ± 0.1 | 12.6 ± 0.1 | 11.34 ± 0.06 | 6.164 ± 0.04 | 5.659 ± 0.01 | 16.71 ± 0.04 |
| 4559.9 | 2193.0 | 13.64 ± 0.1 | 14.3 ± 0.1 | 13.39 ± 0.06 | 7.266 ± 0.04 | 3.869 ± 0.01 | 18.17 ± 0.04 |
| 4600.4 | 2173.7 | 15.11 ± 0.1 | 16.1 ± 0.1 | 15.60 ± 0.07 | 8.38 ± 0.05 | 3.483 ± 0.01 | 15.81 ± 0.05 |
| 4640.0 | 2155.2 | 16.96 ± 0.1 | 17.9 ± 0.1 | 17.79 ± 0.07 | 9.399 ± 0.05 | 3.578 ± 0.01 | 15.93 ± 0.05 |
| 4680.5 | 2136.5 | 18.7 ± 0.1 | 18.9 ± 0.1 | 20.04 ± 0.09 | 10.29 ± 0.06 | 7.276 ± 0.005 | 11.32 ± 0.06 |
| 4720.0 | 2118.6 | 19.36 ± 0.1 | 19.5 ± 0.1 | 21.78 ± 0.11 | 10.96 ± 0.07 | 6.524 ± 0.003 | 8.831 ± 0.07 |
| 4760.5 | 2100.6 | 18.6 ± 0.1 | 19.7 ± 0.1 | 22.40 ± 0.13 | 11.44 ± 0.08 | 5.706 ± 0.001 | 9.392 ± 0.09 |
| 4800.0 | 2083.3 | 16.78 ± 0.2 | 19.3 ± 0.2 | 21.17 ± 0.16 | 11.53 ± 0.1 | 3.133 ± 0.004 | 11.29 ± 0.12 |
| 4840.5 | 2065.9 | 14.02 ± 0.2 | 18.3 ± 0.2 | 18.51 ± 0.2 | 11.25 ± 0.14 | 2.133 ± 0.004 | 13.76 ± 0.13 |
| 4880.1 | 2049.2 | 10.63 ± 0.4 | 17.1 ± 0.2 | 15.21 ± 0.19 | 10.8 ± 0.13 | 1.573 ± 0.01 | 16.47 ± 0.16 |
| 4919.6 | 2032.7 | 7.1 ± 0.7 | 16.1 ± 0.4 | 12.56 ± 0.54 | 10.95 ± 0.41 | 1.336 ± 0.01 | 21.36 ± 0.39 |
| 4960.1 | 2016.1 | 3.6 ± 1.4 | 17.2 ± 1.4 | 14.35 ± 1.88 | 12.34 ± 1.02 | 1.266 ± 0.01 | 27.38 ± 0.59 |
| 4999.6 | 2000.1 | -10.5 ± 2 | 16.7 ± 4.3 | 21.16 ± 3.11 | 9.651 ± 3.19 | 1.567 ± 0.01 | 5.487 ± 2.24 |

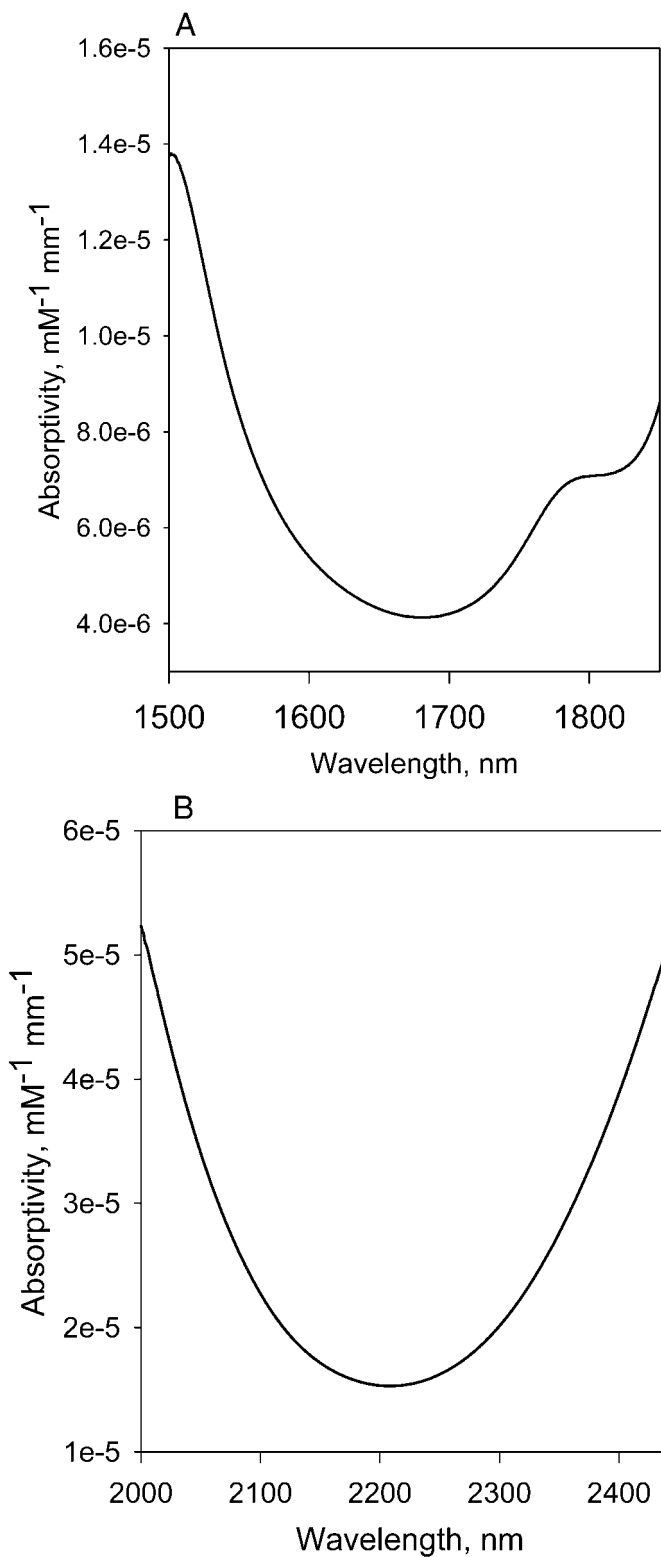


FIG. 1. Molar absorptivity spectra of water at 37.0 ± 0.1 °C over (A) the first overtone and (B) the combination spectral regions of the near-infrared spectrum.

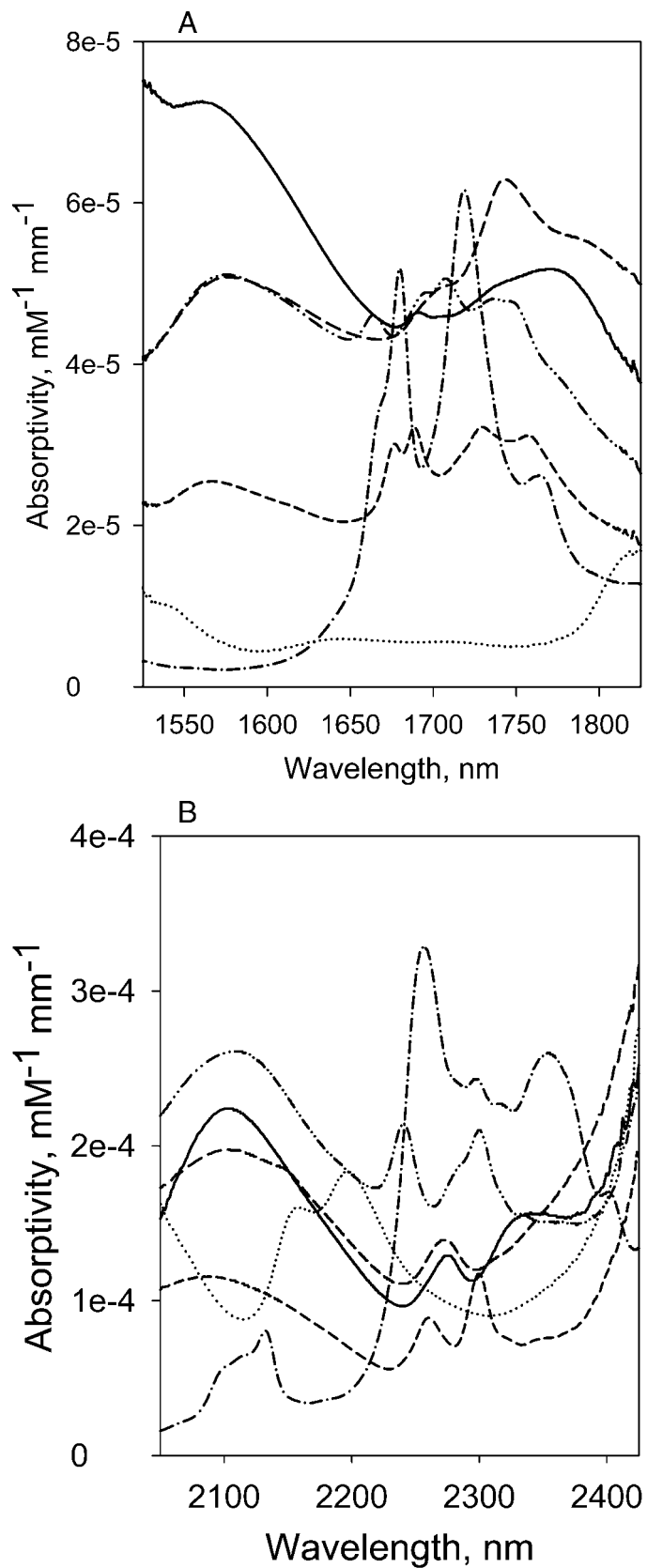


FIG. 2. Molar absorptivity spectra of glucose (solid), alanine (dash-dot-dot), ascorbate (medium dash), lactate (short dash), urea (dotted), and triacetin (dash-dot) at 37.0 ± 0.1 °C over (A) the first overtone and (B) the combination spectral regions of the near-infrared spectrum.

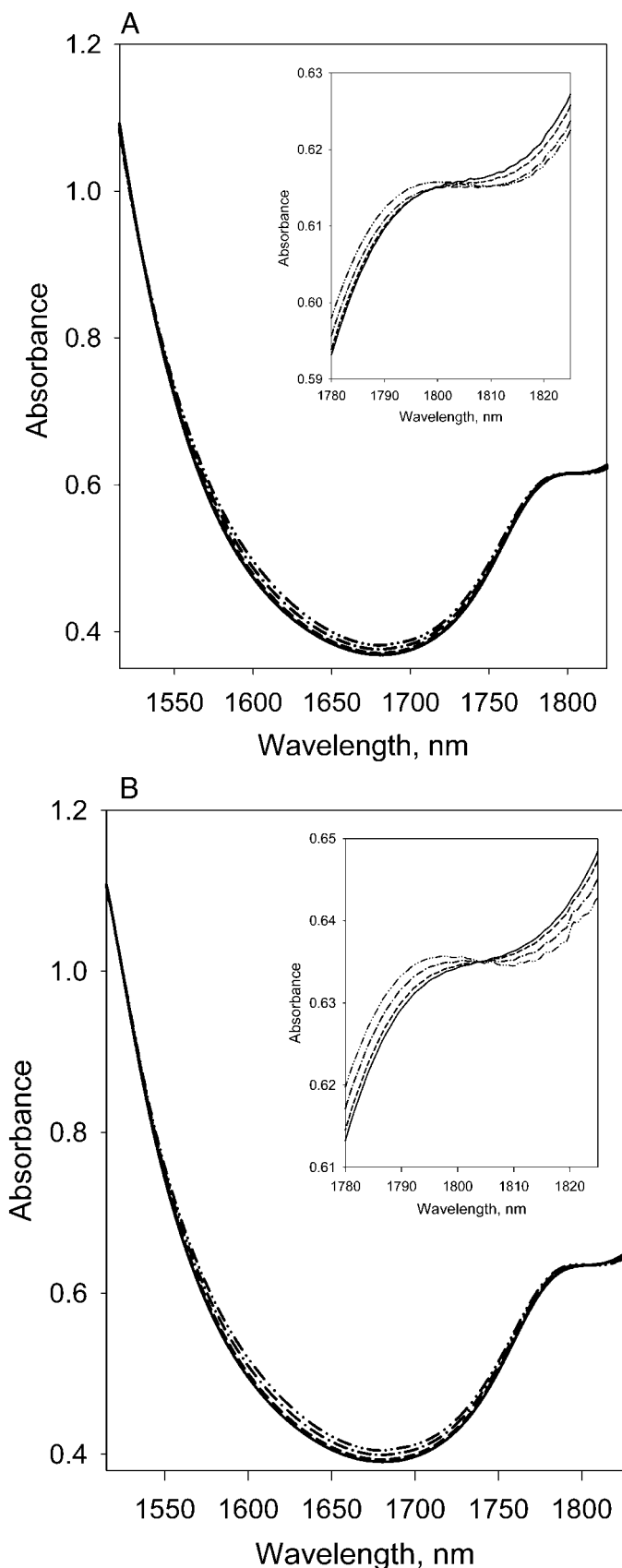


FIG. 3. (A) Actual and (B) calculated absorbance spectra relative to air for 0 (solid), 100 (dash), 300 (dash-dot), and 500 (dash-dot-dot) mM glucose solutions. Inset provides an expanded view over 1780–1825 nm.

increases as a function of glucose concentration, in accordance with the Beer–Lambert law. At wavelengths less than 1520 nm and greater than 1804 nm, however, the absorbance decreases as a function of glucose concentration. This apparent inversion of the Beer–Lambert law is explained by the relative magnitude of the molar absorptivities of water and glucose and the displacement of water molecules by the dissolution of glucose.

Figure 3B shows computed first overtone spectra for an analogous set of solutions composed of 0, 100, 300, and 500 mM glucose. These spectra were generated from Eq. 11 and Eq. 12 with molar absorptivities taken from Table II. The computed spectra match the actual spectra in terms of the increase and decrease in absorbance as a function of glucose concentration. The inset within Figs. 3A and 3B provides an expanded view of the spectral region between 1780 and 1825 nm. The inset highlights an isosbestic point at 1804 nm, which corresponds to the point where the absorptivity for glucose equals the product of the absorptivity for water and the water displacement coefficient for glucose ($\epsilon_s = \epsilon_w \cdot f_w^s$). The same condition holds at 1520 nm. For all wavelengths between 1520 and 1804 nm, the glucose absorptivity is greater than the product of the water absorptivity and the water displacement coefficient ($\epsilon_s > \epsilon_w \cdot f_w^s$). The net effect is a positive absorption of the incident light as a function of glucose concentration. For shorter and longer wavelengths, the absorptivity for glucose is less than the product of the water absorptivity and the water displacement coefficient ($\epsilon_s < \epsilon_w \cdot f_w^s$), thereby resulting in a net decrease in absorbance with an increase in glucose concentration.

Figure 4 shows the relationship between the absorptivity of glucose and the product of water absorptivity and the water displacement coefficient for glucose. The points of equality are evident. A corresponding plot is presented in Fig. 4B for glucose over the combination spectral region. For combination spectra, the glucose absorptivity equals the product of water absorptivity and the water displacement coefficient for glucose at 2066, 2233, 2250, 2286, 2314, 2334, and 2443 nm. These points can be observed in Fig. 4B. Analogous plots are unique for each solute and depend on differences in solute absorptivity values and the water displacement coefficients.

Accuracy of the absorptivity values was also evaluated by comparing measured and calculated absorbance spectra for a mixture of glucose, urea, and lactate dissolved in water. These spectra were obtained over the combination spectral range and correspond to a solution composed of 209 mM glucose, 200 mM urea, and 228 mM lactate. The measured and calculated spectra can be compared in Fig. 5. The excellent similarity in the overall shape and magnitude of these absorbance spectra validates these molar absorptivities. Deviations between measured and computed spectra are less than 1% between 2100 and 2300 nm. Larger variations are noted outside this range. These systematic variations likely correspond to slight differences between the sample and reference spectra, such as slight changes in instrument alignment, source intensity, or sample temperature. No attempt was made to incorporate this type of instrumental variation in the computed absorbance spectra.

It is important to realize that the 1% difference noted

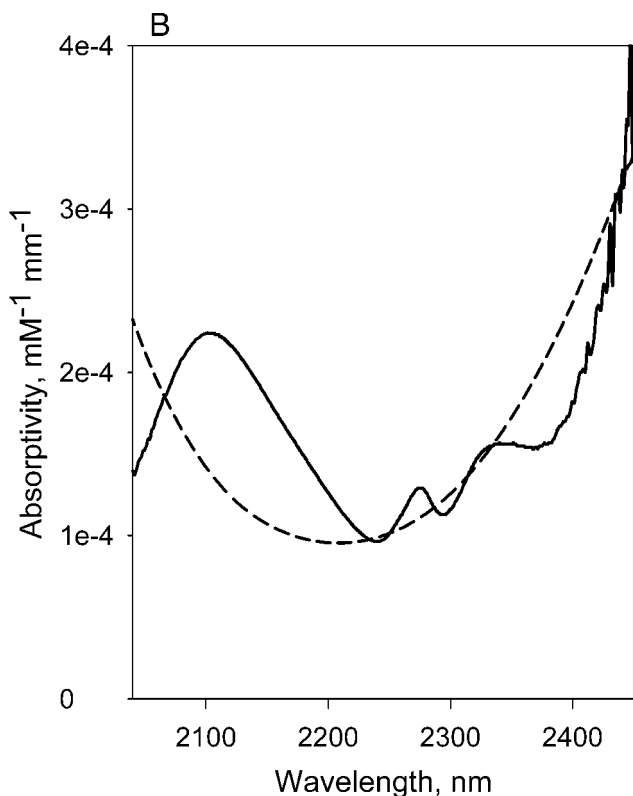
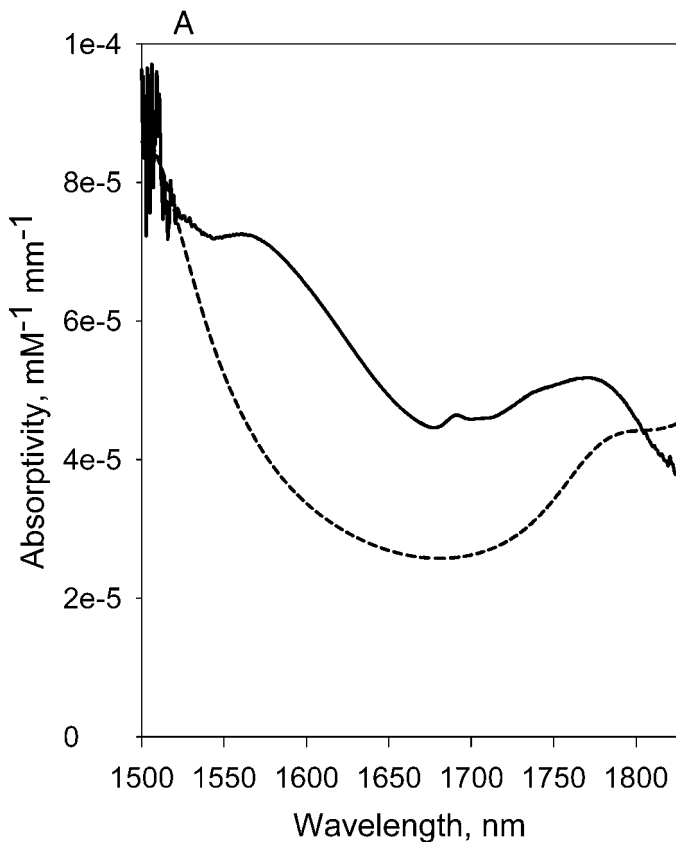


FIG. 4. Absorptivity plots for glucose (solid) superimposed on plots of the product of water absorptivity and water displacement coefficient (dash) for glucose over (A) the first overtone and (B) the combination spectral regions.

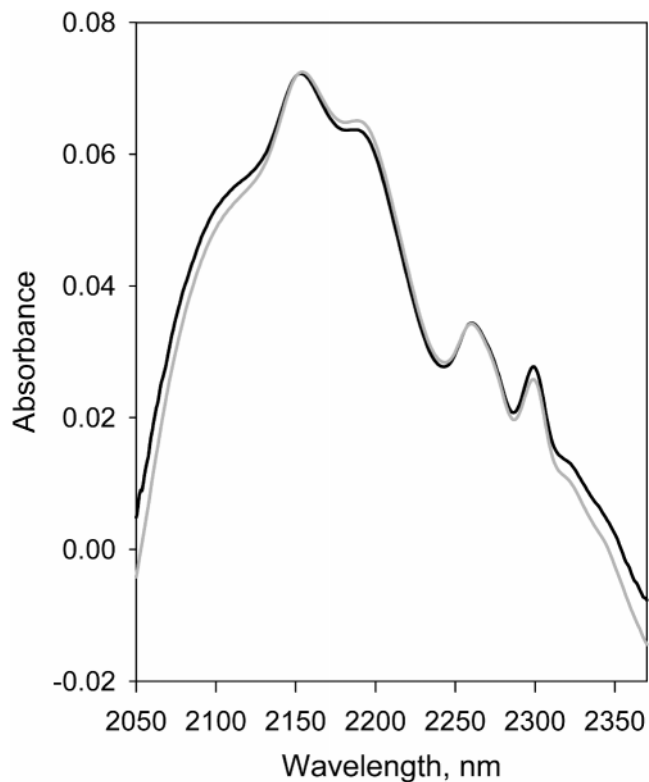


FIG. 5. Measured (black) and calculated (gray) absorbance spectra, relative to water, for a solution composed of glucose (209 mM), urea (200 mM), and lactate (228 mM) dissolved in water.

above between calculated and measured spectra corresponds to the accuracy of the calculation. Although a 1% change in signal can be huge when extracting glucose concentration information from such spectra, the 1% denoted above does not correspond to a change in signal but a difference in spectra. From the standpoint of a near-infrared analysis, the principal feature is the shape of the absorption spectra and the underlying net analyte signal.²⁵ Figure 5 clearly indicates that the calculated spectrum has the same basic shape as the measured spectrum and, most importantly, the calculation accurately accounts for water displacement effects.

CONCLUSION

Molar absorptivities of molecules dissolved in aqueous solutions are critical for the advancement of noninvasive near-infrared spectroscopy. Such values provide a means to model spectra accurately, thereby providing a powerful tool for defining and characterizing the instrumental, physical, and chemical constraints on noninvasive measurements in complex matrices. In addition, this work underscores the importance of water displacement in terms of distinctive features within absorbance spectra collected over these spectral regions. A particularly important feature is the net positive and negative absorption of light, which depends on the relative magnitude of the molar absorptivity of the solute compared to the product of the water displacement coefficient for this solute and the molar absorptivity of water.

The methodologies detailed in this paper can be used to determine molar absorptivities for additional molecules

of biological and clinical significance. Our objective is to establish a library of molar absorptivities that can be used to advance the development of near-infrared spectroscopy for noninvasive analytical measurements in complex biological matrices.

ACKNOWLEDGMENTS

Density measurements were made in Professor John Weincek's laboratory in the Department of Chemical and Biochemical Engineering and his time and efforts are greatly appreciated. In addition, funding from the National Institutes of Health (DK-060657) and the National Aeronautics and Space Administration (NAG8-1835) supported these research efforts and this support is greatly appreciated.

1. J. G. Bayly, V. B. Kartha, and W. H. Stevens, *Infrared Phys.* **3**, 211 (1963).
2. L. Kou, D. Labrie, and P. Chylek, *Appl. Opt.* **32**, 3531 (1993).
3. G. M. Hale and M. R. Querry, *Appl. Opt.* **12**, 555 (1973).
4. J. E. Bertie and Z. Lan, *Appl. Spectrosc.* **50**, 1047 (1996).
5. P. S. Jensen, J. Bak, and S. Andersson-Engels, *Appl. Spectrosc.* **57**, 28 (2003).
6. K. F. Palmer and D. Williams, *J. Opt. Soc. Am.* **64**, 1107 (1974).
7. M. A. Arnold and G. W. Small, *Anal. Chem.* **62**, 1457 (1990).
8. K. H. Hazen, M. A. Arnold, and G. W. Small, *Appl. Spectrosc.* **52**, 1597 (1998).
9. M. J. Mc Shane and G. L. Coté, *Appl. Spectrosc.* **52**, 1073 (1998).
10. M. R. Riley, H. M. Crider, M. E. Nite, R. A. Garcia, J. Woo, and R. M. Wegge, *Biotechnol. Prog.* **17**, 376 (2001).
11. J. Burmeister, M. A. Arnold, and G. W. Small, *Diabetes Technol. Ther.* **2**, 5 (2000).
12. J. Rheims, J. Koser, and T. Wriedt, *Meas. Sci. Technol.* **8**, 601 (1977).
13. M. Medhat, S. Y. El-Zaiat, and M. El-Desouky, *Pure Appl. Opt. J. Eur. Opt. Soc., A* **5**, 967 (1996).
14. P. D. Huijbers, *Appl. Opt.* **36**, 3785 (1997).
15. I. H. Malitson, *J. Opt. Soc. Am.* **52**, 1377 (1962).
16. R. C. Weast, *Handbook of Chemistry and Physics* (CRC Press, Boca Raton, FL, 1986), 6th ed., p. E-371.
17. M. Kohl, M. Cope, M. Essenpreise, and D. Bocker, *Opt. Lett.* **19**, 2170 (1994).
18. J. S. Maier, S. A. Walker, S. Fantitni, M. A. Francheschini, and E. Gratton, *Opt. Lett.* **19**, 2062 (1994).
19. R. C. Weast, *Handbook of Chemistry and Physics* (CRC Press, Boca Raton, FL, 1986), 6th ed., p. D-219.
20. <http://www.s-a-s.org/journal/supplemental>.
21. F. E. Jones and G. L. Harris, *J. Res. Nat. Inst. Stand. Technol.* **97**, 335 (1992).
22. K. H. Hazen, M. A. Arnold, and G. W. Small, *Appl. Spectrosc.* **52**, 1597 (1998).
23. C. V. Eddy and M. A. Arnold, *Clin. Chem.* **47**, 1279 (2001).
24. J. T. Olesberg, B. Armitage, M. A. Arnold, and M. J. Flanagan, *SPIE Proc.* **4624**, 95 (2002).
25. A. Lorber, K. Faber, and B. Kowalski, *Anal. Chem.* **69**, 1620 (1997).