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Near-Infrared Transmission Spectroscopy of Aqueous Solutions: Influence of Optical Pathlength on Signal-to-Noise Ratio

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The optimal choice of optical pathlength, source intensity, and detector for near-infrared transmission measurements of trace components in aqueous solutions depends on the strong absorption of water. In this study we examine under which experimental circumstances one may increase the pathlength to obtain a measurement with higher signal-to-noise ratio. The noise level of measurements at eight different pathlengths from 0.2 to 2.0 mm of pure water and of 1 g/dL aqueous glucose signals were measured using a Fourier transform near-infrared spectrometer and a variable pathlength transmission cell. The measurements demonstrate that the noise level is determined by the water transmittance. The noise levels in the spectral region from 5000 to 4000 cm^{-1} show that the optimal pathlength (0.4 mm) is the same for pure water and 1 g/dL aqueous glucose solutions. When detector saturation occurs it is favorable to increase the pathlength instead of attenuating the light source. The obtained results are explained by an analytical model.

Index Headings: **Near-infrared spectroscopy; FT-NIR; Instrument configuration; Transmission; Optimal pathlength; Scattering; Detector saturation.**

INTRODUCTION

Quantitative transmission near- and mid-infrared spectroscopy of liquids requires careful configuration of the instrumentation when a precise measurement of trace component signals is intended. Historically, quantitative analysis has been carried out with instrumental configurations in which detectors, infrared sources, and the liquid transmission cell pathlength were more or less given. On- and in-line applications of infrared spectroscopic methods for process and quality control has posed new questions concerning the choice of optimal instrumental configuration. The same questions arise when infrared spectroscopic methods are tested in the biomedical field for non-invasive measurements of glucose and other biomolecules. Such applications stress the need for optimization to achieve the necessary ability to measure small concentrations of trace components.

Two key parameters are the wavenumber region and the cell pathlength. Determinations of optimal pathlength and wavenumber region cannot be made independently, however. In a given wavenumber region, the strong absorption features of the solvent determine the pathlengths that may be used for precise measurements. Therefore, the selection of an optimal wavenumber region from spectral data obtained with a fixed cell pathlength in a wavenumber region where strong solvent features are present must be carried out with great care. It is well known by researchers working in the near-infrared region that cell pathlength strongly influences signal-to-noise ratio (SNR).[1–4] The influence of pathlength on signal-to-noise ratio has recently been investigated and reported in the literature. In the work by Hazen et al.,[1] three different pathlengths (2.0, 5.2, and 10 mm) were tried, in the spectral region 6579–5540 cm^{-1}, to determine the optimal settings for the quantification of glucose in water. The wavenumber region, containing first overtone signals from glucose, was selected using an optical bandpass filter, and different light sources were used in the three cases. Based on a chemometric analysis, Hazen et al. concluded that the longest pathlength (10 mm) provided the lowest errors of prediction. These authors managed to determine glucose in very low concentrations; the standard error of prediction was 0.35 mM. Recently, Segtnan and Isaksson[3] studied the influence of pathlength, in the near-infrared region, on measurements of sugar content in water and fruit juice. Their primary interest was to determine the optimal measuring conditions in fluid flow systems. In such systems, the pathlength is of central importance. The authors found the lowest errors of prediction with a 1 mm pathlength using the spectral ranges 9091–5219 and 4695–4255 cm^{-1}. In their conclusion they state that a relationship between pathlength and prediction error has not yet been established and is very much desired. They ask if an optimal pathlength does indeed exist.

Inspired by the work done by these authors, we initiated experimental work to investigate the variation with optical pathlength of the signal-to-noise ratio of water and aqueous glucose absorbance spectra in the near-infrared spectral range. Our primary interest is to know the optimal pathlength for quantitative analysis of low concentrations of biomolecules in aqueous solutions. It is commonly assumed that the highest accuracy in the measurement of an absorbance signal is found when the absorbance is 1/ln 10.[5] In most experiments, this is not exactly true. The optimal pathlength is found when the absorbance is 0.4816. This has been shown by Cole[6] and has recently been discussed extensively by Mark and Griffiths.[7] The cell pathlength may of course be adjusted to provide the optimal absorbance value in a given narrow spectral range. Solvents, traditionally used in FT-IR spectroscopy, are chosen to have negligible absorption, and measurement of small solute concentrations, therefore, requires longer pathlengths in accordance with Beer's Law. For the measurement of trace components in aqueous solutions in the near-infrared, the solvent is strongly absorbing, however. Here, water, not the trace component, is the dominating absorber that determines

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the usable pathlength for a given spectral region. Moreover, the use of cooled sensitive detectors and strong sources of light in the near-infrared region may render short pathlengths useless because of detector saturation or analog-to-digital converter (ADC) overflow.

We determine the noise level at eight different pathlengths from 0.2 to 2.0 mm of measured pure water absorption spectra and of the signals from 1 g/dL glucose in an aqueous solution using a Fourier transform near-infrared spectrometer equipped with a variable pathlength liquid transmission cell. The measurements on pure water demonstrate that the noise level is determined by the water transmittance. Comparison of the noise levels in the spectral region from 5000 to 4000 cm^{-1} for pure water and aqueous 1 g/dL glucose solutions demonstrate that an optimal pathlength exists in both cases. We find that the optimal pathlength in the two cases are identical (0.4 mm) and depends only on the water absorption. Increasing the pathlength to measure small concentrations is therefore not a viable method. When detector saturation is encountered, however, it is demonstrated that it is favorable to increase the pathlength instead of attenuating the light source, thereby lowering the intensity reaching the detector.

We present an analytical model that explains the obtained experimental results. The model shows how an optimal pathlength may be chosen, given the absorption spectrum of water. It yields analytical expressions for the optimal pathlength and for the decrease in signal-to-noise ratio when the pathlength is chosen not to be optimal. The model clearly exposes the tradeoffs involved when detector saturation occurs. The effect of scattering is briefly discussed. Finally, the influence of low spectral noise on the predictive ability of multivariate calibration models is discussed.

THEORY

We assume the solvent, which may be any kind of liquid, to be the dominant absorber in the solution. The validity of this theory when the solution scatters light will be discussed separately below. This means that at a given wavenumber, the absorptivity of the solvent alone determines the SNR of the measurement. Our derivation follows and slightly extends the one given by Venyaminov and Prendergast.[8] The relevant signal in quantitative analysis is the absorbance of the solute. At a given wavenumber $\bar{\nu}$ and pathlength l the absorbance is given by Beer's Law:

$$A_s = \varepsilon_s c_s l \tag{1}$$

where ε_s is the molar absorptivity of the solute and c_s is the molar concentration of the solute.

Note that this proportionality between A_s and l is the only important characteristic of Beer's Law that is used to find the optimal pathlength. One may equally well wish to determine the absorbance of the solvent alone, or the absorbance of solvent and solute. This will change the prefactor $\varepsilon_s c_s$ and thereby, the SNR, but it will not change the pathlength at which the SNR is optimal.

The absorbance is calculated from the measured sample and reference intensities, I and I_0, as:

$$A_s = -\log_{10}(I/I_0) \tag{2}$$

By error propagation,[9] one finds that the noise, or error, σ_{A_s}, in the determination of the absorbance, is given by:

$$\sigma_{A_s}^2 = \sigma_I^2 \left(\frac{\partial A_s}{\partial I}\right)^2 + \sigma_{I_0}^2 \left(\frac{\partial A_s}{\partial I_0}\right)^2 \tag{3}$$

where σ_I and σ_{I_0} are the errors in I and I_0, respectively. This leads to:

$$\sigma_{A_s} = \frac{1}{\ln 10}\left(\frac{\sigma_I^2}{I^2} + \frac{\sigma_{I_0}^2}{I_0^2}\right)^{1/2} \tag{4}$$

In FT-IR spectroscopy the system is detector-noise limited such that the errors σ_I and σ_{I_0} are equal to the detector noise n_d. We note that the sample and reference measurement both contribute in the same way to the error in the determination of the absorbance. The noise of the reference measurement is ignored in common treatments,[5] which leads to an optimal value of the absorbance of 1/ln 10. The work by Cole[6] and Mark and Griffiths[7] shows that inclusion of the reference noise leads to an optimal value of the absorbance which is 0.4816 in situations where the reference is chosen to be an empty beam or an empty cell, such that I_0 may be treated as a constant in Eq. 4. In this case, I_0 is much larger than I, and it is easily seen from Eq. 4 that ignoring the reference noise only changes the optimal value of the absorbance slightly. An important observation is that if one uses an empty beam as reference, this signal has to be measurable. In an optimal setting, the interferogram of the reference just fills out the ADC of the FT-IR instrument. The insertion of an absorbing sample then attenuates the signal, which no longer fills out the ADC. If one is able to use a reference that closely resembles the sample and have sufficient light intensity to fill out the ADC when measuring the signal of this absorbing reference, then the error in determining the absorbance of the sample will be lower. In this case, I and I_0 are both as large as possible and nearly equal. In this case, it is a very good approximation that the two terms on the right hand side of Eq. 4 are equal. The measured absorbance spectra of pure water presented in this paper have been measured with an empty cell as reference. The absorbance spectra of aqueous glucose presented in this paper have been measured with a reference cell containing pure water set at the same pathlength as the sample cell. The reference measurement is therefore not constant in the latter case. In the former case, the results of Cole[6] and Mark and Griffiths[7] apply, and the derivation we present is an approximation. The assumption $I \approx I_0$ yields:

$$\sigma_{A_s} \simeq \frac{\sqrt{2}}{\ln 10}\frac{n_d}{I} \tag{5}$$

Hence, the absorbance noise, σ_{A_s}, is inversely proportional to the intensity reaching the detector, I, and directly proportional to the detector noise, n_d. The intensity reaching the detector, I, is directly proportional to the source intensity, I_{src}, and to the solvent transmittance, T_w, given by Beer's Law as:

$$T_w = 10^{-\varepsilon_w c_w l} \tag{6}$$

where ε_w is the molar absorptivity of the solvent and c_w is the molar concentration of the solvent. The index w has been chosen to reflect that water is the most com-

monly occurring solvent in food analysis and biomedical applications. So:

$$I = I_{\mathrm{src}} \times 10^{-\varepsilon_{\mathrm{w}} c_{\mathrm{w}} l} \tag{7}$$

We may express the SNR $= A_{\mathrm{s}}/\sigma_{A_{\mathrm{s}}}$, by combining Eqs. 1, 5, and 7, as

$$\mathrm{SNR} \approx \frac{\ln 10}{\sqrt{2}} I_{\mathrm{src}} \times n_{\mathrm{d}}^{-1} \times \varepsilon_{\mathrm{s}} c_{\mathrm{s}} l \times 10^{-\varepsilon_{\mathrm{w}} c_{\mathrm{w}} l} \tag{8}$$

By taking the derivative with respect to l and setting the result equal to zero, one finds a maximum at pathlength, l_{opt}, given by:

$$l_{\mathrm{opt}} \approx \frac{1}{\varepsilon_{\mathrm{w}} c_{\mathrm{w}} \ln 10} \tag{9}$$

Using Beer's Law for the absorbance of the solvent:

$$A_{\mathrm{w}} = \varepsilon_{\mathrm{w}} c_{\mathrm{w}} l \tag{10}$$

where A_{w} is the absorbance of the solvent at wavenumber $\bar{\nu}$ and pathlength l, we may write the following expression instead:

$$l_{\mathrm{opt}} \approx \frac{l}{A_{\mathrm{w}} \ln 10} \tag{11}$$

which expresses the optimal pathlength for a given wavenumber in terms of the absorbance at that wavenumber for a different choice of pathlength. Hence, the absorbance of the solvent at the optimal pathlength is:

$$A_{\mathrm{w,opt}} \approx 1/\ln 10 \approx 0.4343 \tag{12}$$

This criterion is identical to the one obtained when one ignores the error in the reference measurement. Inserting the optimal pathlength times a factor f, $f \times l_{\mathrm{opt}}$, in the expression for the SNR, Eq. 8, we obtain:

$$\mathrm{SNR}(f \times l_{\mathrm{opt}}) \approx \frac{\ln 10}{\sqrt{2}} I_{\mathrm{src}} \times n_{\mathrm{d}}^{-1} \times \varepsilon_{\mathrm{s}} c_{\mathrm{s}} \times f \times l_{\mathrm{opt}} \times \mathrm{e}^{-f} \tag{13}$$

Consequently, as the pathlength is changed by a factor f from the optimal, the SNR decreases by a factor:

$$\frac{\mathrm{SNR}(f \times l_{\mathrm{opt}})}{\mathrm{SNR}(l_{\mathrm{opt}})} \approx f \times \mathrm{e}^{-(f-1)} \tag{14}$$

This relation is shown in Fig. 1. We note that the SNR decreases to 74% of the optimal SNR when the pathlength is two times the optimal and to 41% when the pathlength is thrice the optimal. For comparison, the intensity, given by Eq. 7, may be written in a similar manner as:

$$I(f \times l_{\mathrm{opt}}) = I_{\mathrm{s}} \times \mathrm{e}^{-f} \tag{15}$$

yielding a change factor of:

$$\frac{I(f \times l_{\mathrm{opt}})}{I(l_{\mathrm{opt}})} = \mathrm{e}^{-(f-1)} \tag{16}$$

The intensity is seen to decrease more rapidly than the SNR, by a factor of f, as the pathlength is increased beyond the optimal.

Remark About Scattering Solutions. The above analysis assumes that the solution under investigation is non-scattering. Scattering complicates the situation to a considerable degree. In the case of multiple scattering, the pathlength is no longer well defined and the measured signal is very sensitive to the details of the measuring geometry. If, however, we assume that only collimated light is collected and that multiple scattering does not contribute to the collimated light, then the scattering process will introduce only an additional damping term. This means that the factor $\varepsilon_{\mathrm{w}} c_{\mathrm{w}}$ in the above expressions should be replaced by $\varepsilon_{\mathrm{w}} c_{\mathrm{w}} + \mu_{\mathrm{s}}$, where μ_{s} is a scattering coefficient. Under such circumstances, the scattering and absorption of the solvent both have the same effect, namely, damping of the intensity reaching the detector. The measured apparent absorbance spectrum, which now includes a contribution from the scattering, will still have the highest SNR when the measured apparent absorbance of the solvent is 1/ln 10. In any realistic experiment, a certain amount of scattered light is collected. A more precise treatment of this problem requires specification of instrument and sample geometry and optical parameters in each case of interest.

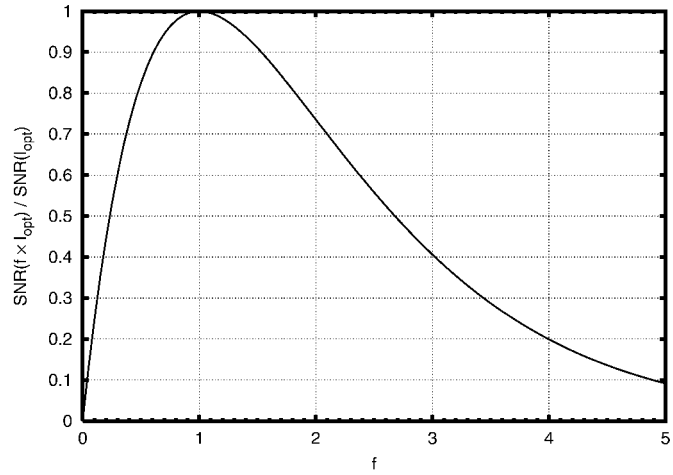

FIG. 1. Decrease factor for the SNR as a function of pathlength change factor f.

Importance of Low Noise in Multivariate Calibration. Multivariate calibration methods, such as principal component regression (PCR) or partial least squares (PLS), predict the concentration of a given solute by multiplication of the sample spectrum with a regression vector. This regression vector is the result of a calibration procedure based on a set of representative spectra. The concentration c of the solute, estimated from the spectrum, may be written as $c = \Sigma_i^n r_i s_i$, where r_i is the regression vector and s_i is the spectrum. Index i refers to the points in the spectrum and n to the number of points. Let us assume that the noise in the spectral points is uncorrelated and that the regression vector is much more accurate than the spectrum. This is reasonable because the regression vector is based on many individual spectra used in the calibration. By error propagation, we obtain that the uncertainty of the concentration σ_{c} is given by $\sigma_{\mathrm{c}}^2 = \Sigma_i^n r_i^2 \sigma_{s,i}^2$. If we simplify this expression by making the assumption that the s_i are about equal, s, that the $\sigma_{s,i}$ are about equal, σ_s, and that the r_i values are about equal, r, we obtain that $\sigma_{\mathrm{c}} \propto \sqrt{n} r \sigma_s$. It is a characteristic of the regression vector that it gives a low weight to points with

high noise in the spectrum since such points show poor correlation with the concentration of the solute. This property of the regression vector makes our simplification less rough than might be thought at first glance. The signal is equal to *nrs* under the same assumption. Therefore the prediction of a concentration from a multivariate calibration should be improved by the square root of the number of spectral points and be inversely proportional to the noise σ_s of the individual points. It is therefore desirable to have many spectral channels with signal from the solute and it is important to have as low a noise level in the measured spectra as possible.

EXPERIMENTAL

Pure Water. Measurement of pure water spectra was carried out using using a Bomem MB155 Fourier transform infrared (FT-IR) spectrometer equipped with a DTGS detector. The source was a 150 W quartz-halogen lamp. Measurements were performed at 8 cm^{-1} resolution. The transmission cell was a Specac 7000 variable pathlength cell with ZnSe windows. Temperature was set at 32 °C and controlled to within ±0.02 °C by a Eurotherm 4208 temperature control unit, which measured sample temperature through the cell-filling port with a PT100 thermo element and heated the cell by a nichrome wire wound around the cell body.

The following measurements were carried out: water was placed in the transmission cell set at a 2.0 mm pathlength and 32 replica spectra with 64 coadditions in each were measured. The cell pathlength was then reduced to 1.5 mm and 32 new replica spectra were measured. The procedure was repeated for pathlengths 1.0, 0.7, 0.5, 0.4, 0.3, and 0.2 mm. There were no other changes in instrument configuration. One measurement was taken on an empty 1.0 mm cell and used as a reference for all the measurements.

At each pathlength, the mean and standard deviation absorbance spectra were calculated from the 32 replica measurements. In this fashion, a statistical estimate of the signal and the noise level were obtained for each wavenumber point in the spectrum. According to Beer's Law, Eq. 1, the signal is proportional to the pathlength. To compensate for this dependency, the mean and standard deviation spectra were normalized by division with the pathlength to obtain equal signals at the eight different pathlengths. This normalization of the signal allows a direct comparison of the noise level at each measured wavenumber point for the eight wavelengths. With equal signal strength at all pathlengths, the SNR is then inversely proportional to the noise level such that the highest SNR is obtained when the noise level is lowest. In the Theory section, the assumption that the noise level is inversely proportional to the water transmittance was used. We wish to verify that assumption. Therefore, noise levels of the normalized data are presented instead of the equivalent SNRs.

To remove spectral variations caused by fluctuations in sample temperature and instrument drift, the above analysis was repeated on the first derivative spectra calculated from the absorbance spectra by finite centered differencing as $dA_i/d\bar{\nu}_i = (A_{i+1} - A_{i-1})/2$; where index i refers to a given wavenumber point.

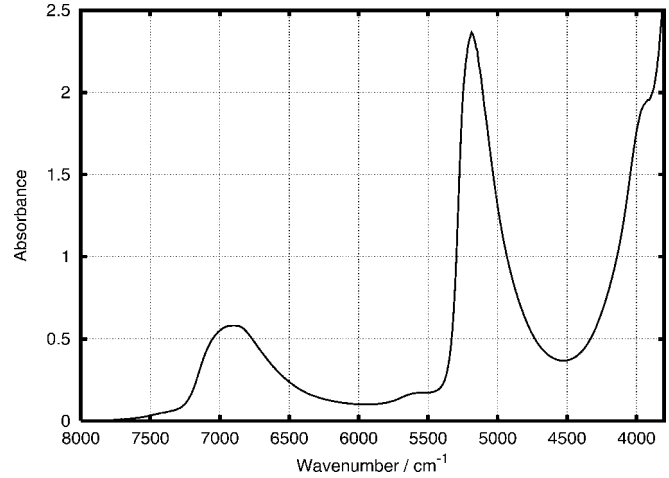

FIG. 2. Water absorbance at a pathlength of 0.4 mm and temperature of 30 °C.

Aqueous Glucose Solution. To demonstrate the decrease in signal-to-noise ratio with increasing pathlength at constant source intensity, in the case where one wishes to measure the small signal of an analyte in an aqueous solution, spectra of aqueous 1 g/dL glucose solutions were measured in the combination band region 5000–4000 cm^{-1} at 32 cm^{-1} resolution. The spectral resolution was chosen to comfortably resolve the spectral structure of the glucose signal, but no higher since this would degrade the SNR (assuming equal measuring times).[10] The source was attenuated by a factor of eight and a Peltier cooled InAs detector was employed. Without attenuation, the analog-to-digital converter of the detector will overflow at pathlengths below 1.0 mm. Thirteen replica spectra with 256 coadditions in each were measured at pathlengths of 2.0, 1.5, 1.0, 0.7, 0.5, 0.4, 0.3, and 0.2 mm. In each case, a pure water reference at the same pathlength was measured prior to the thirteen replicas. To illustrate the effect of increasing the source intensity, a data set (one pure water reference and 13 replica sample measurements) was measured at a 1.0 mm pathlength without attenuating the source. To isolate a glucose signal that obeys Beer's Law and to compensate for matrix effects and minor baseline shifts caused by temperature variations, second derivative absorbance spectra were formed by finite centered differencing as $d^2A_i/d\bar{\nu}_i^2 = (A_{i+1} - 2A_i + A_{i-1})/2$; where index i refers to a given wavenumber point.

For each pathlength, the mean and standard deviation second derivative absorbance spectra were calculated from the thirteen measured replicas. Again, the mean and standard deviation spectra were normalized by division with the pathlength to obtain equal signal for each of the pathlengths, thereby allowing a direct comparison of the noise levels.

RESULTS AND DISCUSSION

Pure Water. Figure 2 shows the near-infrared absorbance spectrum, A_w, of water at pathlength l = 0.4 mm. From this, we may calculate the optimal pathlength as a function of wavenumber using Eq. 11 or using the result of Cole[6] that the optimal absorbance is at 0.4816. The results of this calculation in the two cases are shown in

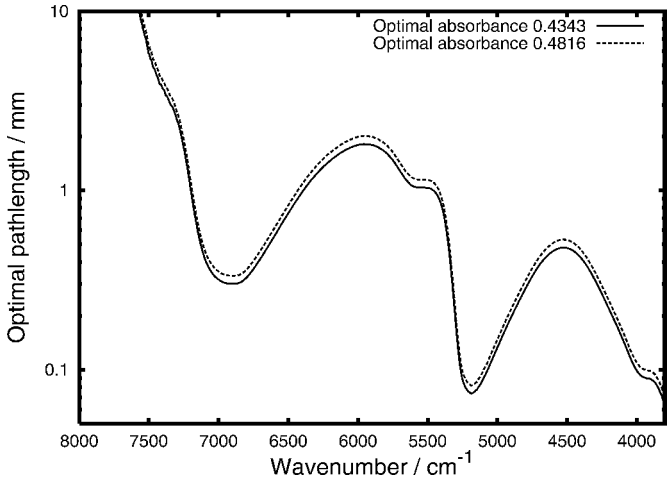

FIG. 3. Optimal pathlength for aqueous solutions as a function of wavenumber calculated from data in Fig. 2. The result is shown in the two cases, optimal absorbance 0.4343 or 0.4816.

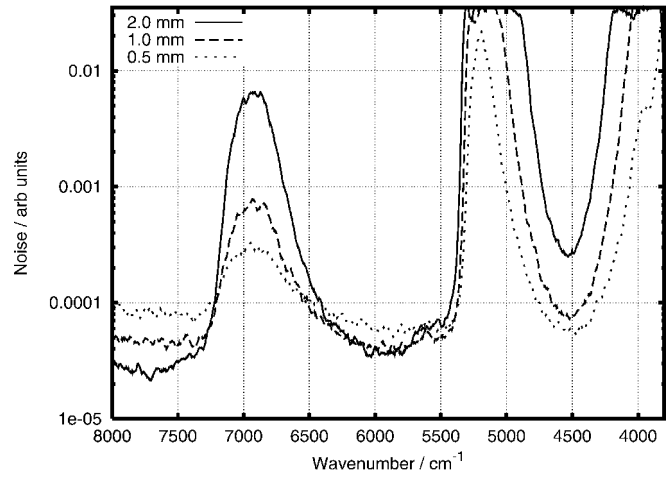

FIG. 5. Noise spectrum of pure water first derivative normalized absorbance spectra at 2.0, 1.0, and 0.5 mm pathlength, smoothed by a 15 point moving average.

Fig. 3 where it is observed that the optimal pathlength spans more than two decades in the spectral region shown. The difference between the two models is small compared with this variation. This figure shows that any single choice of pathlength will result in a very large variation in SNR in the near-infrared region. The variation in SNR can be understood from Fig. 1, which shows the decrease factor of the SNR as one changes the pathlength by a factor f away from the optimal pathlength setting. Figure 4 shows the noise spectra of the normalized absorbance spectra, which are inversely proportional to SNR, for pathlengths of 2.0, 1.0, and 0.5 mm. The spectra have been smoothed by a 15 point moving average for clarity. We observe that none of the pathlengths is optimal in the whole spectrum and that large variations in noise level may be found. Comparison with the absorbance spectrum of water, shown in Fig. 2, reveals that the noise level resembles the water absorbance spectrum as it is plotted on a semi-log scale. The agreement is not perfect, however. This is because the spectral characteristics of the detector and source influence the noise spectrum and, more interestingly, because small temperature changes of the sample between replica measurements alter the water spectrum of the sample. In particular, isosbestic points, such as the one at ~5700 cm^{-1}, have a markedly lower noise level.

The spectral variation caused by the temperature changes are distinguishable from noise in that it has a spectral structure such that the variation in neighboring points is highly correlated. Therefore, one may remove this variation by, e.g., taking the first derivative of the absorbance spectrum. Figure 5 shows the noise spectra of the normalized first derivative absorbance spectra for pathlengths of 2.0, 1.0, and 0.5 mm. Again, the spectra have been smoothed by a 15 point moving average for clarity. The noise spectra now closely resemble the water absorption spectra, in agreement with the assumption used in the Theory section that noise is inversely proportional to the water transmittance. We may use this information to plot the noise level as a function of pathlength at a given wavenumber. The result is shown in Fig. 6 for wavenumber regions 4600–4400, 6100–5900, 7100–6900, and 8000–7500 cm^{-1}. In all four cases, the pathlength with the lowest noise level is consistent with

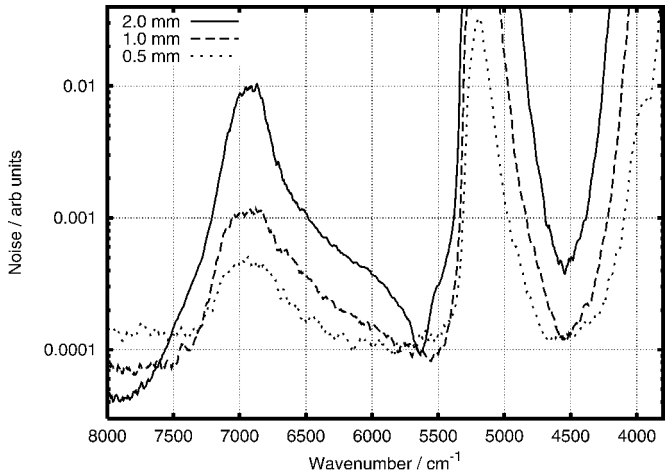

FIG. 4. Noise spectrum of pure water normalized absorbance spectra at 2.0, 1.0, and 0.5 mm pathlengths, smoothed by a 15 point moving average.

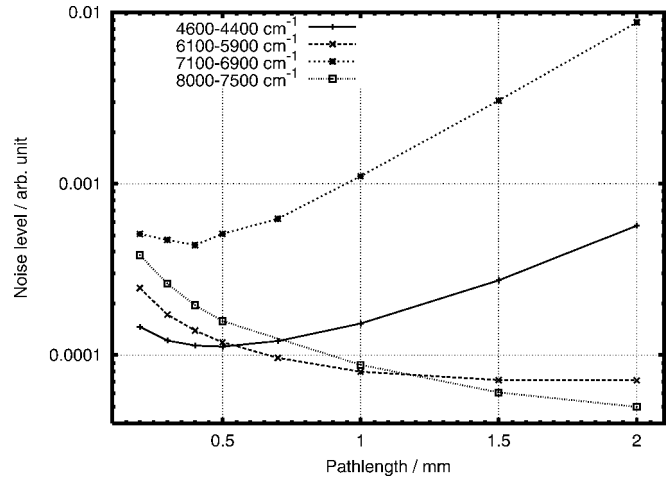

FIG. 6. Noise level as a function of pathlength for spectral regions 4600–4400, 6100–5900, 7100–6900, and 8000–7500 cm^{-1}.

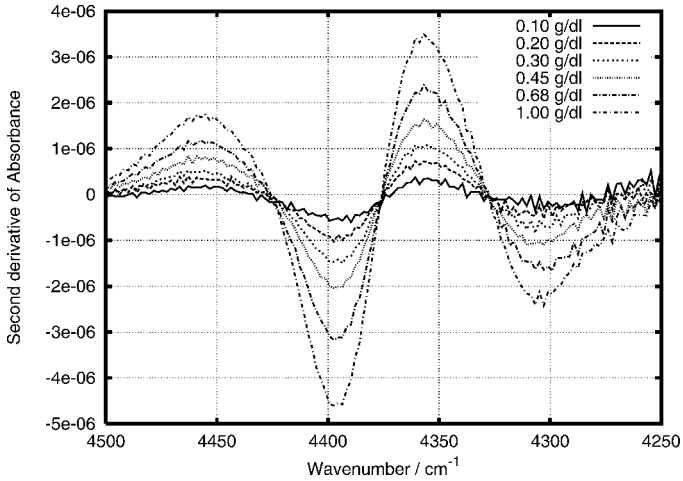

FIG. 7. Second derivative aqueous glucose spectra at 8 cm^{-1} resolution at concentrations of 1.0, 0.68, 0.45, 0.3, 0.2, and 0.1 g/dL measured with a 1.0 mm pathlength.

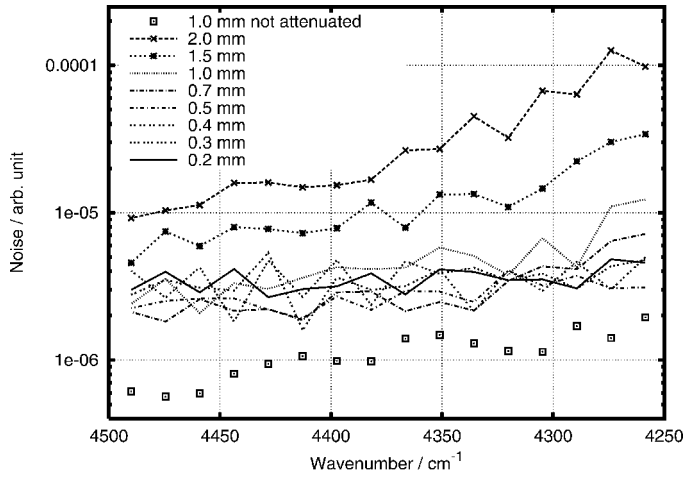

FIG. 8. Noise level of the normalized second derivative glucose absorbance spectra at pathlengths of 1.0 mm with no source attenuation (boxes), and noise level at 2.0, and 1.5 mm (symbol connected with lines), 1.0, 0.7, 0.5, 0.4, 0.3, and 0.2 mm (lines).

the predicted optimal pathlength shown in Fig. 3. In the highest wavenumber region, the investigated pathlengths are all less than the predicted optimal pathlength. The noise level in the spectral region 4600–4400 cm^{-1} is approximately ten times lower than the one in the spectral region 7100–6900 cm^{-1}. Both spectral regions have the same optimal pathlength. The difference is caused by the lower source intensity and detector sensitivity in the high wavenumber region. According to Eq. 13, SNR is proportional to source intensity, I_{src}, and inversely proportional to detector noise, n_d. The same factor of ten may be found by ratioing the intensity of the single-beam spectra in the two spectral regions.

Aqueous Glucose Solution. Figure 7 shows the second derivative glucose aborbance spectrum at a 1 mm pathlength for concentrations in the range 0–1 g/dL with pure water used as a reference. The second derivative glucose signal has positive and negative peaks at 4350 and 4400 cm^{-1}, respectively. This signal is seen to scale with concentration in accordance with Beer's Law.

Figure 8 shows the noise level of 1 g/dL glucose normalized second derivative absorbance spectra at pathlengths of 2.0, 1.5, 1.0, 0.7, 0.5, 0.4, 0.3, and 0.2 mm. The noise level for the glucose spectrum measured at 1 mm without attenuation of the light source is also shown. Plotting the mean noise level of each of the curves in Fig. 8, as shown in Fig. 9, we find that a pathlength of 0.5 mm provides the lowest noise level and therefore the highest SNR. The optimal pathlength in this spectral region predicted by theory is 0.4–0.5 mm according to Fig. 3. The experimentally determined value for measurements on pure water is 0.4 mm.

Comparing Fig. 9 with Fig. 1, which shows the decrease in SNR as the pathlength is changed by a factor f from the optimal, we observe that the noise level at the 2.0 mm pathlength is higher by a factor of ten than the optimal. According to Fig. 1, this means that the optimal pathlength is five times smaller, i.e., 0.4 mm. The difference in noise level between pathlengths of 0.3 and 0.7 mm is small, again in agreement with Fig. 1, which predicts a decrease in SNR of less than 30% when the pathlength is changed by less than a factor of 2 from the optimal. There is thus a good agreement between the noise behavior predicted by theory and the measured values. In particular, increasing pathlength does not improve the signal-to-noise ratio when the source intensity is constant.

We note that the lowest noise level is obtained at a 1.0 mm pathlength with no attenuation of the source. From theory, we expected a noise level that was eight times lower (the attenuation factor used) than the noise level of the attenuated 1.0 mm measurements. Our measurements show approximately a factor of five. The discrepancy may arise because other non-controlled variations begin to influence the measurements at this low noise level. Even so, these measurements show that increasing the pathlength is preferable to attenuating the source if saturation occurs at the optimal pathlength. This is in agreement with the theory. This result may explain the apparent contradiction between the results of Hazen et al. and Segtnan and Isaksson. The former group, working in the spectral region 6579–5540 cm^{-1}, where the optimal path-

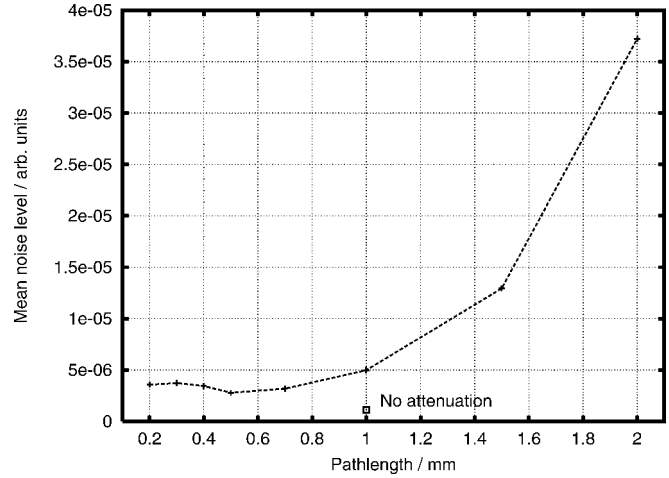

FIG. 9. Mean noise level in the spectral region 4500–4250 cm^{-1} as a function of pathlength calculated from Fig. 8. Source was attenuated for data points connected with lines. The source was not attenuated for the isolated data point (box) at a pathlength of 1 mm.

length is about 1 mm, found the lowest error of prediction for the longest pathlength (10 mm) among the three different pathlengths tried. Hazen et al. have selected a liquid nitrogen cooled, very sensitive detector and isolated the spectral region of interest. With a strong source of light, they would have experienced saturation at the optimal pathlength, but they were able to prevent this by increasing the pathlength to the point where the intensity reaching the detector is sufficiently low. Segtnan and Isaksson, on the other hand, worked in a much wider spectral region. Their instrument is less sensitive and does not saturate. They therefore found an optimal pathlength of about 1 mm, which is a compromise in the chosen spectral region and optimal in the region 6500–5500 cm^{-1}. A pathlength of 10 mm would favor only the highest wavenumbers in their chosen spectral range. In both cases, the best practical pathlength depends on the solvent absorption properties in the chosen spectral region, the light source, and the detector.

CONCLUSION

This article has treated the influence of transmission cell pathlength on SNR for near-infrared measurements of solutes in strongly absorbing solvents. Determination of noise levels in measurements of pure water and 1 g/dL aqueous glucose spectra has been carried out at different pathlengths. The measurements have been compared to an analytical model with good results. In particular, it has been demonstrated that the measurement of the small glucose signal superposed on the water absorption spectrum should be optimized by considering the absorption properties of water in the spectral region containing the glucose signal.

These results show that the water spectrum determines the noise level of a measurement of small solute concentrations in aqueous solutions. Pathlength is a critical parameter which effectively selects the usable spectral regions when measurement of small solute concentrations in strongly absorbing solvents is intended. This implies that a large spectral range, as is usually preferred for chemometric analysis, may be of little use in practice. On the other hand, it clearly shows how pathlength should be chosen when the spectral region containing the signals of interest is known and the source and detector are given. If the signals of interest lie in different spectral regions, the quality of the collected data may be greatly improved if measurements at several different pathlengths are possible. In addition, a comparison between optimal wavenumber regions, found from chemometric analysis, with the absolute value of the measured absorbance may be profitably employed to modify the experimental configuration to provide superior data. One may favor a significant spectral region, found from chemometric analysis, by selecting the pathlength that optimizes that region.

The influence of detector saturation on the choice of cell pathlength has been treated. When saturation occurs at the otherwise optimal pathlength, a reduction of the intensity reaching the detector by an increase in transmission cell pathlength results in better SNR than an attenuation of the light source intensity.

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