Determination of glucose concentration in aqueous solution using FT NIR spectroscopy

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Abstract. NIR spectroscopy is widely used due to its capability to measure a large number of solid and liquid samples, including water-soluble constituents. The measurements of glucose concentrations in aqueous solutions are useful to examine how low concentrations of glucose in water can be measured using near-infrared spectroscopy and its potential applications for non-invasive measurements of glucose level in the blood. This paper describes an alternative approach to the determination of glucose content in aqueous solutions below 1000 mg/dL using Fourier transform near-infrared spectroscopy. This technique has the advantage of being less intensive sample preparation and non-destructive. Glucose in aqueous solutions were carefully prepared with concentrations of 0 - 100 mg/dL at intervals of 5 mg/dL, 110 - 500 mg/dL at intervals of 10 mg/dL and 525 - 1000 mg/dL at intervals of 25 mg/dL. Thus, the total produces 81 samples of standard solutions for both calibration and validation sample sets. PLSR analysis to near-infrared spectra show that glucose content in aqueous solutions can be predicted accurately with a maximum deviation of 6 mg/dL, indicating that the near-infrared prediction model is sufficient to determine glucose content in the aqueous solutions below 1000 mg/dL. The ability of the NIR to detect glucose content below 1000 mg/dL is particularly important when designing a non-destructive glucose level measuring device using a near-infrared light source.

1. Introduction
Near-infrared spectroscopy (NIRS) has gain favor in recent years because of its ease in the workflow analysis and allows measurements of large quantities of samples. It is possible to measure the concentration of one or more constituents, and the single spectrum can be associated with its fingerprint [1]. Vibrations of the O-H, C-H and N-H bonds, where these arise in almost all substances containing covalent bonds of hydrogen, appear in the near-infrared region. The harmonic overtones absorptions of those vibrations are generally broader than their counterparts in the infrared (IR) region, and the intensities continue to decrease as the overtone order rises. Because IR is very sensitive to the O-H vibration mode, the constituents measurements in the aqueous solutions with IR become impossible. NIR allows the measurements of the concentrations of the constituents in aqueous solutions [2,3] since the intensity of the O-H overtone vibration mode decreases significantly.

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compared to the fundamentals in the IR region. On the other hand, the broadening of the overtone absorption bands and the combination of vibrations in the NIR region make the analysis absorption bands to be complicated and require more complex processes. However, the amenability of NIR to chemometrics makes it well-suited processes.

Samples containing functional groups such as OH, CH, and NH are susceptible to NIR because overtones of their fundamental vibrations (R-H) in the IR region correspond to the NIR absorptions. Although the C = C and C-C bonds do not appear in the NIR region, their C-H vibrations frequencies can reflect the C=C and C-C bond. Because all organic materials contain hydrogen bonds, NIR spectroscopy has been used widely in the pharmaceutical field such as discrimination and analysis of the medicinal components [1–3]. Simeone et al. [4] used NIR spectroscopy to measure sucrose, glucose, and fructose of sweet sorghum juice. Yano et al. [004] employed NIR spectroscopy for the simultaneous prediction of glucose and a citric acid aqueous solution of a blood anticoagulant. The validity in measuring blood glucose was then discussed by Zhang et al. [6] using two-dimensional correlation spectroscopy (2DCS) to improve the data analysis. Recently Saleh et al. [005] examined the glucometer design to measure glucose in blood non-invasively using NIR at a single wavelength. This technique is quite promising, but various other organic materials on the tissue affect the accuracy of the determination. In other words, it needs spectrum-based measurements. Efforts to advance non-invasive measurement of blood glucose continue to carry out, starting from a fundamental investigation of the NIR glucose spectrum [7,8]. Various measurement techniques were also developed, including NIR Raman spectroscopy [9,10], direct diagnostics using a chip NIR detector implanted beneath the skin [11,12], and the possibility of long-term continuous observation using wireless detector [13,14]. The NIRS approach with an analysis of various spectral range and other measurement techniques have also been studied [15] and analyzed using chemometric.

In this study, we examined the use of NIRS to determine glucose content in aqueous solution with a physiological range of concentrations from 0 to 1000 mg / dL. This examination is intended to see at the possibility of NIRS as an alternative approach to constructing non-invasive non-invasive blood glucose apparatus.

2. Methods

2.1. Sample Preparation
The D-glucose sample used in this study was ordered from Sigma-Aldrich with 99.5 purity without any treatment before use. A total of 81 samples D-glucose in distilled water were prepared with a concentration of 0-100 mg/dL with intervals of 5 mg/dL, 110-500 mg/dL with intervals of 10 mg/dL and 525-1000 mg /dL with intervals of 25 mg/dL. The D-glucose in each sample solution was completely dissolved by using a magnetic stirrer. The 81 samples were then divided into two groups: (i) samples with even concentration (consists 41 samples) were used for calibration and (ii) samples with odd concentrations (consists of 40 samples) were used for validation. Before uploaded into a 1 mm pathlength cuvette, each sample was stirred again or about 1 minute to ensure that D-Glucose dissolved evenly.

2.2. Data acquisition
The NIR transmission spectrum of each sample solution was scanned using a Fourier transform near-infrared spectrometer (Buchi NIRFLEX 500 solid) in a spectral region of 4000-9000 cm⁻¹ and intervals of 4 cm⁻¹ (thus, each spectrum consists of 1250 data points). During the measurements, the sample temperature was maintained at 26°C. Each sample spectrum was obtained from an average of 32 measurements.

2.3. Data analysis
Both calibration and validation spectra were smoothed using the Savitzky-Golay method employs third order polynomial at a frame size of 21. Spectral normalizations were applied to eliminate multiplicative scattering and baseline variation. The calibration spectra were employed to construct a prediction model using the partial least square regression (PLSR) method. PLSR tries to model
relations between sets of observed variables and latent variables. A detailed description of PLRS has been published elsewhere [3,21]. In the PLSR algorithm, the data matrix was mean-centered in order to remove strong transmittance background from water. Validation spectra were used to cross-validate, by utilizing the PLSR parameters, to predict the concentrations validation samples.

3. Results and Discussions
Glucose dissolves well in water but experienced much less NIR absorption compared to water. Since the main portions of the samples comprise water, their NIR transmission spectra resembled the NIR spectrum of water which is characterized by two strong absorptions; the combination band, and overtone in the area of about 4700-5400 cm\(^{-1}\) and 6500-7500 cm\(^{-1}\). Figure 1 shows the transmittance spectra of the glucose in aqueous solution with a concentration of 0, 60 and 490 mg/dL. The diffuse reflectance spectrum of glucose powder is also shown for comparison. Noted that the transmission spectra of water in the spectral region of 4700-5400 cm\(^{-1}\) were saturated due to the extreme water absorption; therefore, the transmission spectra in that of the particular region were omitted in the subsequent analysis. All spectra appear to be similar even though they represent samples with various concentrations. It is because of the subtle change due to the difference in glucose concentration hindered by a strong water absorption background. However, the NIR spectrum of powder glucose shows a noticeable difference between water and glucose absorptions.

![Figure 1. Near-infrared transmittance spectra of glucose in aqueous solution (black) with concentrations 0, 60, and 490 mg/dL. The diffuse reflectance spectrum of powder glucose (red) is shown for comparison. Note that the intensity of reflectance spectrum for powder glucose is not scaled.](image)

NIR spectra often suffer from baseline variation and multiplicative scattering. Therefore, it is necessary to normalize the spectra by subtracting each spectrum with their corresponding transmittance at 4080 cm\(^{-1}\) followed by dividing the resulted spectrum with its corresponding transmittance intensity at 9350 cm\(^{-1}\). These normalization steps produced spectra with transmittance between 0 and 1. The normalized spectra at a concentration of 0, 60, and 490 mg/dL shown in Figure 2. One sees in the figure that all spectrum are similar and practically overlapped each other. However, when the spectra subtracted with the spectrum from pure water, the difference spectra left behind revealed a typical glucose spectrum with difference transmittance magnitude. They are featuring broad absorption in a region of 7000-8700 cm\(^{-1}\), 5500-6800 cm\(^{-1}\). In Figure 2, the red and green line representing spectra from the samples of 60 mg/dL and 490 mg/dL, respectively, indicating NIRS distinguishes samples with different glucose concentrations.
The singular values decomposition analysis of the spectral data matrix was applied to obtain a region that substantially contributed to the regression model. Calculations of PLSR were then performed using that of a substantial spectral region. Figure 3 shows the loading plot of the first three components in the spectral region of 4000 - 9000 cm\(^{-1}\). The plot suggests that significant contribution to PLSR may arise from the full spectral region.

**Figure 2.** Normalized near-Infrared spectra of glucose in aqueous solution (black) for concentration 0, 60, and 490 mg/dL. Difference normalized spectra, i.e., normalized spectra of glucose in aqueous solution minus normalized spectrum of water, are shown in red and green for glucose concentrations of 60 and 490 mg/dL, respectively. The difference spectra were magnified 100x for clarity.

**Figure 3.** The 1\(^{st}\) (red), 2\(^{nd}\) (blue) and 3\(^{rd}\) (yellow) showing loading plots of the calibration data matrix extracted by singular value decomposition.
The number of the latent variable, $N$, to be used in PLSR calculations were also carefully selected by finding the prediction residual error sum of square (PRESS) to be a minimum. In this case, $N = 8$ provides the minimum PRESS of the validation data set, as shown in Figure 4.

![Figure 4](image)

**Figure 4.** Prediction residual error sum of square (PRESS) of validation data set. Minimum PRESS was achieved at $N=8$.

In the examine the accuracy of the PLSR model, the regression parameters derived from the calibration data set were used as an estimator to predict glucose concentrations of the validation samples set (cross-validation) based on their corresponding NIR spectra. Figure 5 shows the validation plot, comparing observations (ordinate) and NIR predictions (abscissa) of glucose concentrations. This plot shows that NIR predictions are very similar to observation ones. The coefficient of determination of the above plot is given by at $R^2 = 0.99$ with a maximum error of less than 6 mg/dL. These results are equivalent to previous studies [3] showing that NIR spectroscopy with PLSR can be used to
predict glucose concentrations in glucose-water solutions within physiological concentrations of 0-1000 mg/dL. In blood, the measurement of glucose levels certainly more complicated as it contains blood suspended cells, white blood cells, platelets, salt, and dissolved protein. These constituents are sensitive to the NIR so that they require more accurate spectral handling and analysis.

4. Conclusion
Measuring glucose content in aqueous solutions can be done by utilizing NIRS followed by PLSR analysis. The reasonably accurate prediction of glucose concentration in the range of 0-1000 mg/dL was confirmed. The useful spectral region for calibration and detection for glucose content is considerably broad, i.e., 4000-9000 cm⁻¹. In that particular region, NIRS may provide an effective and non-invasive approach to measuring blood glucose levels.

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