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Noninvasive Glucose Monitoring in Diabetic Patients: A Preliminary Evaluation

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Noninvasive monitoring of blood/tissue glucose concentrations has been successfully accomplished in individual diabetic subjects by using near-infrared (NIR) spectroscopy coupled with chemometric methods. Three different spectrometer configurations were tested: *a*) a Fourier-transform infrared spectrometer with an indium antimonide detector; *b*) a grating monochromator equipped with a silicon (Si) array detector, without fiber optics; and *c*) a grating monochromator equipped with an Si detector, with fiber-optic sampling. NIR spectra were obtained from diabetic subjects by transmission through the finger during a meal-tolerance test. The maximum range of observed plasma glucose concentrations obtained from the blood samples was 2.5–27 mmol/L. The NIR spectra were processed by using the chemometric multivariate calibration methods of partial least squares and principal component regression. The best calibration yielded a cross-validated average absolute error in glucose concentration of 1.1 mmol/L. This predictive ability suggests that noninvasive glucose determinations by NIR/chemometrics is a viable analytical method.

Additional Keyphrases: *monitoring therapy · near-infrared spectroscopy · multivariate calibration*

Noninvasive monitoring of glucose concentrations in the blood or tissue of diabetic patients is a long-sought-after goal that has only recently become a real possibility. One promising method that may be able to achieve this goal involves applying chemometric methods to the analysis of near-infrared (NIR) spectra obtained in transmission through or by reflection from blood-containing tissue[3]. The use of chemometric tools, such as multivariate calibration, that are capable of simultaneous analysis of all spectral information has increased the sensitivity, precision, accuracy, and reliability of quantitative infrared spectroscopy. The theory and application of these methods to spectroscopic data were recently reviewed (*1–3*). Chemometrics is rapidly being applied to reagentless medical diagnostics. Multivariate calibration analysis of the data obtained with infrared spectroscopy coupled with attenuated-total-reflectance (ATR) sampling or transmission sampling has been used for measuring cholesterol in serum (*4*) and glucose in whole blood (*5–7*) and plasma (*8–10*).

Our goal was to determine whether noninvasive monitoring of blood/tissue glucose (BT-Glu) concentrations in diabetic subjects was possible by using NIR spectroscopy and multivariate calibration methods. In accomplishing this goal, we investigated several spectrometer configurations that used transmission sampling through the finger of diabetic subjects during meal-tolerance tests.

Materials and Methods

Protocols governing investigation of patients were reviewed and approved by the Human Research Review Committee at the University of New Mexico School of Medicine. Three volunteers with type I diabetes were admitted, having given informed consent, to the General Clinical Research Center at the University of New Mexico School of Medicine. The subjects were fasting and had taken no morning insulin injection. Intravenous access was maintained with a saline infusion to allow for serial collection of 5-mL blood samples. Thus, chemical determination of glucose concentrations could be made concurrently with every NIR reading through the finger. All samples were placed in Vacutainer Tubes containing 50 mg of sodium fluoride and 5 mg of thymol to arrest glucose uptake by erythrocytes. These samples were centrifuged on withdrawal to yield plasma samples. Chemical determinations of the plasma glucose concentrations were made with an Astra-8 (Beckman Instruments., Brea, CA) clinical laboratory instrument that uses glucose oxidase methodology. The resulting glucose concentrations served as the reference values for the partial least squares (PLS) and principal component regression (PCR) spectral modeling. The precision of this instrument ranges from an SD of 0.11 mmol/L at concentrations <8 mmol/L to an SD of 0.3 mmol/L for concentrations ≤28 mmol/L. Because of the automatic dilution of samples >19 mmol/L, the precision of measurements is altered above this glucose concentration.

Subjects were fed a pancake meal containing 493 kcal, 60 g of protein, 104 g of carbohydrate, and 4 g of fat derived from syrup, margarine, grape juice, and wheat cakes. Whole-blood samples of 5 mL were serially drawn at 10–30-min intervals during the next 2 h or until a maximum glucose concentration of ~22 mmol/L was measured by hand-held-glucometer monitoring. At that point, 3–5 USP units of regular insulin was administered intravenously to induce a reduction in blood glucose concentration. Sampling was terminated ~3 h later when blood monitoring demonstrated a return to pre-meal glucose concentrations. NIR spectral data through the finger were acquired immediately after each blood sample was drawn and, depending on instrument configuration, were recorded for 1–2 min to improve the

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[3] Nonstandard abbreviations: NIR, near infrared; ATR, attenuated total reflectance; BT-Glu, blood/tissue glucose; PLS, partial least squares; and PCR, principal component regression.

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signal-to-noise ratio of the resulting spectra. The resulting distribution of glucose values ranged between ~2 and 28 mmol/L. This distribution relates well to the clinically useful range of physiological values. In addition to blood sampling and recording of spectra, surface finger temperatures were monitored in subjects 2 and 3 by using a First Temp Genius Model 3000A pyrometer (Intelligent Medical Systems, Carlsbad, CA). Finger temperatures were measured to ensure that the glucose determinations were not affected by a correlation between glucose concentrations and temperature.

Three instrument configurations were used in these studies. The first was a Nicolet Instrument Corp. (Madison, WI) Model 800 FT-IR instrument equipped with a tungsten halogen source and a liquid-nitrogen-cooled InSb detector. The beam was directed out of the instrument through the accessory port and focused on the finger in a custom-built optical section. In obtaining air background spectra, we partially closed the iris in the spectrometer to avoid saturating the detector. The second and third instrument configurations used a SPEX Industries (Edison, NJ) Model 270M grating spectrometer equipped with a tungsten-halogen source and a Photometrics Ltd. (Tucson, AZ) CCD9000 Si array detector with and without fiber-optic sampling. In the second configuration, the beam was directed with lenses to the finger and to the source. In the third configuration, fiber optics were used both to transmit light to the finger and to collect light from the opposite side of the finger. When fiber optics were not used, the background spectra were acquired by using a cuvette containing whole milk to attenuate the beam and to minimize the influence of water on the spectra. When fiber-optic sampling was used, the background spectra were acquired by using neutral-density filters to attenuate the beam.

We used PLS and PCR calibration methods applied to the spectral data to define an empirical model relating changes in the calibration spectra to the differing reference glucose concentrations of blood samples obtained during the calibration. PLS and PCR are factor-analysis multivariate calibration methods whose properties and relative merits were presented previously (*1–3, 11*). Included in these calibration methods are procedures to estimate the uncertainty of predictions resulting from using these models (*12*).

PLS and PCR calibration models were derived from analysis of an individual subject's plasma glucose concentrations and the corresponding spectral data (*5, 12, 13*). Both the original absorbance spectral data and first derivatives of the spectra were used in developing the calibration models. First derivatives were obtained simply by taking differences between equally spaced successive absorbance intensities. In cases where baselines are smooth but vary widely from one sample to another, first-derivative spectra may be preferable to original spectra for analyses because the derivative spectra deemphasize the influence of the baseline. The results are reported in each case for the method yielding the lowest prediction errors. Cross-validation was used to develop each PLS and PCR calibration model. In the cross-validation procedure, one sample is omitted from the calibration, and the model is redetermined on the basis of this reduced sample set. The concentration of the omitted sample is then predicted by using the multivariate model. This process is repeated until each sample has been left out of the calibration model. Cross validation is required to determine the optimal size of the calibration model, to obtain good estimates of the predictive ability of the multivariate calibration method, and to reliably detect problems in the calibration data (*12*). The optimal size of the model was chosen as the model with the fewest number of factors that were within 1 SE of the minimum value of the predicted error sum of squares (*12*). This method was previously described in more detail relative to our evaluation of IR spectroscopy in the in vitro determination of blood glucose (*13*).

F-ratio tests (*12*) were used to verify the quality of each observation (reference glucose value and associated spectrum). This process of outlier detection is based on assessing statistically significant concentration and spectral *F* ratios calculated during the cross-validation calibration procedure (*12*). A large concentration *F*-ratio is probable if there was an error in the reference concentration determination. A large spectral *F*-ratio might result from spectrometer error, aberrancies in positioning the finger in the instrument, or the presence of components not present in the other calibration samples. In this work, the *F*-ratio tests demonstrated that each calibration spectrum and glucose reference concentration was within the range of the calibration model after a few outlier calibration samples were removed from the models.

Results

Figure 1*A* shows examples of NIR absorbance spectra acquired on the FT-IR spectrometer with light transmitted through the finger. The majority of the spectral variations observed in Figure 1 are the result of spectral baseline variations rather than glucose concentration differences. The fact that the spectral variations due to glucose are subtle relative to other sources of variation emphasizes the need for multivariate analysis. The baseline variations, although undesirable, either are adequately handled by the multivariate model or can be minimized by modeling first-derivative spectra. Figure 2 shows the cross-validated PLS prediction results for BT-Glu as a function of the corresponding reference glucose concentrations obtained from subject 1. The 41 readings were taken over a range of plasma glucose values from 2.7 to 27.7 mmol/L. The best prediction ability was obtained by applying PLS to absorbance spectra rather than to derivative spectra. PCR yielded slightly poorer prediction ability. Use of the entire frequency range from 870 to 1300 nm resulted in the most precise glucose prediction results. The optimal number of PLS factors was 11, and no samples were detected as outliers; the average absolute error of prediction obtained by cross-validation was 1.1 mmol/L.

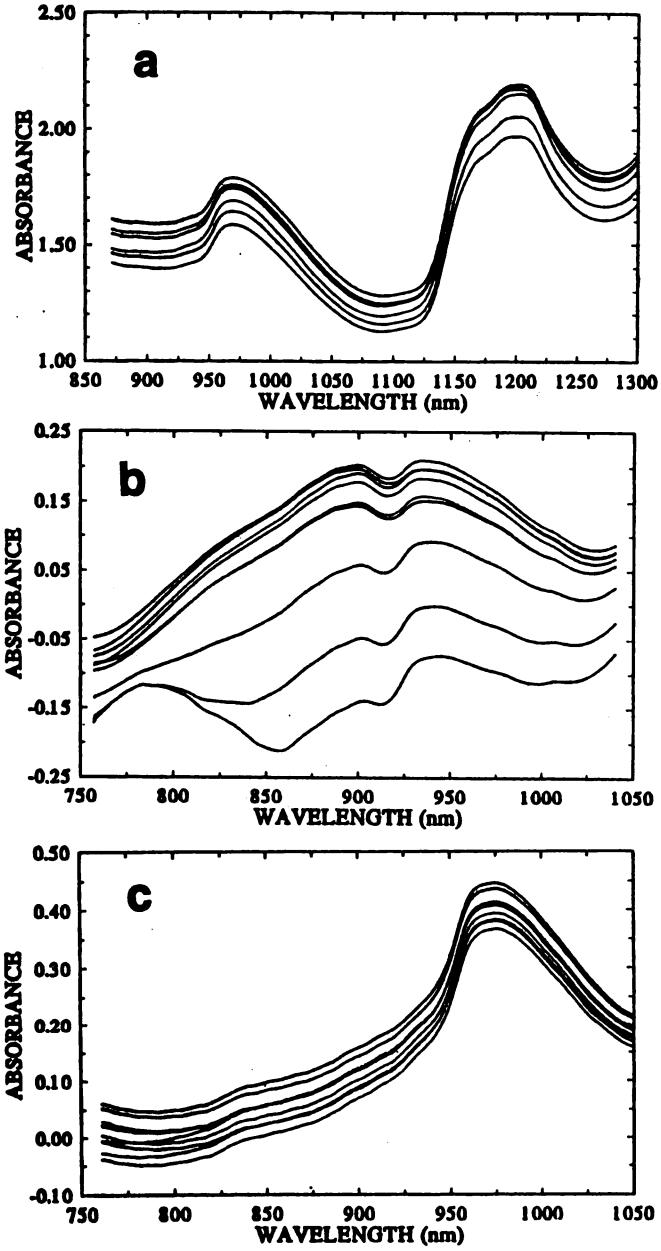

Fig. 1. Representative spectra from subjects

A: Spectra from subject 1 taken with the FT-IR spectrometer and InSb detector; background spectra were taken through air with partially closed iris. *B:* Spectra from subject 2 taken with the grating spectrometer and Si array detector; background spectra were taken through milk to attenuate the beam. *C:* Spectra from subject 3 taken with the grating spectrometer, Si array detector, and fiber-optic sampling; background spectra were taken through air with neutral-density filters to attenuate the beam

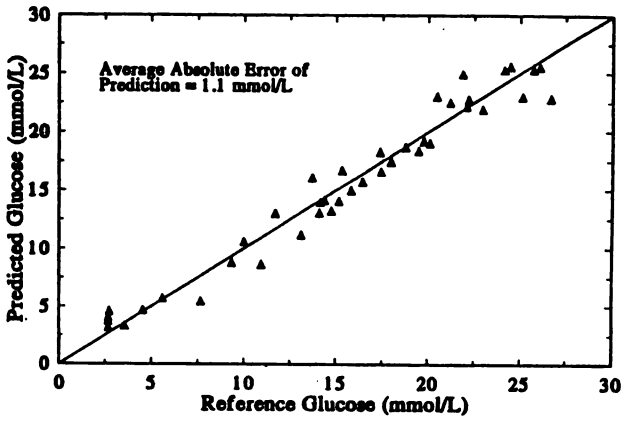

Fig. 2. Cross-validated PLS calibration results for subject 1 during a meal-tolerance test

The solid line indicates the expected relationship, with a slope of 1 and an intercept of 0

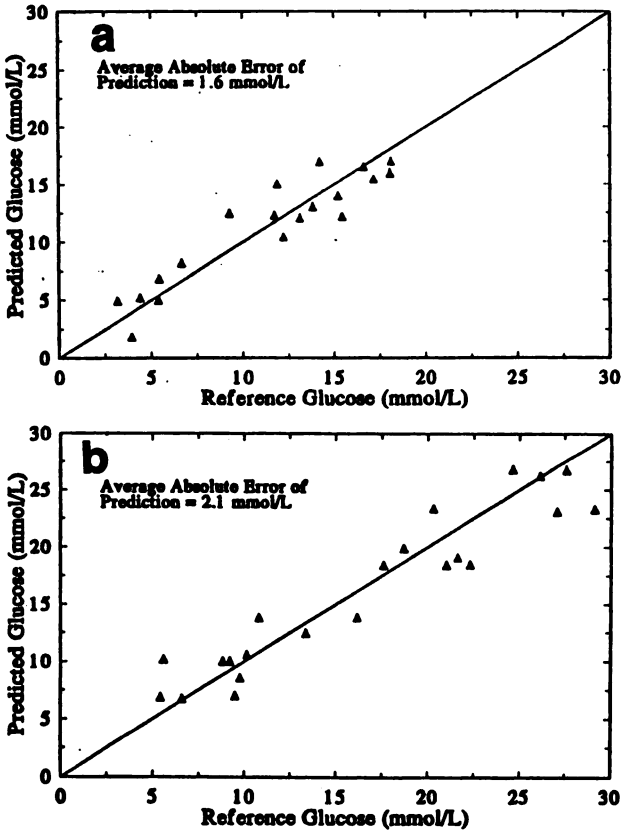

Fig. 3. Cross-validated PCR calibration results for subject 2 (*A*) and subject 3 (*B*) during a meal-tolerance test

The solid line indicates the expected relationship with a slope of 1 and an intercept of 0

Figure 1*B* shows examples of spectra recorded by the grating spectrometer without fiber-optic sampling for subject 2. We obtained 23 readings over a range of plasma glucose values from 2.9 to 18.4 mmol/L. PCR analysis applied to the first-derivative spectra produced the lowest average absolute error, presumably by reducing the harmful effects of large baseline variations. Only a single background was used for all the spectra to eliminate the possibility that changes in the background milk spectra might influence the results. The data from three samples were identified as outlier data, and these samples were removed from the final analysis. Figure 3*A* shows a plot of the cross-validated PCR-determined BT-Glu results obtained for subject 2 as a function of the reference glucose concentrations. The optimal model contained five PCR factors. The absolute average error of prediction for the data shown was 1.6 mmol/L. No consistent correlation between finger temperatures and glucose concentrations was found for subject 2.

Figure 1*C* shows the spectra recorded by the grating spectrometer with fiber-optic sampling. The spectra in parts *B* and *C* of Figure 1 have dissimilar shapes because of the different types of background spectra used for calculating the absorbance values. Again, PCR applied to derivative spectra resulted in the lowest prediction errors. One of the 23 samples was detected as a concentration outlier and was removed from the analysis. The PCR-determined BT-Glu data from 22 samples with values ranging from 5.4 to 29.1 mmol/L are plotted in Figure 3*B* as a function of the reference glucose concentrations. The absolute error of prediction on all samples by a cross-validated PCR four-factor model was 2.1 mmol/L. No correlation between finger temperatures and glucose concentrations was found for subject 3.

Discussion

We present preliminary studies involving three instrument configurations used to explore noninvasive determination of blood/tissue glucose by NIR spectroscopy. Our results demonstrate the potential validity of an infrared–multivariate calibration model derived from trans-finger spectral data in the prediction of BT-Glu concentrations. The sensitivity of this method approaches that required for determining glucose concentrations necessary for diabetes management. Some differences were observed in the predictive abilities of the PLS and PCR algorithms. PLS and PCR are two distinct multivariate-factor analysis methods that use different algorithms. The relative predictive abilities of these methods were examined in detail for the situation when Beer's law is followed (*11*). However, in complex analyses, such as studied here, that involve nonlinearities and other deviations from ideal behavior, we do not fully understand these differences in performance.

The studies demonstrate that BT-Glu can be noninvasively determined in a single subject by multivariate analysis after a calibration model based on 20–30 observations has been developed. The NIR determination is an approximation of plasma glucose and has demonstrated a clinically useful error as low as 1.1 mmol/L in these experiments. Although these studies used NIR frequencies from 600 to 1300 nm, they do not precisely define which wavelengths are necessary or whether additional wavelengths would improve prediction ability. The smaller absolute error of prediction obtained with the configuration comprising the FT-IR spectrometer and the InSb detector may result from the extension of the wavelength region to larger values. Additionally, baseline variations of these data are smaller than for the other instrument configurations, which may have improved the predictive ability. However, the limited number of preliminary experiments performed and the ambiguous effects resulting from the use of different background procedures prevent us from making definite recommendations regarding optimal spectrometer configuration.

Many factors may be critical to the application of this methodology to different subjects or to the same subject at different times. Among these factors are tissue temperature changes, endogenous metabolites, hemoglobin concentration, other species known to absorb in the spectral range analyzed, and repeatable finger positioning within the spectrometer. Changes in concentrations of urea, protein, and hemoglobin were previously recognized by Zeller et al. (*6*) as absorbing significantly in the region of the glucose absorption. Exploring the significance of these factors and whether or not they can be included in a calibration model will be the objective of future investigations. Improvements in our finger-sampling apparatus should help to remove or reduce baseline variations and improve noninvasive glucose prediction results.

In this preliminary study we have not addressed the applicability of a single model to multiple patients. This issue must await a population study with sufficient numbers of patients and samples to consider this question. Nevertheless, our data suggest that NIR spectroscopy coupled with multivariate full-spectrum analysis may have the specificity and sensitivity for glucose determinations through the finger necessary for application to clinical medicine. Further development of this technology could eventually result in a noninvasive home glucose monitor that would improve the quality of life for many people affected by diabetes.

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