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Determination of Glucose Concentration in Whole Blood using Fourier-Transform Infrared Spectroscopy

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Abstract. Fourier-transform infrared (FTIR) transmission spectroscopy has been used for the determination of glucose concentrations in whole blood samples from twenty-eight patients. A four-vector partial least squares calibration model, using the spectral range 950–1200 cm^{-1}, yielded a standard error of prediction of 0.59 mM for an independent test set. For blood samples from a single patient, we found that the glucose concentration was proportional to the difference between the values of the second derivative spectrum at 1082 cm^{-1} and 1093 cm^{-1}, suggesting that these two specific wavelengths can be used for determining glucose concentrations in blood.

Key words: Blood, Fourier transform, glucose, infrared spectroscopy

1. Introduction

The quantification of glucose in blood is an ongoing field of research in clinical analysis. In the laboratory, the determination of glucose concentrations is critical for the study of diabetes, and the monitoring of all patients on critical care wards, particularly premature babies in special care baby units. For this reason, infrared (IR) spectroscopy has attracted much attention for the study of blood or serum. The technique has a number of advantages: no reagents are required, the concentration of more than one analyte can be determined from a single spectrum, and the method is suitable for automation. Previous studies have demonstrated that Fourier-transform infrared (FTIR) spectroscopy can be used for the determination of glucose in blood, either using an attenuated total reflectance (ATR) technique for liquid samples [1–3] or by drying blood or serum onto IR-windows [4, 5]. In contrast, direct transmission measurements in the mid-IR range have long been thought to be a poor technique for blood analysis, owing to the viscosity of blood and its high particle content (whole blood contains about 45% cellular components, and 55% water and dissolved solids). However, despite its high water background ab-

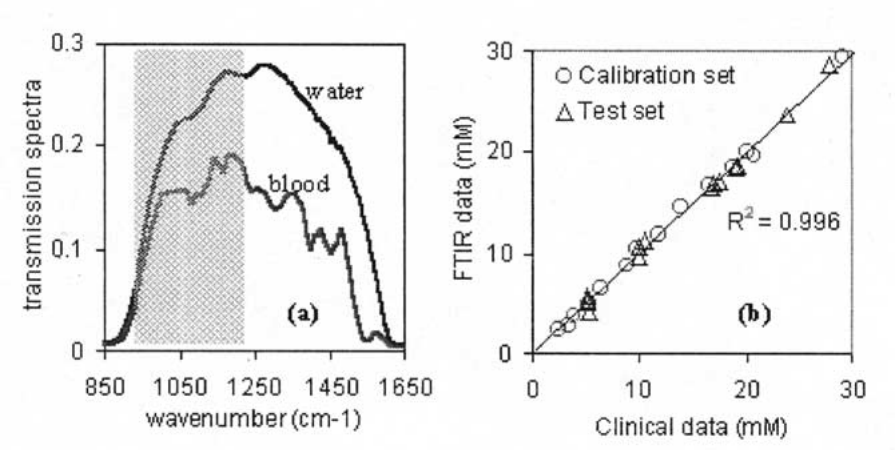

Figure 1. **(a)** FTIR transmission spectra of de-ionized water and whole blood with a glucose concentration of 20.1 mM, the shaded region is the spectral range in which glucose signatures are observed. **(b)** Comparison of predicted (FTIR data) and provided (clinical data) glucose concentration in whole blood, for both calibration and test sets.

sorption and complex blood matrix, direct determination of glucose in whole blood using transmission spectroscopy has been reported [6]. We will present detailed results demonstrating the quantitative determination of glucose concentrations in whole blood samples using FTIR transmission spectra. In addition, we report the direct determination of glucose concentrations using second derivative spectra at two specific wavelengths.

2. Experimental

Blood samples were taken from twenty-eight anonymous patients during routine clinical analysis. The glucose concentrations ranged from 2.4–29.0 mM. The samples were collected into EDTA tubes to prevent coagulation and stored at 4 °C before measurement. All samples were analyzed independently to determine the glucose concentration using a Dade Behring Dimension clinical chemistry system.

All transmission spectra were recorded at room temperature (23 °C) with a Bruker IFS113V spectrometer equipped with a Ge/KBr beam-splitter and a liquid-nitrogen-cooled mercury-cadmium telluride (MCT-316) detector. A liquid cell with KRS-5 windows and a 25μm teflon spacer was used (cell volume 10 μL). For each measurement, 500 μL of the blood sample was first flushed through the liquid cell using a syringe (1 mL). After the measurement, the liquid cell was cleaned with 5 mL of de-ionized water in the reverse direction. Thirty-two scans were performed for data acquisition in the spectral region 500 cm^{-1} to 7000 cm^{-1} (4 cm^{-1} resolution, four-point apodization, and a zero-filling factor of two). All spectra were smoothed with a standard Savitzky-Golay method before being used for further data analysis.

3. Results and Discussion

Figure 1 (a) shows a typical FTIR transmission spectrum of whole blood, together with a water spectrum. In the spectral range 900–1600 cm^{-1} there is approximately a 40% overall decrease in the transmission spectrum of whole blood compared with water. A number of absorption features can be identified from the spectrum of blood, particularly the amide II absorption range (1480–1580 cm^{-1}) corresponding to coupled N-H bending vibration modes and C-N stretching vibration modes of proteins and amino acids. The glucose absorption features around 950–1200 cm^{-1}, however, overlap with other blood components.

For the determination of glucose concentration, a multivariate partial least squares (PLS) calibration model was employed [7, 8]. We investigated different multivariate calibration models, varying the number of PLS vectors, the spectral range, and the data preprocessing steps. The optimum number of vectors was determined to be four, based on second derivative IR spectra in the range 950–1200 cm^{-1}.

Of the twenty-eight patients studied, we used the first fourteen blood spectra as the calibration set and the other fourteen as the test set. As shown in Figure 1 (b), the reference and FTIR data agree very well. The standard error of calibration (SEC) for the calibration set and the standard error of prediction (SEP) for the test set were calculated to be 0.46 mM and 0.59 mM respectively, which are amongst the best results reported for glucose determination in blood [1–6]. We note that by using transmission FTIR spectroscopy and a doping procedure, Vonach et al. [6] obtained an SEP of 0.81 mM when determining the concentration of glucose in blood from eight patients. In our work, all the blood samples are true whole blood samples from different patients and therefore have different blood matrices. Nevertheless both studies show that IR transmission measurements are a convenient and precise tool for determining glucose concentrations in the clinically relevant concentration range of 2.4–29 mM. This technique has advantages over ATR or dried blood film methods in that neither reagents nor sample preparation is necessary.

For practical applications it is generally important to use spectral data at a few specific wavelengths rather than using the whole spectral range. We now show that the second derivative spectrum can be used to give a direct determination of the glucose concentration in whole blood samples from a single patient. To simulate blood samples of desired glucose concentrations from a single patient, we prepared a set of plasma samples with glucose concentration ranging from 7.3–51.2 mM, but in essentially the same plasma pool matrix. Figure 2 shows the second derivative spectra of ten such plasma samples. Before taking derivatives, the spectra were normalized by the spectrum of the plasma sample with the lowest glucose concentration. This has the advantage of automatically compensating for the influence of other blood analytes. As a result, the difference of the spectral values at 1082 cm^{-1} and 1093 cm^{-1} increases linearly with the glucose concentration.

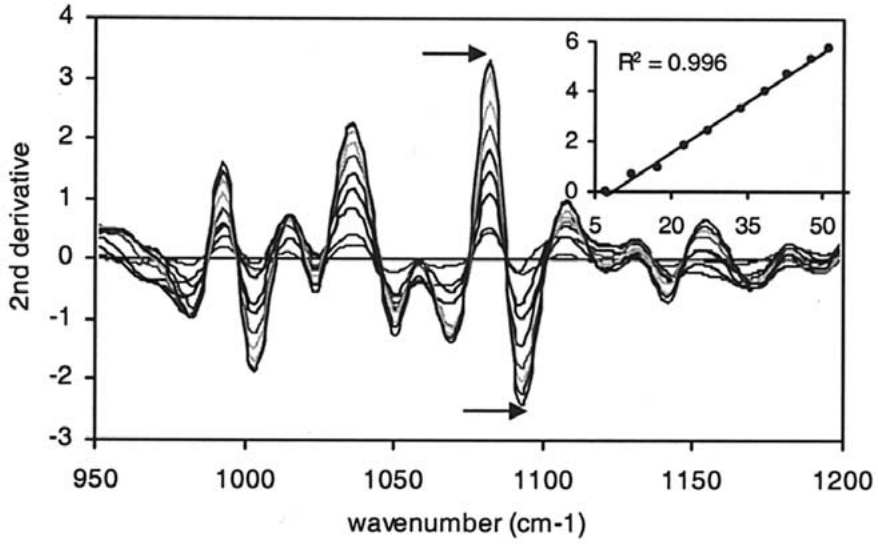

Figure 2. Second derivative spectra of plasma samples. From bottom to top at 1082 cm^{-1} the glucose concentrations are 7.3, 12.5, 17.3, 22.7, 27.2, 33.7, 38.6, 42.8, 47.6, 51.2 mM, respectively. The inset shows the linear dependence on glucose concentrations of the difference between the values at the two peaks indicated by arrows.

This demonstrates that it is possible to determine glucose concentrations in blood plasma using spectra from only a narrow spectral range.

4. Conclusions

In conclusion, we have shown that IR transmission measurements are a convenient and precise tool for the quantitative determination of glucose concentrations in blood. A four-vector PLS calibration model based on the second derivative spectra in the range 950–1200 cm^{-1} provided the best results. However, for blood samples from a single patient the difference between the second derivative spectral values at 1082 cm^{-1} and 1093 cm^{-1} can be used for direct determination of glucose concentrations in blood. One of the benefits of using the latter technique is that one can easily determine whether the blood glucose level is above or below a certain level by simply looking into the spectra at two wavelengths.

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