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The use of Fourier-transform infrared spectroscopy for the quantitative determination of glucose concentration in whole blood

Y C Shen[1], A G Davies[2], E H Linfield[1], T S Elsey[3], P F Taday[4] and D D Arnone[4]

[1] Cavendish Laboratory, University of Cambridge, Madingley Road, Cambridge CB3 0HE, UK
[2] School of Electronic and Electrical Engineering, University of Leeds, Leeds LS2 9JT, UK
[3] Clinical Biochemistry, Addenbrooke's NHS Trust, Cambridge CB2 2QR, UK
[4] TeraView Ltd, 302-304 Cambridge Science Park, Milton Road, Cambridge CB4 0WG, UK

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Abstract

Fourier-transform infrared transmission spectroscopy has been used for the determination of glucose concentration in whole blood samples from 28 patients. A 4-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}. This indicates that spectroscopy at these two specific wavenumbers alone could be used to determine the glucose concentration in blood plasma samples from a single patient, with a prediction error of 0.95 mM.

1. Introduction

The quantification of glucose in blood is an ongoing field of research in clinical analysis. The determination of glucose concentration in blood is critical for the treatment and control of diabetes (Shamoon *et al* 1993, Nathan 1996) and the monitoring of patients on critical care wards, particularly premature babies in special care neonatal units. Infrared (IR) spectroscopy has attracted much attention on the study of blood and serum owing to a number of potential advantages: no reagents are required, the concentrations of more than one analyte can be determined from a single spectrum, and the method is suitable for automation (Hall and Pollard 1992, Hazen *et al* 1998, Shaw and Mantsch 2000, Heise and Bittner 1997, Haaland *et al* 1992, Khalil 1999, Arnold 1996, Heise *et al* 1989, Bhandare *et al* 1993, Ward *et al* 1992).

Previous studies have demonstrated that Fourier-transform infrared (FTIR) spectroscopy can be used in the mid-IR range for the determination of glucose in blood. An attenuated total

reflectance (ATR) technique has been employed for liquid samples which, in combination with a partial least-squares (PLS) calibration model, has produced a standard-error-of-prediction in the range 0.8–1.1 mM (Heise *et al* 1989, Bhandare *et al* 1993, Ward *et al* 1992). The absorption of proteins on the ATR crystal is, however, a shortcoming of this technique. In another approach, therefore, blood or serum samples were deposited onto either a polyethylene card or an IR-window substrate and dried to eliminate water absorption. The standard-error-of-prediction obtained was 0.41 mM for glucose in serum and 1.2 mM for glucose in blood (Budinova *et al* 1997, Shaw *et al* 1998). In these measurements, potassium thiocyanate was added to the blood or serum sample as an internal reference to compensate for variations in the film thicknesses and sample quantity.

Direct mid-IR transmission measurements have long been regarded as a poor technique for the analysis of whole blood, owing to its viscosity and high particle content (whole blood contains about 45% cellular components), together with the high water background absorption. However, despite these problems, direct determination of glucose in whole blood using FTIR transmission spectroscopy has been performed, and a standard-error-of-prediction of 0.81 mM reported for samples from eight patients using a doping procedure (Vonach *et al* 1998).

In this paper, we present detailed results demonstrating the quantitative determination of glucose concentrations in whole blood samples from 28 patients using FTIR transmission spectroscopy and a PLS calibration model. We discuss the effect of the spectral range and the number of vectors in the PLS model on the prediction accuracy. Furthermore, we show that the glucose concentration can be determined directly from the second derivative of the transmission spectra at two specific wavelengths, which might be advantageous for the practical implementation of this technique.

2. Experimental details

Aqueous glucose samples were prepared to various concentrations by appropriately diluting a pre-prepared 32 g/dl D-glucose solution with de-ionized water. The blood samples used were taken from 28 anonymous patients attending routine clinical appointment. These veinous whole blood samples were collected directly into fluoride/EDTA tubes (the fluoride inhibiting glycolysis and the EDTA acting as an anticoagulant), and stored refrigerated at 4 °C before measurement. Glucose concentrations of these whole blood samples were assigned directly by a laboratory reference method (Dade Behring Dimension Analyzer) within 2 h of receipt into the laboratory, and found to range from 2.4 to 29.0 mM. This clinical method has a coefficient of variation within a batch of less than 2% at glucose levels of 5.0 mM and 20 mM.

All transmission spectra were recorded 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 25 μm teflon spacer was used throughout (cell volume 10 μl). For the whole blood measurements, 500 μl of the 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. The temperature of the samples was kept constant (22 ± 2 °C) during all the FTIR measurements, to minimize the spectral variation owing to temperature change. 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 2). All spectra were smoothed with a standard Savitzky–Golay method (13 smoothing points) before being used for further data analysis.

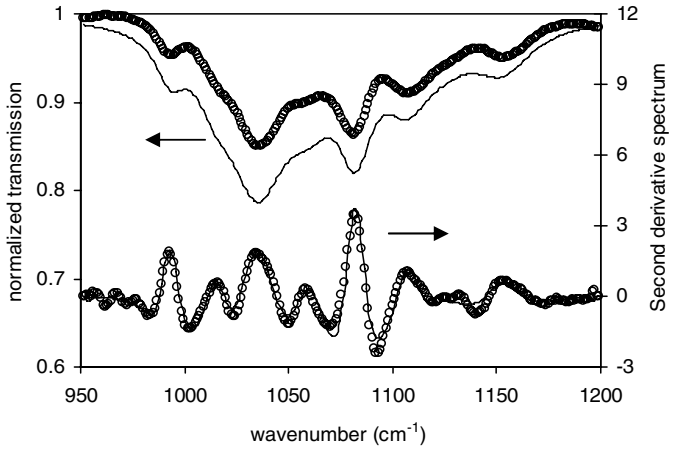

Figure 1. Measured (open circle) and simulated (solid line) FTIR transmission spectra of an aqueous glucose sample (60 mM) and their corresponding second derivatives. Values of the eight sets of fitting parameters (v_j (cm^{-1}), Γ_j (cm^{-1}), S_j) were: (1035, 36.3, 1.19); (1082, 20.3, 0.409); (1107, 25.8, 0.255); (1060, 28.8, 0.217); (1162, 14.8, 0.259); (1015, 21.8, 0.193); (993, 17.1, 0.214); (1123, 41.3, 0.190).

3. Results and discussion

3.1. Aqueous glucose samples

Figure 1 shows the FTIR transmission spectrum of a 60 mM aqueous glucose sample, normalized to a de-ionized water spectrum, over the spectral range 950–1200 cm^{-1}. The absorption features in this spectral range are mainly due to the CC and CO stretching modes as well as the deformations of the OCH, COH and CCH groups (Buslov *et al* 1999, Kacurakova and Mathlouthi 1996). Figure 1 also shows the results of a Lorentz oscillator model which uses a sum over eight oscillators to simulate the glucose optical absorption coefficient α over this spectral range

$$(n + \mathrm{i} \cdot k) = \left(\varepsilon_\infty + \sum_j \frac{S_j v_j^2}{v_j^2 - v^2 - \mathrm{i} v \Gamma_j} \right)^{1/2}$$

$$\alpha = 4\pi v \cdot k$$

where v_j, Γ_j and S_j are centre wavenumber (cm^{-1}), line-width (cm^{-1}) and oscillator strength, respectively, and ε_∞ represents the electronic contribution to the complex dielectric constant. The second derivatives of both measured and modelled absorption spectra are also presented in figure 1; better agreement is observed between the second derivative spectra owing to the elimination of random fluctuations in the baseline and slope, and spectral features can be narrowed and enhanced. However, a clean original FTIR spectrum with a high signal-to-noise ratio is essential to avoid enhancement of noise.

Figure 2 shows the normalized FTIR spectra of four aqueous glucose samples prepared with concentrations in the range 1.0–8.0 mM, together with the second derivatives of the normalized spectra. Owing to the low concentration of these samples, the absorption features in the original transmission spectra are weak and buried in random fluctuations in the baseline and slope of the spectra. However, all glucose absorption peaks are revealed after taking

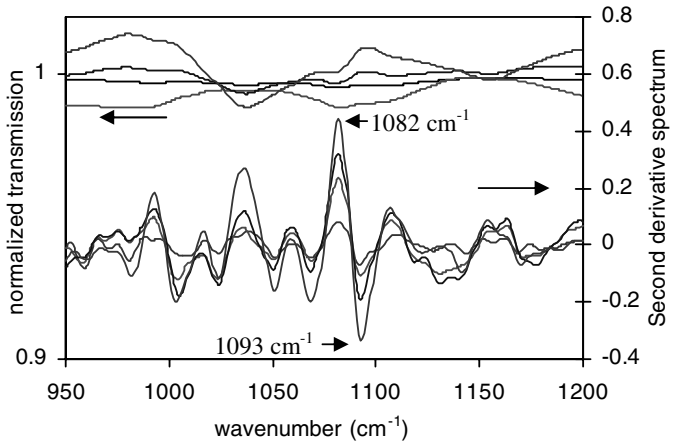

Figure 2. FTIR transmission spectra (normalized to a de-ionized water spectrum) and the second derivative spectra for aqueous glucose samples. For clarity, four out of nine spectra are plotted, and the sample concentrations are 1.8, 3.6, 5.3 and 8.0 mM. The second derivative spectrum with largest peak value (at 1082 cm^{-1}) corresponds to the sample with highest glucose concentration.

the second derivative, as verified by comparison with spectra taken from more concentrated aqueous samples.

A multivariate partial least-squares (PLS) calibration model (Martens and Naes 1991, Beebe and Kowalski 1987) was employed to determine glucose concentration, using the second derivative of the normalized spectra as the basis for the evaluation. For the assessment of the calibration models, we used the standard-error-of-calibration (SEC) and the standard-error-of-prediction (SEP). These values were calculated via

$$\mathrm{SEC} = \sqrt{\sum_{i=1}^{N_C} (C_i - C_{0i})^2/(N_C - 1)}$$

$$\mathrm{SEP} = \sqrt{\sum_{i=1}^{N_T} (T_i - T_{0i})^2/(N_T - 1)}$$

where C_i and C_{0i} are the predicted and true concentrations of the ith sample of the calibration set, respectively, and T_i and T_{0i} are the predicted and true concentrations of the ith sample of the test set, respectively. N_C and N_T are the number of calibrations and test samples. We investigated different multivariate calibration models, varying the number of PLS vectors employed, the spectral range and the data preprocessing steps (Martens and Naes 1991, Beebe and Kowalski 1987). The optimum number of vectors was determined to be 4, based on second derivative IR spectra in the range 950–1200 cm^{-1}.

The PLS model was applied to both the calibration set (shown in figure 2) and an independent test set (a further set of FTIR measurements performed on another nine aqueous glucose samples). The SEC for the calibration set and SEP for the test set were calculated to be 0.01 mM and 0.15 mM, respectively; this SEP is comparable with that of the standard clinical method for the determination of glucose in blood (Petibois *et al* 2001).

To check the efficacy of the established PLS model on a more complicated system, seven mixed aqueous sugar samples were prepared and studied, each comprising a different fructose concentration (in the range 3–22 mM) with a constant glucose concentration (7.0 mM).

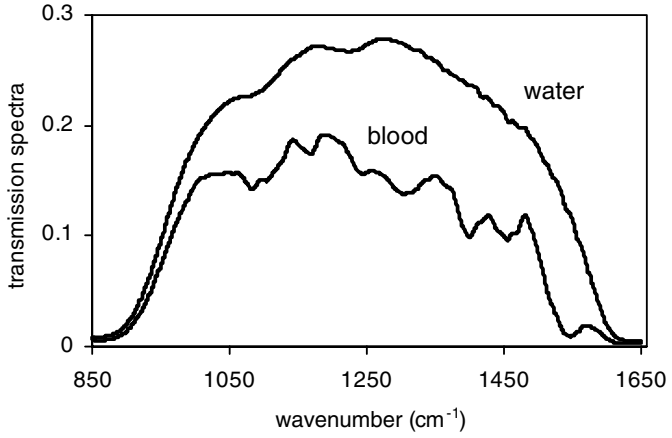

Figure 3. FTIR transmission spectra of whole blood and de-ionized water. The blood is from patient 10 with a glucose concentration of 20.1 mM.

Fructose was chosen since it has a similar molecular structure to glucose and thus similar absorption features. The predicted concentrations have a mean value of 6.7 mM with an SEP value of 0.3 mM, in good agreement with the prepared concentrations. This indicates that the model can quantify the glucose concentration even in the presence of possible interfering analytes such as fructose.

These results show that FTIR spectroscopy has the necessary sensitivity and selectivity for the determination of glucose concentrations in aqueous glucose samples.

3.2. Whole blood samples

FTIR spectroscopy measurements were performed on whole blood samples obtained from 28 anonymous patients with glucose concentrations in the range 2.4–29.0 mM. Figure 3 shows a typical FTIR transmission spectrum of a whole blood sample, compared with a spectrum of de-ionized water. Over the spectral range 900–1600 cm^{-1}, the transmission of whole blood is approximately 40% that of water. In addition, a number of absorption features can be identified in the blood spectrum, particularly the amide II absorption range (1480–1580 cm^{-1}) corresponding to both coupled N–H bending vibration modes and C–N stretching vibration modes of proteins and amino acids (Mantsch and Chapman 1996). The glucose absorption features between 950 and 1200 cm^{-1}, however, overlap with spectral features arising from other blood components (Shaw *et al* 1998).

Of the 28 patients studied, 14 randomly chosen blood spectra were used as the calibration set and the remainder as the test set. The 4-vector PLS calibration model discussed above was employed. Despite the high water absorption background and the complex blood matrix, the reference data and the FTIR data agree very well, as shown in table 1. The SEC for the calibration set and the 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 (Heise *et al* 1989, Bhandare *et al* 1993, Ward *et al* 1992, Budinova *et al* 1997, Shaw *et al* 1998, Vonach *et al* 1998). Using transmission FTIR spectroscopy and a doping procedure (adding glucose to whole blood to obtain samples of the desired glucose concentration), Vonach *et al* (1998) obtained an SEP of 0.81 mM when determining the concentration of glucose in blood from eight patients. In contrast, in our work, each blood sample is from a different patient and hence has a different blood matrix. Both studies show that IR transmission measurements

Table 1. Calibration and test set results for glucose concentrations (in mM) in whole blood.

	Calibration set			Test set	
Patient	Provided	Predicted	Patient	Provided	Predicted
1	2.4	2.4	15	5.4	4.2
2	3.4	2.9	16	5.2	5.8
3	5.3	4.9	17	5.1	5.2
4	3.9	3.9	18	5.1	5.4
5	6.4	6.5	19	10.5	11.3
6	8.9	8.7	20	9.9	9.8
7	13.9	14.6	21	9.9	10.7
8	9.8	10.5	22	16.9	17.0
9	11.8	11.8	23	16.7	16.7
10	20.1	20.0	24	17.5	17.2
11	16.6	16.9	25	19.0	18.5
12	18.7	18.5	26	19.2	18.7
13	20.8	19.6	27	24.0	23.6
14	29.0	29.4	28	27.7	28.7
	SEC = 0.46 mM			SEP = 0.59 mM	

Table 2. Effect of the spectral range used in the PLS model on the SEP (in mM). PLS models with 4- and 5-vectors are listed for comparison.

	SEP for the test set			
Spectral range	Aqueous glucose sample		Whole blood sample	
(cm^{-1})	4-vectors	5-vectors	4-vectors	5-vectors
950–1200	0.20	0.20	0.59	0.68
1015–1045	1.0	0.86	1.1	1.2
1070–1100	0.38	0.37	1.9	2.7
980–1010	0.82	0.96	3.3	2.3

are a convenient and precise tool for determining glucose concentrations in the clinically relevant concentration range of 2.4–29 mM. Furthermore, this technique has advantages over ATR and dried blood film methods in that neither reagents nor sample preparation is necessary (Vonach *et al* 1998).

3.3. Spectral range of PLS calibration model

To understand the underlying factors limiting glucose determination in whole blood, we evaluated a number of different multivariate PLS models by varying the spectral ranges and the number of PLS vectors employed. Three spectral ranges (980–1010 cm^{-1}, 1015–1045 cm^{-1} and 1070–1100 cm^{-1}) were chosen because the absorption features at these frequencies dominate the second derivative spectrum of aqueous glucose sample. The results for both aqueous glucose and whole blood samples are summarized in table 2.

For aqueous glucose samples, the 1070–1100 cm^{-1} spectral range provided the best prediction results. This is because the absorption peak at 1082 cm^{-1} has a narrower line-width and is also the largest peak in the second-derivative spectrum. As there is no interference from other analytes, the SEP is determined by the performance of the FTIR spectrometer. For whole blood samples, however, the PLS model based on the 1070–1100 cm^{-1} range gives a large SEP, about five times that for aqueous glucose samples. This is because other blood

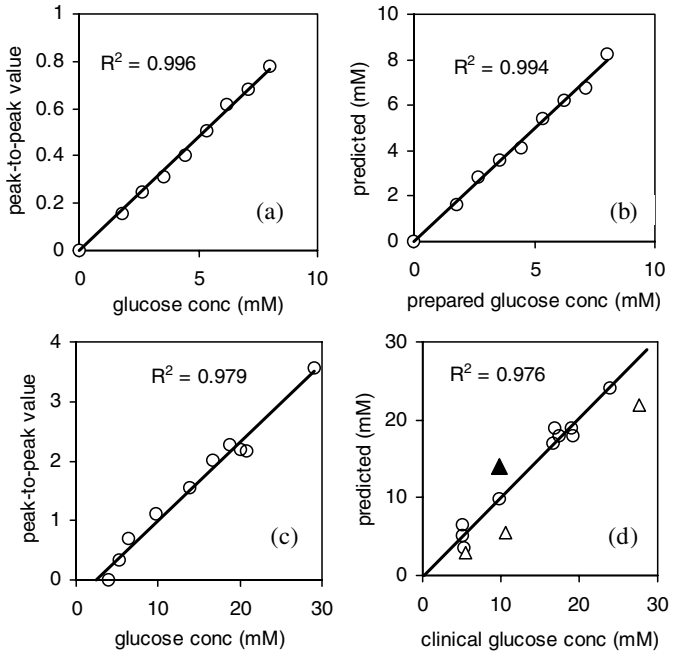

Figure 4. The peak-to-peak value of the second derivative spectrum as a function of glucose concentration in (a) an aqueous glucose calibration set, (b) an aqueous glucose test set, (c) a whole blood calibration set and (d) a whole blood test set. The solid lines in each case represent the best-fit results. Four data points in (d) (triangles) were not included in this fitting process.

analytes contribute significantly to the spectrum over this range. The PLS model based on 1015–1045 cm^{-1}, however, provides much better results with the SEP for whole blood samples very close to that for aqueous glucose samples. This indicates that over this specific spectral range, the SEP is, once again, mainly limited by the performance of the spectrometer.

A PLS model containing all three absorption bands (950–1200 cm^{-1}), however, gives the best overall prediction results for both aqueous glucose and whole blood samples. We believe that the SEP of 0.59 mM achieved here can be further improved by optimizing the measurement parameters (aperture size, spectral resolution, and scan velocity, *inter alia*). This will make the FTIR technique competitive with the standard clinical methodology (Petibois *et al* 2001).

3.4. The second derivative spectra for glucose determination

For practical applications it is convenient to use spectral data obtained at a few specific wavelengths rather than the whole spectral range. Furthermore, it is of importance for monitoring diabetes to know if the blood glucose level is above or below certain limits (Shamoon *et al* 1993, Nathan 1996). Here we show that one of the key benefits of using the second derivative spectra for data analysis is that one can easily judge whether the blood glucose concentration is above or below a certain level, with reasonable accuracy, by looking at the transmission at a few specific wavelengths only.

The peak-to-peak values (at 1082 cm^{-1} and 1093 cm^{-1}) of the second derivative spectra of figure 2 were calculated and are plotted as a function of glucose concentration in figure 4(a). Owing to the linear dependence of this calibration set, the concentrations of an independent test set of aqueous glucose samples can be determined and the best-fit line has an *R*-square

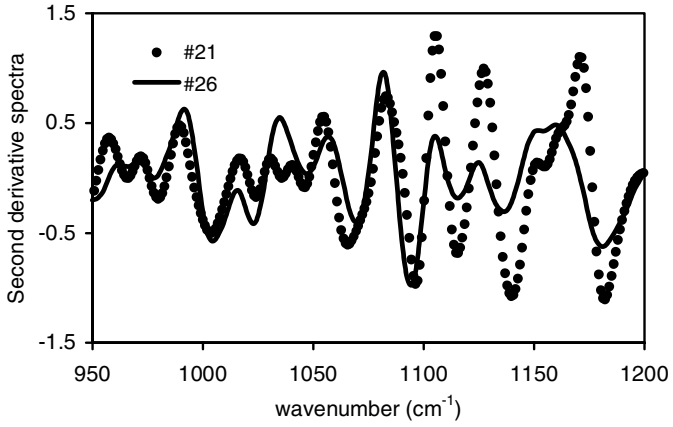

Figure 5. Second derivative spectra of the whole blood samples from (a) patient 26 and (b) patient 21. Note the difference in the spectral shape above 1100 cm^{-1}.

value of 0.994 (figure 4(b)). The equivalent results for whole blood samples are shown in figures 4(c) and (d), but note that the data in figure 4(c) were obtained after first normalizing the transmission spectra to the spectrum of the blood sample with lowest glucose concentration, in an attempt to reduce interference from other components in the blood matrix. Based on this linear dependence, the glucose concentrations in whole blood samples from 14 patients (the test set) were calculated and plotted in figure 4(d). The predicted and provided concentrations agree well for most samples, although there are a few discrepancies which we conjecture could be a result of interference from other blood analytes. Figure 5 compares a typical second derivative spectrum (patient 26, 19.2 mM) with an 'anomalous' spectrum (patient 21, 9.9 mM), which leads to one of the outlying points in figure 4(d) (solid triangle), showing the difference between the spectra particularly above 1100 cm^{-1}. The 'anomalous' spectrum of patient 21 has a larger discrepancy than the spectrum of patient 26 when compared with the spectra of aqueous glucose samples shown in figures 1 and 2. Further investigations are needed to understand the causes fully.

The second derivative spectrum can be used to give a direct determination of the glucose concentration in a whole blood sample from a patient. To simulate blood samples of different glucose concentrations from a single patient, we prepared a set of plasma samples with glucose concentrations ranging from 7.3 to 51.2 mM, using the same plasma pool matrix. Figure 6(a) 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, which automatically compensates for the influence of other blood analytes because in this particular case the plasma matrix of all the samples is identical. As can be seen in the inset of figure 6, the peak-to-peak difference of the spectral values at 1082 cm^{-1} and 1093 cm^{-1} increases linearly with glucose concentration (the best-fit line has an R-square value of 0.996). The glucose concentration could thus be predicted from the FTIR spectral data and the SEC was determined to be 0.95 mM.

Figure 6(b) shows the second derivative spectra of the same plasma samples, but in this figure, all spectra were normalized with the average of the ten spectra, before taking derivatives. The averaged data then represents the spectrum of a plasma sample with a glucose concentration of 30 mM. All spectra with negative values at 1082 cm^{-1} and positive values at 1093 cm^{-1} (solid lines) in figure 6(a) therefore correspond to plasma samples of glucose

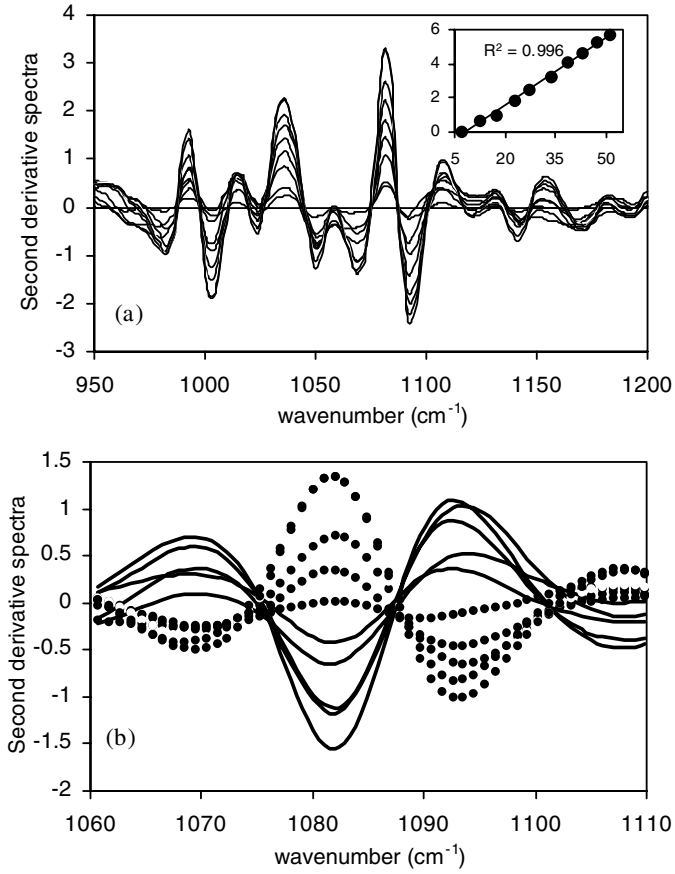

Figure 6. (a) Second derivative spectra of ten plasma samples with concentrations of 7.3, 12.5, 17.3, 22.7, 27.2, 33.7, 38.6, 42.8, 47.6 and 51.2 mM. The plasma sample with the lowest glucose concentration was used as a reference for normalization, and the second derivative spectrum with the largest peak value corresponds to the blood sample with highest glucose concentration. The inset shows the linear dependence of the peak-to-peak value on glucose concentration. (b) Same as (a) but the averaged spectrum was used as the reference for normalization.

concentration <30 mM. Similarly, spectra with positive values at 1082 cm^{-1} and negative values at 1093 cm^{-1} (dotted lines) correspond to plasma samples of glucose concentration >30 mM. Thus, the second derivative spectra at two specific wavelengths can determine whether the blood glucose concentration is above or below a certain level. This concept should also apply to near-infrared spectroscopic techniques, which offer the prospect for a non-invasive glucose monitor (Khalil 1999, Arnold 1996).

4. Conclusions

We have shown that IR transmission measurements are a convenient and precise tool for the quantitative determination of glucose concentrations in blood. A 4-vector PLS calibration model based on the second derivative spectra in the range 950–1200 cm^{-1} provided the best results. For blood plasma 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 crucial level by simply looking into the spectra at two wavelengths.

Acknowledgments

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