Two-wavelength carbon dioxide laser application for *in-vitro* blood glucose measurements

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Abstract. To develop a fast and easy clinical method for glucose measurements on whole blood samples, changes in glucose spectra are investigated varying temperature, glucose concentration, and solvent using attenuated total reflection Fourier transform infrared (ATR- FTIR) measurements. The results show a stability of the spectra at different temperatures and wavelength shifts of the absorption bands when water is replaced by blood. Because the ATR measurements are influenced by sedimentation of the red blood cells, a two-wavelength CO_2 laser is used to determine the glucose concentration in whole blood samples. For this purpose, the first laser wavelength λ_1 is tuned to the maximum of the glucose absorption band in blood at 1080 cm^{-1}, and the second laser wavelength λ_2 is tuned to 950 cm^{-1} for background measurements. The transmitted laser power through the optical cell containing the whole blood sample at λ_1 and λ_2 is used to determine the ratio. This signal correlates well with the glucose concentration in the whole blood samples. The CO_2 laser measurement is too fast to be influenced by the red blood cell sedimentation, and will be a suitable method for glucose determination in whole blood. © *2008 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.2870093]

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1 Introduction

The concentration of glucose in the capillaries of the skin is a very important parameter in the field of medical diagnostics. Glucose plays a major role within the complex nutritive supply system of the tissue. In healthy persons, the concentration of glucose is stable within a narrow range (dependent on age and fasting: 70 to 110 mg/dl). In patients with diabetes, the regulation system is disordered. The disease causes the metabolism to malfunction, and without therapeutic support, the probability of survival is low. Diabetic therapy requires that blood glucose concentration be measured several times a day. More than 10% of the costs in a clinical chemistry laboratory result from tests used to determine blood glucose concentration, thereby being the most frequently measured parameter. The standardized laboratory measurement methods include several work steps, and are therefore time- and cost-intensive methods.

A minimally invasive, fast, and reliable method for the determination of blood glucose is still of high economic relevance, because such a method has not yet been found. The development of a method for the quantitative determination of glucose in blood by means of infrared spectroscopy has been the subject of recurrent research and publication. Such research has involved investigations not only on dried blood,[1–3] but also on serum and whole blood[4] utilizing both transmission spectroscopy and ATR spectroscopy.[5,6] Cylindrical ZnSe crystals have been employed for these experiments. However, this approach has various practical shortcomings, such as the adsorption of proteins on the surface of the crystal.

In other methods, blood samples have been applied dropwise on polyethylene cards and dried before measurement to overcome the interference of water absorption.[7] One early report about the state of the art[8] and a recently published review[9] have discussed the use of analytical instrumentation for glycaemic control. For an overview of various other laboratory assays, see Heise and Abel.[10] Multivariate methods[11] and Raman spectroscopy have also been used to determine glucose concentration *in vivo* in blood.[12] A recently published article used a system based on liquid-core optical fiber Raman and absorption spectroscopy, followed by partial least square regression to determine chemical concentrations in blood serum or urine.[13] Glucose in water has proved to be a challenge to analysts in the past, because water has strong absorption bands in the infrared wavelength range of interest. The new FTIR spectrometers allow the detection of glucose quantitatively within a range of 1300 to 1000 cm^{-1}. Whole blood shows bands of other substances within the range of interest such as proteins, urea, cholesterol, and triglycerides, in addition to glucose absorption. Moreover, there is an additional loss of light caused by erythrocyte scattering.

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Vonach et al. examined the direct determination of glucose in whole blood, without any sample preparation, using FTIR spectroscopy.[14] The measurements were carried out in a flow cell. The whole spectral region from 1182 to 964 cm^{-1} had to be analyzed for determination of glucose concentration. A clear correlation could be obtained between the measured data and clinically determined blood glucose concentration.

In the investigations described in this work, FTIR measurements were used to investigate the influence of temperature on glucose in water, the influence of concentration on glucose in water and in whole blood, and the influence of the surrounding media (water versus blood) on glucose spectra. The results were compared to glucose determination carried out by the application of a two-wavelength CO_2 laser.

2 Materials and Methods

2.1 *Material*

Glucose samples were prepared by dissolving nine different amounts of glucose (Merck KGaA, Darmstadt, Germany) in 100-ml water. The sample concentration ranged from 40 to 400 mg/dl.

Blood samples of healthy volunteers were used in the experiments. Glucose was added prior to testing, and the concentration of these samples was determined in the laboratory of clinical chemistry using the glucose-UV-fluid test (GLUC, Hitachi 747, Biomed, Oberschleißheim, Germany). In healthy volunteers, the normal blood glucose concentration ranges between 60 and 117 mg/dl, and can be slightly increased by ingesting pure glucose.

2.2 *Fourier Transform Infrared Spectrometer*

A FTIR spectrometer (Vector 22, Bruker Optik GmbH, Bremen, Germany) with a ZnSe ATR crystal was used for the analysis of aqueous glucose solutions and whole blood samples. The measurements were carried out at different temperatures between 15 and 28 °C. The measurements were carried out in ATR mode, and therefore the ATR crystal and the samples were surrounded by a heating system. The temperature was measured before and after each measurement with a digital thermometer GTH 1200 A (Greisinger Electronics, Regenstauf, Germany).

2.3 *Two-Wavelength CO_2 Laser*

A two-wavelength CO_2 laser was developed and manufactured at the Institute of Applied Physics in Nishny Novgorod, Russia. The two laser wavelengths were selected at $\lambda_1 = 1080$ cm^{-1} and $\lambda_2 = 950$ cm^{-1}, because λ_1 is located within the glucose absorption band and λ_2 is used for background corrections.

The two laser beams with λ_1 and λ_2 were superposed in the optical cell containing the blood sample (Fig. 1). The transmitted light was detected separately for each wavelength behind the sample. Part of both laser beams was detected on a reference detector. The ratio of the laser power detected at λ_1 and λ_2 representing the glucose absorption was determined and transferred to data analysis.

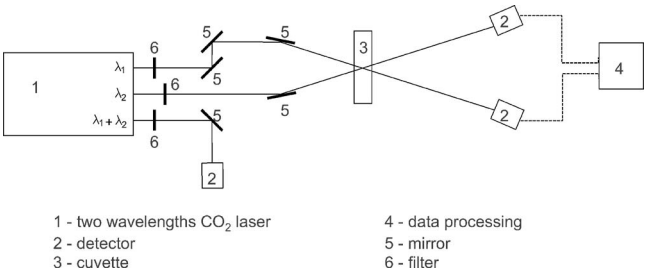

Fig. 1 Scheme of the two-wavelength CO_2 laser setup.

The optical cell consisted of two CaF_2 plates with a spacer of 50 μm. The cell was filled with the blood sample using capillary action. The blood volume required for the measurements was about 1 μl.

3 Results

3.1 *Fourier Transform Infrared Attenuated Total Reflection Measurements*

The absorbance values of nine different concentrations of glucose in water in the region from 40 up to 400 mg/dl are presented in Fig. 2. The typical glucose absorption bands are shown at 1080, 1036, 1059, and 980 cm^{-1}. A continuous increase in the absorbance could be detected with increasing glucose concentration. Sufficient signal could be detected even for a 40-mg/dl glucose solution. No dependence on glucose concentration could be observed at 950 cm^{-1}.

The relationship between the absorbance and the glucose concentration of the solvents is demonstrated for the three main bands at 1036, 1059, and 1080 cm^{-1} in Fig. 3. All three absorption bands show a linear dependence and could be used for the determination of glucose concentration in water.

The influence of temperature on glucose absorption was determined in the range from 15 to 28 °C for the glucose band. The results have shown that the glucose bands from 1160 to 980 cm^{-1} are not significantly influenced by temperature changes.

When analyzing a blood sample containing 92-mg/dl glucose, it was found that the glucose spectrum changes from water to blood. If glucose was added to a whole blood sample, its spectrum changed with time and resulted in a constant spectrum after 2 min. The spectra of glucose in pure water, and of glucose added to a whole blood sample, measured at different times are presented in Fig. 4. The glucose band at 1034 cm^{-1} has its maximum intensity in water (curve a). In blood, this absorption decreases (curve b), and after 100 s, the highest intensity was obtained at 1080 cm^{-1} (curve c). Furthermore, the ATR signals changed when the red blood cells started to sediment. To investigate this in more detail, a blood sample was left to sediment and different parts of the sample were measured. Figure 5 shows a spectrum of the pure supernatant, the plasma, which gave the lowest absorbance (curve a). The sediment, consisting of the red blood cells (Rbc), resulted in a spectrum with the highest absorbance (curve d). A mixture of plasma with 5% red blood cells was measured directly after application on the crystal (curve b), and 2 min later (curve c). The measurement taken immediately after ap-

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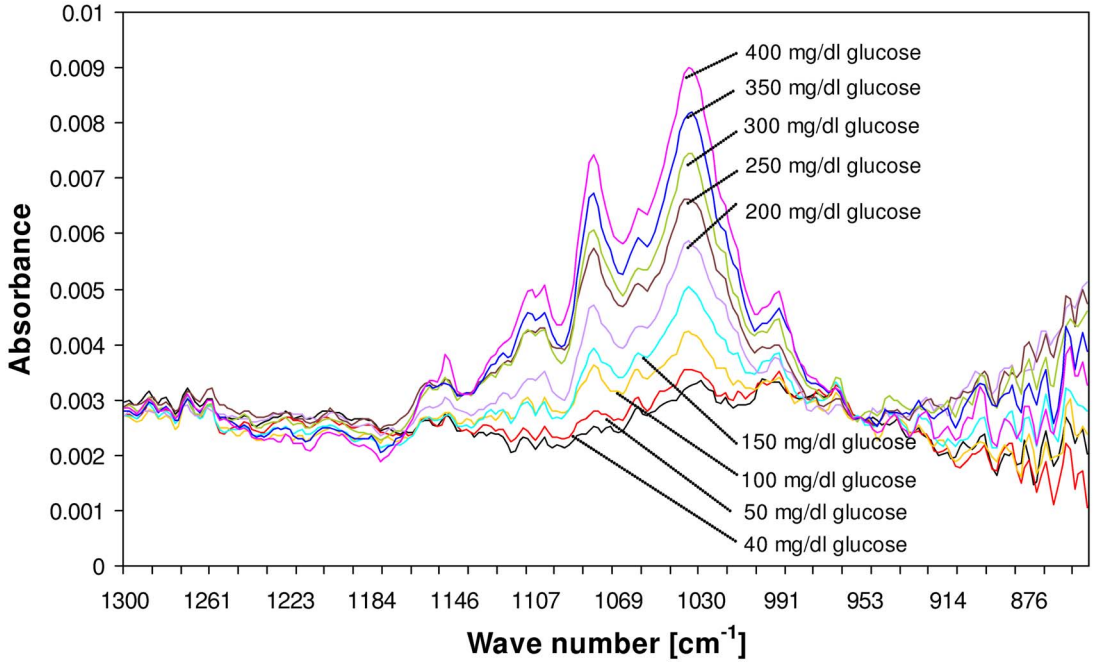

Fig. 2 Mid-infrared spectra for nine aqueous glucose concentrations between 40 and 400 mg/dl obtained by ATR-FTIR spectroscopy.

plication showed a spectrum slightly higher than the plasma spectrum, whereas the one taken after 2 min came close to that of the high-concentrated red blood cells.

3.2 *Two-Wavelength CO_2 Laser Measurements*

The applicability of a two-wavelength laser for the quick determination of glucose was tested in blood. First of all, measurements were performed on aqueous glucose solutions and analogical to the ATR measurements, the glucose concentration exhibited a linear dependency (not shown here). Then whole blood samples were investigated at different glucose concentrations. As a result of the FTIR measurements, one laser wavelength was tuned to 1080 cm^{-1} where the maximum absorption for glucose was found in whole blood. The other wavelength was tuned to the isosbestic point at 950 cm^{-1} where no dependence on glucose concentration

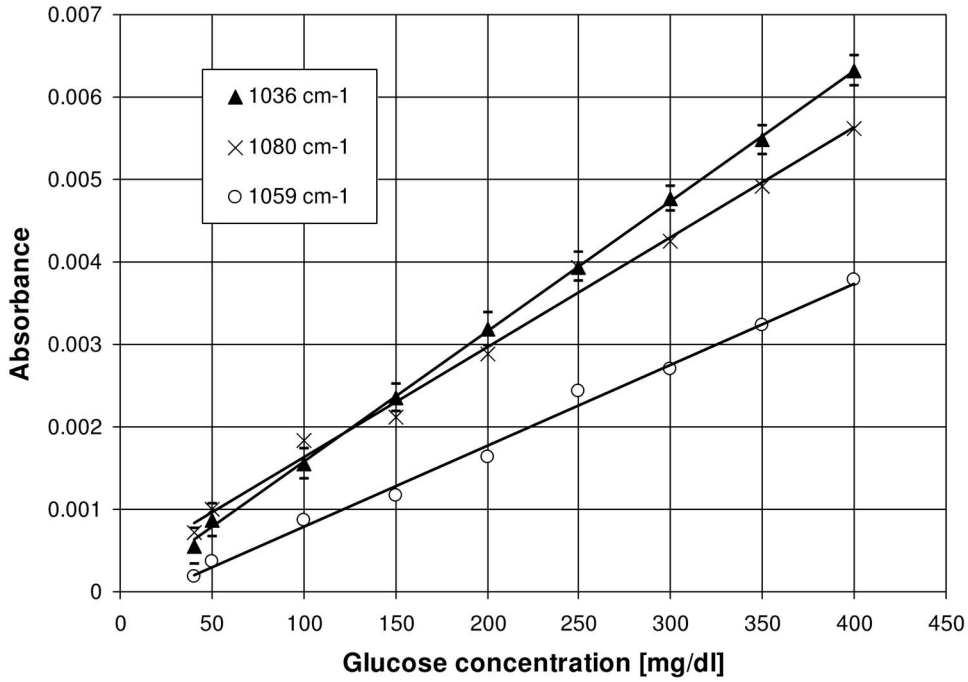

Fig. 3 Linear correlation of three glucose absorption bands obtained by ATR-FTIR spectroscopy with the glucose concentration.

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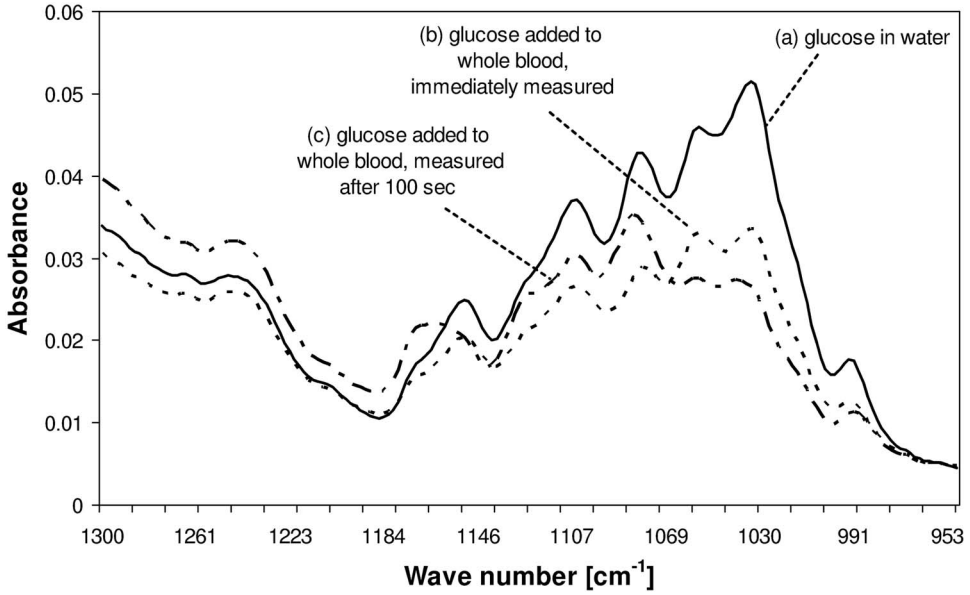

Fig. 4 ATR-FTIR absorbance spectra for 92-mg/dl glucose in water (curve a), 92-mg/dl glucose added to whole blood and measured immediately after application (curve b), and glucose added to whole blood and measured after 100 sec (curve c).

could be observed. Glucose concentrations of whole blood samples were determined in the Dept. of Clinical Chemistry. The samples were then analyzed using the two-wavelength CO_2 laser system. The correlation between the quotient of the signal intensity of λ_1 and λ_2, and the glucose concentration determined in the Dept. of Clinical Chemistry, is presented in Fig. 6. A correlation factor of $R^2=0.997$ was obtained.

4 Discussion

In contrast to NIR measurements, the investigations in the MIR spectral range have shown that the intensity of glucose absorption bands around 1035 cm^{-1} does not change with temperature between 15 and 28 °C. This indicates that the absorption can be correlated with the glucose concentration and could be used for glucose determination. The linear dependency of the ATR signal could be shown for selected wavelengths. The intensities of the absorption bands changed when water was substituted by blood plasma. The changes were due to changes in the glucose structure induced by the different surrounding media.[15]

The maximum glucose absorption was found in whole blood samples at 1080 cm^{-1}. The background was determined

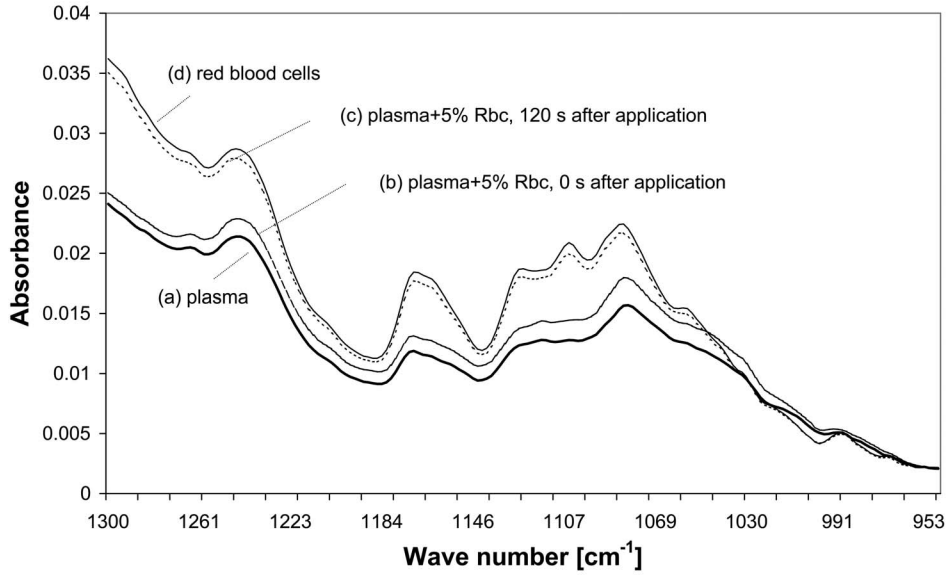

Fig. 5 ATR-FTIR absorbance spectra from one blood sample (glucose concentration 92 mg/dl); plasma and red blood cells were separated. Spectra are shown from (a) pure plasma, (b) the same plasma with 5% red blood cells (Rbc) added and measured immediately after application onto the ATR crystal, (c) the same sample as (b) but measured 2 min after application onto the ATR crystal, and (d) red blood cell fraction.

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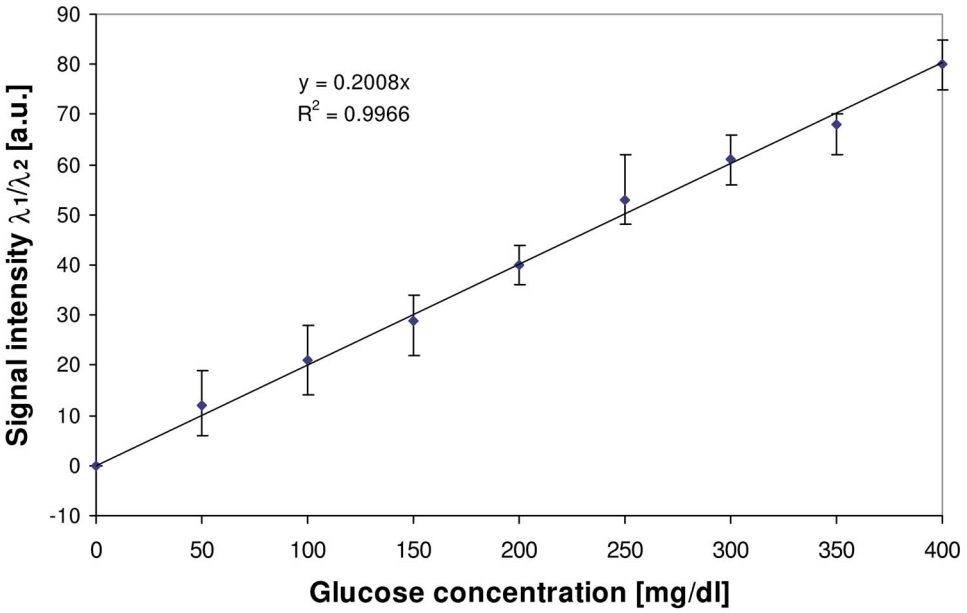

Fig. 6 The quotient of the signal intensities at wavelengths 1080 and 950 cm^{-1}, obtained using the CO_2 laser correlated with the glucose concentration in the range of 40 to 400 mg/dl.

close to the glucose absorption band at the isosbestic point at 950 cm^{-1}. These two wavelengths could be used for the spectroscopic determination of glucose concentration in whole blood samples. The influence of water absorption could be neglected, if this background is subtracted from the glucose absorption.

In contrast to the CO_2 laser measurements, the ATR measurements show a strong dependency on red blood cell sedimentation. A part of the glucose is located in the red blood cells. This is known from the HbA1c value, which provides information about blood glucose level over the last 4 to 6 weeks. Hemoglobin is connected to glucose, a relationship that is dependent on the concentration of glucose in blood plasma. The glucose connected to hemoglobin could render the blood glucose determination faulty. To use this method for routine measurements, a very strict time schedule would be necessary, and a dependency on the hematocrit is anticipated. This strong time dependency was not observed when using the CO_2 laser. Nevertheless, a time dependency due to red blood cell sedimentation could also be expected for this method, albeit to a lesser extent. The influence on this method due to red blood cells is mainly related to the scattering behavior of red blood cells. But the scattering of the cells is similar for both of the applied wavelengths and can be corrected using a ratio of the two. As a result, no marked dependency on the hematocrit is to be expected. The difference in these signals is only correlated to the glucose absorption and not influenced by changes in laser radiation intensity.

Measurement of low glucose concentrations using the CO_2 laser is faster than using the ATR, as this requires several scans before a reliable signal-to-noise ratio can be attained. The measurements can be carried out on a ms time scale by using small blood samples of less than 10 μl. In contrast to methods in the literature that require several preparation steps, such as separation of plasma and blood cells, diluting or drying the sample, this method provides a one-step procedure by filling whole blood into the cuvette or capillary. To develop a routine device based on the two-wavelength CO_2 laser method, interindividual influences should be tested using blood from different persons.

5 Conclusion

The investigations using ATR-FTIR spectrometry show that glucose in water is linearly dependent on absorbance. The absorbance maximum at 1036 cm^{-1} decreases when glucose is added to whole blood. All intensities change and the absorption band at 1080 cm^{-1} is the most prominent absorption band in whole blood. The signal intensity at 950 cm^{-1} is not dependent on glucose, and can be used for background correction such as water and red blood cells. Based on these results, a fast and easy method could be established without any prior preparation of whole blood by using a two-wavelength CO_2 laser, and tuning the laser to the wavelengths 1080 and 950 cm^{-1}. The laser method is not affected by red blood cell sedimentation, and can be used to measure the glucose concentration in the range of interest 40 to 400 mg/dl on a ms timescale.

Acknowledgments

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References

1. W. Petrich and G. Werner, "Erkennung von krankheitsmustern im infrarotspektrum von blutseren," *Physikalische Blätter* **55**, 49–51 (1999).
2. G. Budínová, J. Salva, and K. Volka, "Application of molecular spectroscopy in the mid-infrared region to the determination of glucose and cholesterol in whole blood and in blood serum," *Appl. Spectrosc.* **51**, 631–635 (1997).
3. D. Rohleder, G. Kocherscheidt, K. Gerber, W. Kiefer, W. Kohler, J. Mocks, and W. Petrich, "Comparison of mid-infrared and Raman spectroscopy in the quantitative analysis of serum," *J. Biomed. Opt.*

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10, 031108 (2005).
4. H. Zeller, P. Novak, and R. Landgraf, "Blood glucose measurement by infrared spectroscopy," *Int. J. Artif. Organs* **12**, 129–135 (1989).
5. R. J. Robinson and S. D. McDonald, "Glucose-sensitive membrane and infrared absorption spectroscopy for potential use as an implantable glucose sensor," *ASAIO J.* **38**, M458–M462 (1992).
6. Y. Mendelson, A. C. Clermont, R. A. Peura, and B. C. Lin, "Blood glucose measurement by multiple attenuated total reflection and infrared absorption spectroscopy," *IEEE Trans. Biomed. Eng.* **37**, 458–465 (1990).
7. H. M. Heise, R. Marbach, T. H. Koschinsky, and F. A. Gries, "Multicomponent assay for blood substrates in human plasma by mid-infrared spectroscopy and evaluation for clinical analysis," *Appl. Spectrosc.* **48**, 85–95 (1994).
8. H. Brauner and G. Müller, "Determination of glucose – a state of the art report," *Biomed. Tech.* **25**, 26–32 (1980).
9. V. R. Kondepati and H. M. Heise, "Recent progress in analytical instrumentation for glycemic control in diabetic and critically ill patients," *Anal. Bioanal. Chem.* **390**, 125–139 (2008).
10. H. M. Heise and P. U. Abel, "Clinical analysis: glucose," in *Encyclopedia of Analytical Science*, P. Worsfold, A. Townshend, and C. Poole, Eds., Elsevier Science, Amsterdam (2005).
11. B. Eppich and G. Müller, "Mehrbandenfilteranalysator zur bestimmung kleinster konzentrationsänderungen in mehrkomponentensubstanzgemischen," DE 100 18941 A1 (17.04.200).
12. G. Müller, "Nicht invasive glucosemessung," DE 195 38 372 A1 (14.10.95).
13. D. Qi and A. J. Berger, "Chemical concentration measurement in blood serum and urine samples using liquid-core optical fiber Raman spectroscopy," *Appl. Opt.* **46**(10), 1726–1734 (2007).
14. R. Vonach, J. Buschmann, R. Falkowski, R. Schindler, B. Lendl, and R. Kellner, "Application of mid-infrared transmission spectroscopy to the direct determination of glucose in whole blood," *Appl. Spectrosc.* **52**, 820–822 (1998).
15. A. Pintar, J. Batista, and J. Levec, "In situ Fourier transform infrared spectroscopy as an efficient tool for determination of reaction kinetics," *Analyst (Cambridge, U.K.)* **127**(11), 1535–1540 (2002).

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