Journal of Biomedical Optics 7(4), 613–617 (October 2002)

Using two discrete frequencies within the middle infrared to quantitatively determine glucose in serum

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Abstract. Tight glucose monitoring is essential for the reduction of diabetic complications. This research investigated the changes of absorption spectra observed in serum at three prominent glucose absorption peaks in the middle infrared using a demountable liquid, transmission cell. Two frequencies of light were used to determine the glucose absorption: one at 1193 cm^{-1} to determine the background water absorption and the other at one of the characteristic peaks (1035, 1080, and 1109 cm^{-1}). The peak at 1035 cm^{-1} was best for quantitative determination with a standard of error of 20.6 mg/dl (1.1 mmol/L). While interference from other serum constituents could cause problems, urea and albumin—two constituents known to have close absorption peaks—were determined to have no effect on the ability to determine the glucose levels at 1035 cm^{-1}. [DOI: 10.1117/1.1501893]

Keywords: glucose sensor; infrared spectroscopy; artificial pancreas; transmission.

Paper JBO 01047 received July 16, 2001; revised manuscript received Mar. 22, 2002; accepted for publication Apr. 25, 2002.

1 Introduction

Diabetes mellitus is a metabolic disease whereby the human body cannot properly control its levels of glucose.[1] Past research has established a clear understanding of how the normal body controls its glucose.[1] Currently researchers are focused on methods of monitoring the glucose levels, both invasively and noninvasively. Ultimately, a glucose monitoring system could be used in the design of an artificial control system. The Diabetes Control and Complications Trial Research Group (DCCTRG) conducted a study between 1983 and 1989 to determine if the severity and frequency of complications related to insulin-dependent diabetes mellitus could be decreased with tight monitoring of glucose levels.[2] By performing at least four self-monitoring blood glucose treatments a day, the DCCTRG conclusively found a 76% reduction in the development of retinopathy, a 54% reduction in the progression of retinopathy, a 40%–50% reduction of nephropathy, and a 60% reduction of clinical neuropathy.[2] Therefore, tight glucose monitoring is essential for the reduction of diabetic complications. Since Clark's oxygen electrode was first used in the 1950's to detect glucose,[3] many attempts have been made to monitor glucose levels, ranging from other enzymatic techniques[4–24] to optical techniques.[25–39]

The use of the glucose oxidase enzyme has been well documented and can provide reliable short-term monitoring.[3–24] However, as a potential implantable device, the lifetime of the device is too short, and the electrodes are difficult to consistently reproduce. This is illustrated by several recent studies wherein the reliability of several enzymatic devices ranged from only 24 h to 11 days.[17,20,21]

Coté summarized several optical techniques for glucose monitoring in a recent paper.[25] Absorption spectroscopy techniques, both in near infrared (NIR) and the middle infrared (MIR), were discussed. There are relative advantages and disadvantages to each frequency range. Water absorbs more in the MIR, but the peaks are less distinct in the NIR. Thus, multivariate analysis is often used with NIR because of peak smearing and absorption interference from other blood constituents.[25] Also, the path length must be smaller for MIR because of the higher water absorption. While Heise et al. first proposed multivariate analysis for MIR, most of the recent research is focused on NIR.[30,31] Polarimetry, Raman spectroscopy, and fluorescence techniques for glucose monitoring were also discussed.[25]

Optical methods are advantageous as there is no dependence on chemical reactions or enzymatic lifetimes.[5,34] Also, a fluorescent molecule relating specifically to glucose is not required.[25,32] Various methods explored for potential implantation have included reflectance, light scattering, and transmission.[23] The approach under investigation in this paper is transmission within the MIR range.

The variables that exist with the light transmission approach are: (1) the concentration of the glucose within the sample—of particular interest for practical use, and (2) the path length, or the distance, that the light travels through the sample—a determining factor for the power requirement of the source and for the interference due to the water absorption peak. The concentration of other materials within the sample will affect the level of light transmission at certain frequencies as well. The proper frequencies for the determination of glucose were investigated by researching previous literature and performing new experiments.[33,34,36]

Zeller, Novak, and Landgraf, Kajiwara et al., and Back and Polavarapu have identified several characteristic frequencies in the MIR glucose absorption spectra.[33,34,36] Specific frequencies identified in blood by Zeller and co-workers using a de-

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mountable liquid cell with a 25 μm path length were 1365, 1152, 1109, 1080, and 1035 cm^{-1}. Only the 1035 cm^{-1} peak was limited to glucose in the context of other blood constituents. For example, the 1365 cm^{-1} peak was identified in albumin, hemoglobin, and other constituents; the 1080 cm^{-1} peak was identified in albumin and hemoglobin; and the 1109 cm^{-1} peak was identified in hemoglobin. However, if interstitial fluid is used for glucose detection, thus eliminating the effects of hemoglobin, then the 1109 cm^{-1} peak may be used. In addition, the presence of large peaks near a characteristic peak may mask its appearance. For example, a strong urea peak at 1160 cm^{-1} masked the characteristic glucose peak at 1152 cm^{-1}. While albumin will still cause added absorption at the 1080 cm^{-1} peak, the frequency is potentially usable because changes in albumin concentration have little effect on the absorption spectra.[34] Therefore, more than one peak could determine the glucose concentration. However, Zeller, Novak, and Landgraf used a range of glucose concentrations that were mostly higher than physiological levels, and even then the spectra had to be magnified 20-fold in order to resolve the differences at some of the characteristic peaks. The apparent frequency of the peaks will shift slightly depending on the resolution used and the exact temperature of the sample according to Lambert-Beer's law.[34] While there has been discussion of a decrease in water absorption when samples are taken at body temperature (37 °C) versus room temperature (25 °C), the effects are expected to be minimal.

There are several sample media that have been explored for the determination of glucose within the human body. For noninvasive external monitoring techniques, blood and tissue have been investigated. Other semi-invasive external techniques draw out interstitial fluid for measurement. The technique described in this paper is designed for use with extracellular fluid, particularly interstitial fluid, whether it is eventually implanted in the peritoneal cavity or used externally. Interstitial fluid closely resembles plasma with similar glucose values.[1] The only distinct difference between plasma and interstitial fluid is the decrease in protein in interstitial fluid, about sixfold.[1] Plasma and serum are similar as well except that plasma contains fibrinogen, whereas serum does not. Thus, serum was an appropriate substitute for interstitial fluid for experimental purposes. Also, Wientjes and Schoonen have demonstrated that the measurement of glucose in interstitial fluid and blood are similar.[24] According to Guyton and Hall, the glucose levels of interstitial fluid are similar to those of plasma.[1] However, Coté suggested that measurement of glucose in interstitial fluid is several orders of magnitude less than in plasma.[25] Guyton and Hall had also noted that intracellular fluid contains a significantly lower level of glucose than the extracellular fluid.[1] This may be the reason for the contrary opinion.

The primary objective of this study was to investigate the use of two frequencies of light within the middle infrared, one detecting the base line water absorption and the second detecting glucose, to determine the glucose levels in human blood serum samples. While other multivariate approaches have been used, it was hypothesized that a calibration curve using only two frequencies can be constructed. Since other constituents in the interstitial fluid can have an effect on the absorption spectra, a secondary objective was to determine the effects of high and low concentrations of urea and albumin (protein) on glucose absorption. The quantitative relationship between physiological glucose concentrations and the absorption spectra will be demonstrated.

Table 1 Glucose depleted human blood serum assay and referenced value ranges from literature.

Constitutents	Measured values	Normal range[a]
Glucose (mg/dl)	3	70–105
Albumin (g/dl)	5.7	6–7.9
Urea (mg/dl)	3	6–19
Cholesterol (mg/dl)	128	100–200
Triglyceride (mg/dl)	123	40–150
*p*H	7.2	6.5–7.8
Calcium (mg/dl)	0.9	9.5–10.5
Chloride (mEq/L)	96	100–110
Potassium (mEq/L)	2.5	4–5.4
Sodium (mEq/L)	138	138–148

[a]From literature (Guyton and Hall, Ref. 1; ABT).

2 Materials and Methods

Glucose concentrations ranging from 0 to 450 mg/dl in 50 mg/dl intervals were obtained by the addition of anhydrous glucose (Fisher Scientific) to glucose depleted base human serum (ABT, Sequin, TX). The glucose depleted base human serum assay is documented in Table 1.

The two serum constituents with the most prominent absorption near the glucose peaks of interest are urea and albumin. Albumin is the main protein in serum.[1] Urea and albumin (Sigma-Aldrich) were added to the serum, the samples were mixed thoroughly, and the samples were analyzed (UAB Clinical Outreach Lab) to confirm the amount of urea and albumin in the solution. Samples prepared with urea and albumin are identified in Table 2. The spectra for high and low glucose concentration samples with high and low levels of urea or albumin or both will be compared to control glucose concentration samples.

Absorption spectra from 1250 to 1000 cm^{-1} were collected at 25 °C using a Mattson Research Series 1000 Fourier transform infrared (FTIR) spectrometer (Mattson Tech. Inc., Fremont, CA). While a slightly higher absorption difference may be noticed when samples are run at 37 °C (body temperature), the focus of this research was to demonstrate the technique at room temperature. The spectra were collected at a 0.75 cm^{-1} resolution over 128 sample scans. The spectrometer utilized a liquid nitrogen cooled mercury–cadmium–telluride detector. The transmission cells utilized CaF_2 crystals for windows, as CaF_2 is resistant to aqueous solutions, acids, and alkalis and, more importantly, translucent in the spectral range of interest (Specac, Inc., Smyrna, GA).

As the path length of the transmission cell increases, the absorption due to the materials within the cell will also increase. The strongest absorbers of light within this region are

Table 2 Constituent values of urea, albumin, and glucose in high and low glucose human blood serum samples.

	Glucose	Albumin	Urea
Glucose depleted serum	3	5.8	2
Urea added serum	3	5.7	48
Albumin added serum	3	7.2	3
Urea and albumin added serum	3	7.4	28
Glucose added serum	512	5.8	3
Urea added serum	495	5.9	37
Albumin added serum	523	7.8	2
Urea and albumin added serum	509	7.8	31

water and glucose. Eventually, water absorption peaks will completely mask all resolvable glucose peaks if the path length is continuously increased. A compromise must be reached whereby a path length large enough to allow for resolvable differences between physiological concentrations of glucose and small enough to observe a minimum level of glucose (in this case 5 mg/dl) is reached. There must also be a significant difference between the absorption at 5 mg/dl and the absorption at 450 mg/dl, so that there are enough resolution points for quantitative determination.

Only 6 and 12 μm tin spacers were available for this liquid cell (Specac, Inc., Smyrna, GA). Use of up to three 12 μm spacers resulted in a minimum detection limit of 5 mg/dl. The addition of a 6 μm spacer or a 12 μm spacer resulted in a minimum detection limit of 50 and 100 mg/dl, respectively, due to increased water absorption. Thus, three 12 μm spacers were used to construct the liquid cell.

Spectra of the empty cell was obtained to validate the path length. By utilizing the fringe patterns resulting from constructive and destructive interference, the nominal path length was calculated according to Eq. (1)

$$d=(n*10^4)/[2*(f_1-f_2)], \tag{1}$$

where f_1 and f_2 are the frequencies (cm^{-1}) of the two maxima from interference fringes observed in the spectra of the empty cell, n is the number of maxima between f_1 and f_2, and d is the path length.[30]

The path length of the empty cell was determined to be 39 μm. Due to the tolerance value for the thickness of the spacers, the path length was slightly larger than the expected 36 μm. The nominal path length was used to calculate the K coefficient of absorption according to Eq. (2)

$$K=(\ln 10^A)/d=(A*\ln 10)/d=\sim 2.3*(A/d), \tag{2}$$

where A is the absorption value according to the spectra taken, and d is the calculated path length.

Following the determination of the path length, spectra were collected for each of the samples. Three data points were collected for each of the human blood serum samples at the specific concentration intervals. Between each spectra, the cell was purged with distilled water several times in order to remove any possible residue from the previous test. Raw absorption values were recorded at 1035, 1080, 1109, and 1193 cm^{-1}. The 1193 cm^{-1} absorption point had no observable change across all spectra of glucose concentrations nor appeared to be related to any other constituent, so it was used as a base line determination of water absorption since the base line of a spectra can vary due to the spectrometer. All raw absorption glucose values were subtracted by the value at 1193 cm^{-1}. This difference absorption value, which is related to the relative glucose change, was then converted into a K coefficient of absorption value with Eq. (2).

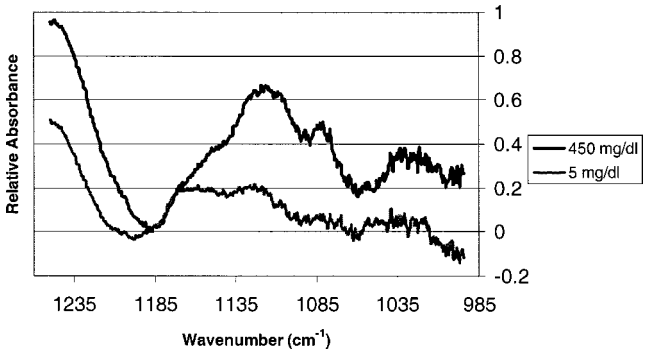

Fig. 1 Relative absorption change between high and low glucose human blood serum samples following water subtraction.

Statistical analysis was performed using StatView software (Cary, NC). Linear regression analysis provided the R^2 values. P values were calculated for the first, second, third, and fourth order equations. Residual plots were also obtained to determine if the data exhibited randomness about the fitted equations. The estimated error in the regression analysis was determined from the root mean squared residual error values.

3 Results

Figure 1 presents a comparison between spectra at 5 and 450 mg/dl. All water absorption has been subtracted from each spectra to clearly illustrate absorption due to serum constituents, specifically glucose. A relative increase in absorption due to glucose changes is noticeable at the three frequencies of interest: 1035, 1080, and 1109 cm^{-1}.

The K coefficient of absorption difference, or the absorption due to glucose, is plotted versus the concentration of glucose in Figure 2. Each concentration point is the average of three data points with each error bar representing one standard deviation.

The statistical analysis of the data is presented in Table 3. The R^2 values suggest potential linearity of the data presented in Figure 2. P values for the coefficients of the first order term were less than 0.05, thus indicating a linear relationship. P values for the coefficients of the second order equation were greater than 0.05 for the 1035 and 1080 cm^{-1} frequencies indicating that the second order term can be neglected. Similarly, the P values for the third and fourth order equations were greater than 0.4 indicating the higher order terms could also be neglected. The residual plots conducted for the first order fitted lines exhibited a randomness of the data. The estimated error of prediction for glucose concentrations ob-

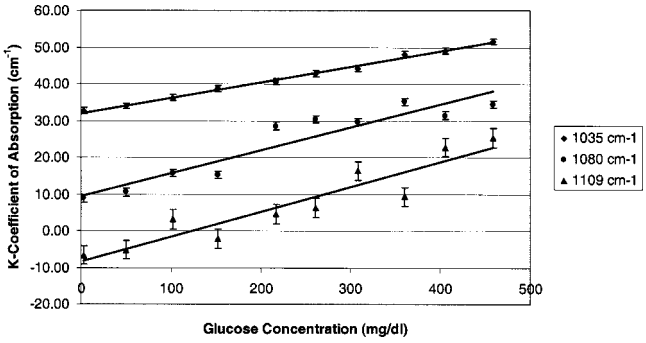

Fig. 2 Observed change in *K* coefficient of absorption with changes in glucose concentration. Error bars represent one standard deviation from each data point average.

tained using the 1035 cm^{-1} frequency was significantly lower than the estimated error for the other two frequencies.

Urea and albumin changes did not result in notable absorption changes in the high or low glucose spectra. The predicted glucose values that were determined for each of the spectra with varying urea and albumin values were within the estimated error presented in Table 3 for each potential frequency to be used for glucose determination (1035, 1080, and 1109 cm^{-1}). The values were random about the clinically determined value and did not exhibit any relationship due to an increase in either urea or albumin.

4 Discussion

A quantitative relationship between the glucose concentrations and the *K* coefficient of absorption values was determined (Figure 2). The relative change in absorption between high and low glucose values within the physiological region, when water is subtracted, is clearly observed (Figure 1). While there is a larger difference observed at 1109 and 1080 cm^{-1}, the 1035 cm^{-1} frequency is more quantitative. A possible cause is that water is a stronger absorber near the 1109 and 1080 cm^{-1} frequencies and thus causes less discernible results.

The 20.6 mg/dl (1.1 mmol/L) error at the 1035 cm^{-1} frequency is comparable to other research. Coté summarized that multivariate NIR research produced error values of 1.67–2.78 mmol/L (30–50 mg/dl).[25] Also, high and low concentrations of urea and albumin did not affect the quantitative determination of glucose in serum at 5 or 500 mg/dl concentrations.

Table 3 Statistics for the quantitative determination of glucose.

	1035 cm^{-1}	1080 cm^{-1}	1109 cm^{-1}
R^2 values	0.982	0.880	0.821
P values			
First order (*x*)	0.0001	0.0001	0.0001
Second order (*x*)	0.005	0.580	0.001
Second order (x^2)	0.163	0.240	0.027
Estimated error (mg/dl)	20.6	53.4	65.2

While Zeller and co-workers demonstrated a qualitative ability to detect glucose using transmission techniques, this study demonstrated the ability to quantitatively determine glucose in serum.[33] Most of the concentrations used by Zeller and co-workers were higher than normal levels and revealed the apparent saturation of absorption due to glucose. The results from this study exhibited no saturation within physiological levels and suggested a linear relationship. However, the quantitative ability of the technique is most important, regardless of linearity.

The next step will be to transition from the FTIR system to a breadboard design consisting of frequency specific lasers and detectors. The calculated cross section of absorption for the glucose molecule at 1035 cm^{-1} ($\sigma_a = 2.2 \times 10^{-16}$ cm^2) is acceptable for this transition. The required sensitivity of the detector for a successful, breadboard device could be estimated using the values of the path length (d), the glucose concentration, the cross section of absorption coefficient (σ_a), and the excitation power of the required infrared source (~10 mW). According to this calculation of the sensitivity requirement (hundreds of μW), commercial detectors are available and thus allow a transition to a breadboard design. Currently, a quantum cascade laser will be used in conjunction with a pyroelectric detector. Both of these devices operate at room temperature and could be the beginning of a potentially implantable device. Other spectroscopic techniques besides transmission will also be considered, such as optical cavity ring-down spectroscopy (CRDS).[40] CRDS has been reported to be six orders of magnitude more sensitive than FTIR for gas detection.[40] Studies are ongoing to determine the applicability of this technique for the detection of glucose.

5 Conclusions

A quantitative relationship between glucose concentrations and corresponding absorption spectra changes within normal physiological ranges was demonstrated. The 1035 cm^{-1} frequency was the best predictor of glucose with a 20.6 mg/dl error. Additions of urea and albumin at high and low glucose concentrations had no effect on the ability to detect glucose. While this research is a basic spectroscopic study of glucose, it demonstrates the feasibility of using two frequencies of light, one to detect the water background absorption and the other to detect changes, to quantitatively determine the glucose concentrations in serum samples.

Acknowledgments

A Whitaker Foundation Special Opportunity Award provided a partial graduate student stipend for this research. The initial support and guidance of Micah D. Schmidt, Jim L. Davidson, Duco E. Jansen, and Robert L. Galloway (Vanderbilt University) during a senior design project is also acknowledged.

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