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Advances in Photoacoustic Noninvasive Glucose Testing

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We report here on in vitro and in vivo experiments that are intended to explore the feasibility of photoacoustic spectroscopy as a tool for the noninvasive measurement of blood glucose. The in vivo results from oral glucose tests on eight subjects showed good correlation with clinical measurements but indicated that physiological factors and person-to-person variability are important. In vitro measurements showed that the sensitivity of the glucose measurement is unaffected by the presence of common blood analytes but that there can be substantial shifts in baseline values. The results indicate the need for spectroscopic data to develop algorithms for the detection of glucose in the presence of other analytes.

The noninvasive measurement of body analytes is of considerable interest and importance to both the medical community and the general public. As concern grows regarding the spread of diseases, such as HIV and hepatitis, may be transmitted by bodily fluids, the need for noninvasive measurement techniques that eliminate the risk of contamination also increases. In addition, the elimination of pain in the measurement process and the potential for continuous measurement are also key factors. The noninvasive measurement of blood glucose in particular is of great individual and economic importance because of the large population of diabetics who require regular and accurate information regarding their blood glucose concentrations.

In 1997, it was reported *(1)* that there were more than 120 million diabetics world-wide; this number is predicted to increase to more than 220 million by the year 2010. Diabetes mellitus is a complicated and serious condition and is one of the most prevalent noncommunicable diseases in the world. Successful management of diabetes involves knowledge of the current blood glucose concentration to allow the diabetic to compensate by diet, oral medication, or insulin injections. Without knowledge of the blood glucose concentration, the correct treatment is not possible and serious complications affecting internal organs, circulation, and eyesight may occur.

The current methods of measuring blood glucose concentrations require the diabetic to obtain a blood sample for analysis by a test strip. According to a recent 9-year study, The Diabetes Control and Complications Trial *(2)*, optimum treatment for a diabetic requires regular blood glucose measurements; however, the discomfort experienced by users means that present methods are not being used by diabetics with sufficient regularity. There are indications *(3)* that the use of spot check measurements does not provide adequate information for optimal glucose control, and there is a strong case for more frequent or "continuous" measurements. This type of measurement would also yield the rate of change of glucose, which would allow planning of the appropriate insulin dose or dietary content.

The measurement of blood glucose by any technique is inherently complex because of the wide range of potentially interfering components. For a noninvasive technique, not only are there many analytes within human blood that could interfere with the measurement, but there are also other problems such as the variability and inhomogeneity of human skin and the constantly changing human physiology.

Research into new, less painful methods of measurement has been carried out for several decades, and several reviews on the subject have been published *(4–6)*. The two most popular techniques being attempted are measurement of the change in optical transmission or polarization rotation attributable to the presence of glucose. In transmission configurations, the intensity of light transmitted at several wavelengths is used to determine glucose concentrations. Transmission spectroscopy is susceptible to light scattering in tissue and is technically limited

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by the requirement of fixed, diametrically opposed, positioning of light source and detector. Transmission measurements have been carried out on the skin *(7)* and on animal and human eyes *(8)*, making use of the correlation between blood glucose concentration and the glucose concentration of the aqueous humor in the eye. Optical rotation of polarized light in the aqueous humor of the eye *(9–12)* has also been investigated. Other researchers have carried out in vitro tests using Raman spectroscopy *(13–16)*, fluorescence studies *(17)* and Fourier-transform infrared spectroscopy *(18–20)*. The human lip has been used as a location for glucose measurements using techniques including diffuse reflectance *(21)* and attenuated total reflectance *(22)*. The latter technique has also been used for tests on the finger *(23)*.

Other noninvasive approaches attempt to use a correlation between glucose content in interstitial fluid and capillary blood *(24)*. The use of a patch or reservoir on the skin surface to collect glucose has also been explored *(25)*.

In this report, we discuss the alternative technique of pulsed laser photoacoustic spectroscopy. In this technique, short pulses of laser light are directed into the tissue. The resulting acoustic signal depends on the optical and physical characteristics of the sample and may be the basis of determining blood glucose concentrations noninvasively.

Recent developments in optoelectronic technologies give the photoacoustic method of measurement the potential for portability and long component life with the use of piezoelectrics and compact electronics and diode lasers with levels of optical radiation that are several orders of magnitude below pain or tissue damage thresholds.

Background

The photoacoustic process involves the conversion of optical energy into acoustic energy by a multistage energy conversion process. The fraction of incident optical energy that is absorbed is determined by the optical absorption coefficient. The absorbed energy may then be dissipated by radiative processes in which light is re-emitted from the sample or by nonradiative processes in which the absorbed energy leads to localized heating of the sample. In the latter case, this produces a small temperature rise, which is characterized by the specific heat capacity and in turn leads to volumetric thermal expansion of the optical interaction region. The resulting dimensional change and associated pressure pulse are the basis of the thermoelastic photoacoustic generation. This ultrasonic pressure pulse propagates from the generation region and can then be measured by a piezoelectric detector.

The photoacoustic pulse consists of an isolated pressure wave comprising a compressive pulse followed by a rarefaction. Generally, the peak-to-peak amplitude of the detected photoacoustic pulse is used for analysis and is directly proportional to the absorbed energy. Accordingly, an energy measurement is used to normalize the peak-to-peak measurement. A typical photoacoustic signal is shown in Fig. 1. The lower trace is the optical signal, and the upper trace is the photoacoustic signal. A time delay between the optical pulse and the photoacoustic pulse is evident. This time delay is attributable to the propagation of the photoacoustic pulse from the acoustic source to the detector and can be used for velocity of sound measurements or to determine the location of the acoustic source in imaging techniques.

The generated pressure in the photoacoustic process can be described by the following wave equation, where electrostriction *(26)* is ignored:

$$\left[\frac{1}{v^2}\frac{\partial^2}{\partial t^2} - \nabla^2\right] p = \frac{\alpha\beta}{C_p}\frac{\partial I}{\partial t} \tag{1}$$

where I is the intensity of the laser light, v is the sound velocity in the medium, α is the optical absorption coefficient, β is the coefficient of volumetric thermal expansion, C_p is the specific heat capacity, and $p(r,t)$ is the acoustic pressure.

For a weakly absorbing sample, the peak pressure P can be described *(27, 28)* by the following equation:

$$P = k\frac{\beta v^n}{C_p}E_0\alpha \tag{2}$$

where k is a system constant, E_0 is the incident laser pulse energy, and n is a constant between one and two, depending on the particular experimental conditions.

In some cases, the combination of physical parameters gives an enhancement of the photoacoustic response compared with the response in conventional spectroscopy, and this can be exploited, for example, in the detection of hydrocarbons in water *(29, 30)*. The response to glucose, however, does not give substantial photoacoustic signal enhancement.

Photoacoustics have been used to obtain information about the concentrations of species, including the moni-

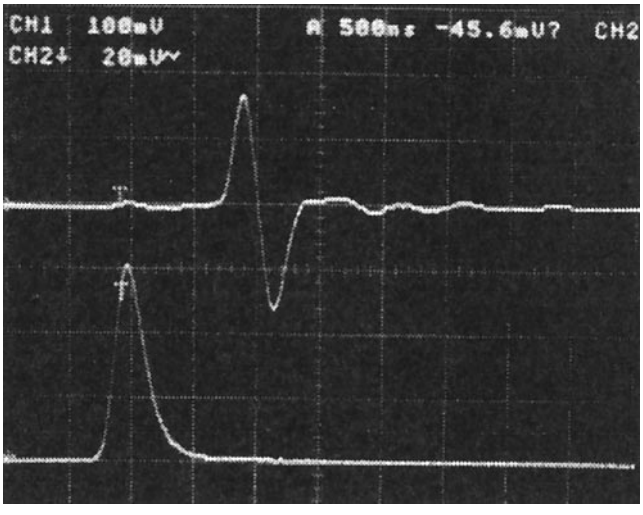

Fig. 1. Typical photoacoustic and optical signal.

toring of trace gas concentrations *(31–34)*, medical applications such as the detection of cancer *(35, 36)*, and investigations into skin structure *(37)* and chemical penetration in the skin *(38)*. Photoacoustics has also been used for lateral and depth imaging *(35, 36, 39–44)* and has applications in other medical and biological areas, including the determination of melanin in human hair *(45)* and investigations into the diffusion of chromophores in human skin *(46)*.

Experimental Studies

To assess the feasibility of photoacoustic noninvasive blood glucose detection, the technique was first used for in vitro studies in the mid-infrared region with both aqueous glucose solutions and human whole blood *(47)*. Within the wavelength region of 9.564–9.694 μm, it was shown that the optimum wavelength for glucose detection was 9.676 μm. At the wavelength 9.676 μm, aqueous glucose solutions of 8.3–426 mmol/L (149–7670 mg/dL) were investigated, and at glucose concentrations <33 mmol/L (600 mg/dL), a linear relationship was shown.

Although previous work in the mid-infrared region demonstrated the potential of photoacoustics as a method of measuring glucose concentrations, this wavelength region is not regarded as viable for human tissue studies because of the high water absorption that reduces penetration depths to microns. This penetration may not be sufficient to investigate blood constituents within human tissue, although interaction with interstitial fluid yields measurements that correlate with blood glucose concentrations but with a time shift *(48)*.

The spectral region that shows the most promise for absorption by the analytes within blood is within the "tissue window", around the 1–2 μm region *(49)*. Although measurements within this region are advantageous for tissue studies, they coincide with a region of lower glucose absorption. Initial results with pulsed photoacoustics achieved glucose concentration measurements within this region in aqueous solutions *(50)*, gelatin phantoms *(51)*, and blood samples *(52)*.

Aqueous glucose solutions in the concentration range 1.7–33 mmol/L (30–600 mg/dL) have been used for in vitro measurements in this tissue window spectral region *(49)*. The percentage of change in the photoacoustic response of the glucose solutions was compared with that of water at a wavelength of 1700 nm. A linear relationship, $y = 0.21x - 0.02$ with a correlation coefficient of 0.99 was obtained, where x is the glucose concentration and y is the percentage of change of the photoacoustic response from that of water.

Photoacoustic measurements in a gelatin-based tissue phantom *(51)* have also shown a linear correlation when a wavelength of 1.064 μm is used to investigate glucose concentrations between 1 and 100 mmol/L (180 and 18 000 mg/dL). At this wavelength, there was a 71% change in the photoacoustic signal over the above concentration range.

In vitro photoacoustic investigations of blood plasma and human whole blood samples within the glucose concentration range 0.56–10 mmol/L (10–1800 mg/dL) have also been investigated *(47, 53)* and gave a linear correlation between the glucose concentration and the change in the photoacoustic response at 1180 and 1700 nm.

Preliminary in vivo studies have also been undertaken to assess the feasibility of photoacoustics as a noninvasive technique on humans *(49)*.

Eight consenting volunteers were studied under a protocol, which was approved by the local ethics committee. Four nondiabetic subjects, two type 2 diabetics (non-insulin-dependent), and two type 1 diabetics (insulin-dependent) were studied.

The non-diabetic and type 2 volunteers fasted from the previous evening, and the type 1 diabetic volunteers omitted their morning insulin. Non-diabetic and type 2 diabetic volunteers received a standard 75-g D-glucose load orally. The blood glucose concentration of the type 1 diabetics was controlled by administration of insulin, and all subjects with higher than normal blood glucose concentrations were monitored until their blood glucose concentration fell to normal values. The subject was seated throughout the procedure with the right index fingers immobilized in a mount. The laser pulse was incident on the side of the finger through an optical fiber, and the photoacoustic signal was detected from the pad of the finger, which was in contact with the piezoelectric transducer.

A near-infrared pulsed laser source *(30)* was utilized in the in vivo studies. A piezoelectric transducer detected the photoacoustic pulses, and the resulting signals were acquired with a similar set-up as described in the in vitro studies discussed later. The mean photoacoustic signals were acquired over a 5-s period at intervals of 5 min.

Reference blood samples were taken at 10-min intervals, and blood glucose concentrations were measured using a Yellow Springs Instruments analyzer (YSI 2300G). In addition, blood glucose concentrations from the diabetic subjects were monitored periodically during the tests using an Exactech Companion 2 meter and test strips.

The photoacoustic data presented in Fig. 2 are based on the measurement of the peak-to-peak amplitude of the photoacoustic signal, with a correction for linear system drift, which may be attributable to physiological factors as a result of the subject being immobilized throughout the observations. The CV in the reference measurement of the blood glucose concentration was 3%, and that in the photoacoustic response varied between 1% and 5%, depending on the initial instrument gain settings.

In Vivo Results

Three key results from these tests, which were previewed earlier *(52)*, are shown in Fig. 2, which shows the change in both the glucose concentration and the photoacoustic response throughout the duration of the test. In each case,

there was good correlation (r^2 >0.84) between the photoacoustic measurement and the measured glucose concentration. The gradient and the offset differed in each case, which may have been attributable to a combination of both instrumental settings and person-to-person variables, which will be investigated in future work. The results, however, did allow a predicted glucose concentration to be calculated from the photoacoustic measurements, using the individual linear regressions.

The results for all of the measurements, at 10-min intervals, when both clinical and photoacoustic measurements were available for all eight subjects are shown in Fig. 3. This shows a linear correlation of 0.96 between the predicted and the clinical glucose concentration.

We recognize that this is a favorable presentation of the results, and a more realistic representation of the data can be seen through analysis of the percentage of change of the predicted glucose concentration from the clinical measurement. The results of this analysis are presented in Fig. 4, which shows that ~91% of the data points lie within ± 20% of the clinical glucose concentration, corresponding to zone A in a Clarke *(54)* error grid analysis.

Current In Vitro Studies

In the preceding sections, the only variable under consideration was glucose. To explore the more realistic physiological picture, an in vitro investigation of the effect of other blood analytes was undertaken. The in vitro studies described here were designed to investigate the wavelength dependence of the photoacoustic signals from glucose in the presence of several common blood analytes. There are numerous analytes within the blood, and three were chosen for this initial study, sodium chloride, cholesterol, and bovine serum albumin (BSA).

A tunable MOPO-710 laser system (Spectra Physics), pumped by the third harmonic of a Nd:YAG laser, was used as the optical source. This provided nearly continuously tunable pulsed optical energy from 400 to beyond 2000 nm, with a pulse duration of approximately 7 ns at a repetition rate of 10 Hz. The optical output was launched into a 2-m length of optical fiber (diameter, 1000 μm), using a 6-cm focal length lens. The fiber output was directed through a beam splitter at an angle of 45° to create an energy monitoring channel, and each optical channel was focused into a photoacoustic cell using a 10-cm focal length lens. The sample to be analyzed was contained within the first photoacoustic cell, whereas the presence of a second reference photoacoustic cell provided energy monitoring. For the experiments under consideration here, the energy monitor was provided by the second photoacoustic reference cell containing distilled water, which allowed compensation for variations in incident optical energy and the photoacoustic response to the water spectra.

The voltage pulses from the piezoelectric detector were amplified with a precision AC 9452 amplifier (1MHz bandwidth; 100 MΩ input impedance; 1 kΩ output im-

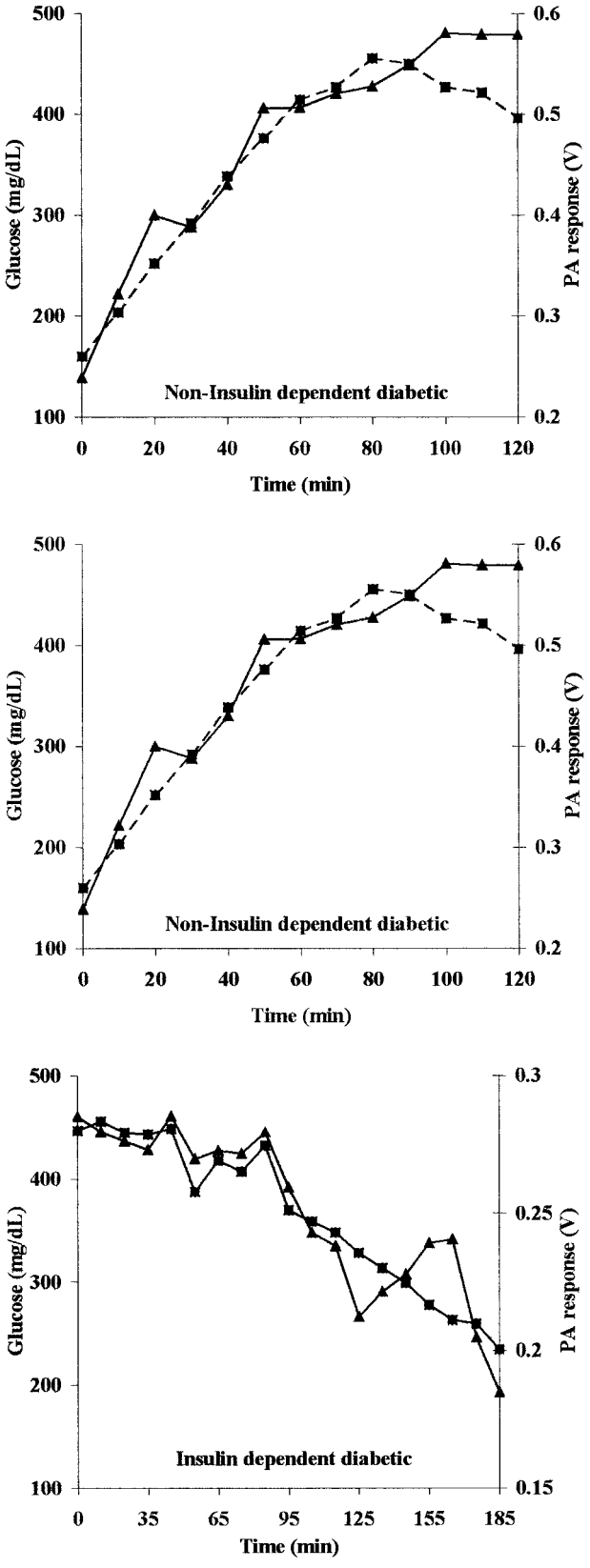

Fig. 2. Results of oral glucose tolerance tests on three subjects. ■, glucose (mg/dL); ▲, photoacoustic response (V). To convert mg glucose/dL to mmol/L, divide by 18.

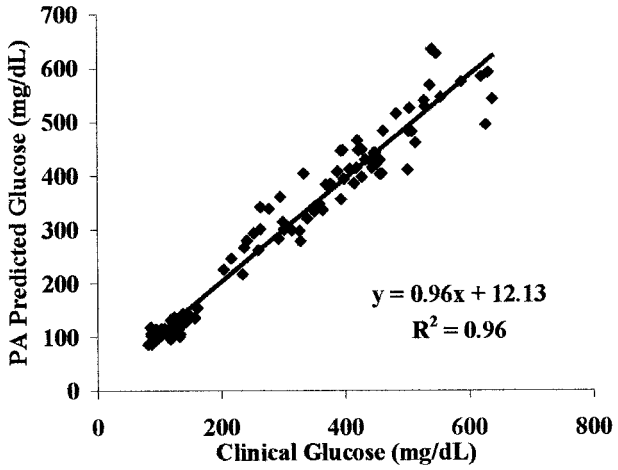

Fig. 3. All results from all 53 volunteers.
To convert mg glucose/dL to mmol/L, divide by 18.

pedance; Brookdeal/EG & G). The resulting signals were digitized by a 125 million samples/s, dual channel CompuScope CS2125 PC plug in card (GaGe Applied Sciences), where the signals were recorded and analyzed. Typically, one photoacoustic measurement resulted from 100 pulses averaged in the software to reduce random noise.

Glucose solutions were prepared by dilution of a 1.67 mol/L (30 g/dL) D-glucose stock solution. The glucose concentrations were also cross-referenced with an Abbott-Vision™ clinical chemistry analyzer (Abbott Laboratories).

The spectral region of 800-1200 nm was chosen for these investigations after initial work showed the largest changes with glucose concentration occurred at ~1040 nm.

Photoacoustic responses from the sample and baseline water samples were measured alternately to separate long-term system drifts from changes attributable to the sample concentration. The spectrum of distilled water and a 500 mmol/L (9 g/dL) glucose solution can be seen in Fig. 5. To investigate the changes from the water spectra, the percentages of difference of all the spectra from the baseline water spectrum were calculated. In each of the following studies, the letters a, b, c, d, and e indicate the sequence in which the spectra were obtained.

The results of this procedure for the glucose spectrum of Fig. 5 can be seen in Fig. 6. The absolute values of the peak wavelengths are inexact in this type of low-resolution experiment, but spectral features may be noted that relate to established spectroscopy of glucose in solution *(4)*. The greatest percentage of change in the photoacoustic response from the glucose sample is in the region of the C—H second overtone at 1126 nm, with a further peak in the region of the second O—H overtone at 939 nm. These assignments are tentative at this stage and require further investigation. Similarly, in Fig. 6, the reduction in photoacoustic response at 825 nm is within the resolution of the experiment and may be attributable to a displacement effect, but this also requires further investigation.

To investigate the possible contributions of other blood analytes to the total photoacoustic response from blood, solutions of sodium chloride, cholesterol, and BSA were investigated. In this preliminary study, the percentages of change of the photoacoustic signals of the analytes compared with a baseline from water were investigated within the same spectral region as the glucose study. As can be seen from the results, sodium chloride (Fig. 7), cholesterol (Fig. 8), and BSA (Fig. 9) each exhibited a different photoacoustic response.

The photoacoustic response of the sodium chloride solution was distinctly different from the corresponding response of the glucose solution, as shown in Fig. 6. The sodium chloride solution gave a significant increase in the photoacoustic response from 800 to 975 nm. The peak of

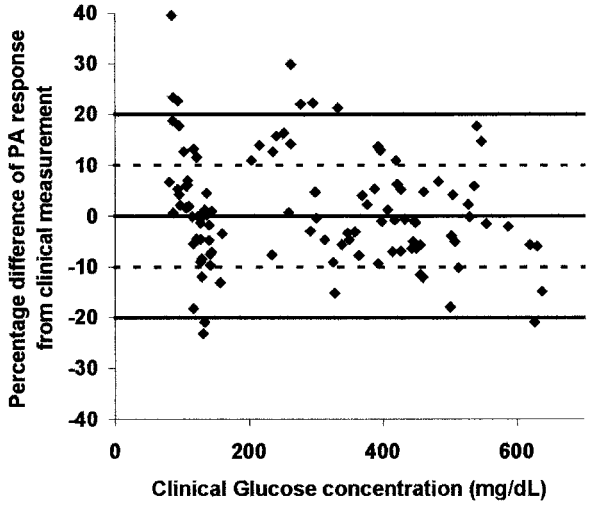

Fig. 4. Percentage difference plot of all data.
To convert mg glucose/dL to mmol/L, divide by 18.

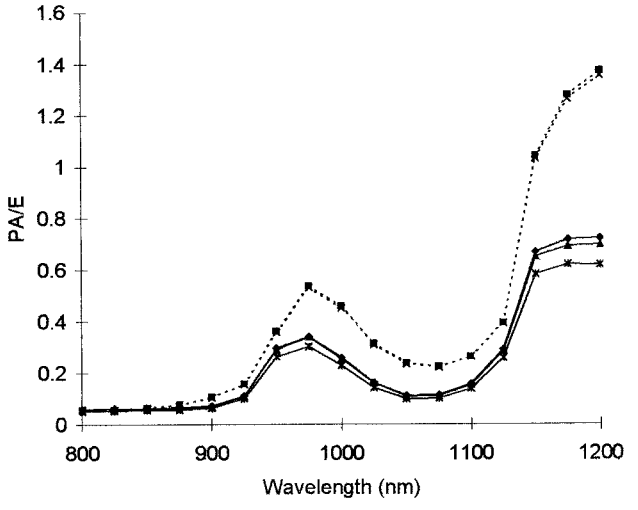

Fig. 5. Photoacoustic spectra of distilled water and a 500 mmol/L (9 g/dL) glucose sample.
♦, water (sequence a); ■, glucose (sequence b); ▲, water (sequence c); ×, glucose (sequence d); ∗, water (sequence e).

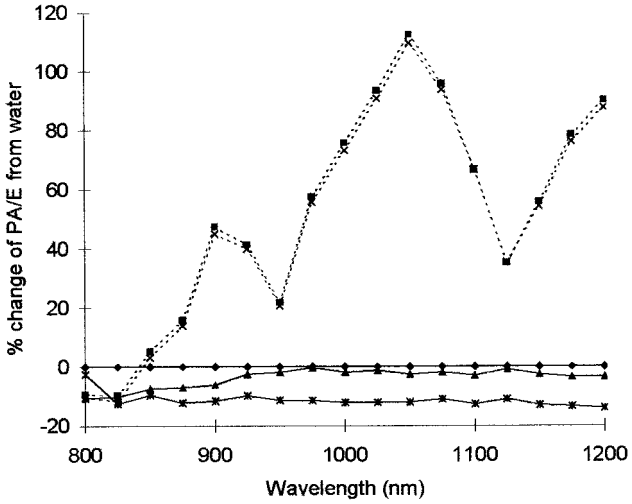

Fig. 6. Spectral percentage of change of 500 mmol/ L (9 g/dL) glucose from water.

♦, water (sequence a); ■, glucose (sequence b); ▲, water (sequence c); ×, glucose (sequence d); ∗, water (sequence e).

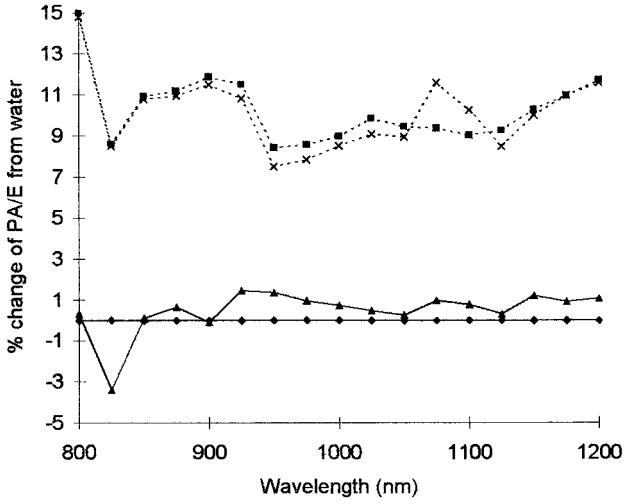

Fig. 8. Spectral percentage of change of 24 mmol/L (926 mg/dL) cholesterol from water.

♦, water (sequence a); ■, cholesterol (sequence b); ▲, water (sequence c); ×, cholesterol (sequence d).

this response coincides with an overtone band in the water spectrum at 970 nm *(55)*, and the dissolution of NaCl is known to cause a shift of such water bands to longer wavelengths *(56)*. It may also be that the changed physical parameters related to the presence of sodium chloride enhance such changes.

The difference in the cholesterol photoacoustic response showed the least spectral features of all four samples, with a basically flat response. However, the sensitivity and resolution in this experiment may be insufficient to show the weak features that have been observed in previous work *(57)*.

The photoacoustic response from the BSA solution showed a small feature at 1050 nm and a significant increase in the photoacoustic response at wavelengths below 900 nm that had not been observed in previous studies *(58)* and may be attributable to coloration or other contaminants in the sample.

This initial study is a precursor to the detailed investigation that will be required before selecting a set of wavelengths on a clear spectroscopic basis that, with appropriate algorithms or other analysis to compensate for the background response from other analytes, may allow the unique determination of glucose concentrations.

Discussion

There are many analytes within human tissue and body fluids that could interfere with the determination of glucose concentrations in the blood. Three such analytes

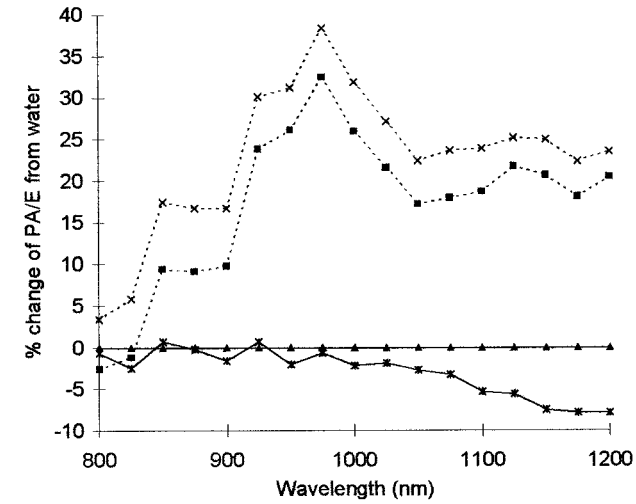

Fig. 7. Spectral percentage of change of 9 g/L sodium chloride from water.

■, salt (sequence a); ▲, water (sequence b); ×, salt (sequence c); ∗, water (sequence d).

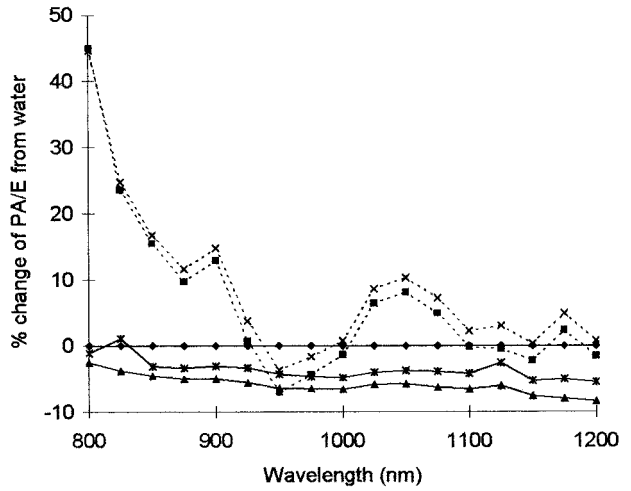

Fig. 9. Spectral percentage of change of 256 g/L BSA from water.

♦, water (sequence a); ■, BSA (sequence b); ▲, water (sequence c); ×, BSA (sequence d); ∗, water (sequence e).

have been investigated here, sodium chloride, cholesterol, and BSA. From these studies and additional studies of other blood analytes, it may be possible to select wavelengths for discrete glucose concentration measurements. Specific analysis techniques for wavelength selection have not been developed here because the spectroscopic study is not yet complete and we felt that analysis of a complicated system such as tissue and blood based on only a few body analytes would not be fruitful. However, the photoacoustic spectra obtained here show a good indication for the potential use of several wavelengths to specifically monitor glucose in the presence of other body analytes. Previous work *(50)*, as illustrated in Fig. 10, has shown that changes in the photoacoustic response as a result of changes in glucose concentration are insensitive to the presence of specific blood analytes, although the baseline changes substantially. This aspect indicates that multiple wavelength sources will be essential in a viable instrument to give both selectivity of response and baseline correction.

Because of the complicated nature of both the photoacoustic generation process and the structure and composition of human tissue, it is inevitable that further analysis techniques will be required to eliminate these influences. At present, the analysis of data is carried out using the peak-to-peak value of the photoacoustic response, although the entire signal is recorded. Other methods are under consideration that use the recorded temporal signal, such as Fourier and wavelet analysis, as well as general data analysis techniques including chemometrics.

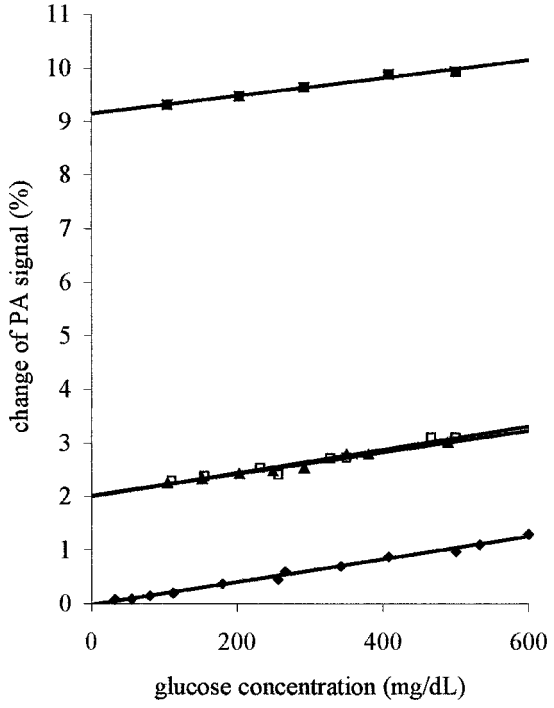

Fig. 10. Response to glucose in the presence of other analytes.

♦, glucose; □, glucose + NaCl (3 g/L); ▲, glucose + cholesterol (1.8 g/L); ■, glucose + BSA (63 g/L). To convert mg glucose/dL to mmol/L, divide by 18.

The absolute noninvasive determination of blood glucose concentrations may also require the combination of the photoacoustic technique with other optical techniques or physical measurements.

Conclusion

The feasibility of the photoacoustic measurement of blood glucose has been explored in aqueous solutions and whole blood and plasma samples as well as in a gelatin phantom and noninvasively with human subjects. The in vitro study *(52)* has shown that the sensitivity of response to glucose is unimpaired by the presence of other analytes, but it also highlights the need for high-quality spectroscopic data to eliminate the photoacoustic response produced by blood analytes other than glucose. This in vivo study has demonstrated that glucose trends can be tracked by the photoacoustic technique and may have potential for the development of a noninvasive instrument for the monitoring of blood glucose concentrations.

Current work is focusing on improving the repeatability and sensitivity of photoacoustic measurement to that required by the diabetic community as well as investigating possible detection sites on the human body and sensor design geometry for such body sites.

The overall conclusion from this initial study is that although the photoacoustic method has several promising aspects, it is possible that additional data may be required from the fund of interesting work in other areas that is being pursued in the challenging quest for the noninvasive measurement of blood glucose.

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