FEASIBILITY STUDY ON NON-INVASIVE MEASUREMENT OF BLOOD GLUCOSE CONCENTRATION USING INSTANTANEOUS DIFFERENTIAL NEAR INFRARED SPECTROPHOTOMETRY

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Abstract: A novel method, called *Pulse Glucometry*, for non-invasive measurement of blood glucose concentration (BGL) using instantaneous differential near infrared spectrophotometry is described. The method uses the analysis of sequential temporal differences of infrared transmittance spectra from the light intensity (I^{λ}) emerging from a fingertip containing an arterial pulse component (ΔI^{λ}) produced by pulsatile arterial blood volume changes occurring throughout the cardiac cycle. A new spectrophotometer was developed, covering the wavelength range from 900 to 1700 nm, scanning at a maximum spectral rate of 1800 spectra/s, with a minimum exposure time of 20 μs. Calculation of sequential spectral differences then allowed the derivation of spectra originating only from the pulsatile blood component, thus eliminating influences of interfering factors including the basal blood volume, skin, muscle and bone. *In vivo* tests were carried out with a glucose tolerance test in 2 healthy volunteers, in whom blood samples were collected from the cephalic vein simultaneously with light intensity measurements in the fingertip every 10 min before and after oral administration of glucose solution for 120 min. Results show a reasonable accuracy of BGL predicted with a PLS calibration model as compared to colorimetric assay data from sampled blood.

Introduction

Considerable effort has been directed over several decades towards developing methods for the measurement of blood glucose concentration (BGL), primarily for use in diabetic subjects where the method must ideally be convenient and painless. The methods that have emerged include invasive and semi-invasive techniques such as implantable glucose sensors[1, 2] and skin micro-poration using laser or miniaturized lancets[3]. Transdermal measurement of BGL by the extraction of interstitial fluids through the skin of a forearm has been reported both with iontophoresis[4, 5] and with sonophoresis [6] techniques. A BGL monitoring device using iontophoresis, the GlucoWatch Biographer[7], has recently been made commercially available (Cygnus Inc., USA). Although this measurement approach could be more favourable for diabetic patients than implantable devices or techniques based on sampling of blood the occurrence of dermatitis due to extraction of interstitial fluids from the skin has been pointed out as a frequent problem.

In addition to the development of invasive and semi-invasive techniques there have been numerous attempts to develop truly non-invasive BGL measurement techniques, particularly using infrared spectroscopy (see review[8]). Although such optical methods seem very attractive, because they avoid the problems of implantation or blood sampling, to date results have not been acceptable. In principle spectrophotometry has the potential to allow non-invasive measurement of glucose but such measurement is confounded by other optically absorbing and scattering elements such as skin, muscle and bone[8].

We have recently developed a novel non-invasive BGL measurement technique, named *Pulse Glucometry*, which is capable of removing influences of such confounding factors by obtaining optical data from a blood-only compartment in the tissue using instantaneous differential near infrared spectrophotometry[9].

Basic Principle of the Measurement

The essence of our new method is to utilise a blood volume change in a tissue segment under optical interrogation, such as a finger, and then with a subtraction process we remove the influences of basal interfering elements. We assume a simplified model of the tissue

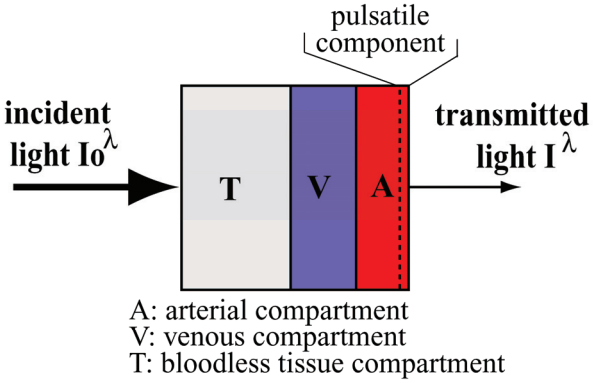

Figure 1: Simplified Optical Model of Biological Tissue Comprising of Three Compartments, Arterial, Venous and Bloodless Tissue

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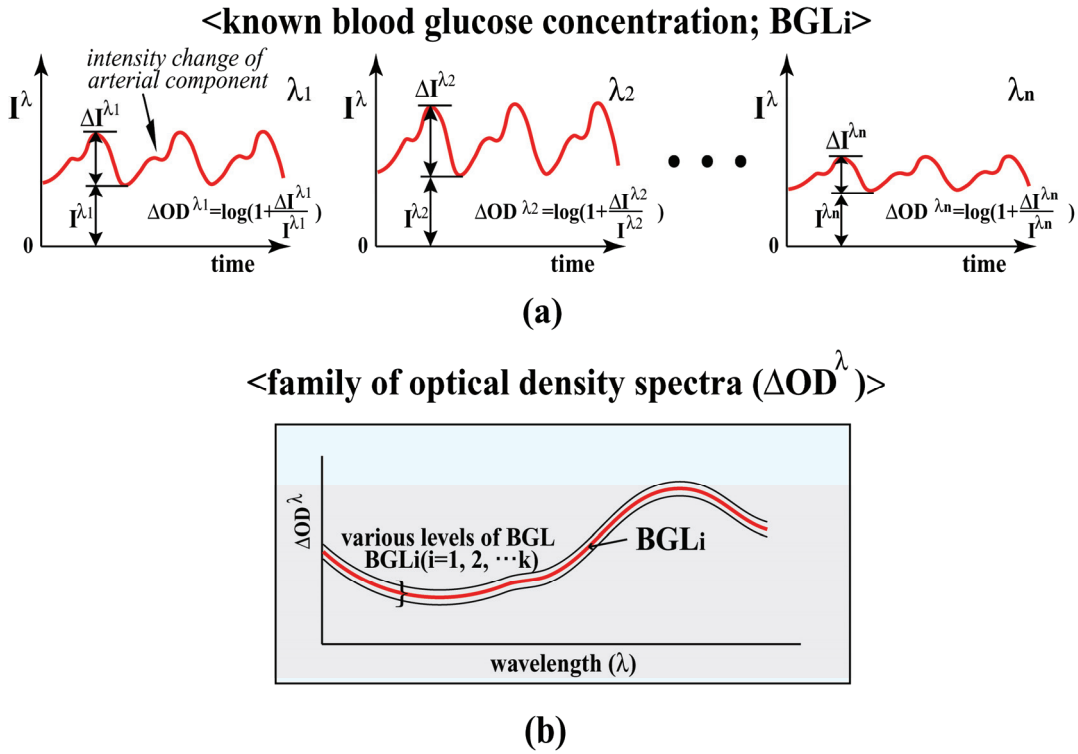

Figure 2: Schematic Diagram of Pulsatile Intensity Change (ΔI^{λ}) Superimposed on the Transmitted Light Intensity (I^{λ}) for Different Wavelengths λ_1, λ_2, $\cdots\lambda_n$, with a known BGL (BGLi) **(a)**, and a family of Spectra (ΔOD^{λ}) Calculated from I^{λ} and ΔI^{λ} for Different BGLi Levels **(b)**.

under interrogation, as shown in Figure 1**.** The model can be seen to comprise of three compartments: arterial; venous; and bloodless tissue. The optical interrogation of the tissue is carried out with a broad-spectrum light source having incident light intensity I^{λ}_{0}. Following absorption and scattering in each compartment we have transmitted light intensity, I^{λ}. The arterial volume changes throughout the cardiac cycle, thereby producing very small changes in the transmitted intensity, ΔI^{λ}. If the transmitted intensity, I^{λ} , is detected periodically at time intervals Δt during the cardiac cycle, we can obtain the time series data of I^{λ} every Δt (I^{λ}_{t1}, I^{λ}_{t2}, •••, I^{λ}_{tn}; $\Delta t=t_{i+1}-t_i$). Therefore, the difference between the optical densities at t_i ($OD^{\lambda}_{ti}=log(I^{\lambda}_{0}/ I^{\lambda}_{ti})$) and t_{i+1} ($OD^{\lambda}_{ti+1}= log(I^{\lambda}_{0}/ I^{\lambda}_{ti+1})$), where $I^{\lambda}_{ti+1}= I^{\lambda}_{ti}+\Delta I^{\lambda}_{ti}$, can be obtained. This has the effect of removing the venous and the tissue contributions to yield only the change in intensity due to the pulsating arterial blood compartment.

Theoretically ΔI^{λ}_{ti} can be derived from any time sampling points within each complete cardiac cycle. Figure 2 (a) illustrates the transmitted intensity change (ΔI^{λ}) due to the arterial pulse component for different wavelengths, λ_1, $\lambda_2 \ldots \lambda_n$. These data can also be viewed as spectra, for example over a near infrared range, with ΔOD^{λ} plotted against wavelength, λ. If such spectra are derived at different levels of blood glucose concentration, BGLi, we will obtain a family of spectra, as is illustrated schematically in Figure 2(b)**,** which may then be used for a multivariate analysis.

It is common to use a Partial Least Squares (PLS) calibration model with multi-regression analysis for spectrophotometric BGL measurements[8]. If this is done, then measured spectra of ΔOD^{λ} *vs* λ for the unknown BGLs derived from *in vivo* measurements on, for example, a finger can be compared with the PLS-derived calibration model and predicted BGL values thereby calculated.

Materials & Methods

To carry out a "proof of principle" study we have developed a new spectrophotometer system capable of obtaining instantaneous spectra of the light transmitted through tissue, such as a finger. This system comprises of a broad-band light source, a fibre optic bundle to deliver the incident light to the tissue and a single optical fibre for receiving the transmitted light, a spectrometer, a linear InGaAu photodiode-array, and a conventional PC with an appropriate interface. The spectrometer covers the wavelength range from 900 to 1700 nm with a resolution of less than 8 nm, and scans at a maximum spectral rate of 1800 spectra/s with a minimum exposure time of 20 μs.

To test the ability of the complete system to provide estimates of blood glucose levels a glucose tolerance test was carried out in 2 healthy male volunteers, 22 and 27 years old. The subjects were asked to abstain from any food and alcohol from 9 pm on the previous day until the end of the experiment on the next morning. The experiment was conducted at 9 am in a temperature controlled room at 25 ºC. During the experiment the subjects were seated quietly in a chair whilst blood samples were taken at the same time as optical meas-

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urements were made in a fingertip. The blood samples (about 3 ml) were collected from the cephalic vein of the left forearm and the light intensity measurements were made in the left index fingertip every 10 min before and after oral administration of glucose solution (75g/225ml: Trelan-G75; Shimizu Seiyaku, Co. Ltd., Japan) for 120 min. The BGL in the samples (measured BGL) was determined using an automatic blood analyzer (DRI-CHEM 7000; Fujifilm Medical Co. Ltd., Japan). For the optical measurements, the fingertip was placed carefully in a space between the ends of the transmitting and receiving optical fibres so as to ensure gentle contact between fibre and skin. The optical measurements were made for about 20-30 s which corresponded to about 20-30 cardiac pulses, spectra being gathered at a rate of 100 s^{-1}.

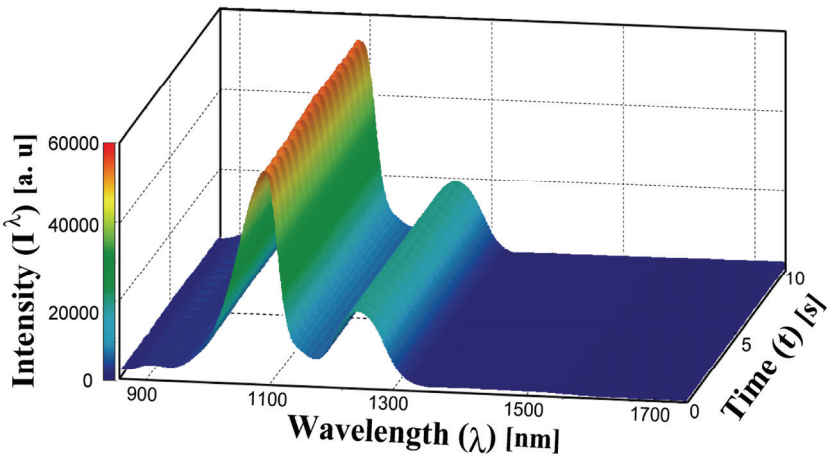

Figure 3: An Example of 3-dimensional Display of Transmitted Light Intensity (I^{λ}), Wavelength (λ) and Time (t) Measured in the Fingertip.

13 sets of optical intensity measurements were made in each subject during the 120 min study period, whilst the BGL was changing. The beat-by-beat spectral data of blood (ΔOD^{λ}) against a measured BGL were calculated to obtain the averaged ΔOD^{λ} of 20-30 consecutive cardiac beats during the measurement interval of 20-30 s. Thus 26 paired data sets of the measured BGL and the averaged ΔOD^{λ} were obtained for constructing an optimal PLS calibration model using the PLS_Toolbox 3.5 with MATLAB™ (Eigenvector Inc., USA). The leave-one-out cross validation method[8] was applied to compare the predicted and the measured BGL values.

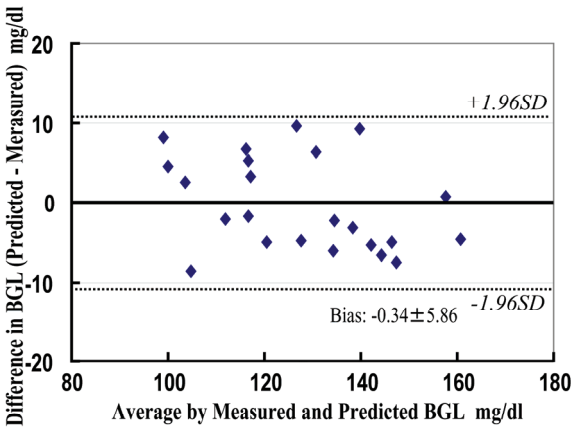

Figure 4: Difference between Predicted and Measured BGL Values Plotted Against Their Average (Bland-Altman Plot)

Results

The optical measurements made during each 10-s sampling period allow transmittance spectra to be plotted and for the temporal changes through each cardiac cycle to be seen. Figure 3 shows an example of a 3-dimensional representation of the transmitted light intensity (I^{λ} : vertical axis, y), *vs* wavelength (λ: horizontal axis, x) and *vs* time (*t*: horizontal axis, z) measured in the fingertip of one of the subjects. In this plot the time period is 10 s and therefore the pulsatile cardiac-related intensity changes cannot be perceived. However, such pulsatile changes can be clearly seen when the appropriate time-base is used.

From the transmittance spectra shown in Figure 3 it can be seen that the intensity exhibits a peak around 1100 nm and it is considerably decreased beyond approximately 1200 nm due to large absorption by the water component in the tissue including blood.

Using these spectra the optical density of the blood component, ΔOD^{λ}, could be calculated successively over time as BGL changed. Predicted values of BGL were subsequently calculated using the PLS model. Figure 4 is the Bland-Altman plot of the measured and predicted BGL values in the two subjects during the glucose tolerance tests. The total of 26 paired data sets of these BGL values were analyzed by the Bland-Altman method[10]. The bias and precision of the measurements obtained by the new method as compared with direct blood analysis averaged -0.34±5.86 mg/dl, for a range from about 90 to 160 mg/dl of BGL levels. The error grid analysis[11] was also carried out, showing that all paired data fell within less than 20 % error lines (zone A[11]). These results indicate that the new method was able to produce BGL estimates with reasonable accuracy.

Discussion

The development of a reliable and convenient non-invasive method for blood glucose monitoring has been the focus of intense efforts for several decades. Optical methods have seemed attractive since Ivan Amoto proposed the idea of non-stick optical blood monitoring using a human finger[12], but to date there has been no report of an entirely successful optical instrument[8, 13]. *In vivo* spectrophotometric analysis is confounded by a number of factors, especially the influences of absorption and scatter by numerous tissue

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components, such as water, bone, muscle, skin and the very many chemical constituents in blood other than glucose. Our new approach is to utilise a change in blood volume of an interrogated tissue segment, such as a fingertip, produced by the cardiac-related pulse in order to eliminate the basal interfering components. This approach is similar to the widely used method of pulse oximetry with two wavelengths for oxygen saturation monitoring[14]. In this connection, Heise et al[15] suggested the possible use of diffuse reflectance spectroscopy for glucose measurement on human oral mucosa and that pulsatile absorbance spectral measurement might be further investigated. In fact, they tried to measure pulsatile spectra on the lip, but in vain due to serious problems of the spectral SN ratio as well as poor time-resolved spectroscopy.

We have designed a measurement system which is capable of deriving transmittance spectra from an adult human finger at sufficiently high speed to reveal cardiac-related pulsatile spectral changes. In combination with a PLS calibration model our instrument appears to be capable of yielding blood glucose estimates having sufficient precision and accuracy for clinical use. In this paper we have presented results from just two subjects in whom blood glucose was varied by means of a glucose tolerance test. More extensive testing will be required to assess fully the performance of our instrument in a larger group of subjects. The present instrumentation is laboratory-based and further development is required to investigate the feasibility of designing a small portable device suitable for patient use to achieve self-monitoring. It is also possible that the method could have the potential for non-invasive blood analysis of such substrates as triglyceride, albumin, cholesterol, glycated albumin, and so on. Further considerable investigations have to be carried out to exlore such possibilities.

Conclusion

A high-speed optical instrument has been successfully developed for gathering transmittance spectra from the adult human finger over the wavelength range of 900 to 1700nm. We have been able to determine cardiac-related pulsatile changes in optical density and, using a PLS calibration model for glucose, we have subsequently been able to derive blood glucose estimates non-invasively in two healthy adult subjects during glucose tolerance tests. Further work is required to determine fully the performance of the instrument and also to assess the real potential of this method, which we name *Pulse Glucometry.*

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