BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, August 2014. Vol. **11**(2), 593-602

Measurement of Glucose Concentration using Amplitude Modulated Ultrasound with Infrared Technique in Intralipid Phantoms and Human Whole Blood Mixed Intralipid Phantoms of Healthy and Diabetic Subjects

Anuj Srivastava, Md Koushik Chowdhury, Shiru Sharma and Neeraj Sharma

School of Biomedical Engineering, Indian Institute of Technology
(Banaras Hindu University), Varanasi, Uttar Pradesh - 221005, India.

doi: http://dx.doi.org/10.13005/bbra/1311

(Received: 11 April 2014; accepted: 10 May 2014)

The present paper describes the results of experimentation, carried out on intralipid phantoms and human whole blood mixed intralipid phantom samples to measure various concentration of glucose. In this technique, the amplitude modulated ultrasonic waves were applied to excite the samples. As a result, different constituent molecules oscillate at their specific response frequency depending upon their physical and chemical properties. These typical signature specific oscillations are detected using infrared techniques. The output response signal is in the form of modulated light signal. It carries information about the concentration of the different constituents. This resultant output signal data is collected using infrared detector. The signal acquiring algorithm detects the glucose specific signature signals to extract the information about the glucose concentration level in different types of human whole blood mixed intralipid phantoms. Hence, this method provides a new technique for the design of amplitude-modulated ultrasound & infrared technique based noninvasive glucometer.

Key words: Intralipid phantom, Human whole blood, Noninvasive, Amplitude modulated ultrasound, Infrared technique.

Diabetes and its related complications are prominent nowadays. More than 140 million people worldwide suffer from it. As it is reported[1-3], this number will increase up to 300 million by the year 2025. The disease develops due to either lack of the hormone insulin created by pancreas in the organism or inability of cells to accept it. These two mechanisms cause two kinds of diabetes (I and II respectively). It could lead to increased amount of glucose in blood resulting in coma and even death of an individual. That is why determination of glucose content in blood and skin is of great importance. Currently, glucose diagnostics had been carried out mostly by finger prickling. Such unpleasant procedure can prevent diabetic patients from monitoring the glucose content as often as necessary. In order to avoid psychological problems, novel non-invasive, in particular, optical methods should come into practice[4-6].

Many optical approaches have been suggested: infrared absorption[7,8], near infrared scattering[7,9], Raman spectroscopy[7,10], fluorescent[7,11],

* To whom all correspondence should be addressed.
Mob: 09454631925.
E-mail: anuj.srivastava100@gmail.com

thermal gradient spectroscopies[7,12], polarimetric[7,13], polarization heterodyning[14], photonic crystal[15], photoacoustic[7,16], photothermal[17], optical coherence tomography (OCT) techniques[7,18] and ultrasound-modulated optical technique[19]. However, all of them suffer from the lack of sensitivity within the physiological range of glucose (100-500 mg/dl). The major difficulty in optical technique based blood glucose sensor is associated with the very low intensity signal produced by glucose molecules[6, 20, 21]. The present paper describes the role of amplitude-modulated ultrasound and infrared techniques for detection of glucose in Intralipid based samples. Intralipid is an intravenous nutrient consisting of an emulsion of phospholipids micelles and water. Intralipid is turbid, having strong absorption bands in the visible region of the electromagnetic spectrum. Intralipid is readily synthesizable as well as available in market; and could be reliably used as phantom for tissue simulating experiments[22].

MATERIALS AND METHODS

Preparation of Tissue Phantom

Phantoms which resemble the optical characteristics of the biological tissues (human, animal) were used to perform various experiments. These phantoms find application in (i) testing, (ii) optimizing, (iii) comparing, and (iv) Quality optimization for recently developed instruments. Tissue based phantoms were prepared for daily and robust experimentations. Phantoms of eminence properties are required for various lab investigation purposes.

To match the optical characteristics of phantom with that of tissues it is required to consider following parameters:

(a) Understanding of key physical and chemical properties of the tissues involved.

(b) The magnitude of scattering angle degree phenomenon.

(c) Relation between absorption coefficients, scattering coefficient, and anisotropy coefficient of the tissue.

In this current work the tissue phantom by means of optically related to human finger were prepared as described in Ref.23, 24.

Preparation of Intralipid Phantom and intralipid based whole blood samples

The water medium based tissue phantoms like Intralipid (sterilized) had been utilized here for resembling biological tissue complex based optical properties. Obtainable as 10%-Intralipid and 20%-Intralipid- (10% lipid signify 10 g of lipid for every 100 ml of suspension). The constituents of 10%-Intralipid in a 1000 ml container according to the maker had been shown in Table 2.

Variation in optical property of Intralipid occurs from bottle to bottle. Hence, standardization & calibration of the Intralipid sample before experimental work is essential[23, 24].

The intralipid phantom had been designed to mimic the scattering and absorption properties of the human finger in the near-infrared domain. Whereas absorption is accounted for the direct use of intralipid mixed whole blood samples[25]. Both components are mixed to mimic the blood–tissue compound and are inserted into a sample holder. During the experimentation following three physiological parameters have been explored:

(a) The concentration of glucose is varied and controlled in intralipid based tissue phantoms by direct addition of glucose in the range of 500 to 1500 mg/10ml.

(b) Whole blood from healthy and diabetic subjects were collected in vacuum-based blood collecting vials where K_2EDTA is present as anticlotting agent

(c) Deoxygenating is induced through nitrogen bubbling of the whole blood samples for 45 minutes. The variability of the hematocrit and oxygen concentrations hinders the glucose induced effect in the output data. This property indicates the impact of physiological changes on the acquisition of the glucose signature based signals.

(d) Phosphate buffer solution (PBS) has been used to maintain the pH level of the whole blood samples during the experimental procedures.

EXPERIMENTAL

To determine the role of amplitude modulated ultrasound and infrared techniques the pilot study for detection of glucose in Intralipid

based samples were performed.

A 940nm IR LED light was chosen as the light source because the wave-length dependence of the optical coefficient of the human blood is known. The infrared light (max.5milli watt) delivered to the sample has been used here. Fig.1 describes the block diagram of the experimental system. The experimental system had been based on amplitude modulated ultrasound wave (standing wave) and near infrared (IR) system. The generator block provides square wave pulses to the light source (IR LED). Light from the IR LED is directed to the sample holding unit. The modulator unit is supplied with modulating sine wave and high frequency carrier waves to generate amplitude modulated signals. The ultrasound transmitter unit is connected to receive such amplitude modulated signal from the modulator unit. The ultrasound transmitter unit passes these signals to the sample holder unit, which detects the magnitude and frequency of amplitude modulated ultrasound wave (standing wave). The directions of the light and ultrasound waves were perpendicular in their orientations. The infrared light beam is illuminated over the ultrasonic focus zone to obtain the maximum value of the amplitude modulated ultrasound light signal. The resultant signal as acquired by the detector undergoes processing for result verifications.

The effect of Amplitude Modulated Ultra Sound wave (standing wave) on molecules in Intralipid phantom medium and its optical detection

The presented investigations aimed to enhance infrared technique for non-invasive blood glucose detection by Amplitude modulated ultrasonic manipulation of body fluid specially blood constituents. Hybrid approach of this principle has the potential of new measurement concepts for use in non-invasive detection of glucose concentration in the human whole blood.

Amplitude modulated ultrasonic force provides the benefits to determine the molecule specific infra red typical signatures in the target area. The variation in frequency controls the molecular location with reference to the sensitivity in the infrared domain[26-32].

Ultrasonic manipulation of molecules in Intralipid phantoms

When amplitude modulating ultrasonic standing waves are applied to the liquid medium, resultant radiation waves got generated, which cause variations in between the suspended molecules[26-28]. The suspended molecules were subjected to thinning phenomenon due to the impact of the acoustic pressure generated over it. The thinning phenomenon occurs specially in the nodes of the standing wave (Amplitude Modulated Ultra Sound wave)[26, 31, 32].

The material property, speed of ultrasonic sound, mass density of the medium and its components determines the way of force which has been governed by the compressibility factor. The coefficient factor called as acoustic contrast were used to express this relation[26]. Depending upon their physical property the molecules attains a specific position in the sound field. The dimension factor prevails here, bigger molecules faces stronger attractions[26, 31, 32].

The oscillations generated between these molecules depend on their physical property and the magnitude and degree of force involved in the ultrasound waves[26-32]. The infrared light sensor picks up these molecular oscillating changes and the output signal is acquired. The pressure amplitude expresses the antinodal and nodal values two times over a distance of a unit wavelength. Specific molecules attain a position dependent acoustic potential energy due to their presence in the sound field which causes a discontinuity in the propagating phase[27, 31, 32].

When the ultrasonic wavelength is larger compared to the molecular diameter, the 'primary' radiation force (F_r) acts on the molecular volume (V_c) positioned from the pressure node at a distance (z). It had been calculated from the gradient function of the molecular acoustic potential energy[26-32]. It had been expressed as:

$$\mathbf{F_r = -\left[\frac{\pi p_0^2 V_c \beta_w}{(2\lambda)}\right] . \phi(\beta,\rho) . \sin(4\pi z/\lambda)} \quad ...(1)$$

The peak acoustic pressure amplitude denoted as (P_o) and λ represents the sound wavelength in the liquid medium of the tissue phantom, where compressibility has been expressed as λ_w [26-32]. This phenomenon has been expressed as:

$$\phi(\beta,\rho) = \left[\frac{5\rho_c - 2\rho_w}{2\rho_c + \rho_w} - \left(\frac{\beta_c}{\beta_w}\right)\right] \quad ...(2)$$

Where β_c indicates the compressibility

of the intralipid phantom based whole blood molecules, ρ_c and ρ_w represents the molecular density and the suspending phase (blood medium) respectively[26-32].

Secondary forces[27-32] drive the concentrated molecules to the local minima of the pressure amplitude, within the pressure nodal planes, to give regions of molecule concentration that appear as columns of clumps striated at half-wavelength separations[26-32].

Acquisition of absorption spectra

The intrinsic property of the substances used to vary the intensity of the light path by a specific wave number. The Lambert-Beer Law has been applied assuming light absorption as (A) and wave number as (v). The equation is as follows:

$$\mathbf{A(v) = -\log I(v) / I_o(v)} \quad ...(3)$$

Here I_0 represents the intensity of the surrounding medium, I denotes the intensity at the specific wave number v of the valid measurement[26,31,32].

Spectral data for wavelength selection and transducer properties

The absorption band of glucose molecule superimposes with the absorption of the water molecules. The situation becomes more complicated due to the varied nature of the human blood and its biological tissues optical property. For this reason wavelength characterization must be specific and glucose detection oriented[33, 34]. Water coefficient of absorption and the molecular extinction coefficients of skin pigment like melanin, hemoglobin in oxygenated form are described in Figure 2. Tissue optical property changes the wavelength that depends largely on the concentration of water, oxy-hemoglobin, hemoglobin, skin pigments like melanin, etc. The absorption pattern of these biologically active absorbers is less sensitive in the near infrared and red wavelength spectrum. All biological cells and tissue are apparently transparent in the wavelength region of 700nm to 1100nm. This spectral region has been referred as optical window. The light penetration also increases within this spectral band[33, 34].

In Fig.no.3, the absorption spectral pattern of glucose and water molecule at wavelength 1037nm is illustrated[33, 34].

The absorption pattern of oxygenated haemoglobulin and deoxygenated haemoglobulin in the wavelength spectra of 1000nm to 1200nm are different. But this dissimilarity pattern is lower in the range of 900nm to 980nm as seen in the absorption phenomenon graph in the red and near infra red spectral regions in fig.4.[35]. Blood oxygenation concentrations vary the light absorption profiles[35, 36]. Taking in to the phenomenon of the tissue optical window, the wavelength around 940nm had been considered. The ultrasonic transducers utilized here (transmitter and receiver) operate at the frequency of 40 kHz. With capacitance of 2000pF, 20Vrms capacity for maximum input voltage and sensitivity of 67dB @ 40 ± 1.0 kHz.

The IR light source of 940nm and 40 kHz ultrasound transmitter is utilized for our experimentations. All tissue phantom based experiments were conducted using this experimental setup.

Signal Processing Algorithm

The resultant output signal had been processed through the indigenous algorithm for data analysis and result interpretations. The Fast Fourier Transform (FFT) based algorithm had been used for measuring the various concentration of glucose in Intralipid phantoms and human whole blood mixed Intralipid phantoms of healthy and diabetic subjects.

Clinical status of Subjects

The subjects were five non-diabetic subjects (normal healthy) adults (three male, two female, aged 33.8±4.2 years, of height 164.8±8.8 cm, weight 56.6±13.1 kg, and a random BGL of 94 ±10.0 mg/dl), and five diabetic subjects (three type-I male, two type-II male, aged 42.3±11.9 years, of height 159.6±7.9 cm, weight 50.1± 14:5 kg, and a random BGL of 377 ± 50 mg/dl). The aim of the experiment were explained to the subjects and consent were obtained after confirmation that they fully understood the course of the experiments. The IMS-BHU ethical committee approved the study.

RESULT AND DISCUSSION

At present, various techniques are available for non-invasive blood glucose determinations. Research related to tissue optical

properties had increased due to innovations in the fields of tissue modeling, better signal to noise ratios instruments, invitro experiments, etc. Matthias Kohl *et al.,*[37] described the experimental and theoretical investigations for the existence of glucose effects upon the optically sensitive scattering medium. Matthias Kohl *et al*.[37] explain the presence of glucose induced effects on the scattering tissues. The glucose sample in higher amount than the biological levels has been needed for obtaining the considerable glucose induced effects in the tissue phantoms. Zuomin Zhao *et al*.[38] discussed the efficacy of the photo acoustic method to detect 100mg/dl of glucose concentrations in tissue phantoms and whole blood samples. Zemansky MW *et al*.[39] demonstrated that the NIR pattern of glucose molecules as compared to water molecules varies by ±20% at 905nm, 1550nm. Alexey N. Bashkatov *et al*.[40-42] reports the use of higher amount of scatters in phantoms to mimic the real life biological tissues. Matti kinnunen *et al.,*[41,42] determines the property of glucose molecule at 1064nm and 532nm in pig blood and intralipid phantom samples. The optical property of glucose indicates instrumentation with high accuracy and precision were needed. Our paper shows the utilization of amplitude modulated ultrasound and IR technology to enhance the glucose detection capability.

The experiments were performed on three stages.

Stage I

Invitro experimentation on different concentration of dextrose (glucose) with Intralipid phantom were performed.

In this stage the effect of various concentration of dextrose (glucose) with intralipid suspension had been evaluated with amplitude modulated ultrasound and infrared technique unit. Peak to peak voltage amplitude value had been plotted as obtained by the FFT of the observed signal. The voltage amplitude values start increasing when concentration of Dextrose (glucose) concentration had been increased from 0 mg to 1500mg in the intralipid phantoms.

Stage II

Table No: 4 shows the Invitro experiments on different healthy human whole blood mixed intralipid phantom samples.

Stage III

Table No: 5 shows the Invitro experiments on different diabetic human whole blood mixed

Table 1. Showing Different constituents of 10% Intralipid suspension[23,24]

(Soybean oil or glycine max)	100 g	107.88 ml
Lecithin from egg yolk	12 g	11.64 ml
Glycerin ($C_3H_8O_3$)	22.50 g	17.84 ml
Water (H_2O)	861 g	862.66 ml
Total Volume	995.5 g	1000 ml

Table 2. Setting used for the experiment

Parameter	Value
1. Infrared LED operating wavelength.	940nm
2.Ultrasound Operating frequency	40.0±1.0kHz
3.Interval between two readings	09sec
4.Total Time	10min

Table 3. Invitro experiments on different concentration of dextrose with Intralipid phantom.

S No.	Medium	Frequency (kHz)	Amplitude (mV)
1.	Intralipid suspension as Blank (0.03dl)	16.45	6.7
2.	Intralipid suspension (0.02 dl) + 0.01dl of dextrose (Glucose) solution which is taken from the stock preparation of 500 mg of glucose in 0.l dl of distilled water	16.45	7.9
3.	Intralipid suspension (0.02 dl) + 0.01dl of dextrose (Glucose) solution which is taken from the stock preparation of 1000 mg of glucose in 0.l dl of distilled water	16.45	8.5
4.	Intralipid suspension (0.02 dl) + 0.01dl of dextrose (Glucose) solution which is taken from the stock preparation of 1500 mg of glucose in 0.l dl of distilled water	16.45	10.1

intralipid phantom samples.

In II and III stage different healthy and diabetes subjects blood samples with the intralipid phantom as blood tissue compound is evaluated using amplitude modulated ultrasound and infrared technique unit respectively. Peak to peak voltage amplitude values had been plotted as obtained by the FFT of the observed signal. At the same time, when the signals were recorded using amplitude modulated ultrasound and infrared technique unit,

Table 4. Invitro experiments on different healthy human whole blood mixed intralipid phantom samples

S. No.	Medium	Frequency (kHz)	Amplitude (mV)	Random *BGL mg/dl value as measured by established invasive procedure
1.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Healthy Subject 1	16.45	22.0	102.0
2.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Healthy Subject 2	16.45	17.6	98.0
3.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Healthy Subject 3	16.45	23.7	85.0
4.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Healthy Subject 4	16.45	20.4	92.0
5.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Healthy Subject 5	16.45	25.8	90.0

* BGL= Blood Glucose Level

Table 5. Invitro experiments on different diabetic human whole blood mixed intralipid phantom samples

S. No.	Medium	Frequency (kHz)	Amplitude (mV)	Random *BGL mg/dl value as measured by established invasive procedure
1.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Diabetic Subject1	16.45	38.3	285.0
2.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Diabetic Subject2	16.45	32.9	298.0
3.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Diabetic Subject3	16.45	36.5	282.0
4.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Diabetic Subject4	16.45	39.3	300.0
5.	Intralipid suspension (0.02 dl) + 0.01dl of whole blood sample of Diabetic Subject5	16.45	37.4	299.0

* BGL= Blood Glucose Level

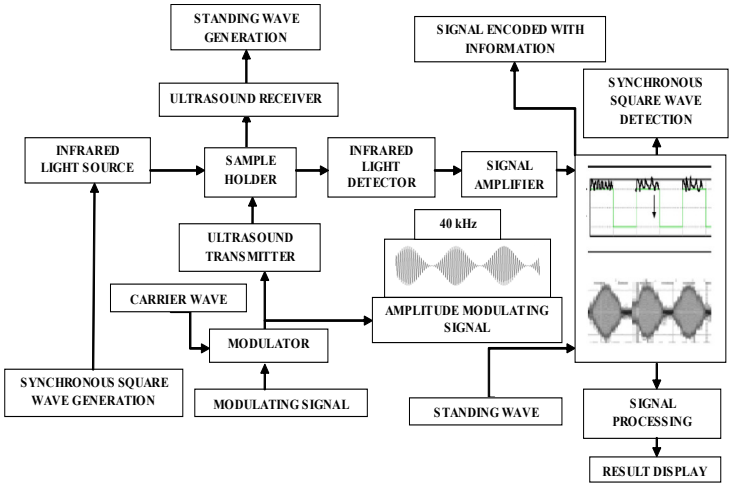


Fig.1. Block diagram of the Experimental System

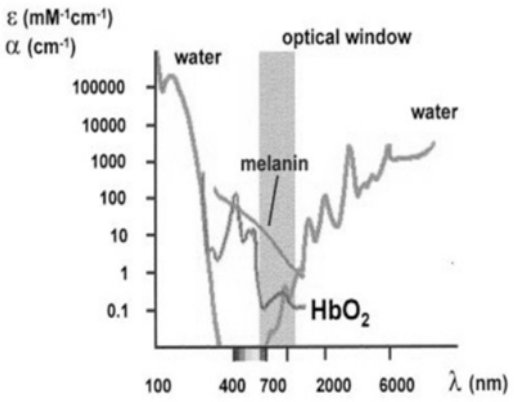


Fig. 2. Water coefficient of absorption and the molecular extinction coefficients of skin pigment like melanin, hemoglobin in oxygenated form are described here[33]

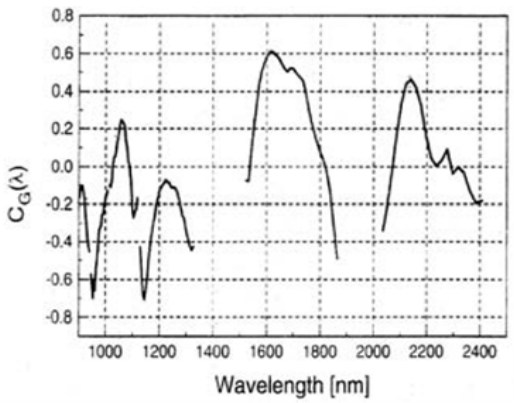


Fig. 3. The absorption spectral pattern of glucose and water molecule at wavelength 1037nm is illustrated[34]

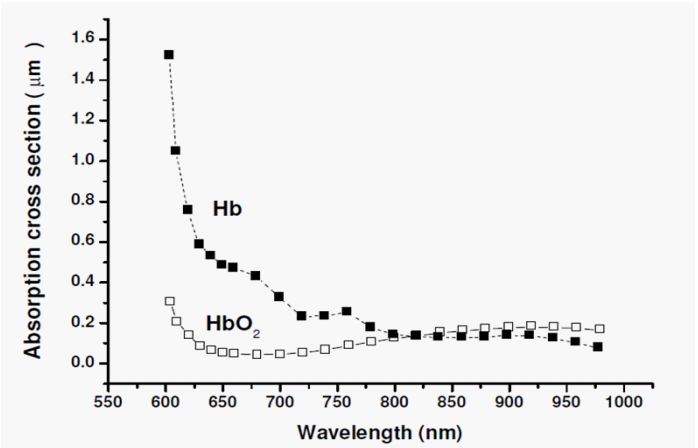
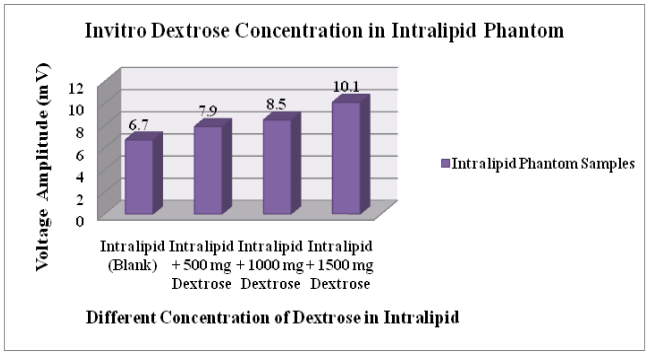
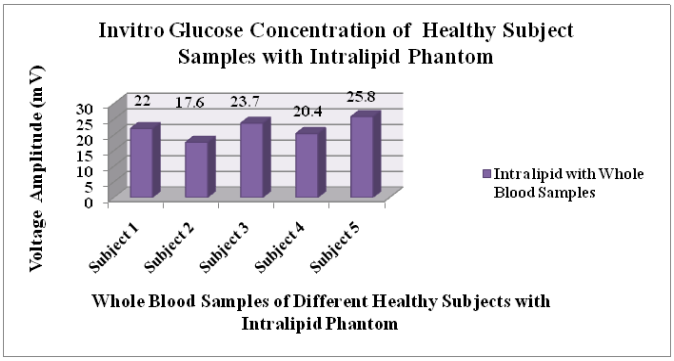


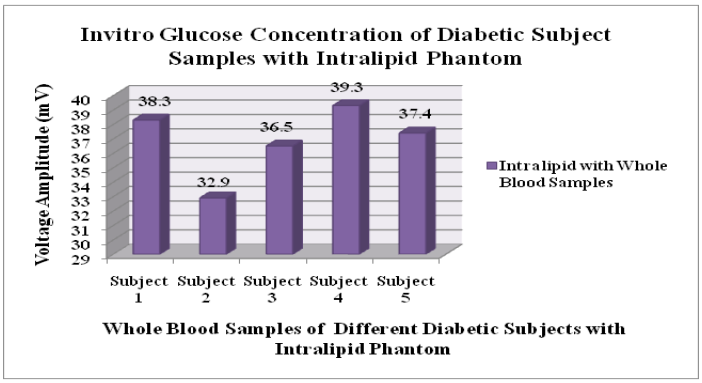
Fig. 4. Absorption pattern of Oxy-Hemoglobin and Reduced Hemoglobin at Red and NIR region[35]



Graph 1. Graphical plot of different concentration of dextrose with Intralipid phantom



Graph 2. The graphical plot of healthy human whole blood mixed intralipid phantom samples



Graph 3. The graphical plot of different diabetic human whole blood mixed intralipid phantom samples

the invasive blood glucose determination had also been performed. The corresponding voltage amplitude and invasive blood glucose level is recorded.

In stage I, II and III, the trend that had obtained indicates that voltage amplitude values of the signal increases with increase in dextrose (glucose) concentration. It indicates that increase in dextrose (glucose) concentration changes the optical character of the medium, thereby altering the sample's photon distribution. This in turn serves to change the energy density of the light source, so varying the amplitude modulated ultrasound based light signal accordingly.

CONCLUSION

Glucose measurements using amplitude modulated ultrasound and infrared technique were conducted in Intralipid and human blood samples to

ascertain how glucose affects the optical properties of Intralipid and human blood samples at 940nm. It had been found that the glucose-induced change in the peak-to-peak values of the signal was significant in Intralipid and human blood samples. In the FFT response of the signals, the voltage amplitude variation of Intralipid and human blood samples (both diabetic and nondiabetic) was noteworthy. The results demonstrate the capability of the amplitude modulated ultrasound and infrared technique unit used in studying dextrose (glucose)-induced absorption pattern changes in the Intralipid and human blood mixed phantom samples. Thus, this technique proved to be potential methodology for noninvasive blood glucose monitoring in the near future.

ACKNOWLEDMENTS

The Authors would like to thank Coordinator, Administrative and Laboratory Staff of the School of Biomedical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi, for supporting this research work.

REFERENCES

1. G. Danaei, M. M. Finucane, Y. Lu, G. M. Singh, M. J.Cowan, C. J. Paciorek, J. K. Lin, F. Farzadfar, Y. H.Khang, G. A. Stevens, M. Rao, M. K. Ali, L. M. Riley,C. A. Robinson, and M. Ezzati, 'National, regional, and global trends in fasting plasma glucose and diabetes prevalence since systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2•7 million participants', *Lancet* 1980 ; **378**: 31 (2011).
2. "The fourth edition of the IDF Diabetes atlas", *International Diabetes Federation,* 2009.
3. S. Wild, G. Roglic, A. Green, R. Sicree, and H. King, "Global Prevalence of Diabetes. Estimates for and projections for 2030." Diabetes Care, 2000; **27**, 2003, pp 1047–1053
4. Peter J.Watkins, ABC of Diabetes, (Fifth Edition), London : BMJ Books, 2003.
5. http://diabetes.webmd.com/ (12/03/2014)
6. Tuchin V.V. (ed.) [Handbook of Optical Sensing of Glucose in Biological Fluids and Tissues]. CRC Press, Taylor & Francis Group, London 2009; 41-64.
7. A. Tura, A. Maran and G. Pacini, "Non-invasive glucose monitoring: Assessment of technologies and devices according to quantitative criteria", *Diabetes Research and Clinical Practice*, 2007; **77**: 16-40
8. J. Tenhunen, H. Kopola, and R. Myllyla, "Non-invasive glucose measurement based on selective near infrared absorption: requirements on instrumentation and special range," *Measurement*, 1998; **24**: 173–177.
9. J.S. Maier, S.A. Walker, S. Fantini, M.A. Franceschini, and E. Gratton, "Possible correlation between blood glucose concentration and the reduced scattering coefficient of tissues in the near infrared," *Opt. Lett.,* 1994; **19**: 2062–2064.
10. A.M.K. Enejder, T.G. Scecina, J. Oh, M. Hunter, W.-C. Shih, S. Sasic, G.L.Horowitz, and M. S. Feld, "Raman spectroscopy for noninvasive glucose measurements,"*J.Biomed. Opt*., 2005; **10**: 031114.
11. J. Lakowicz and B. Maliwal, "Optical sensing of glucose using phase modulation fluorimetry,"*Anal.Chim.Acta,*1993; **271**: 155-164.
12. S-j Yeh, C. F. Hanna, S. Kantor, et al., "Differences in thermal optical response between intact diabetic and nondiabetic human skin," *Proc. SPIE*, 2003; **4958**: 213–224.
13. S. Bockle, L. Rovati, and R.R. Ansari, "Polarimetric glucose sensing using the Brewster-reflection off-eye lens: theoretical analysis," *Proc. SPIE*, 2002; **4624**: 160-164.
14. C. Chou, C.Y. Han, W.C. Kuo, Y.C. Huang, C.M. Feng, and J.C. Shyu, "Noninvasive glucose monitoring in vivo with an optical heterodyne polarimeter," *Appl. Opt.,* 1998; **37**: 3553–3557.
15. V.L. Alexeev, A.C. Sharma, A.V. Goponenko, S. Das, I.K. Lednev, C.S. Wilcox, D.N. Finegold, and S.A. Asher, "High ionic strength glucose sensing photonic crystal," *Anal. Chem*., 2003; **75**: 2316-2323.
16. Z. Zhao, Pulsed Photoacoustic Techniques and Glucose Determination in Human Blood and Tissue, doctoral thesis, University of Oulu, Finland, 2002.
17. S.A. Telenkov, D.P. Dave, and T.E. Milner, "Low-coherence interferometric detection of photothermal and photoacoustic effects in tissue," *Proc. SPIE,* 2003; **4960**: 142–146.
18. K.V. Larin, M.S. Eledrisi, M. Motamedi, R.O. Esenaliev, "Noninvasive blood glucose monitoring with optical coherence tomography: a pilot study in human subjects," *Diabetes Care*, 2002; **25**(12): 2263–2267.
19. L.Zhu, J.Lin, B.Lin, H.Li., "Noninvasive blood glucose measurement by ultrasound-modulated optical technique", *Chinese Optical Letters*, 2013; **11**(2): 021701-1to 021701-5.

20. Md.K.Chowdhury, A.Srivastava, N.Sharma, S.Sharma, "Challenges & Countermeasures in Optical Noninvasive Blood Glucose Detection", *International Journal of Innovative Research in Science, Engineering and Technology* (IJIRSET), 2013; **2**(1): 324-329.
21. A.Srivastava, Md.K.Chowdhury, S.Sharma, N.Sharma, "Blood Glucose Monitoring Using Non Invasive Optical Method: Design Limitations and Challenges", *International Journal of Advanced Research in Electrical, Electronics and Instrumentation Engineering* (IJAREEIE), 2013; **2**(1): 615-620.
22. Brian W. Pogue, Michael S. Patterson," Review of tissue simulating phantoms for optical spectroscopy, imaging and dosimetry", *Journal of Biomedical Optics* 2006; **11** (4): 041102(1-16) (July/August 2006)
23. ST Flock, SL Jacques, BC. Wilson, WM Star, MJC van Gemert, "Optical Properties of Intralipid: A phantom medium for light propagation studies,"*Lasers in Surgery and Medicine* 1992*;* **12**: 510-519.
24. HG van Staveren, CJM Moes, J van Marle, SA Prahl, MJC van Gemert, "Light scattering in Intralipid-10% in the wavelength range of 400-1100 nanometers," *Applied Optics* 1991; **30**: 4507-4514.
25. Orna Amir, Daphna Weinstein, Silviu Zilberman, Malka Less, Daniele Perl-Treves, Harel Primack, Aharon Weinstein,Efi Gabis,Boris Fikhte, and Avraham Karasik. "Continuous Noninvasive Glucose Monitoring Technology Based on Occlusion Spectroscopy", *Journal of Diabetes Science and Technology*, 2007; **1**(4): 463-469.
26. S. Radel , M. Brandstetter, B.Lendl, 'Observation of particles manipulated by ultrasound in close proximity to a cone-shaped infrared spectroscopy probe', Ultrasonics 2010; **50**: 240–246.
27. W. Terence Coakley, 'Ultrasonic separations in analytical biotechnology', *Trends in Biotechnology* 1997; 506-511.
28. L.V. King, 'On the acoustic radiation pressure on spheres', *Proceedings of the Royal Society of London* 1934; 212-240, A147.
29. K. Yosioka, Y. Kawasima, 'Acoustic radiation pressure on a compressible sphere', *Acustica* 1955; **5**: 167–173.
30. F. Petersson, A. Nilsson, C. Holm, H. Jonsson and T. Laurella, 'Separation of lipids from blood utilizing ultrasonic standing waves in microfluidic channels', *The Analyst, The Royal Society of Chemistry*, 2004; **129**: 938-943. doi: 10.1039/b409139f.
31. Md. K. Chowdhury, A. Srivastava, N. Sharma, & S.Sharma, 'The influence of blood glucose level upon the transport of light in diabetic and non-diabetic subjects'. *International Journal of Biomedical and Advance Research*, 2013; **4**(5): 306-316. doi:10.7439/ijbar.v4i5.357.
32. A. Srivastava, Md. K. Chowdhury, S. Sharma, N. Sharma, 'Optical Clearance Effect Determination of Glucose by near Infrared Technique: An Experimental Study using An Intralipid Based Tissue Phantom', *International Journal of Advances in Engineering & Technology* (IJAET), 2013; **6(**3): 1097-1108.
33. K. Konig, "Multiphoton microscopy in life sciences", *Journal of Microscopy,* 2000; **200**-2: 83-104.
34. J. Tenhunen, H. Kopola, and R. Myllyla, "Non-invasive glucose measurement based on selective near infrared absorption: requirements on instrumentation and special range," *Measurement,* 1998 ; **24**: 173–177.
35. O.W. Assendelft, Spectrophotometry of Hemoglobin Derivates, Royal Vangorcum Ltd., Assen, 1970.
36. Y.Mendelson, "Pulse oximetry: theory and applications for noninvasive monitoring*," Clin. Chem.,* 1992; **38**: 1601-1607.
37. M. Kohl,M. Cope,M. Essenpreis, and D. Böcker, "Influence of Glucose Concentration on Light Scattering in Tissue-Simulating Phantoms," *Opt. Lett.,* 1994; **19**: 2170–2172.
38. Zhao Z and Myllylä R, "Photoacoustic blood glucose and tissue measurement based on optical scattering effect" Saratov Fall Meeting, Proceedings of SPIE 2001; **4**: 707-16.
39. Zemansky MW., Heat and Thermodynamics: an Intermediate Textbook. 5th edition, McGraw-hill Kogakusha, Tokyo, 1968.
40. Alexey N. Bashkatov, Elina A. Genina," Measurement of Glucose Diffusion Coefficients in Human Tissues". Valery V. Tuchin (editor.), Handbook of Optical Sensing of Glucose in Biological Fluids and Tissues, CRC Press, Taylor & Francis Group,6000 Broken Sound Parkway NW, Suite 300 Boca Raton, FL33487-2742. pp.no.587-622.
41. M. Kinnunen and R. Myllyla, "Effect of glucose on photoacoustic signals at the wavelength of 1064 and 532 nm in pig blood and Intralipid," *J. Phys. D: Appl. Phys.,* 2005; **38**: 2654–2661, doi.10.1088/0022-3727/38/15/018.
42. Kinnunen M, Popov AP, Plucinski J, Myllylä R & Priezzhev AV., Measurements of glucose content in scattering media with time-of-flight technique; comparison with Monte Carlo simulations. Proceedings of SPIE, Saratov Fall Meeting 2003: *Optical Technologies in Biophysics and Medicine* V 2004 ; **5474**: 181–191.