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ORIGINAL ARTICLE

The Viability of Intralipid optical Phantom for Developing Noninvasive Blood Glucometer

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ABSTRACT
This research article focuses on the critical analysis about the viability of Intralipid optical tissue resembling phantoms for developing noninvasive blood glucometer. Utilization of the intralipid phantoms in Photo-Acoustic Spectroscopy (PAS), Pulsed Photo-Acoustic (PA) Techniques, OCT (Optical Coherence Tomography), TOF (Time of Flight) based Monte Carlo Simulations, Ultrasound-Modulated Optical Techniques, Polarization Techniques, Occlusion Spectroscopy, Raman Spectroscopy for noninvasive glucometer development has been discussed in this research article. Moreover, Standard Oral Glucose Tolerance Test (OGTT) conducted over 03 normal human subjects by our indigenously developed MUS-IR (Modulated Ultra Sound-Infra Red) unit for determination of blood glucose levels has been reported here. Actually, the blood plasma samples have been collected during the fasting stage of the subjects and every 30minutes after 75gm glucose consumption up to the total time period of 2hr and 30minutes. Blood plasma samples have been mixed with intralipid phantom samples and processed through indigenously developed MUS-IR unit for respective sample glucose concentration determinations. Moreover, for cross validation of results obtained, readings have been compared with the findings of the Digital spectrophotometer respectively. Outcome of the results indicates intralipid phantom samples serves as a feasible option in invitro experimentations for designing and developing noninvasive glucometer.
Key words: *Intralipid, Phantom, Infrared, Noninvasive Glucometer, OGTT, Spectrophotometer.*

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INTRODUCTION

Diagnosis of diabetes mellitus is a significant topic worldwide. The estimated numbers of diabetic patient according to the report of World Health Organization (WHO) has been above 140 million persons [1-3]. The WHO provided the calculated number of diabetes persons, predicted to be around 300 million in the year 2025[1-5]. Diabetes mellitus indicates the physiological changes in person with deficient in insulin hormone or incapability to recognize the insulin by the glucose sensing cells [3-5]. Depending upon the mechanisms of complications, they categorized it into two types known as Type I and Type II Diabetes Mellitus [4, 5]. It may cause coma condition or even death when the glucose level rises in uncontrolled manner. Monitoring the glucose levels numerous times per day is essential for perfect regulation of insulin-based therapy [3-5]. The routine protocol for invasive blood glucose analysis is to puncture the fingertip to obtaining the blood samples. It then reacted with enzymatic chemicals to obtain the desired results [3-5]. Invasive techniques for obtaining blood glucose predictions, the person usually suffers from many painful procedures, skin injuries and from high risk of infections [3-5]. In the continuation of developing noninvasive techniques, for pain free attitude, the need of non-invasive blood glucose meter arises [3-6]. This paper describes the novel optical procedures like Optical Coherence Tomography (OCT) [8,14], Photo Acoustic Spectroscopy (PAS) [8], Time-Of-Flight (TOF) [8], Ultrasound-Modulated Optical Technique [9], Polarization Technique [10,15,16], Raman Spectroscopy [11,15,16], Occlusion Spectroscopy [12] where intralipid has been utilized as the tissue phantom medium for the measurement of blood glucose concentrations. Various simulation and software models techniques like Monte Carlo had

also been applied for optical noninvasive blood glucose measurements [13].Depending on the optical properties of the intralipid as tissue phantom; all the light based techniques provide blood glucose concentrations.

UTILIZATION OF INTRALIPID AS A PROMINENT TISSUE PHANTOM MODEL IN VARIOUS TECHNOLOGIES AS FOLLOWS

(a) Photo-Acoustic Spectroscopy (PAS)

The photo acoustic spectroscopy deals with the optical and sound wave energy. The LASER (Light Amplification Simulation Emission Radiation) light energy beam of short pulse duration had been focused on the intralipid tissue phantom based samples. By this phenomenon the heat of the target region increases. Consequently, the absorbed light in intralipid based samples emits thermo elastic waves. The piezoelectric sensor detects that generated thermo elastic waves. Intralipid with sample provides definite scattering profiles that distribute the absorbed light and pressure energy waves. This physical phenomenon had been utilized for designing and developing noninvasive blood glucometer [8].

(b) Pulsed Photo-Acoustic (PA) Technique

Glucose induced changes in the pig blood mixed 1% Intralipid samples had been studied utilizing the photo acoustic (PA) techniques. The Nd :YAG LASER of 1064 and 532 nm had been applied here as a light source energy. The results shows that glucose induced changes were 11.4% and 1.35% per 500mg/dl of pig blood and1% Intralipid samples respectively. The Intralipid phantom plays a vital role in Glucose characteristics determinations in invitro samples by Photo-Acoustic Technology [7].

(c) OCT (Optical Coherence Tomography)

The Optical Coherence Tomography (OCT) technique had been based on utilization of infrared based back scattered light. The collected back scattered light were calculated and images were formed for diagnostic purposes. Intralipid as tissue phantom had been utilized in this technique to mimic the biological tissues based light back scattering properties. The glucose concentration of 0-200mg in 1ml of intralipid sample had been utilized in this method. The glucose concentration covers the physiological range of hypoglycemia (below 80mg/dl), normal (between 80 to 120mg/dl) and hyperglycemic levels (above 140mg/dl). The general width of the intralipid scatters were about 3.5 μm as predicted by the confocal microscope. The glucose induced back scattering light changing pattern in the intralipid based tissue phantom were recorded and analyzed through this technique [8].

(d) TOF (Time of Flight) Based Monte Carlo Simulations

When a beam of LASER light propagates through the sample medium usually two phenomenons takes place. The LASER light got reflected from the surface of the sample medium or it penetrates the sample medium. The degree of refraction had been based on the sample medium incident and reflection indexes. Moreover due to the inhomogeneous nature of the sample medium, the LASER light beam propagates in three other pathways. They are (i) straight path (ii) zigzag path (iii) multi scattered path. Depending on the path utilized, the Time of Flight varies respectively. The resultant light signals were attenuated and broadened due to the impact of the absorption and scattering phenomenon inside the sample medium respectively. Measuring the variation in the LASER pulse might reveal the scattering properties of the sample medium. The intralipid sample of 1% to 5 % concentrations had been utilized to study the light absorption and scattering profiles. Moreover 2 % intralipid samples with 0-2000mg of glucose samples were varied to study the glucose induced variation in the intralipid samples. The Monte Carlo based simulation were performed to analyze the light scattering properties inside the glucose mixed intralipid samples. The LASER pulse increases with increase in the glucose concentration in the intralipid samples [8].

(e)Ultrasound-Modulated Optical Technique

In this technology, the 20% intralipid suspension had been mixed with bovine hemoglobin solution to replicate the blood. Glucose powders were mixed with the distilled water samples to change the glucose concentration of the samples. The intralipid based samples shows variation in the scattering properties with respect to the glucose concentrations [9].

(f) Polarization Techniques

Various samples of glucose mixed intralipid units prepared according to the physiological conditions (hypo, normal, hyper blood glucose levels) to simulate and study the glucose-induced polarization based optical properties. The intralipid suspensions with known optical and scattering values were utilized here as a tissue phantom. The 20% Intralipid with scattering coefficient (μ's) of 10cm^{-1}, 0.8 value of anisotropy factor (g) had been utilized here [10].

(g) Occlusion Spectroscopy

In this technique the intralipid samples had been mixed with the Red Blood Cells (RBC) to simulate the pattern of blood-tissue complex. The intralipid and RBC mixed samples were collected inside the rectangular cuvette. The hematocrit level had been maintained between 30 to 50%. Similarly, the nitrogen bubbling for deoxygenating the RBC had been performed in these experiments. The glucose concentrations were varied in the range between 0 to 1000mg/dl in the rectangular cuvette and its optical characteristics variation were analyzed and studied here [12].

(h) Raman Spectroscopy

In these experiments, the India ink and Intralipid samples were used as major absorber and scatterer to mimic the tissue optical properties in the NIR range. The scattering property of intralipid samples varies from 24 to 130 cm^{-1} at 830nm. Similarly, the absorption property varies from 0.08 to 1.3 cm^{-1} at 830nm. Moreover, the anisotropy parameters (g) had been within 0.8 to 0.9 as near to the human living tissues [11].

PRINCIPLE OF INDIGENOUSLY DEVELOPED MUS-IR UNIT (MODULATED ULTRA SOUND AND INFRA RED UNIT)

The ultrasonic waves modulating in nature when travels through the human blood plasma mixed intralipid based sample medium, it initiates the process of vibration within that medium [17-25]. Its potential force of radiation [17-25] applied over the molecules present in human blood plasma mixed intralipid based sample medium are expressed as follows:

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

Here **(F_r), (V_c), (z), (P_o)** and **(λ)** expresses the radiating force, volumes of the molecules, pressure node distances, acoustic pressure peak amplitude and the wavelength of ultrasound respectively [17-25]. With the introduction of the compressibility factors **(βw)** of the medium in this phenomenon [17-25], the equation represented as follows:

$$\boldsymbol{\phi}(\boldsymbol{\beta}, \boldsymbol{\rho}) = [\frac{5_{\rho_c} - 2_{\rho_w}}{2_{\rho_c} + \rho_w} - (\frac{\beta_c}{\beta_w})] \quad \textbf{(2)}$$

Here **(β_c)** expresses the compressibility of the molecules, **(ρ_c)** and **(ρ_w)** refers the molecular densities of both the suspending molecules and the medium respectively [17-25]. When light beam were directed over this human blood plasma mixed intralipid sample medium, it obeys the typical Lambert-Beer law [17-25]. This observable fact had been represented as follows:

$$\mathbf{A}(\mathbf{v}) = -\log \mathbf{I}(\mathbf{v})/\mathbf{I_0}(\mathbf{v}) \quad \textbf{(3)}$$

Here **(A), (v), (I_0)** and **(I)** means phenomenon of absorption, wave number of light, the light intensity of the background and the light strength after travelling through the human blood plasma mixed intralipid sample pathway respectively[17-25].

DESCRIPTION OF MUS-IR UNIT (MODULATED ULTRA SOUND AND INFRA RED UNIT)

(a)LED Wavelength and Ultrasound Operating Frequency

In these experimentations a LED of 940nm has been chosen, as it falls under the tissue optical window spectrum (700nm -1100nm) [26-28]. Moreover, the impact of water, hemoglobin and oxyhemoglobin were fairly less in that very range of light spectrum [26-28]. The main operating frequency of ultrasound has been 40 kHz as selected. It is widely available and safe for human use [25, 26].

(b)MUS-IR Unit Block Diagram

In MUS-IR unit, the ultrasonic block produces amplitude modulating waves to the sample holder. These waves cause vibration in the human blood plasma mixed intralipid sample medium. Different molecules vibrate accordingly to their respective physical and chemical orientation properties. The glucose molecule specific vibrations were captured by the infrared light and detector unit. The captured signals were analyzed and decoded for blood glucose level information extraction. The Figure No.1 describes the Block level diagram of the MUS-IR Unit.

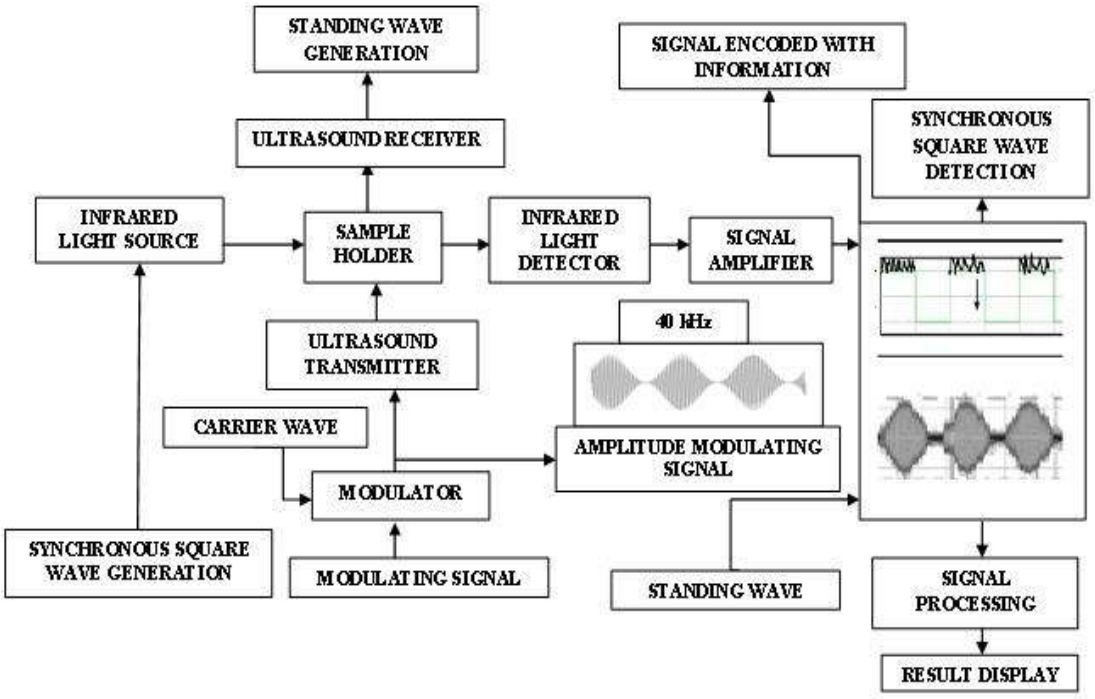


Figure. 1. MUS-IR unit Block level Diagram.

(c)Study Volunteers

The Group of 03 subjects (02 males and 01 female) age between 24 to 29 years allotted for this pilot study. The Subjects were fit and fine in health conditions. Moreover, the objectives of the experiments were discussed with the participating subjects and they provided their positive consent. The institutional ethical clearance for the pilot study had been obtained.

(d)Preparation of Intralipid Suspension Samples (Tissue Phantom)

The famous intravenous nutrient known as Intralipid suspension primarily composed of water and phospholipids micelles. The nature of the suspension is chemically inert, typical, uniform and turbid in appearances [7, 29-31]. Phospholipids micelles size ranges from 25nm to 675nm [7, 29-31]. The skin tissue light scattering properties were similar to intralipid suspensions scattering properties [7,29-31]. The light absorption profile of intralipid must be maintained accurately to obtain identical absorption properties [7, 29-31]. The ultrasonic impedance of pure water resembles the diluted intralipid suspensions [7, 29-31]. The standard Intralipid suspension as described in Reference No. [30, 31] has been prepared in the lab as given in the Table No.1 respectively.

Table No. 1. Composition of Intralipid suspension [30, 31].

Soybean oil	100 g	107.88 ml
Lecithin	12 g	11.64 ml
Glycerin	22.50 g	17.84 ml
Water	861 g	862.66 ml
Total	995.5 g	1000 ml

(e)Preparation of Human Blood Plasma Samples

The 05 ml of blood sample has been collected from each human subject in vacuum based collecting vials containing EDTA inside it as an anti clotting agent. Vials containing the blood samples were centrifuged for 10 minutes. Supernatant part of fluid called as blood plasma had been collected after the centrifugation process. After that the samples were stored for research purposes [32].

RESULT AND DISCUSSION

To evaluate the working of the indigenously developed MUS-IR experimental setup, the standard protocol of the Oral Glucose Tolerance Test (OGTT) [3-5] has been conducted over 03 healthy subjects. The OGTT conducted as given below:

The trials were held in the morning and the subjects were instructed to fast (water is allowed) for 8–12 hours prior to the tests. The OGTT tests were started in the morning and the subjects were demonstrated to fast (water allowed) for 8-10 hours before the experimental procedures.

Step A. Fasting blood glucose samples of the subjects were obtained at 00min for our indigenously developed MUS-IR unit and for Digital spectrometer based measurements.
Step B. 75gm of glucose in 100ml of water [35] has been provided to the subjects for drinking in a time span of 5 min after the step A.
Step C. This part involves postprandial sample collections for MUS-IR unit and Digital spectrometer every 30 minutes up to 2 hours and 30minutes respectively. The data obtained from the OGTT (Oral Glucose Tolerance Test) experimentations at different time intervals using MUS-IR unit has been shown in Table No.2 to 7 and in Graph No. 1 and 2 respectively.

Table No. 2. Shows the Fasting condition OGTT sample values obtained after 00 minute from the normal subjects (1-3).

Serial No.	Tissue Phantom Medium with prepared subject samples	Values Obtain by MUS-IR Unit (mV)	Values Obtain by Digital Spectrophotometer (mg/dl)
1.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 1	6.0	68.0
2.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 2	5.6	60.0
3.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 3	7.3	76.0

Table No. 3. Shows the Postprandial condition OGTT sample values obtained after 30 minutes from the normal subjects (1-3).

Serial No.	Tissue Phantom Medium with prepared subject samples	Values Obtain by MUS-IR Unit (mV)	Values Obtain by Digital Spectrophotometer (mg/dl)
1.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 1	8.2	80.0
2.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 2	6.3	70.0
3.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 3	8.0	86.0

Table No. 4. Shows the Postprandial condition OGTT sample values obtained after 1 hour from the normal subjects (1-3).

Serial No.	Tissue Phantom Medium with prepared subject samples	Values Obtain by MUS-IR Unit (mV)	Values Obtain by Digital Spectrophotometer (mg/dl)
1.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 1	10.3	108.0
2.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 2	9.9	102.0
3.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 3	10.6	110.0

Table No. 5. Shows the Postprandial condition OGTT sample values obtain after 1 hour 30 minutes from the normal subjects (1-3).

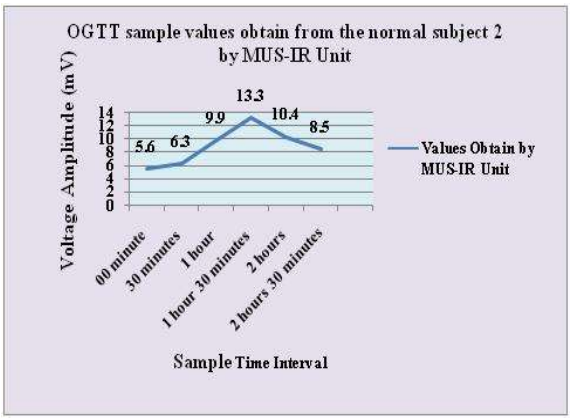
S. No.	Tissue Phantom Medium with prepared subject samples	Values Obtain by MUS-IR Unit (mV)	Values Obtain by Digital Spectrophotometer (mg/dl)
1.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 1	12.2	128.0
2.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 2	13.3	140.0
3.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 3	12.9	132.0

Table No. 6. Shows the Postprandial condition OGTT sample values obtain after 2 hours from the normal subjects (1-3).

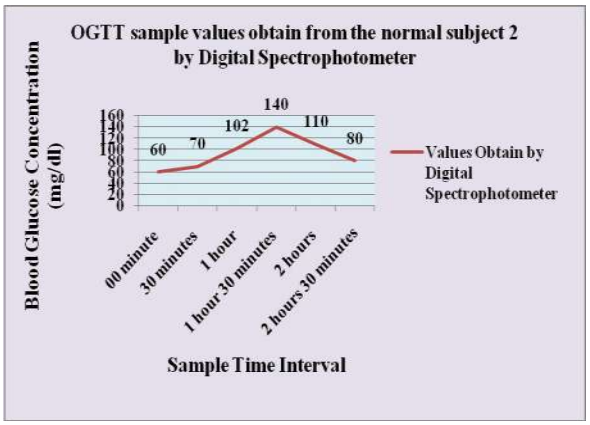
S. No.	Tissue Phantom Medium with prepared subject samples	Values Obtain by MUS-IR Unit (mV)	Values Obtain by Digital Spectrophotometer (mg/dl)
1.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 1	9.1	90.0
2.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 2	10.4	110.0
3.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 3	9.8	109.0

Table No. 7. Shows the Postprandial condition OGTT sample values obtain after 2 hours 30 minutes from the Normal subjects (1-3).

Serial No.	Tissue Phantom Medium with prepared subject samples	Values Obtain by MUS-IR Unit (mV)	Values Obtain by Digital Spectrophotometer (mg/dl)
1.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 1	6.6	63.0
2.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 2	8.5	80.0
3.	3 ml Intralipid Phantom + 1 ml Plasma sample of subject 3	7.7	79.0



Graph No.1. Depicts the OGTT sample values of the normal subject 2 by the MUS-IR unit.



Graph No.2. Depicts the OGTT sample values of the normal subject 2 by the Digital spectrometer.

The data obtained from the Table No 2 to 7 reveals that the voltage amplitude values of MUS-IR unit changes with respect to the change in blood glucose levels during fasting and postprandial OGTT samples as obtained from the normal subjects (1-3) respectively. These results were also confirmed by the readings obtained from the established digital spectrometer method. These entire phenomenons provide evidence that our indigenously developed MUS-IR unit had been working accurately for detecting blood glucose levels in intralipid phantom based medium.

IMPORTANT ASPECTS FOR NONINVASIVE BLOOD GLUCOSE MONITORING TECHNOLOGY

Worldwide plenty of researchers, scientists were utilizing numerous scientific and technological ideas to pave the successful invention of non-invasive blood glucose monitoring device. The essential criteria about the noninvasive glucometer device must be stable clinically, user friendly, easily portable and cost efficient [33, 34]. The noninvasive blood glucose determining technology had been at the stage of development now days. The weak blood glucose signals, over lapping with water absorption spectra, weak signal to noise ratios were the main hurdles in the path of realization for noninvasive blood glucose detection technology. Large number of diabetic population worldwide had anticipated the steep rise in demand for noninvasive technology. Various quantitative analytical tools like multivariate approaches provide results based on data available.

For blood glucose predictions, subject related specificity; individual result calibration approach needed exploitation for successful results [33, 34].Factors like blood glucose level variation in different parts of the body at a time, temperature fluctuations, skin pigmentations, contact area pressure related glucose variation issues, physiological status of the subject concern, metabolic rate, body fluid circulations, must be considered in optical technology based noninvasive blood glucose monitoring devices [33, 34]. Due to large variation factors, universal approach for noninvasive blood glucose suffers real life setbacks. The noninvasive technology might be successful when subject based individual calibration would be targeted [33, 34].

CONCLUSION

For blood plasma glucose predictions, intralipid phantom based tissue specificity; result calibration based approaches needed to exploit for successful results. Our MUS-IR unit might be the useful technology for predictions of blood glucose levels.

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