

# OPTICAL CLEARANCE EFFECT DETERMINATION OF GLUCOSE BY NEAR INFRARED TECHNIQUE: AN EXPERIMENTAL STUDY USING AN INTRALIPID BASED TISSUE PHANTOM

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## ABSTRACT

*The present paper describes the results of experimentation, carried out on intra-lipid phantom to study the optical clearing properties of glucose. For this intra lipid phantom with different concentration of dextrose levels have been used and the measurement based on selected mathematical parameters have been obtained using indigenously designed amplitude modulated ultrasound & infrared system. The results shows that dextrose minimizes the refractive index dissimilarity between scatterers and their surrounding media, leading to a smaller scattering coefficient, consequently, a shorter optical path. Hence, it is concluded that light clearing effect in relation with dextrose concentration can be principally utilized for the design of amplitude modulated ultrasound & infrared based non-invasive blood glucose meter.*

**KEYWORDS:** *Optical Clearing, Dextrose, Intralipid Based Tissue Phantom, Amplitude Modulated Ultrasound, Infrared Techniques.*

## I. INTRODUCTION

Tissue phantoms must possess the scattering and absorption phenomenon similar to that of living human tissues in all respect along with their wavelength detection criteria. Tissue phantom refractive index properties are required to match with that of living tissue refractive index. The functional and tissue mimicking properties must be steady enough and not to be influenced by temperatures, humidity, photo bleaching & other environmental conditions. The tissue phantoms with low cost of production, easy processing, greater stability, high optical tissue resemblance, and logistics friendly are popular now a day. The above mentioned features are needed in tissue phantom to model blood glucose properties of human body, and same is required for the design and development of non invasive bio-sensing of blood glucose in human body [1]. The major difficulty in the development of a clinical application of optical noninvasive blood glucose sensors is associated with the very low signal produced by glucose molecules [2, 3].

A realistic non invasive blood glucose sensing tissue phantom is required to satisfy the following requirements[1] : (i) It should model the physiological features which include geometry and optical properties of the living tissue for light transport phenomenon (ii) Tissue phantom composition must be stable for providing better chemical stability and better spectroscopic properties (iii) Sample composition must provide unique & reproducible idea about radiation transport measurement (iv) The physical factors of the phantom sample should be temporally stable from evaporation, diffusion, and aging must be independent of environmental influence (v) The construction of inhomogeneous samples by stacking phantom slabs or by elaborate molding techniques must be allowed by tissue phantom compositions (vi) Easy, safe, handy and faster sample preparation procedures.

### 1.1. The Effect of Glucose on the Optical Properties of Tissue Phantom

Presence of glucose in an aqueous suspension of inert scattering particles can vary a number of physical parameters such as absorption, scattering and transmission (a brief detail of these factors dependency on glucose is summarized in table 1), further, the alteration in these properties effects the propagation of light in the scattering medium[4,5]. Glucose reduces the absorption coefficient ( $\mu_a^w$ ) of the water in the aqueous solution because it displaces water (i.e. reduces the molar concentration of water molecules). At the same time it adds the intrinsic glucose absorption coefficient ( $\mu_a^g$ ) (table 1. (a), (b)). The refractive index 'n' of the aqueous solution increases with the glucose concentration [table 1 (c)], resulting in a reduced velocity of light and a changes of the scattering properties of particles [scattering coefficient ( $\mu_s$ ) phase function (p) and (g) value] suspended in the solution (table 1(d)-(f)). In Tissue-Simulating Phantoms when glucose is added Light Transport property like Transmittance (T) and Phase shift ( $\phi$ ) increases (table 1 (g)-(h))[4,5].

**Table No.1.** A summary of the effect of glucose upon the basic optical properties of a tissue phantom and the light transport within this tissue phantom [4, 5].

No.	Effect of Glucose on Basic Optical Properties of Tissue Phantom [4,5]		
I.	Change in Absorption Properties	Notations	Effect
	(a)Water Absorption Coefficient	$\mu_a^w$	Decreases
	(b)Intrinsic Glucose Absorption Coefficient	$\mu_a^g$	Increases
II.	Change in Scattering Properties		
	(c)Refractive Index of Suspending Medium	$\Delta n$	Increases
	(d)Scattering Coefficient	$\mu_s$	Decreases
	(e)Phase Function (P)	g value	Increases
	(f)Modified Scattering Coefficient	$\mu_s' = \mu_s(1 - g)$	Decreases
III.	Effect of added Glucose on Light Transport in Tissue-Simulating Phantoms		
	(g)Transmittance	T	Increases
	(h) Phase Shift	$\phi$	Increases

**The present paper is organized as follows:** Section II describes the materials and methodology in term of instrumentation system based on amplitude modulated ultrasonic waves and infrared light, and its working to detect the optical clearing effect of glucose in intralipid based tissue phantoms. Section III provides the experimental results and discussion. Section IV provides conclusion of the paper. Finally in section V plan about future work is presented.

## II. MATERIALS AND METHODS

Our method utilizes amplitude modulated ultrasound (40 KHz) & Infra Red (940 nm wavelength) technique. Amplitude modulated ultrasonic waves are used to excite the intralipid phantom (tissue based), as a result different constituent molecules vibrates at their specific response frequency depending upon their weight, shape & size, these specific vibrations are detected using light, the output response signal is in the form of modulated light signal, that carries information about the concentration of different constituent molecules. This modulated light response signal is collected using photo-sensor, and suitably processed using indigenously developed signal processing algorithm to extract the information about the glucose concentration in tissue phantoms.

Block level description of the instrumental scheme has been shown in figure1. System consists of an amplitude modulation module which provides modulated signal to 40 kHz ultrasound transmitter attached to sample holder.

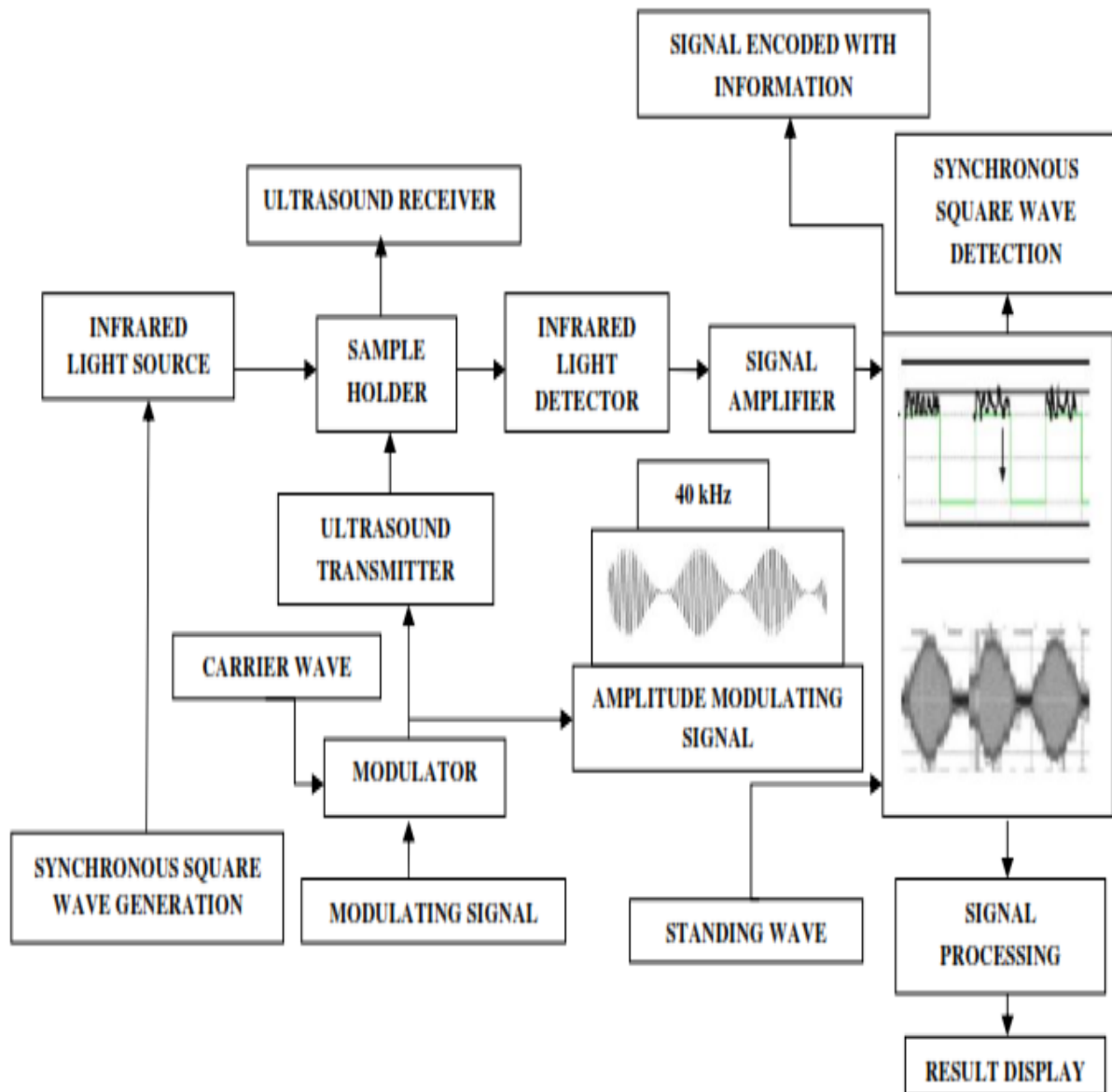


Figure 1: Block diagram of the system.

Ultrasound receiver is used for cross verification of the standing wave. Light source controlled by square wave generation module is directed to the sample holder followed by light detector, signal processing & result display.

### 2.1. 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 ultrasonic manipulation of intralipid phantom (tissue based) molecules. The combination of these techniques has the potential of new measurement concepts for use in non invasive detection of blood glucose. Local increase of molecular concentration brought about by ultrasonic force could facilitate measurements of molecular-specific infrared spectra of the suspending phase (intralipid phantom medium) and molecules independently [6-7]. By changing the frequency of modulating wave used to generate Amplitude Modulated Ultra Sound wave (standing wave), it is possible to control the position of molecules in respect to the optically sensitive region of the infrared spectra [9-10].

## 2.2. Ultrasonic Manipulation of Intralipid Phantom (Tissue Based) Molecules

When molecules in intralipid phantom medium are subjected to an Amplitude Modulated Ultra Sound wave (standing wave), so-called radiation forces exerted on the suspended molecules permit their manipulation. The origins of these forces are the spatial gradients of the sound wave's acoustic pressure; therefore the nodes within an Amplitude Modulated Ultra Sound wave (standing wave) are regions where molecular aggregation (or thinning) can be observed. The direction and strength of the forces is influenced by the compressibility-which itself is a function of the material properties speed of sound and mass density – of both components of the dispersion. The coefficient representing this dependency is called acoustic contrast; molecules typically travel into the pressure nodes of the sound field. Furthermore, the phenomenon is strongly dependent on the diameter of the suspended molecules: forces exerted on larger molecules are stronger [11-12].

## 2.3. Separation Principle

In an Amplitude Modulated Ultra Sound wave (standing wave), the pressure amplitude has maximum (antinodal) and zero (nodal) values twice over a distance of one wavelength. A discontinuity, in the propagating phase, for example specific molecules acquires a position-dependent acoustic potential energy by virtue of being in the sound field. Suspended molecules therefore tend to move towards and concentrate at positions of minimum acoustic potential energy. For molecules, these localized regions are generally close to pressure nodes, which are separated from each other by distances of half a wavelength. For the case where the molecular diameter is small compared to the ultrasound wavelength, the 'primary' radiation force,  $F_r$  acting on a molecule of volume  $V_c$  located at a distance  $z$  from a pressure node is derived from the gradient of the molecule acoustic potential energy[13], and is given by:

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

Where  $P_o$  is the peak acoustic pressure amplitude and  $\lambda$  is the wavelength of sound in the aqueous suspending phase, which has a compressibility  $\beta_w$ . The function equals to

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

Where  $\beta_c$  is the compressibility of the molecule,  $\rho_c$  and  $\rho_w$  are the densities of the molecule and the suspending phase (intralipid phantom medium) respectively.

Secondary forces 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 [10, 14].

## 2.4. Acquisition of Absorption Spectra

Many influences are to be considered when looking at an IR spectrum as every substance present in the light path changes the intensity at a certain wave number. The absorption  $A$  at a given light wave number  $\nu$  is then calculated by the **Lambert–Beer law**,

$$A(\nu) = -\log I(\nu) / I_o(\nu) \quad (3)$$

Where  $I_o$  denotes the intensity of the background,  $I$  denotes the intensity at the respective wave number  $\nu$  of the actual measurement, i.e. when the sample is additionally present in the light path [9, 15].

## 2.5. Preparation of Tissue Phantom

To mimic the properties of human or animal tissues, the use of tissue simulating objects plays a significant role. These phantoms are generally used for testing, optimizing, comparing, quality control of the newly designed systems. They are well calibrated for routine system evaluation and standardization. For all reasons good quality phantoms is essential for research purposes. To match the optical characteristics of tissues with phantom preparations, it depends directly on understanding of key physical and chemical properties of the tissues involved. To achieve average cosine of the scattering angle, the relation between the absorption coefficients, the scattering coefficient and the

anisotropy coefficient angle must be retained. In the present work the finger phantom with optically similar to finger has been prepared as proposed in Ref. [16, 19, 20].

## 2.6. Intralipid as Tissue Phantom

Intralipid is an aqueous suspension of lipid droplets that is sterile and used as phantom for mimicking tissue optical properties. Available as Intralipid-10% and Intralipid-20% (10% lipid indicates 10 g of lipid per 100 ml of suspension). The constituents of Intralipid-10% in a 500 ml bottle according to the manufacturer are [17, 18, 21]:

**Table 2.** Showing Different constituents of 10% Intralipid suspension [17, 18, 21].

Soybean oil	50 g	53.94 ml
Lecithin	6 g	5.82 ml
Glycerin	11.25 g	8.92 ml
Water	430.5 g	431.33 ml
Total	497.75 g	500 ml

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

## 2.7. Uses of Intralipid

- As a phantom for mimicking tissue optic properties.
- Non-pyrogenic fat product prepared for intravenous administration, as a source of calories and essential fatty acids [22, 23, 24].
- **Chemicals Used:** Dextrose anhydrous, purified powder from Merck specialties private limited.

## 2.8. In Vitro Experimental Setup

In order to test the optical clearing effect in intralipid phantom (tissue based) with respect to different glucose (dextrose) concentration, intralipid phantom has been prepared with the above mentioned procedure. The dedicated instrument and the mathematical parameters (absolute, integral, square value) based algorithmic concept as shown in figure 2 was designed and developed. Our method utilizes amplitude modulated ultrasound & Infrared techniques for detecting this optical clearing effect of Dextrose in intralipid tissue phantoms based on various mathematical parameters. The intralipid phantom was prepared with the help of Soya bean oil, lecithin, glycerol, Distilled water. The 100ml of this Tissue phantom is used to carry out experimentation with different concentrations like Blank (0 mg), 500 mg, 1000 mg and 1500 mg of Dextrose anhydrous purified powder to verify optical clearing property of glucose (dextrose). The prepared phantom is placed for measurement in indigenously developed instrument. The signal acquired is processed in indigenously developed software for data analysis. The Mathematical Function with absolute, integral and square function was used here. The value obtained was interpreted to validate the acquired data with respect to sample concentration.

2.9. Algorithm concept

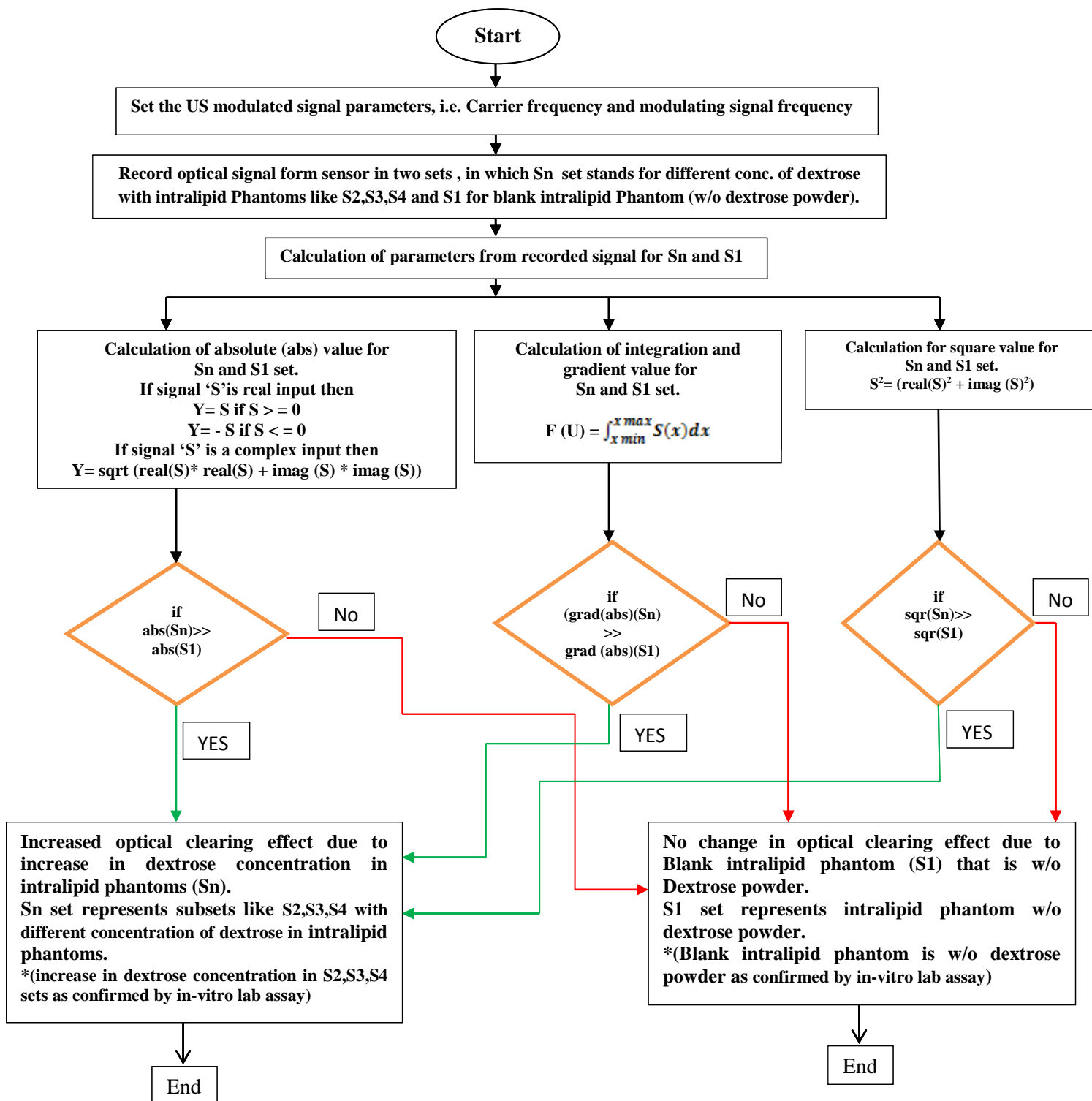


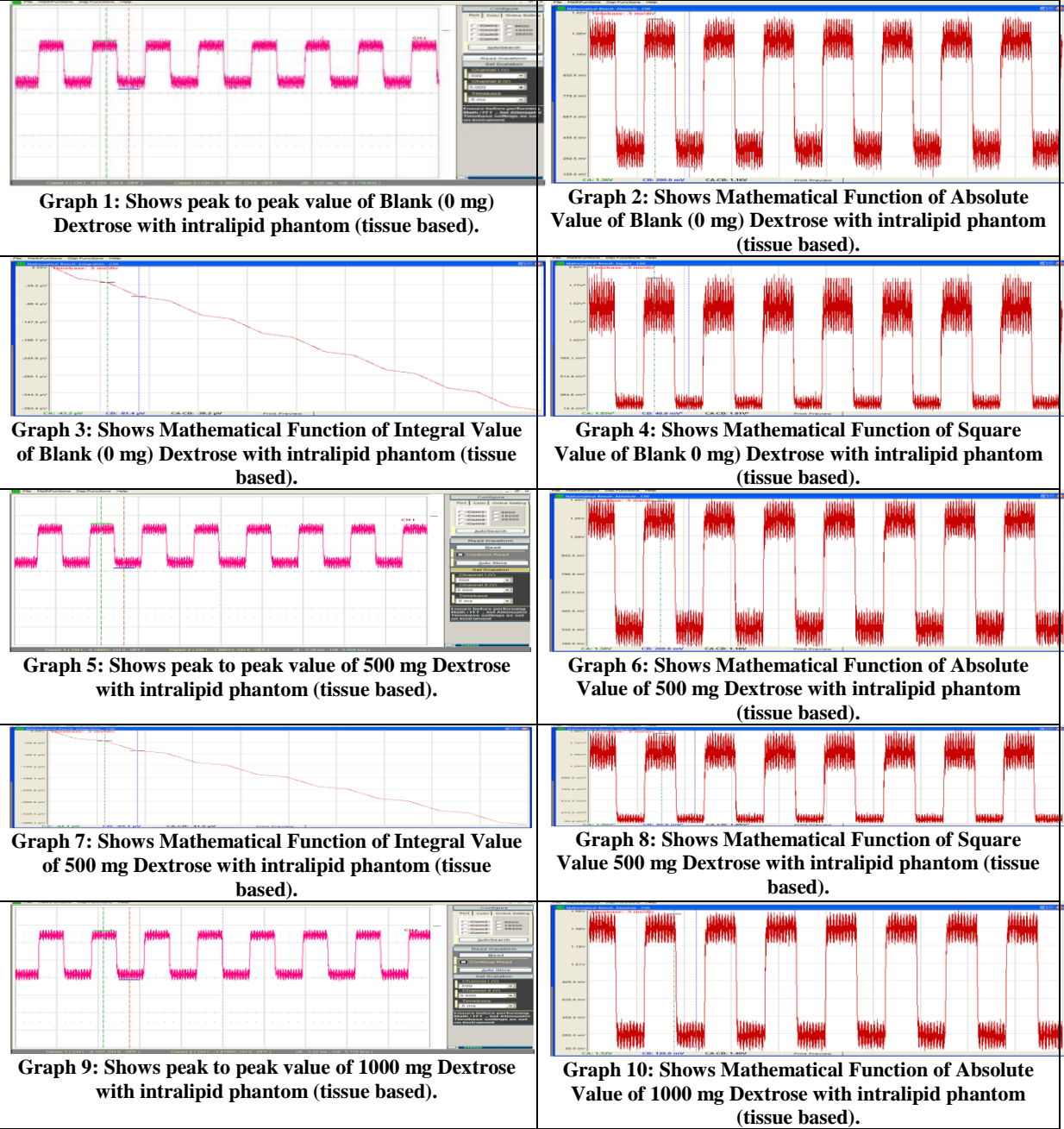
Figure 2: Flowchart of the algorithmic concept.

III. RESULT AND DISCUSSION

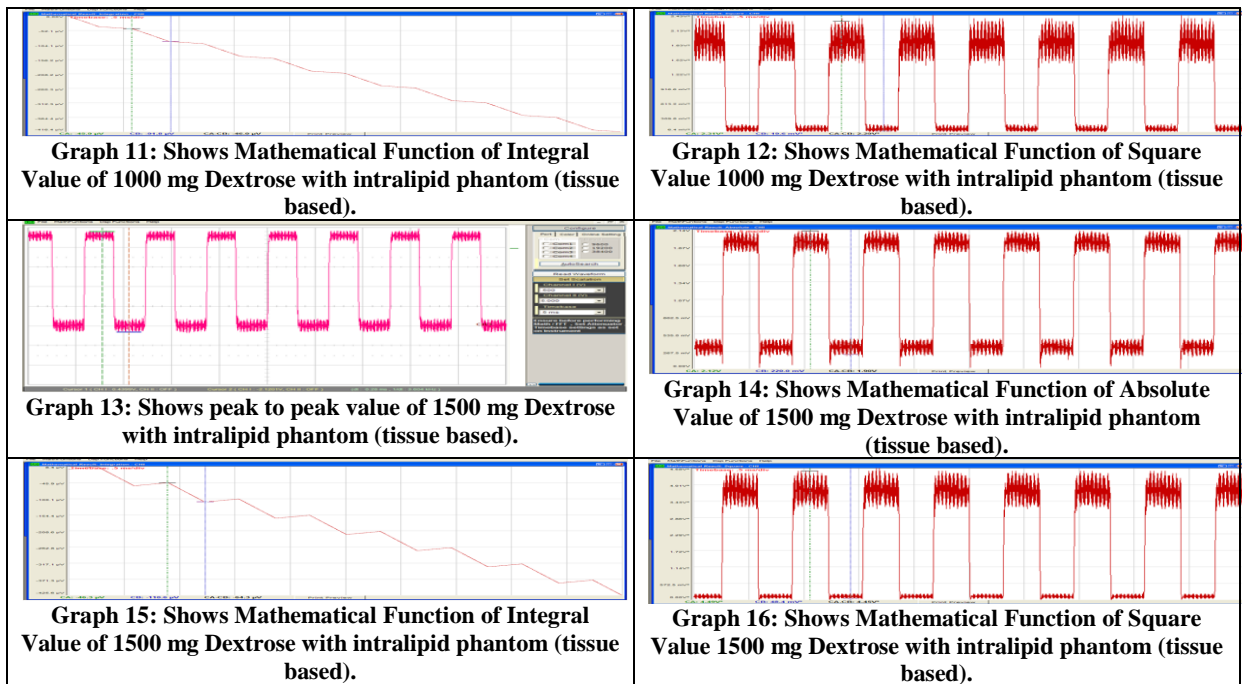
3.1. In Vitro Measured Spectra

In the present work intralipid phantom (tissue based) with optically similar to finger has been prepared as proposed by HG van Staveren *et al*<sup>14</sup>. Light transport data based on mathematical function (Absolute value, Integral value, Square value) of different concentration of Dextrose [Blank 0 mg,

500 mg, 1000 mg and 1500 mg] in intralipid phantom (tissue based) (w/v) are shown in Graphs (1-16) respectively. Dextrose minimizes the refractive index dissimilarity between scatterers and their surrounding media, leading to a smaller scattering coefficient and, consequently, a shorter optical path. As a result, with the growing concentration of dextrose, fewer photons are absorbed and the light intensity increases [4, 5]. Table 3-6 shows the mathematical function values of various intralipid phantoms (tissue based). In various mathematical functions (absolute value, integral value, square value) the magnitude of amplitude (light transport) increases with increase in dextrose concentration at different frequency positions. Thus optical clearing effect increases with increase in dextrose concentration in intralipid phantoms (w/v). The corresponding mathematical function (absolute value, square value) of different intralipid phantom samples as obtained by the developed software are shown in Graphs 17 and 18 respectively. Graph 19 expresses the different concentration of dextrose in different samples of intralipid phantom (w/v) (ILPsp1-ILPsp4). The above stated experimentation is processed and analyzed by developed instrumentation system and algorithm.







Graph 1-16: Shows Mathematical Function (absolute, integral and square value) of various concentration of Dextrose with Intralipid (Tissue Phantom).

### 3.2. Peak to Peak Amplitude Calculation

Peak-to-peak amplitude is the change between peak (highest amplitude value) and trough (lowest amplitude value, which can be negative). With appropriate circuitry, peak-to-peak amplitudes oscillations can be measured by meters or by viewing the waveform on an oscilloscope. Peak-to-peak is a straightforward measurement on an oscilloscope, the peaks of the waveform being easily identified and measured against the graticule. This remains a common way of specifying amplitude [25]. If 'S' is the real or complex signal input then the peak to peak amplitude value for the signal 'S' is calculated as

$$S = S_{max} - S_{min} \tag{4}$$

Where  $S_{max}$  stands for highest amplitude value and  $S_{min}$  stands for lowest amplitude value.

Table 3: Showing Mathematical Function Value of Blank with intralipid phantom (tissue based).

Peak-to-peak amplitude calculation of Dextrose (0 mg) intralipid phantom (tissue based)	Time & Frequency (dt: 0.27ms, 1/dt: 3.774 KHz)		
	Mathematical Function	$S_{max}$	$S_{min}$
Absolute Value	1.36 V	200.0 mV	1.16 V
Integral Value	-43.2 $\mu$ V	-81.4 $\mu$ V	-38.2 $\mu$ V
Square Value	1.85 $V^2$	40.0 $mV^2$	1.81 $V^2$

Table 4: Showing Mathematical Function Value of 500 mg Dextrose with intralipid phantom (tissue based).

Peak-to-peak amplitude calculation of Dextrose (500 mg) intralipid phantom (tissue based)	Time & Frequency (dt: 0.28ms, 1/dt: 3.604 KHz)		
	Mathematical Function	$S_{max}$	$S_{min}$
Absolute Value	1.38 V	200.0 mV	1.18 V
Integral Value	-44.1 $\mu$ V	-85.1 $\mu$ V	-41.0 $\mu$ V
Square Value	1.90 $V^2$	40.0 $mV^2$	1.86 $V^2$

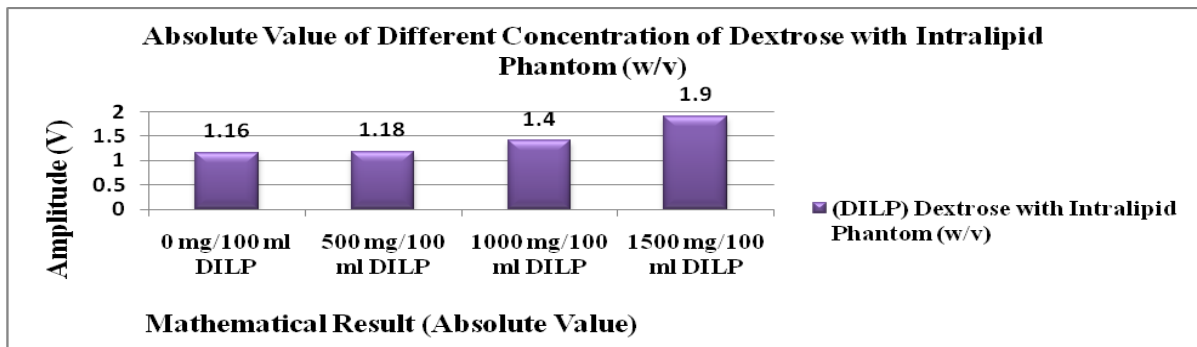


**Table 5:** Showing Mathematical Function Value of 1000 mg Dextrose with intralipid phantom (tissue based).

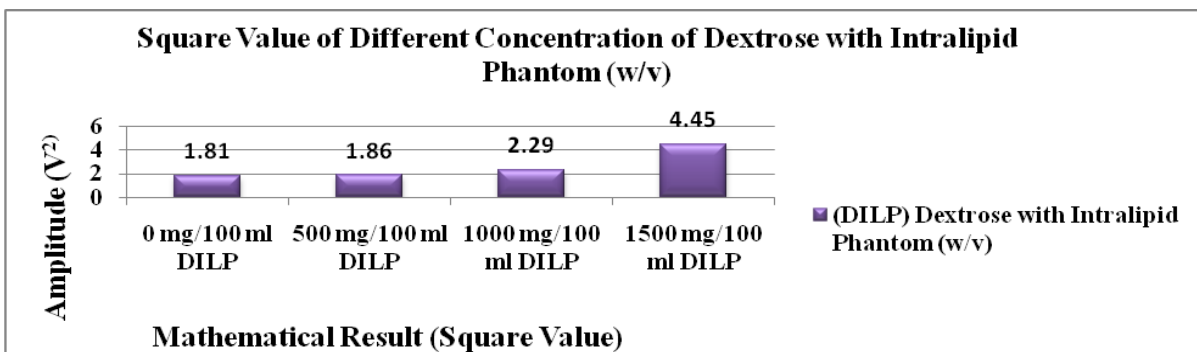
Peak-to-peak amplitude calculation of Dextrose (1000 mg) intralipid phantom (tissue based)	Time & Frequency (dt: 0.32ms,1/dt: 3.175 KHz)		
	Mathematical Function	S <sub>max</sub>	S <sub>min</sub>
Absolute Value	1.52 V	120.0 mV	1.40 V
Integral Value	-45.9 μV	-91.8 μV	-46.0 μV
Square Value	2.31 V <sup>2</sup>	19.6 mV <sup>2</sup>	2.29 V <sup>2</sup>

**Table 6:** Showing Mathematical Function Value of 1500 mg Dextrose with intralipid phantom (tissue based).

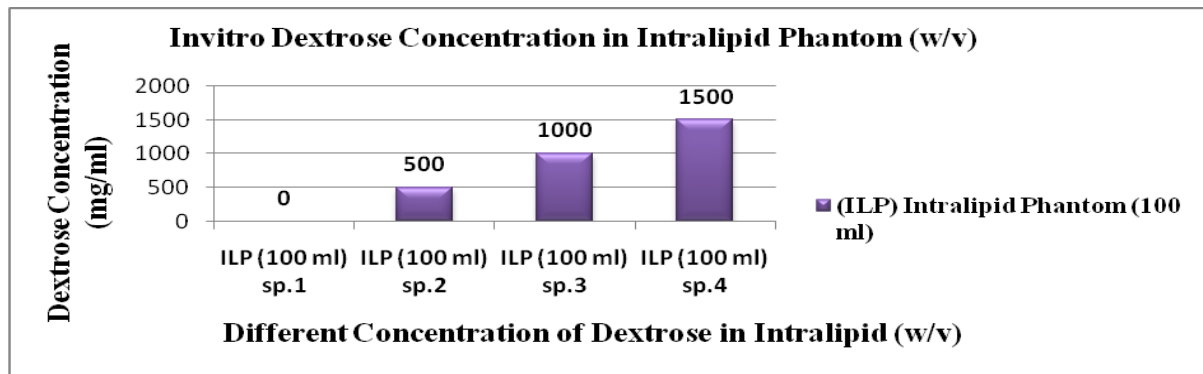
Peak-to-peak amplitude calculation of Dextrose (1500 mg) intralipid phantom (tissue based)	Time & Frequency (dt: 0.28ms,1/dt: 3.604 KHz)		
	Mathematical Function	S <sub>max</sub>	S <sub>min</sub>
Absolute Value	2.12 V	220.0mV	1.90 V
Integral Value	-46.3 μV	-110.6 μV	-64.3.9 μV
Square Value	4.49 V <sup>2</sup>	48.4 mV <sup>2</sup>	4.45 V <sup>2</sup>



**Graph 17:** Shows Mathematical Function of Absolute Value of Different Concentration of Dextrose with intralipid phantom (tissue based) (w/v).



**Graph 18:** Shows Mathematical Function of Square Value of Different Concentration of Dextrose with intralipid phantom (tissue based) (w/v).



**Graph 19:** Shows in vitro Value of Different Concentration of Dextrose with intralipid phantom (tissue based) (w/v).

### 3.3. Discussion

Zemansky MW et al. [26] (Zemansky MW *et al.* 1968) studied the near-infrared absorption spectroscopy of glucose and established that the absorption coefficient of glucose differs approximately by  $\pm 20\%$  from that of water at 905 nm and 1550 nm ( $0.007 \text{ mm}^{-1}$  and  $0.98 \text{ mm}^{-1}$ , respectively). Matthias Kohl et al. [5] (Matthias Kohl *et al.* 1994) described the experimental and theoretical investigations for the existence of glucose effects upon scattering media. The most significant of these effects was the modification of the reduced scattering coefficient and even this scattering effect was small. Glucose concentrations of an order of magnitude greater than physiological levels were needed to obtain measurable effects in tissue phantoms. It is hoped that the glucose effect in tissue is much greater than in these phantoms. Alexey N. Bashkatov et al. [27] (Alexey N. Bashkatov *et al.*) discusses some aspects of tissue-like phantoms their optical properties, especially phantoms with a high concentration of scatterers which corresponds to real tissues. Zuomin Zhao et al. [28] (Zuomin Zhao *et al.* 2002) proposed that photo acoustic apparatus could detect the minimal glucose concentration of 100 mg/dl in whole blood samples or in tissue phantoms. Decades of research indicate instrumentation with high specificity and sensitivity is required for non invasive blood glucose determination. In present work to increase the sensitivity of the analytical technique modulated ultrasound and infrared technique is used. Here amplitude modulated ultrasonic waves are used to excite the intralipid phantom, as a result different constituent molecules vibrates at their specific response frequency depending upon their weight, shape & size, these specific vibrations are detected using light, the output response signal is in the form of modulated light signal, that carries information about the concentration of different constituent molecules. This modulated light response signal is collected using photo-sensor, and suitably processed using signal processing algorithm to extract the information about optical clearance induced by dextrose concentration in tissue phantoms.

## IV. CONCLUSION

In this experimental work we have used an intralipid based tissue phantom to investigate the glucose induced optical clearing effect. A phantom that models the tissue properties is needed to evaluate and optimize techniques and procedures for noninvasive measurement of glucose. Dextrose minimizes the refractive index dissimilarity between scatterers and their surrounding media, leading to a smaller scattering coefficient and, consequently, a shorter optical path. Our method utilizes amplitude modulated ultrasound & Infrared techniques for detecting this optical clearing effect of Dextrose in intralipid tissue phantoms based on various mathematical parameters. Experimental results suggest that this optical phenomenon must be considered for noninvasive blood glucose measurement. Hence, this proposed technique can be principally utilized for the design and development of non-invasive blood glucose meter. Further, optical clearing may increase effectiveness of a number of therapeutic and surgical methods on a target area hindered in depth of a tissue.

## V. FUTURE WORK

The experimental study presented in this paper provides a possible methodology using intralipid based tissue phantom for development of noninvasive blood glucose measurement using amplitude modulated ultrasound and infrared techniques. Promising results have been obtained using the developed system. Further for improving accuracy, the other factors like skin pigmentation, melanin content of skin, tissue and skin contours, pressure dependent variation in blood flow, finger positioning alignment schemes are also needed to be considered for design and development of intralipid based finger phantoms. Investigation and experimentation related to these points will be considered in our future research work.

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