

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction:

Now a day, most common diseases prevalent in our society is Diabetes. Diabetes mellitus, commonly referred to as diabetes, is a group of metabolic disorder which results due to high blood sugar levels for a prolonged period in the human body [Newman *et al.* (2005)]. Improper management and lack of treatment worsens the situation leading to extremely serious medical complication. The result can be fatal with worse pathological conditions and increased death rate. Figure 2.1 depicts long-term medical complications of diabetes which include mainly cerebrovascular disease, retinopathy, neuropathy, nephropathy, diabetic foot ulcers, vascular micro and macro angiopathy, etc. Diabetes Mellitus is also a significant factor in rapid narrowing and solidifying of the arteries to cause strokes, which may lead to coronary heart disease as well as blood vessel related diseases [www.dreamstime.com (2016); Tuchin (2009); Khalil (2004)].

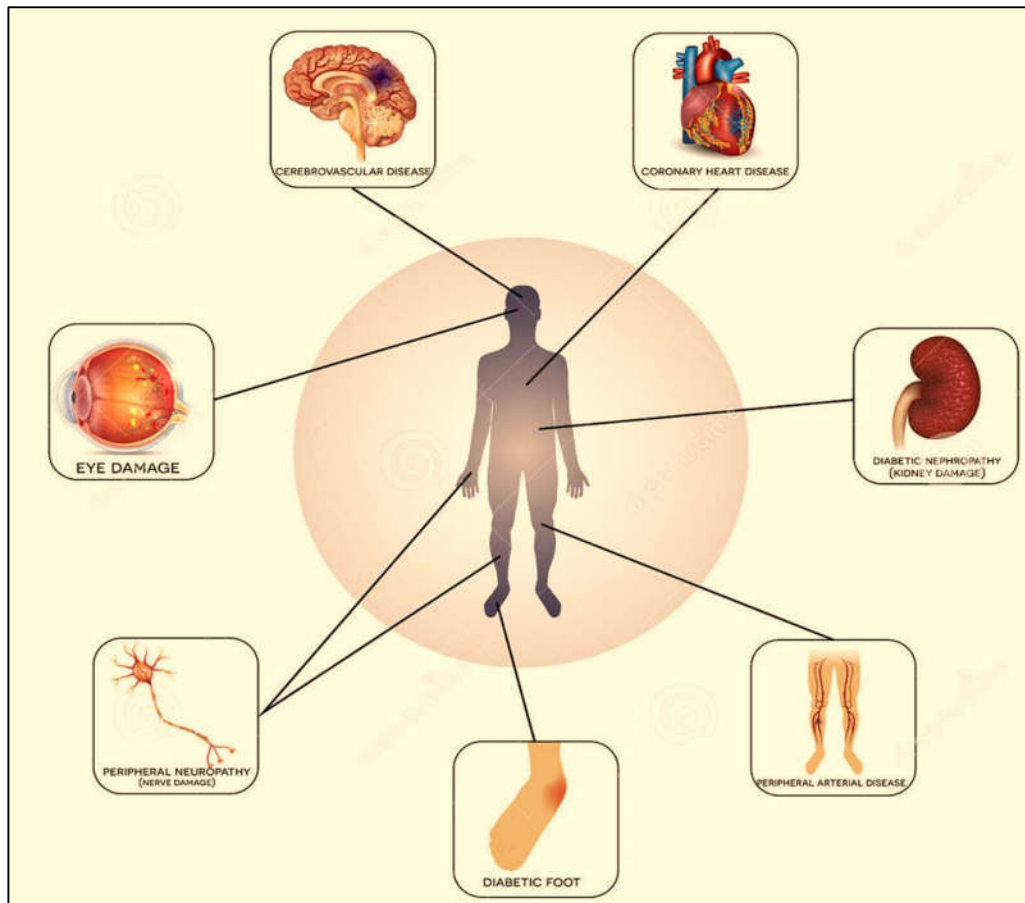


Figure 2.1: Long term complications of Diabetes.

To have proper control over this disease, it is important to keep a check over blood glucose level regularly. Most of the methods available to self-check the blood glucose level, use invasive technique in which the patient has to puncture the skin to take out blood which eventually can be tested. This process is quite painful over prolonged time-period and stressful too.

2.1.1 Diabetes Mellitus:

Diabetes Mellitus is a condition described by prevalence of excess amount of glucose in blood and tissue of the human body. Insulin is a hormone produced by pancreas in human body to regulate glucose level which in turn is the energy source of the body. In diabetic condition, the pancreas stops or lowers the insulin secretion leading to metabolic irregularity, hence irregular blood glucose level [Tuchin (2009); Li *et al.* (2007)^a].

There are three main types of diabetes:

- Type I diabetes
- Type II diabetes and
- Gestational diabetes

2.1.2 Type I Diabetes:

It is recognized as insulin-dependent diabetes, which affects almost 10% of human population with diabetes.

Occurrence: It rarely occurs in the first nine months of life but prevalent at the age of twelve and between 20 to 35 years [Heise (2000)].

Cause: The major cause of this type of diabetes is destruction of beta-cells in the pancreatic islets of Langerhans which results in insufficient production of insulin leading to hyperglycemia. The damage to islets of Langerhans starts at a very early age but the symptoms appear later.

Symptoms: A person with this type of disease may have various symptoms like:

- Polydipsia (increase thirst)
- Polyuria (frequent urination) with presence of glucose
- Electrolyte imbalance due to excess fluid loss leading to muscle cramps
- Weight loss (because deficiency of insulin caused break down of proteins and fats)
- Blurred vision because of accumulation of glucose in eye lenses

- Increased appetite
- Nausea, vomiting and abdominal pain

2.1.3 Type II Diabetes:

It is recognized as non-insulin dependent diabetes which affects almost 90% of major diabetic population.

Occurrence: It is generally detected in middle aged and older generations.

Cause: This is serve diabetic condition because it is a mixture of irregularities and disorders. The major disorder includes.

- Resistance offered due to presence of peripheral tissues like muscle and fat cells to insulin action.
- Insufficient insulin secretion by cells of pancreas.
- Inability of insulin to check glucose production in liver.

Symptoms: includes

- Polydipsia
- Polyuria
- Increased appetite
- Fatigue
- Erectile dysfunction
- Frequent/slow-healing wounds and infections [Newman *et al.* (2005)].

2.1.4 Gestational Diabetes:

It is a condition that occurs during pregnancy. It affects 2-4% of all pregnancies, developing in mother and child both [Marbach (2005)].

Occurrence: During pregnancy, risk of developing in both mother and child.

Cause: During pregnancy, the placenta produces a large number of hormones including insulin. Most women do not suffer from this diabetes, but in case of malfunctioning of pancreas, a women may develop such diabetes when the blood glucose levels remains high.

2.2 Structure of glucose and its physiological concentration:

The D-glucose molecular formula and weight are $C_6H_{12}O_6$ and 180.157 respectively. The glucose is most basic form of sugar and is abundant in surrounding environment existing both in its open and combined form. Photosynthesis help in

producing glucose in plants which is further transformed into monosaccharide, disaccharides and polysaccharides in different plants and animals [Zhao (2002)].

D-glucose can be classified into two types according to their cyclic structure: the first one is defined as α -D glucose and the second one is β -D-glucose. Due to hydrophilic nature of glucose, it is generally present in the form of water solution. Five anomeric D-glucose forms are shown in figure 2.2 : on the basis of percentage, 62.6% β -D-pyranose-glucose, 37.3% α -D-pyranose-glucose, 0.1% β -D-furanose-glucose and a very small concentrations of acyclic D-glucose and α -D furanose-glucose [Collins *et al.* (1987)]. Therefore, if a fresh anomer of glucose is mixed with water, it will carry on mutarotation until an equilibrium state is reached among the anomers. This whole process of mutarotation plays an important role in studying time-dependent infrared spectra of this glucose solution [Libnau (1994)].

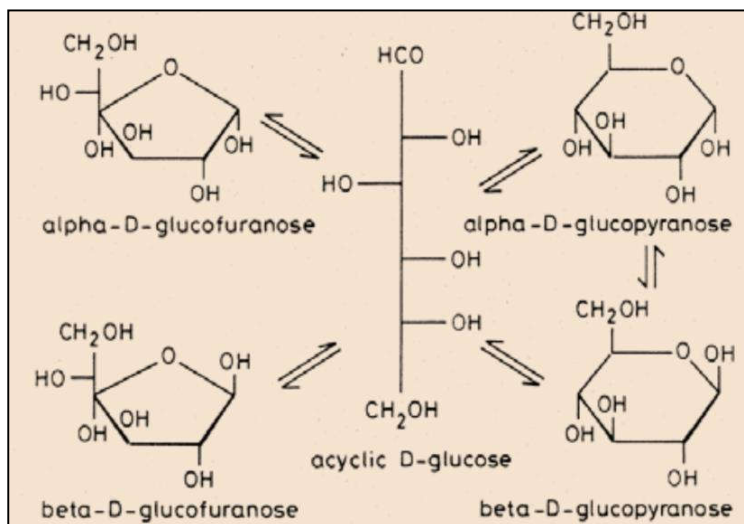


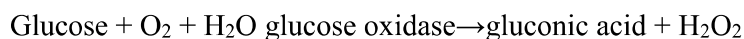
Figure 2.2: Anomers of D-glucose present in aqueous solution [Collins *et al.* (1987)].

The mechanism of energy production in human body starts from the conversion of food into sugar. Different tissues and organs utilize this energy through blood circulation. D-glucose being the chief constituent of human blood sugar, which is present as water base of blood plasma [Marshall (1989)]. Physiological glucose level in human blood varies in the range of 18 to 450 mg/dl. Blood collection through capillaries and arteries by pricking a body part like fingertip gives the average glucose levels in healthy and diabetic subjects.

The glucose molecule is also present in other body fluids like interstitial fluid, intracellular fluid, sweat, humour, saliva and urine besides the blood sample. Researchers found that the glucose concentration in humour associate robustly with the glucose present in the blood sample, while the glucose concentration level in sweat, urine and saliva are not more adequate [Zhao (2002)].

2.2.1 Oxidation technique of glucose:

Established glucose measurement technique depends on oxidation of glucose, which is catalyzed by glucose oxidase (GOD) method. It may be expressed by



Oxidation of β -D-glucose to D-gluconic acid as well as hydrogen peroxide is catalyzed by this method. This method has no effect on α -D-glucose since it is highly specific for β -D-glucose. Still overall glucose level can be monitored by utilizing glucose oxidase method. It is because, after consuming β -D-glucose, α -D-glucose is changed to β -form by mutarotation at equilibrium state. Glucose content has a linear relation with ensuing production of gluconic acid or oxygen utilized or hydrogen peroxide (H_2O_2). Estimation of free glucose present in human body fluids is performed by this method [Auses *et al.* (1975)]. High sensitivity and accuracy of this method also ensures this method to be reliable. Estimation of the glucose content during glucose oxidation reaction can also be done by using electrochemical methods like amperometry or potentiometry [Zhao (2002); Eggins (1996)].

2.3 Glucose determination techniques can be mainly categorized in three categories:

2.3.1 Invasive method based glucose sensing:

Majority of the old methods for blood glucose sensing utilizes a test strip doped with a chemical for oxidation of glucose. [Goldstein *et al.* (2004); Stewart *et al.* (1983)]. These strips were read visually for a color change. Such methods were mainly used in hospitals for blood and urine analysis [Clement *et al.* (2004)]. Currently such strips are used only by those who don't want to buy a glucose meter. Strips are coated with an enzyme and dye to react with glucose to give a colour product [Sherwood *et al.* (1983)]. This colour is compared with a reference chart to give concentration of glucose. The readings were prone to human error. In 1970's light reflectance meters were used to remove human error in each reading. Such meter were

used in hospitals as well as homes also. In 1980's, these meters and strips got replaced with sensors based on electrochemistry [Goldstein *et al.* (2004); Turner *et al.* (1999)]. The test strips were coated with glucose oxidase to oxidize glucose and produce gluconic acid and hydrogen peroxide. This hydrogen peroxide gets oxidized at a platinum electrode, producing a current detected amperometrically. This gives a digital reading of glucose concentration [Kramer (2005)].

Currently all sensors have been used for self-monitoring. However, the inconvenience and discomfort involved in obtaining a drop of blood remain back [Goldstein *et al.* (2004)]. Often a needle is used to puncture the finger tip (finger stick method). In recent years, sensors have improved and small lancets have been made to draw less blood but the pain remains the same. The cost of sensors strips are also high making its too expensive for people who have to use it twice or thrice a day.

This discomfort and cost involved in each time measurement hinders people to use it frequently, especially difficult for people with Type I and Type II diabetes those have to manage and control their insulin dose and diet regularly [Bloomgarden (2002); Harris (1995)].

Research is going on to combat such problems. One alternative to these methods is the use of an implantable insulin pump which is placed in abdomen and releases insulin according to user defined schedule [Cefalu (2004)]. Some researchers have designed glucose sensors to be attached with this pump. In this case the pump will release insulin only when the sensor detects abundance of glucose in the body. This would be very useful for Type 2 diabetes patients who can decrease their risk by using this and hence stay fit from long-term health issue.

The discomfort arising from the use of lancet forces the patient to avoid glucose monitoring. Hence researchers are trying to find out a less invasive or non-invasive method of blood glucose monitoring [Henry (2002); Henry (1998)]. Even though no such device has been developed which can challenge the accuracy of invasive methods still, several analytical techniques are in progress to evolve some minimally invasive and non-invasive approaches [Kramer (2005)].

2.3.2 Minimally invasive method based glucose sensing:

Some of the existing blood glucose monitoring systems are not invasive or noninvasive in true sense but they are designed in such a manner that they tend to lessen the overall pain discomfort suffered by the patient. Such devices are based on

the technique that measures the glucose levels in the interstitial fluid under the skin with the help of an implantable sensor. The glucose molecules travel the interstitial fluid while moving in blood vessels and entering the cells. Therefore glucose concentration in such matrix is directly related to blood glucose levels. Other examples are such as wearing sensor based contact lenses which are doped with glucose sensing fluorescing reagents also under study [Badugu *et al.* (2005); Kramer (2005); Badugu *et al.* (2004)].

2.3.2.1 Implantable sensors:

Several types of researches are going on for developing electrochemical sensors which would replace the finger stick measuring system [Ginsberg (1992); Wilson *et al.* (1992)]. A few of such sensors are available but they can be used only under the supervision of a physician. The problems associated with such sensors are lack of biocompatibility resulting in shorter life span of device. The body rejects the sensor assuming it a foreign body. As the sensor gets coated with a layer of protein, there is a decrease in its sensitivity. One such example is a glucose sensor manufactured by MiniMed[®]. It gives the reading every 5 minutes over 3 days of time. The data then needs to be taken to a physician for diagnosis which is then a tedious job. Hence, the patient is encouraged to conduct the usual finger prick measurement [Kramer (2005)].

Implantation of gelatinous spheres beneath the skin is also proposed by some researchers [Ye *et al.* (2003); Fang *et al.* (2002); D'Auria *et al.* (1999)]. These spheres contain glucose-sensing fluorescent reagents. Contact with glucose molecule cause change in colour of fluorescence. Host-friendly and biocompatible material is a topic for study. Another method is implantation of optically sensitive reagents within contact lenses [Badugu *et al.* (2005); Badugu *et al.* (2004); Alexeev *et al.* (2003)]. Change in fluorescence with change in glucose concentration in the tear fluid can be measured. The disadvantage of such a system is time difference between concentration of glucose in blood and in eye [Badugu *et al.* (2004); Kramer (2005)].

2.3.3 Non-invasive optical method based glucose sensing:

All methods discussed till now, require extraction of glucose from body which is painful and discomfortable. Several researchers tried to develop a non-invasive methods where the optical beam interacts with glucose within the body. The generated signals are analyzed and diagnosed. Since such methods are less painful,

several researchers are going on in this field [Waynant *et al.* (1998); Cote (1997); Heise (1996)]. The non-invasive optical techniques are based on near-infrared light, wavelength ranging from 600 to 2300 nm because this light can penetrate the human tissue or fluid from few hundreds micrometers to several millimeters. When the light is transmitted through target areas (such as finger tip, forearm, abdomen, thigh, ear lobe, lip, anterior chamber of eye), many parameters of light are altered by the tissues. The diffused light creates an optical signature or fingerprint of the penetrated tissue content. The amount of light absorbed and scattered depends on the tissue structure (like thickness, layers, tissue fluid, location, blood vessel distribution) and its chemical composition (like water, fat, hemoglobin, glucose, and melanin).

The concentration of glucose levels in the tissue can be diagnosed by analyzing the changes in the strength, polarization and wavelength of the optical sign, which is related with interstitial fluid, capillary blood and intracellular fluid in the lighted volume. However the composition of body fluids (plasma, interstitial, intracellular) fluctuates by factors such as action level, eating regimen or hormone variances, additionally by blood circulation, metabolic movement, body temperature change and medication. Day to day changes in vasculature and tissue texture, ageing process, all these factors influence the optical parameters and consequently the stability of glucose monitoring [Zhao (2002)].

2.4 Tissue optical phantom and its properties:

It is important to understand biochemical and physical characteristics of tissue pertaining to optical interactions [Pogue *et al.* (2006); Jacques (1992)]. Thus, complete understanding of properties is helpful in matching tissue properties in phantoms. The properties which have to be matched for 1mm small scale application are absorption coefficient ($\mu_a(\lambda)$), scattering coefficient ($\mu_s(\lambda)$) and anisotropy coefficient (g).

For the large distances, scattering length is defined as the reciprocal of scattering coefficient.

$$\text{Scattering Length} = 1 / \mu_s \quad \text{Equation (2.1)}$$

The reduced scattering coefficient is also called transport scattering coefficient. It is defined as:

$$\mu'_s = (1 - g) \mu_s \quad \text{Equation (2.2)}$$

Thus, it is observed in neutral particle scattering that over multiple scattering event lengths, there is no difference between anisotropic scattering process and isotropic scattering process with a decreased values of effective scattering coefficient [Arridge *et al.* (1992); Patterson *et al.* (1991); Wyman *et al.* (1989)]. In case of transmission through a thick tissue, it is entirely feasible to imitate the effective attenuation coefficient of the tissue. It is only possible to mimic the system where diffusion theory is applicable in the range of

$$\mu_{eff} = 3\mu_a\mu_s'^{-1/2} \quad \text{Equation (2.3)}$$

and wavelength regime is clearly defined. It is quite feasible because the stable condition attenuations in homogeneous medium are directly related to alteration in scattering or absorption. The diffusive procedure emerges to be attenuated exponentially with this single coefficient above the large distances. The only changes that occur are when there is introduction of temporal or boundary signals and thus, there is a significant difference between ' μ_a ' and ' μ_s '. The off matching can frequently be quite adequate when the goal is imitating the tissue transmission [Pogue *et al.* (2006)].

Other than that, most of the tissue spectroscopy experimentations where the aim is to separate and to permit spectral fitting, the tissue need to have representative values similar to these parameters (μ_a and μ_s). In the overdue 1980's, an outstanding summary of tissue optical characteristics was compiled by Welch, Prahl and Cheong [Cheong *et al.* (1990)] and it was updated in the year of 1995 [Cheong (1995)]. Since that period various new spectra have been proposed a dozens of diverse tissue categories, consisting of brain [Cooper *et al.* (1996)], cervix and breast [Shah *et al.* (2001); Cubeddu *et al.* (2000); Suzuki *et al.* (1996)], skin and esophagus [Sternborg *et al.* (1995); Lynn *et al.* (1993)].

2.4.1 Tissue optical phantom basic requirements:

The Boltzmann transport equation describes the light propagation and scattering through a tissue in the visible range. Description of the transport phenomenon associated for optical characteristics of the tissue is given by scattering coefficient μ_s , absorption coefficient μ_a and scattering phase function [Pravdin *et al.* (2002)].

These characteristics form the foundation of phantom modeling. The light propagation through synthetic tissue mimics the biological tissue in the given range of similar parametric values of μ_s , μ_a and anisotropy factor (as an approximation for phase function) g of the phantom. Typical values for the coefficients in the soft tissues are, $\mu_s \approx 0.2$ to 400 cm^{-1} , $\mu_a \approx 0.5$ to 5.0 cm^{-1} and $0.8 < g < 0.99$ in the ranges of visible and near infrared.

In case of diffusion approximation to Boltzmann equation, and modeling of parameters for any shape of phase function, the necessary parameters to mimic the light transport are μ_a and the transport scattering coefficient, $\mu'_s = (1 - g) \mu_s$. Coherence between the optical properties of the tissue and phantom may be on the similar scale of diffusion coefficient which is defined as:

$$D = \mu_a / \mu_{eff}^2 \quad \text{Equation (2.4)}$$

μ_{eff} = effective attenuation coefficient, and

$$\mu_{eff}^2 = 3\mu_a[\mu_a + \mu_s(1 - g)] \quad \text{Equation (2.5)}$$

Thus, leveled phantom models can be generated and analyzed in which light scattering imitates the biological system. To mimic the function of the organ or tissue with complex architecture, it is advisable to reproduce the macroscopic geometry of the natural object. The use of layered tissue structures are the most common method to mimic the biological system. It has been already used in the studies of skin [Sathyam *et al.* (1997)], human head [Wolf *et al.* (1999); Okada *et al.* (1998); Saidi *et al.* (1990)] and the cervix [Chernova *et al.* (1999)].

The refractive index of multiple layers must be estimated in the complex structure design of phantoms. Thus, multiple layers should have similar refractive indices as a mismatch at interfaces may result in the light propagation within the tissue. The value of refractive index should be carefully chosen in the range of 1.33 to 1.50 for the multilayer soft tissue [Bolin *et al.* (1989)]. A basic approach for the modeling of the optical characteristics of tissue is to generate the absorption and scattering coefficients independently.

2.4.2 Phantom utilization as a tissue-simulating process for medical purpose:

The utilization of tissue-simulating substance to imitate the characteristics of human tissue or animal tissues has constantly been studied for the development and

improvement of entire diagnostic systems. These “phantoms” are commonly utilized for [Seltzer *et al.* (1988); Cohen (1979); Olsen *et al.* (1995)]:

- Primarily examine the system plan.
- Enhancing the acquired signal and suppressing the noise in the present system, hence increasing signal to noise ratio.
- Regularly executing the quality control for the purpose of calibration
- Evaluating the performance of different systems.

Once a system is established, for its validation some general recommendations are needed for quality control phantoms in a routine medical investigation. These needed recommendations were made by regulatory bodies like American College of Radiology (ACR), the American Association of Physicists in Medicine (AAPM) and the Canadian Organization of Medical Physicists (COMP). These recommendations are made to fulfill the minimum criteria of a newly designed system or regular analysis of pre-existing systems. The advantage of these recommendations provide a standard platform [Pogue *et al.* (2006)].

In the early period of 1980s, the research for tissue mimicking phantoms started with optical tissue imaging and near-infrared spectroscopy. [Linford *et al.* (1986); Drexler *et al.* (1985); Watmough (1982)]. During 1990s, researchers investigated spectroscopy using light in spatially resolved, time resolved and frequency domain. This further motivated researchers to design tissue phantoms for different applications. [Wilson *et al.* (1990); Patterson *et al.* (1989); Delpy *et al.* (1988)].

During the past few years, there has been a considerably increase in applications of the light in medicine. The advancement of processing of tissue phantom became highly important for optical analysis. Keen research techniques include photodynamic therapy, dosimetry [Star *et al.* (1988); Grossweiner (1986); Wilson *et al.* (1986)], near-infrared tomography [Hebden (2003); Gratton *et al.* (1997)], optical coherence tomography [Fujimoto *et al.* (2000)], luminescence imaging [Walenta *et al.* (2002); Contag *et al.* (2002)] and fluorescence molecular imaging [Sevick-Muraca *et al.* (2002); Ntziachristos *et al.* (2002)] along with other applications. In support of every reason as discussed above, the standardization and improvement of tissue optical phantoms is very necessary and inevitable.

2.4.3 Optical phantom structural orientations and physical properties:

Maximum of the early observation in the area of tissue phantoms had their focus on making regular-shaped things that imitate the tissue reduced absorption ‘a’ and scattering ‘s’ at precise wavelengths. However, in the earlier decade, researchers focused on providing the phantoms that represented the tissue properties covering the complete spectrum of the tissue. The advantages of selecting NIR spectrum are its low energy, high penetration depth and suppressed background fluorescence. Effective excitation of biomolecules can be done in NIR region. The absorption profile of Intralipid, fat, hemoglobin, skin melanin and water in NIR region is shown in figure 2.3 [Shih (2007)].

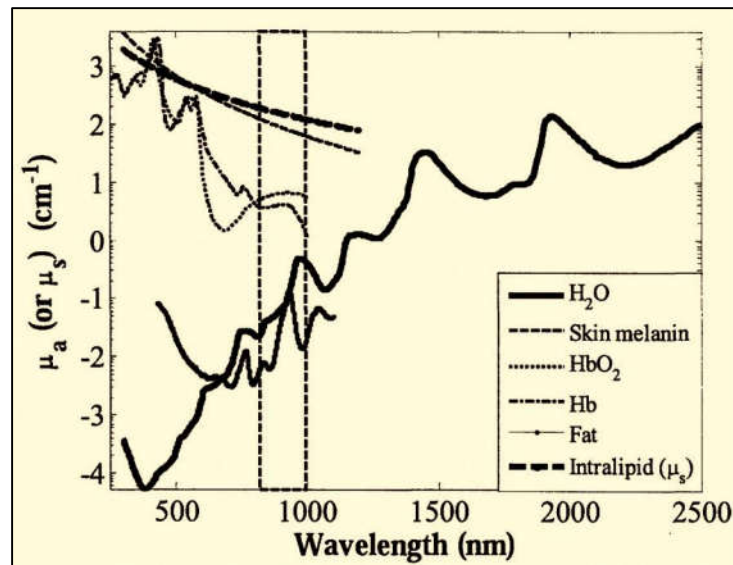


Figure 2.3: Absorption profile of Intralipid, fat, hemoglobin, skin melanin and water in NIR region [Shih (2007)].

A significant interest has been shown in emergence of biologically and biochemically well-matched tissue phantoms, which can practically and effectively utilize the biologically significant molecules for instance melanin, hemoglobin, or the endogenous fluorophores [Durkin *et al.* (1993)]. Developing of the hybrid type phantoms with recognized precise properties in support of the multimodality imaging including electrical properties [Li *et al.* (2003)], thermal properties, water/lipid concentrations and biochemical properties [Merritt *et al.* (2003)], magnetic resonance properties and elastic properties [Jiang *et al.* (2003)], in addition with the optical

characteristics, has turned out to be increasingly more useful and convenient [Pogue *et al.* (2006)].

2.4.4 Selection of tissue optical phantom constituents:

Important parameters in deciding an appropriate phantom material and design include wavelength range of spectrum as well as the geometrical design parameters such as heterogeneities, thickness, container and feasible machining features. Spectral sensitivity to molecular features of tissues being one of the important features of the optical and near-infrared NIR spectroscopy, poses an immediate need to develop reliable phantoms that precisely mimic the tissue chemistry. Hence, biologically compatible structures like collagen matrixes, agar or gelatin that permit the entry of cellular components (fluorescent molecules and fat or blood such as FAD, NADH, porphyrins) [Drezek *et al.* (2001); Mang *et al.* (1993)], as well as exogenous organic luminescent molecules [Troy *et al.* (2004); Bremer *et al.* (2003); Ebert *et al.* (2001)] are required to replace solid non-organic polymers and silicone phantoms.

2.4.5 Tissue optical phantom purposes and their assessment criteria:

The purposes of tissue optical phantoms are broadly classified as:

- Authenticating the simulation as well as validating the physical prototype.
- Performance evaluation and optimization of instrument.
- Calibrating the instrument to test its stability and reproducibility.
- Comparing and standardizing amongst laboratories.

The precise application of the ideal phantom depicts its characteristics. For example, precise characterization of validation phantoms is essential as compared to reproducibility and stability of phantoms during inter-laboratory comparisons [Pogue *et al.* (2006)]. The properties of ‘ideal’ phantom are listed below as:

- Different tissues have different absorption and scattering properties.
- Dependence of the wavelength characteristics is similar to the absorption and scattering properties of tissue.
- The properties of tissue as well as environmental conditions are must be stable such as humidity, photo bleaching and temperature.
- The refractive index of phantom and that of tissue are similar.
- Regions with different optical properties can be mimicked for example inclusions layers mimicking skin.

- Surface and mechanical properties of phantom and tissue are similar for example Young's modulus nearly 4 to 20 kPa [Krouskop *et al.* (1998)].
- Capability to incorporate Brownian motion and flow in the phantom.
- Capability to exhibit thermal characteristics identical to the tissue properties.
- Simplicity in manufacturing.
- Low or reasonable production cost.
- Ease of Transport

In practical applications, some of the above properties can be neglected.

2.4.6 Spectrum of the Intralipid™ scattering coefficients:

The enormous cited studies of Intralipid™ was done by [Van Staveren *et al.* (1991)]. For estimating scattering spectrum he used Mie theory calculations, observations from optical transmission and electron microscopy. They also coined a relation between wavelength and reduced scattering coefficient called simple power law. The standard stock solution of 10%, the formulas for anisotropy and scattering coefficients are as:

$$\mu_s(\lambda) = 16\lambda^{-2.4} \quad \text{Equation (2.6)}$$

the units of mm^{-1} , where λ is present in microns, and

$$g(\lambda) = 1.1 - 0.58\lambda, \quad \text{Equation (2.7)}$$

In the equation, the resultant of reduced scattering coefficient are described as:

$$\mu'_s(\lambda) = 9.3\lambda^{-1.4} - 1.6\lambda^{-2.4} \text{ units of } \text{mm}^{-1} \quad \text{Equation (2.8)}$$

Across the visible range of complete spectrum, insertion of Rayleigh scattering is probably required; also have needed a third term expression including the standard λ^{-4} for power function, excepting the requirement of fitting for the coefficient. Many investigations holds initial term of this latter formula, and fits $\mu'_s(\lambda)$ to the functional form described as:

$$\mu'_s(\lambda) = a\lambda^{-b}, \quad \text{Equation (2.9)}$$

where a and b as representing free parameters. Where fitting is restricted for the near infrared range, this may be logical assumption above the minimum wavelength range [Pogue *et al.* (2006)].

2.4.7 Lipid based scatterers assessable commercially:

Phantoms prepared from milk [Waterworth *et al.* (1995)] and emulsified oil suspension for the optical diagnostic technique as well as spectroscopy were replaced

by Intralipid™ (commercially prepared lipid suspension) [Pogue *et al.* (2006); Sukowski *et al.* (1996); Flock *et al.* (1992)]. The detail information are listed in the Table 2.1. The easy availability of the commercial calibrated lipid solutions because of their manufacturing for the intravenous feeding [Sayeed *et al.* (1986)]. Trade name of the product varies from manufacturer to manufacturer. The manufacturer's names are Erlangen Germany, Clayton, Pharmacia, Upjohn, Intralipid™ Kabi-Pharmacia, New Jersey, Stock-holm, Kabi-Vitrum Incorporated, Sweden is the most cited word, by the other versions it has called as Quebec, Nutralipid™ Pharmacia, Montreal, Canada and Liposyn II™ Abbott Labs Incorporated. The solution is easily accessible in all the hospital pharmacy with an appropriate uniformity between batches. Proper mixing of a suspension provides a homogeneity which stands for few hours.

Table 2.1: Outline of lipid suspension-based phantoms.

Scatterer Medium	Role	Limitations	Stability	References
Oil/fat/lipid	Used to custom-make scattering and lipid/water phantoms	Must be emulsified and blended reproducibly	Hours	Waterworth <i>et al.</i> (1995); Drexler <i>et al.</i> (1985)
Milk	Readily available	Not highly reproducible between samples	Hours	Merritt <i>et al.</i> (2003)
Intralipid/ Nutralipid	Reproducible source of lipid solution	Stability better than 10%	Days	Flock <i>et al.</i> (1992); Van Staveren <i>et al.</i> (1991)

2.4.8 Biomedical application Intralipid™ suspension:

Lipid suspension also termed as fat suspension endorsed in 1962, originated by Professor Arvid Wretling, Sweden (the initial trade name being as Intralipid™). It is a fat suspension utilized for the human purpose. It was further approved in United States in 1972. It is helpful for patients with nutritional deficiency. Main constituents of this suspension are soybean oil, egg phospholipids and glycerin. It is commercially available in market in 10%, 20% and 30% of concentrations, which is shown in figure 2.4 [www.fresenius-kabi.co.in (2016)].



Figure 2.4: Commercially available 10%, 20% and 30% Intralipid™ medium.

Apart from these constituents Intralipid™ also provide omega-3 fatty acid, an omega-6 fatty acid, linoleic acid (LA), alpha-linolenic acid (ALA).

2.4.9 Medical uses:

2.4.9.1 Antidote:

Lipid suspension can treat the overdose anesthetic drug (like bupivacaine), overdose of other fat-soluble medicines and have significant effect on people insensitive to the usual resuscitation methods. [Picard *et al.* (2006); Weinberg *et al.* (1998)]

2.4.9.2 Tissue optical phantom:

Intralipid™ is generally utilized as a part of optical trials to reproduce the scattering properties of organic tissues. Proper concentration of Intralipid™ solution may be arranged to firmly emulate the reaction of human or biological tissue. The light with wavelength range in red and infrared reaches a depth where tissue is extremely dispersing yet has a low absorption coefficient [Driver *et al.* (1989)].

2.4.9.3 Cardio protective agent:

Recent studies of Intralipid™ explores its role as a cardio protective agent and can also treat ischemic reperfusion injury [Li *et al.* (2012)].

2.5 Utilization of Intralipid™ based tissue phantom for developing various non-invasive optical techniques for glucose concentration monitoring:

2.5.1 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Near-infrared transmission and reflectance spectroscopy technique:

Jeon *et al.* (2004) presented noninvasive estimation of bio-fluid materials in human body based on the optical spectroscopy. They observed glucose specificities by examining the features in the partial least squares regression models for both instances of transmittance and reflectance estimations. For the sample, Glucose-Intralipid™ solutions were utilized with variable scatterer's concentrations. In this work, authors utilized Intralipid™ concentrations of 4%, 4.08% and 4.16% in the solution at temperature 30°C throughout whole experiment and found that measured values were similar to tissue scattering. Factors studied for reflectance information did not demonstrate glucose absorption factors and the characteristic were extremely noisy especially in the combination band. It is considered that light does not need enough data of glucose because the path length in reflectance is extremely short. Then again, the factors acquired from the Partial Least Square examination of transmittance revealed glucose signatures. Authors found that transmittance estimation is favored for in vivo glucose checking than reflectance estimation.

Jeon *et al.* (2006) presented the method of glucose estimation using Near-IR spectroscopy for the estimation of transmittance and reflectance. Range of the wavelength was the 1100 to 2500 nm that incorporated both the conjunction and overtone bands of glucose absorption. Intralipid™ solutions have been utilized as samples, in which the glucose levels shifted somewhere around 0 and 1000 mg/dl. Sample thickness for the reflectance purpose is 10 cm and 1-mm as well as 2-mm-thick samples have been utilized for transmission. Partial least-squares regression (PLSR) examinations were conducted to anticipate glucose concentrations. The standard errors of calibration in 2-mm-thick transmittance is found to be similar to that of reflectance. Loading vector investigation for reflectance does not demonstrated glucose assimilation characteristics. Reflected light might not need enough data of glucose because a significant part of identified light includes a short optical path length. Likewise, expectation turns out to be more reliant on medium scattering as opposed to glucose, weighed against transmission estimation. Loading vectors acquired from a PLSR transmittance examination have glucose assimilation profiles.

The 1-mm-thick specimens give preferable results over the 2-mm-thick specimens for equally prediction and calibration of models. The transmittance setup has been prescribed for noninvasive glucose measuring.

Liu *et al.* (2008) represented the non-invasive blood glucose concentration detected by the near-infrared spectroscopy, the signal to noise ratio value of the used framework was found to be extremely low. In this work, firstly the author introduced hypothesis expression method of traditional background correction. Further, to avoid the impact from the variance of optical features in the samples and the move in the optical framework, the proportional background correction techniques were planned. The *in-vitro* studies of real absorption medium and scattering medium have been performed to validate the outcomes. The outcomes demonstrated that, for the glucose in the blood plasma solution as well as Intralipid™ 2% solution, after the modification of the background that was having the comparable optical features with the samples, the prediction accuracy of multivariate model for the glucose concentration level have been enhanced by 25.9% and 40.1%, correspondingly.

Kakuta *et al.* (2008) reported the techniques that utilize near-infrared spectroscopy and multivariate investigation to estimate the temperature of turbid watery solutions. The estimation guideline used the fact that the peak wavelength of the water assimilation band, having its peak at almost 1440 nm, alters with temperature. This concept was utilized to estimate the temperatures for 1 mm thick specimens of watery solution including Intralipid™ (2%) that are regularly utilized as optical phantom for natural tissues because of comparable scattering features. Temperatures of real water and watery solution including glucose (100 mg/ml and 200 mg/ml) were likewise estimated for comparison. In support of the turbid Intralipid™ solution, the process of absorbance spectrum shifted sporadically with time because of the alteration in scattering features. Then again, by utilization of the variation amid the absorbance at 1412 nm along with the temperature free absorbance at 1440 nm, they acquired standard error of prediction of 0.3°C and 0.2°C through univariate linear regression and the partial least squares regression, correspondingly.

Yang *et al.* (2010) presented an issue that considered about the accuracy of non-invasive blood glucose estimation using NIR Spectroscopy due to weak signals of glucose and variations in background. Authors reported the presence of the radial reference position in a floating-reference technique, to address this issue. They

deduced local state of the reference point theoretically using infinite diffusion theory. For the experiment, Intralipid™ solutions were constructed to testify the existence of the reference point. For the validation, authors used Monte Carlo simulations to determine the diffused light distribution. All the reference points presented in three-layer skin model recorded at the wavelength range of 1200 – 1700 nm. For validating the investigation, the presence of reference point and the allocation of diffuse reflected light have been distinguished in the 10% Intralipid™ solutions with various glucose concentrations.

Zhang *et al.* (2012), suggested a method for extracting the net analyte signal (NSA) based on the floating reference hypothesis. In this method, the noise background subspace has been diffused by spectrum at the position of floating radial reference point, and after that, the spectra at the estimation position are anticipated on the subspace. Furthermore, the glucose levels in Intralipid™ solutions were examined through Monte Carlo simulation and experimentation. The partial least squares (PLS) models with and absence of NAS investigation has been assembled. The root mean square lapses of prediction and calibration reach to 28.87% and 27.33%, correspondingly. The outcomes affirm the presence of data incited by glucose concentration variances and the authenticity of the floating reference hypothesis. The 10% Intralipid™ solution with various glucose concentration level was utilized to simulate the individual tissue, with various glucose level from 100 to 2000 mg/dL at 100 mg/dL interims.

2.5.2 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Occlusion spectroscopy technique:

Amir *et al.* (2007) presented that a noninvasive glucose-detecting device could revolutionize diabetes therapy by prominent enhanced consistence with suggested glucose levels, accordingly lowering the long term complications and expense of diabetes. Thus, author exhibit the technology and assess the effectiveness of a genuine noninvasive device for the continuous blood glucose monitoring, the NBM (Or Sense Ltd.). Intralipid™ phantom medium was used for *in-vitro* examination to validate the technology and algorithms purpose. *In-vitro* investigation illustrated a strong correlation between determined and actual glucose concentrations. This study shows the potential utilization of Or Sense's NBM device as a noninvasive sensor for constant blood glucose assessment. The device was safe and all around endured. The

finger phantom is intended to imitate the absorption and scattering properties of the finger in the red and near infrared spectral band. The figure 2.5 showing a finger phantom and potential photon path [Amir *et al.* (2007)].

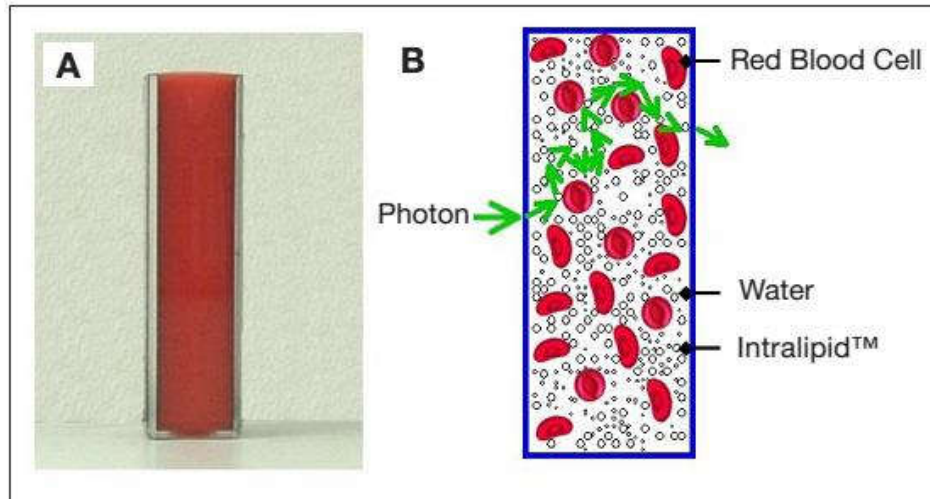


Figure 2.5: (A). Finger phantom and (B). Potential photon path in phantom [Amir *et al.* (2007)].

Tissue scattering is modeled by usage of an watery suspension of lipid droplets (Intralipid™), while absorption is represented by direct utilization of red blood cells (RBC). Both parts are mixed to imitate the blood–tissue substance and are embedded into a rectangular cuvette. During the investigations, three physiological parameters were investigated. (i) The concentration of glucose is altered and controlled by directly adding of glucose in the range of 0 to 1000 mg/dl. (ii) The hematocrit level is estimated by the adding of different RBC concentrations, having the hematocrit levels from 30 to 50%. (iii) Nitrogen bubbling stimulated de-oxygenation in RBC suspension.

The finger phantom is illuminated with white light (Spectra Physics), and the transmitted light is assessed utilizing a spectrometer (Princeton Instruments). Estimations are performed for various concentrations of glucose with concurring varieties in the hematocrit and oxygenation.

2.5.3 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Spatially resolved diffuse reflectance measurement technique:

Chen *et al.* (2007), authors proposed a novel technique, based on floating-reference and using the fact that decrement in the impact of the physiological

background alteration on the blood glucose estimation by differentially preparing two signals from reference point and estimating point. In this work, authors used the reference point, where in practical the diffuse reflected light was not sensible to the variance of glucose level, was hypothetically demonstrated. Further, Monte Carlo simulations have been implemented to examine the radial allocation of the diffuse reflectance at various glucose concentrations for the skin. In addition, the analyses was built to estimate the radial allocation of the diffuse reflectance through the IntralipidTM solutions with various glucose levels. Both the outcomes from simulation and examination validated the presence of floating-reference point. By hypothetically examining the background noises and their troubling mode on the blood glucose identification, an original information preparing strategy taking into account the reference point was proposed to adequately extract the blood glucose data. In addition, it is available from their preparatory examination with IntralipidTM samples that, this information preparing technique can decrease the impact of background variance on the extraction of actual glucose signal and subsequently upgrade the determining ability on glucose concentration.

The significant engineering of floating-reference system is to find the reference point and get suitable strategy to deduct the physiological noise in light of the inner reference point. In this report, the present state of the reference point was concluded. At that point, scientist conducted Monte Carlo simulation on the human tri-layer skin model and built the studies with 10% IntralipidTM solution to confirm the area of reference point. Moreover, they hypothetically examined the data processing technique in view of the reference point for extraction of glucose data. And further appropriate investigations were performed to validate the impact of this system to increase precision in glucose estimation.

Liu *et al.* (2009) represented a highly efficient noninvasive technique for glucose measurement. In this work, the data processing technique have been taken into account for the data of reference point to improve the accuracy of glucose detection. The diffuse reflectance of the IntralipidTM solution with various glucose concentrations in numerous source-detector ranges were acquired using Monte-Carlo simulation. For the simulation of actual measurement condition, the linear drift and random noise were included on the simulated spectra. Further, the similar optical probe was developed by dissemination of light strength in the radial distance and an

in-vitro experimentation about Intralipid™ solution with various glucose levels was performed to validate the impact of the information correction basis on the data from the reference point. Results demonstrated that, three various estimating regions must be identified in the wavelength range of 1100 nm - 1700 nm as indicated by the wavelength feature for reference point. In addition estimating region ought to be around 0.2 - 0.3 mm far from the reference region. For the simulation along with *in-vitro* investigation, after the improvement by the data from the reference point, the forecast error for glucose was decreased to 46.2% and 23.2%, correspondingly.

2.5.4 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Frequency-domain reflectance technique:

Cletus (2010) presented a wavelength process as tunable frequency domain spectrometer which was developed by modulating the yield strength of a titanium: sapphire laser utilizing an acousto-optic modulator. A small noise avalanche photodiode module along with a lock-in enhancer was utilized to estimate the amplitude attenuation along with phase lag within a turbid specimen. The spectrometer was used for assessing the precise and accurate optical characteristics of a significant tissue simulation phantom, Intralipid®, at a consultant wavelength 790 nm. The outcomes demonstrated that the spectrometer examine the assimilation with a precision of 10%. The device investigated the reduced scattering coefficients and absorption with an accuracy of 3% and 6%, correspondingly.

The spectrometer has been used to estimate the optical temperature coefficient of the Intralipid®, investigating its potential as a noninvasive temperature checking device. The estimated little alteration in the absorption coefficient proposed a least recognizable temperature change of $\pm 4^{\circ}\text{C}$, which further require advancement for high sensible applications. The impact of glucose on the optical characteristics of Intralipid® demonstrated that the absorption coefficient diminishes consistently at 730 nm up to 1000 mg/dL. The reduced scattering coefficient also reduces with enhancing glucose level at the majority of the wavelengths.

Amplitude and phase estimations were acquired in a 1% Intralipid® solution (w/w), in the wavelength range between 710 to 850 nm. The main arrangement of estimations was done at low glucose concentration level in Intralipid® : 200, 400 and 600 mg/dL. Decreased scattering coefficients and absorption were evaluated from the

amplitude and phase estimations at every glucose concentration. A new Intralipid® solution (1%) was arranged and the estimations were rehashed at three glucose concentrations of 1000 mg/dL, 2000 mg/dL and 4000 mg/dL.

2.5.5 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Polarimetry technique:

Mehrubeoglu *et al.* (1998) presented the possibility of utilizing diffuse reflectance polarimetry to recognize alterations generated by various concentrations of molecular compounds in tissue-simulating phantoms. The impacts of glucose, β -alanine and l-lysine at various concentrations in turbid media had been explored independently. This methodology was used for taking into account the impact of optical characteristics on the polarization condition of light. Further, the outcomes demonstrated that this procedure is having potential for estimating alterations in molecular concentrations in extremely scattering biological medium for polarized images.

Researchers arranged a liquid phantom with identified optical properties (absorption coefficient, μ_a and decreased scattering coefficient, μ_{0s}) much like those of natural tissue. The phantom contained Intralipid™ suspension as well as trypan blue dye dilute in the distilled water, where in fact the Intralipid™ was utilized as scatterers as well as trypan blue dye as absorbers. The optical characteristics of the phantom were define as $\mu_{0s} = 10 \text{ cm}^{-1}$ and $\mu_a = 0.1 \text{ cm}^{-1}$ acquired from a combination of 500 ml of the distilled water, 165 μl of the trypan blue dye, and 14.3 ml of 20% Intralipid™ suspension.

Cameron *et al.* (2007) authors presented the capability to estimate glucose concentration by noninvasive methodologies. In that work, Polarization-based optical methodologies have obtained significant interest due to their potential therapeutic applications. They showed the glucose, as a chiral molecule, having the capability to turn around the plane of the linearly polarized light, regularly known as optical action, and also altering the refractive index of the medium, which subsequently influences the general scattering coefficient in a specified media. Observations examined that how each one of these impacts is associated to glucose concentration in a physiological range for extremely scattering natural media.

2.5.5.1 Techniques:

An indigenously imaging polarimeter was utilized to image extremely scattering Intralipid™ based medium including various concentrations of glucose. Model development and glucose prediction were conducted through the utilization of partial least squares (PLS) regression. Further perception in to the variations among polarization-based image estimations and encoding of glucose data was made available through the utilization of principal component analysis (PCA).

2.5.5.2 Result:

When combined with PLS regression, the *in-vitro* polarization estimations yielded extremely associated glucose predictions in equally validation and calibration, 0.999 and 0.998, correspondingly. Through the utilization of PCA, it emerges that the most of the image-based signal producing the absolute significant glucose data is due to changes in the general scattering coefficient because of glucose concentration and to a smaller degree, impacts of optical action.

2.5.5.3 Conclusion:

This observation demonstrated how polarimetric-based imaging combined with PLS regression can be utilized to measure glucose level in extremely scattering medium. Such techniques might feasibly have the ability to increase the utilization of noninvasive *in vivo* polarimetric estimations, typically obtained in the anterior chamber of a person's eye, to different ideal detecting sites for example the skin.

Intralipid™ suspensions (Kabi Clayton, NC) with identified optical characteristics and scattering point of a referred size dispersion were utilized as a tissue phantom. The scattering coefficient of the phantom is $\mu_s = 10 \text{ cm}^{-1}$, that has been acquired by accordingly diluting the 20% Intralipid™ suspension. The factor of anisotropy (g) of the specimens is around 0.8. Out of this base collection of Intralipid™ solution, an aggregate of 16 different 100-ml glucose tests were arranged. These samples were made by doping every sample with a suitable measure of glucose from a concentrated store solution. All the 16 specimens included glucose concentration levels which range between 50 to 4000 mg/dl with 11 specimen having glucose concentrations less than 1000 mg/dl. Concentrations over the usual physiological range were selected with keeping in mind a goal to completely examine and show the optical action and scattering impacts of glucose.

2.5.6 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Raman spectroscopy technique:

Barman *et al.* (2009) investigated significant problem of measureable biological Raman spectroscopy, especially as related to transcutaneous Raman spectroscopy estimations. In this work the Raman spectral data has been twisted by different absorption and scattering phenomenon in the encompassing medium, resulting in decreasing the expectation capacity of the calibration model. To record for these distortions, authors provided a novel analytical technique, which is called as turbidity-redressed Raman spectroscopy (TCRS), based on the photon migration method and utilizing diffuse reflectance and Raman spectra accessed alternatively. Authors also presented that, by using TCRS, the generally differing Raman spectra seen from a set of tissue phantoms having exactly the same concentration of Raman scatterers however with different turbidities tends to collapse onto an individual spectral profile. Besides, in a forthcoming study that utilizes physical tissue models with changing turbidities and randomized concentration levels of Raman scatterers and meddling agents, a 20% diminishment in forecast error is acquired through the use of the turbidity correction strategy to the observed Raman spectra. The diffuse reflectance spectra were aligned utilizing a reference standard of 20% Intralipid™ solution. Tissue phantom reports were conducted for (1) calibration of the instrument, (2) authenticate the TCRS formalism, and (3) analyze its execution in forthcoming expect.

The tissue phantom were arranged by pipetting glucose (Sigma Aldrich), Intralipid™ (Baxter Healthcare), creatinine (Sigma Aldrich), Speedball Art Products Co.) , India ink (Super Black India Ink), and distilled water into clean glass vials. Aliquots of those tissue models were occupied in a fused silica cuvette for spectroscopic estimations that have been executed at room temperature. Among every estimation, the cuvette was rinsed three times with distilled water and air dried. The phantom were put away in the fridge at 4°C to maintain a strategic distance from defilement. Glucose and creatinine, both of which may have specific Raman spectral profiles, worked as the analyte of fascination and spectral meddling agents, respectively. Each of the three-tissue phantom were studied for (1) calibration, (2) acceptance, and (3) potential predictions were executed various times on diverse days to analyze the reproducibility of the TCRS approach.

In this work calibration and acceptance reports, 20 and 36 tissue phantom were developed, separately. The Raman scatterer (glucose) concentration was kept steady at 500 mM. The flexible absorption and scattering coefficients were changed arbitrarily in the ranges formerly mentioned. In both calibration and acceptance reports, creatinine was not utilized, in light of the fact that the goal was to characterize the viability of TCRS in removing the natural line shape and strength information of glucose Raman spectrum range gained from the turbid medium.

Dingari *et al.* (2011) presented an informative study, chance correlations in Raman spectroscopy centered calibration model for glucose estimations. That are examined for both *in-vitro* (physical tissue models) and *in vivo* (human subject and animal model) cases. Numerous false glucose concentration profiles are assigned to the Raman spectra obtained from physical tissue models, where in fact the glucose concentration is deliberately held steady.

In their work, concisely, 36 tissue phantoms were arranged in distilled water, where in fact the glucose concentration was kept consistent (500 mM) while fluctuating alternate analytes (like IntralipidTM and India ink). Specifically, the IntralipidTM and India ink concentrations were fluctuated to reproduce the normal absorption (0.08 to 1.3 cm⁻¹) and scattering coefficients (24 to 130 cm⁻¹) of natural tissue in the tissue phantoms. A diode laser (830 nm) was utilized as the excitation supply and an f/1.4 spectrograph along with a liquid nitrogen cooled charge coupled device (CCD) was utilized to gather Raman spectra from these tissue phantoms. It ought to be noticed that the spectra were obtained randomly concerning the concentration of tissue phantom constituents. Surely, the connections between the concentrations of any two tissue phantom in gradients or between an ingredient concentration and the specimen run request are seen to be under 0.1. The gained spectra has been subjected to curvature modification, vertical binning and cosmic beam evacuation preceding calibration model. Spectra from 360 - 1600 cm⁻¹ are utilized for all our information examination (lower wave number spectral regions are not regarded due to the overlap with the notch filter effect and higher wave number spectral regions have problems like disintegration of CCD quantum effectiveness).

2.5.7 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Optical coherence tomography technique:

Kirillin *et al.* (2004), authors presented the peculiarities of light transportation in Intralipid™ solutions and the impact of glucose on light scattering characteristics of the solution at two distinctive Intralipid™ concentrations levels. That were examined with optical coherence tomography (OCT) method *in-vitro*. An outside OCT frame work with a super luminescent light source having focus wavelength = 830 nm was utilized. 5% Intralipid™ solutions were utilized to imitate a natural tissue (skin) in their investigation. Glucose concentration levels at the physiologically important level were included with Intralipid™ solutions. Raising Intralipid™ concentration raising the scattering coefficient of the media then raising glucose concentration raising the refractive index of the media and decreases the scattering coefficient of the media. The test information were compared to Monte Carlo simulations. And similarly they made simulations for 2% Intralipid™ solution. The outcomes demonstrated that glucose present in 2% and 5% Intralipid™ solutions altered their scattering characteristics, which was demonstrated by a reduction in the slope of the OCT signal.

Kinnunen *et al.* (2006), authors utilized the optical coherence tomography (OCT) to estimate glucose-induced alterations in Intralipid™ and in mouse skin specimen's *in-vitro*. In their work Mouse skin specimens cultured in a CO₂ incubator before estimations are made with diverse amounts of included glucose concentrations. The outcomes demonstrated that the glucose-induced alterations in the OCT slope value change somewhere around 20% and 52%/30 mM glucose in diverse mouse skin specimens. This alteration was found to be much bigger than the alteration in 2% Intralipid™ (2.1%/30 mM) and in 5% Intralipid™ (0.86%/30 mM). Henceforth the outcomes demonstrated that OCT have ability to monitor glucose-induced alterations in tissues *in-vitro*.

Kinnunen *et al.* (2007) reported the optical coherence tomography (OCT) as an effective device for imaging tissue structure. By averaging depth level scans, a strength profile can be framed as a function of depth. The slope of a straight line close-fitting to the OCT signal depth profile included informative data on light attenuation in the specimen at different depths. This slope can be utilized to detect alterations in the scattering characteristics of the specimen, particularly in one single

scattering region. In this informative article, the impact of fitting the line at different depths on recognition sensitivity was examined in Intralipid™ phantoms with various concentrations. Various glucose concentration levels were additionally utilized with 5% Intralipid™ samples. Various depths were examined as the depth of the dermis and the widths of skin layers in human skin change in various body regions. The outcomes demonstrated that the tenderness of identifying variations in the scattering characteristics of Intralipid™ is much better at a depth relating to that particular of the dermis in the human arm (0.166 - 0.276 mm) than at a depth paralleling the dermis in the fore finger (0.441 - 0.579 mm).

Li *et al.* (2007) have designed a noninvasive technique to check the glucose concentration in tissue phantom utilized the OCT model which was on the basis of the Extended Huygens-Fresnel (EHF) principle. The variations of the optical characteristics, such as, the scattering coefficient μ_s , the anisotropy factor g and the efficient scattering coefficient μ_s with the raise in concentration of glucose in 10% Intralipid™ solution were acquired. The outcomes demonstrated that the scattering coefficient μ_s was reduced with increase in glucose concentration in the solution, which is accorded with the conclusions by others. On the other hand, factor g raised when the glucose concentration was high. Therefore, the coefficient μ_s is fall with the expansion of the glucose concentration. Comparing with the acquired relative values of scattering coefficient μ_s , the most essential point of interest of this model is that they acquired the practical value of μ_s , g and μ_s . These demonstrated that the OCT EHF model as a promising approach that would be used to identify the glucose concentration in solution.

Feng *et al.* (2008) represented the capability of optical coherence tomography (OCT) for noninvasive glucose estimation in tissue replicating phantoms and natural tissues. Speckle noise considerably decreases the precision of the measurements with this method. The speckle noise can be suppressed by averaging of thoroughly scans. They examined the suppression of speckle noise for precise estimation of back scattering signal and the precision of glucose estimation in Intralipid™ suspensions with the OCT procedure. The outcomes demonstrated that the accuracy of glucose estimation can accomplish ± 4.4 mM for 10% Intralipid™ and ± 2.2 mM for 3% Intralipid™ following the averaging of 50,000 scans.

The accuracy of glucose estimation with OCT has been determined not only by alteration in OCT signal slope but also by precision of OCT signal slope. The OCT procedure was having high signal-to-noise ratio (SNR). Nevertheless, in scattering media the coherent character of radiation and inter ferometric estimating of the scattered light result in creation of speckles. The speckles pose a remarkable obstruction to estimation of OCT sign slope with the OCT system. Without spot averaging, it is very difficult to quantify the OCT signal slope in tissue precisely.

All investigations were conducted with concentrations of 3% and 10%. Samples were arranged by addition of distilled water to 20% Intralipid™ suspension. The ingredients of Intralipid™ 20%, as per the manufacturer, are: soybean oil 20%, glycerin 2.2%, lecithin 1.2% and water 76.6%. The glucose concentrations (500 mg/dl, 1000 mg/dl, 1500 mg/dl, 2000 mg/dl, 2500 mg/dl, 3000 mg/dl, 3500 mg/dl, 4000 mg/dl, 4500 mg/dl and 5000 mg/dl) were utilized to examine the alteration of OCT signal slope.

Solanki *et al.* (2012) proposed the application of optical coherence tomography signal strength for estimation of glucose concentration which has been investigated in tissue phantom and blood specimens from human subjects. The diffusion mathematical statement based calculations and in-vivo OCT signal estimations confirms the cyclic association of signal strength with glucose concentration and scatterer size. Their essential objective was to observe the association between the amplitude of OCT signal and concentration of glucose. An efficient estimations were made with the following specimens; (i) tissue phantom based as Intralipid™ and (ii) blood tests from deliberate human subjects. Author utilized Intralipid™ as a tissue phantom that gives the backscattered constituent. Normal measurement of scatterers in Intralipid™ measured utilizing confocal microscope is found to be 3.5 μm . Following a standard methodology, they made glucose concentrations of 0 – 200 mg/dl in 1ml sample tissue phantom for examination perceptions. This range cover up hypoglycemic (< 80 mg/dl), normal (80 - 120 mg/dl) and in addition hyperglycemic (>120 mg/dl) conditions. A completion time of 2 minutes was given after adding up of glucose to the Intralipid™ solution so that the included glucose could change the scattering properties of the medium. All the while, an alteration in refractive index of the solution is likewise observed utilizing Abbe's refractometer.

Estimation was done by using a fixed volume 1ml of tissue phantom diluted to 0.01% in the distilled water (100 ml) and concentrated glucose solution were prepared by dissolving 200 mg glucose in 100 ml distilled water. The concentration level of glucose in Intralipid™ is raised in steps of 10 mg/dl utilizing U-TEK Chromatography syringe with least count of 5 µl. To ensure the alterations in the optical properties of the solution with raising glucose concentration in Intralipid™, Abbe refractometer was utilized to get the refractive index of tissue phantom and obtained to be 1.407. As examined before, adding of 50 µl glucose to Intralipid™ solution of volume 1ml raised the glucose concentration by 10mg/dl. In the meantime, it decreased the index matching within the scatterer and the medium. The estimations of refractive index and scattering coefficients was made with the help of Abbe refractometer and OCT, at the same time to prevent the temporal fluctuations in values.

Solanki *et al.* (2012)^a proposed the application of optical coherence tomography signal amplitude for the estimation of concentrations that has been examined in tissue phantom and in blood samples from human subjects. The diffusion mathematical statement based measurements and additionally in-vivo OCT signal estimations affirm a cyclic association of signal strength with glucose concentration and scatterer size. Estimation was done by utilizing exact volume (1 ml) of tissue phantom and dilute to 0.01% in distilled water (100 ml) and concentrated glucose solution arranged by dissolving 200 mg glucose in 100 ml distilled water. The investigational perceptions with tissue phantom likewise demonstrated a cyclic relationship between glucose concentration and OCT signal amplitude and an alteration in the peak amplitude in hypothetical and investigational results could be seen. Both the curves show related character and affirms the utility of the present hypothetical measurements in relating them with blood glucose levels in human subjects. The optical coherence tomography signal amplitude of tissue host and also blood samples have now been measured and found that cyclic variety in OCT signal happens with raising glucose concentrations.

Zhang (2013) measured the glucose concentration of the turbid media based on optical coherence tomography (OCT). The impacts of scattering coefficient as well as anisotropic factor on heterodyne effectiveness factor were investigated by the Monte Carlo simulation and analog computation. Another system was produced to

acquire the optical properties utilizing Extended Huygens-Fresnel principle for the instance of the specular reflection. The absorption and scattering properties were acquired by measuring the Intralipid™ solution of the various concentration. Furthermore, the association of scattering coefficient with the concentration of glucose in Intralipid™ were examined.

2.5.8 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Fluorescence technique:

Gryczynski *et al.* (2003) presented the novel technique to deal with sensor design, on the basis of the utilization of an interior standard with suitable spectral properties, gave new conceivable outcomes to design easy devices for fluorescence detecting. Detection of consolidated emission from the reference and an analyte sensitive fluorophore has been accomplished in various estimations in cuvettes and in high throughput formats. These strategies have been utilized with a long-lifetime reference to assess glucose, O₂, pH, pCO₂ and calcium by means of modulating sensing technique and by the utilization of oriented films as the reference for polarization detecting of glucose, oxygen, pH and lactate. Polarization detecting has additionally been created with visual identification to quantify the concentration of rhodamine B and pH. Modulation and polarization was found to be successful in exceedingly scattering media, for example, Intralipid™ or tissue.

2.5.9 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Photoacoustic technique:

Kinnunen *et al.* (2005) presented the utilization of the pulsed photoacoustic (PA) system to examine glucose-incited alterations in pig whole blood and 1% Intralipid™ utilizing a Nd:YAG laser with wavelengths of 1064 and 532 nm as the optical vitality source. Scattering properties of the sample significantly influence the laser-induced pressure waves. Glucose was found to influence both the absorption and scattering properties of the samples. The outcomes demonstrated a rise of 11.4% /500 mgdl⁻¹ included glucose in the peak to peak value of the PA motion in blood at 1064 nm, while the relating increment was just 1.35%/500 mgdl⁻¹ in 1% Intralipid™. At a wavelength of 532 nm, the glucose raise the peak to peak of the PA signal by 6.0% /500 mgdl⁻¹ included glucose in blood. In general, the pulsed PA procedure proved an efficient tool for the study of glucose-induced alterations in blood and tissue phantoms *in-vitro*.

Kinnunen *et al.* (2006)^a presented the photoacoustic and optical estimation methods, which were utilized as a part of this report to examine glucose-induced alterations in Intralipid™ suspensions. Optical vitality was given by a Nd:YAG laser at 1064 as well as 532 nm and a picosecond laser module at 906 nm. As detectors, the setup utilized a photoacoustic detector, PIN diode, and streak camera. Raising the glucose content of the suspensions also raised the refractive indices of the studied media. This served to reduce the refractive index incompatible between the background and scattering particles and led consequently to reduced scattering, which then influenced both the peak to peak value of the photoacoustic signals also the transmitted optical pulses. At 1064 nm, glucose altered the peak to peak value of the photoacoustic signal more in the 1% than in 2% Intralipid™. Furthermore, the glucose-induced alteration in the transmitted pulse amplitude was less discernible at 532 than at 1064 nm in 1% Intralipid™.

Zhao *et al.* (2006) investigated the utilization of the scattering photoacoustic (SPA) system for feebly absorbing substances in suspensions. This method permitted the scattering and photoacoustic (PA) properties of feebly absorbing materials to be analyzed at the same time. Authors investigated decreased scattering coefficients of milk, polystyrene and Intralipid™ suspensions with and without glucose. It is proved that in milk the SPA technique is having higher recognition affectability for fat rather than for glucose. However, glucose was observed to have a larger impact on scattering in light milk and Intralipid™ 1% bases than in without fat milk and polystyrene-0.5% bases, in spite of the fact that the scattering impact contributed small to the PA signal produced in the suspensions.

Kinnunen *et al.* (2007)^a examined the frequency spectrum of photoacoustic signals produced in Intralipid™ and water. Intralipid™, which is a surely understood tissue mimicking phantom, was utilized with concentrations of 1% and 2%. A portion of the studies included the adding of glucose (5000 mg/dl). Estimation results exhibited that, at 1064 nm, the intensity of the acquired spectra is higher in water than in Intralipid™. Likewise the states of the acoustic spectra assessed for water and Intralipid™ were disparate. The relative portion of low frequency components was found to be bigger in Intralipid™ than in water. Then again, a definite intensity optimum was found in the frequency range of water, while in Intralipid™, intensity reduced towards higher frequencies from a greatest of under 0.5 MHz. In addition,

water had a more extensive and smoother photoacoustic signal spectrum. 1% and 2% Intralipid™ concentrations varied mostly as far as acoustic wave amplitude, while the glucose had an irrelevant impact on the acoustic spectra of these solutions. The determined spectral instances, in particular, difference of the power spectrum, kurtosis and skewness, were no preferred indicators for glucose over the peak to peak value.

2.5.10 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Time-of-flight technique:

Kinnunen *et al.* (2004) presented the study of the pertinence of Time-Of-Flight (TOF) estimation procedure for glucose recognition in 2% Intralipid™ solutions *in-vitro*. Glucose samples having concentrations of 100 mg/dl, 200 mg/dl, 300 mg/dl, 500 mg/dl, 1000 mg/dl, 2000 mg/dl, 4000 mg/dl and 8000 mg/dl are examined. Laser pulses with $\lambda = 906$ nm and FWHM = 30 ps was utilized as a part of the analysis to examine scattering properties of Intralipid™. 1-5% Intralipid™ suspensions were utilized to imitate scattering properties of various skin layers in the NIR spectrum region. Estimations were executed with a slab cuvette, with 300- μ m step index form fibers, and with 100- μ m gradient index form fibers. Light transmission in the aqueous solutions is likewise examined by the Monte Carlo simulation. The simulations and the estimation results appeared to correlate and connect very well for Intralipid™ suspensions. Definite relationships of pulse parameters as a function of Intralipid™ concentrations were obtained. Gradient index form fibers were observed to be better selection for detecting glucose over the step index form fibers and slight variations of time delays of pulse maxima as well as the pulses broadening as a function of the glucose concentration were revealed.

Scattering of light was examined utilizing Intralipid™ solutions with the concentrations of 1%, 2%, 3%, 4%, and 5%. Samples were prepared with the addition of distilled water to 10%-Intralipid™ (Fresenius Kabi AB, Uppsala, Sweden) solution. The ingredients of Intralipid™ 10% as indicated by manufacturer were: soybean oil 10%, glycerin 2.2%, lecithin 1.2% and water 86.6% by mass. The impact of various glucose concentrations (100 mg/dl, 200 mg/dl, 300 mg/dl, 500 mg/dl, 1000 mg/dl, 2000 mg/dl, 4000 mg/dl and 8000 mg/dl or 0.1%, 0.2%, 0.3%, 0.5%, 1%, 2%, 4% and 8% by mass, respectively) on the scattering properties of 2% Intralipid™ was also examined. Glucose produced by Merck Euro lab was utilized. 2% Intralipid™

suspension was utilized in light of the fact that the scattering properties of such solution are much like epidermis of human skin in NIR spectral region.

Kinnunen *et al.* (2006) presented a review on non-invasive blood glucose monitoring technique. Three methods, pulsed photoacoustic system, the optical coherence tomography and the time-of-flight method were investigated in more detail. IntralipidTM was utilized as a tissue-mimicking phantom to compare these procedures. Furthermore, glucose-induced alterations in the optical properties of IntralipidTM were assessed with these procedures. The glucose-induced alterations in the signals assessed from IntralipidTM are dependent on the estimation setup and the method. The impact of 1,000 mg/dl glucose on 2% IntralipidTM was recorded as including a 3.9% variation in the OCT signal slope value, a 4.7% variation in the PA signal peak to peak value and a 5.8% variation in the TOF pulse amplitude.

Popov *et al.* (2005) presented the significance of glucose molecule as of various individuals suffering from diabetes. In this work, laser pulses propagation in a sample of watery IntralipidTM solution with glucose, is simulated by Monte Carlo technique. Impact of glucose, based on refractive index has been taken into account. The temporal profiles of femtosecond pulses (906 nm) diffusely scattered inside of a 2-mm thick plain glass cuvette through a skin phantom are enlisted in reverse direction by fiber-optics detectors 0.30 mm in measurement with numerical apertures of 0.19, 0.29, and 0.39. It was revealed that glucose content inside of the physiological range from (100-500 mg/dl) can be identified in view of the impact of glucose on the peak pulse strength and on the range under the pulse temporal profile (vitality of the enlisted pulse).

The researcher examined a probability of glucose detection within an IntralipidTM 2% skin phantom with femtosecond pulsed laser radiation (acceptable estimate for delta function shaped pulses) at a NIR wavelength of 906 nm. Identifying the back scattered radiation with an optical fiber, variations in the glucose level inside of the physiological range was recognized.

Popov *et al.* (2012) examined the sensitivities of the two optical modalities, optical coherence tomography (OCT) and laser pulse time-of-flight (TOF) for detecting glucose inside the range of 0-1000 mg/dL in flowing blood and tissue-imitating fluid (IntralipidTM). They demonstrated that TOF method was more responsive than OCT, for blood as well as for IntralipidTM measurements.

2% Intralipid™ water suspension was utilized for estimations as a part of this study. 20 ml of 20% Intralipid™ (Fresenius Kabi AB, Uppsala, Sweden) was mixed with 155 ml of distilled water. The dilution was then separated into five flasks each including 35 ml of the suspension. After this, proper levels of D-glucose were diluted with 5 mL of distilled water into five dosage. A load of glucose were 0 g, 0.1 g, 0.2 g, 0.3 g, and 0.4 g. Dosage of glucose with distilled water were then mixed with each 35-ml flask of 20% Intralipid™ and distilled water. In this manner, five samples of 40 ml of 2% Intralipid™ with glucose concentrations of 0 mg/dl, 250 mg/dl, 500 mg/dl, 750 mg/dl, and 1000 mg/dl were prepared.

Before starting the estimations, the tubes and the cell were rinsed with water and Phosphate-buffered saline (PBS) for blood and with distilled water for Intralipid™. At that point the first test sample containing 0 mg/dl of glucose was pump into the system and the estimation started. For estimating TOF, thirty images each containing of 100 exposures gradually in the range of 500 ps were recorded. Averaging of OCT images (more than 60 images) was selected so that durations of estimations by both systems remain same (around 6min). It guaranteed suitable picture quality also. Figure size of 600×500 pixels (1.650×1.610 mm) was utilized for OCT. Every concentration was assessed five times to determine the estimation error. The pumping pace was 10% of the maximum. Pumping was ceased just for the time of changing the specimen. The tubes and the cell were rinsed between every sample with water (three times) and Phosphate-buffered saline (PBS) or distilled water (onetime), contingent upon the specimen (rinsed RBCs). The temperature of the specimen in the collecting bottle was checked within the estimations with a thermocouple (Lutron TM-903, Lutron, Taiwan), further the room temperature and the relative moisture were observed with an inherent arrangement of the room. Each sample was shaken periodically before estimations to keep the glucose mixed and to avoid sedimentation of the blood samples.

2.5.11 *In-vitro* Intralipid™ phantom based study for glucose monitoring by Ultrasound-modulated optical technique:

Zhu *et al.* (2013) presented a technique for the noninvasive estimation of blood glucose levels, which is identified by the ultrasound modulated optical technique. The technique is on the basis of the optical scattering coefficient. An affectability investigation of the ultrasound modulated light signals in a scattering

medium is performed. Glucose concentrations in Intralipid™ and the hemoglobin solutions are measured utilizing the modulation depth of ultrasound modulated scattered light. The impacts of occurrence light strength and sample temperature on the ultrasound modulated signals are likewise evaluated. Preparatory investigational results recommended that the proposed technique is a promising procedure for noninvasive blood glucose estimation.

In this investigation, researchers added various amounts of 20% Intralipid™ suspension in 350 ml distilled water for the alteration of the scattering coefficient of the sample. The normal level of human fast and prandial blood glucose is around 3.9 - 6.1 mmol/l. Researchers analyzed diabetes by utilizing the following criteria: fast and prandial glucose level >7 mmol/l and post-prandial 2-h glucose level >11.1 mmol/l. The ultrasound modulated optical method is utilized to noninvasively assess glucose-induced alterations in turbid medium. The outcome demonstrates that modulation depth linearly raises with rising glucose concentrations in Intralipid™ solution, with high estimation affectability. Considerable glucose-induced alterations in optical properties are found under high hemoglobin concentrations.

2.5.12 Conclusion:

Significant research carried out by various researchers which we have discussed in our review for *in-vitro* Intralipid™ phantom based study for glucose monitoring. The work discussed on *in-vitro* Intralipid™ phantom based study in our review are: near infrared transmission and reflectance spectroscopy, occlusion spectroscopy, spatially resolved diffuse reflectance measurements, frequency domain reflectance technique, polarimetry, Raman spectroscopy, optical coherence tomography, fluorescence technique, photoacoustic technique, time of flight technique and ultrasound modulated optical technique.

These techniques established the wide scale application of Intralipid™ phantom for validating blood glucose monitoring techniques, which have motivated us to apply Intralipid™ based optical phantom in this present thesis for validating our indigenously developed technology. The upcoming chapters deal with such experiments and their results implies that Intralipid™ based optical tissue phantoms provides significant information and directions for research for developing blood glucose monitoring techniques.