CHAPTER 1

INTRODUCTION

1.1 Background:

The purpose and goal of this thesis work is use of *in-vitro* Intralipid^{TM} phantom for design and development of the non-invasive blood glucose-monitoring device. Primary objective is to give the patients a pain free blood glucose-monitoring device. Regular monitoring and control over fluctuating blood glucose levels will provide a tool to improve and regulate the lifestyle of the diabetic patient significantly. The glucose molecules produce very weak signals and this is a major difficulty in its measurement. The weak sensitivity and specificity of glucose molecules as well as overlapping of spectral response of water with the glucose molecule is an important issue. There is a need to address the design carefully and develop an optical based noninvasive glucose-monitoring device. Moreover, for the application of the non-invasive based optical techniques, the skin tissue based scattering, transmittance and reflection pattern plays the main role and these parameters should be carefully analyzed [Srivastava *et al.* (2013)].

The present thesis significantly examines and analyzes the Intralipid^{TM} phantom based experimental aspects of amplitude-modulated ultrasound and infrared techniques for non-invasive blood glucose level observing procedures. The following essential points are-targeted:

(i) Preparation of Intralipid^{TM} phantom for mimicking the physiological environment and to analyze the efficiency of our proposed technique to measure various glucose concentration levels in it.

(ii) Estimation of blood glucose levels in different stages of oral glucose tolerance test, fasting, postprandial and random stages *in-vitro* human blood sample mixed with Intralipid^{TM} phantom samples.

(iii) Estimation of glucose concentration in different optical phantom mediums such as water, commercialized milk, chicken breast tissue and human whole blood mixed in different concentration of *in-vitro* dextrose solutions.

Diabetes mellitus refers to the state of patho-physiological cum metabolic disorder where increased blood glucose levels exist in an unrestrained state. Millions of Indian residents and other global inhabitants have been suffering from this metabolic disorder since long time [Wild *et al.* (2004)].

As of now, different types of viable invasive glucometer exist in the market, which have been used for examining the blood glucose levels [Tura *et al.* (2007);

Wild *et al.* (2004)]. There are various different symptoms like increase in appetite, dehydration and frequent urination, which indicate the high glucose levels. Diabetes mellitus is classified into two main categories that are recognized as Type-1 and Type-2 diabetes mellitus [Wild *et al.* (2004)]. In the case of Type-1 diabetes or juvenile diabetes, the beta cells of the pancreas are damaged owing to certain pathological status. That causes absolute insulin scarcity and the insulin insufficiency disturbs the normal blood glucose regulations. This Type-1 diabetes is very frequently genetically inherited [Tura *et al.* (2007)]. Nearly 50-60% of the juvenile diabetic people belong to the group of Type-1 Diabetes. Insensitivity to the insulin production generates the symptoms, which are known as the Type-2 Diabetes. The prime reason behind the Type-2 Diabetes is the sedentary lifestyle of the patient [Khalil (2004)]. Generally, those people who have a daily routine of elevated physical activity, energetic lifestyle and who do not suffer from smoking habits are less prone to the ills associated with diabetes. With the advent of the invasive glucometer, now people can monitor their respective blood glucose levels at their home only. The invasive blood glucometer monitoring practice is extremely painful and very expensive at the same time. For an effective blood glucose managing, a very tight blood glucose regulation is desired. The continuous blood glucose measurement is regularly hindered by the pain factor and the cost related factor [Daneman (2006)]. The non-invasive harmless physiological approach is coming up in the future with a clinically efficient blood glucose monitoring [Khalil (2004)].

1.2 Problem statement and motivation:

The major problem faced by people suffering from Type 1 and Type 2 diabetes is the management of constant glucose level in the body. To overcome this problem, domestic glucose testing facilities are available. With the help of such device, the diabetic person regularly investigates blood to acquire an exact value of their blood glucose level at a particular time. If the blood glucose level is too high, the person can take appropriate measure of insulin by injecting it and if the blood glucose level is in lower side, then the person can improve it by intake of food [Cunningham *et al.* (2009)]. It is important to note that glucose levels of a diabetic patient are extremely variable and continuous monitoring of blood glucose levels can provide adequate information which can help them maintain their normal blood glucose level.

According to ADA (2014), a clinical investigation revealed that the diabetic subjects should perform five-blood glucose test on an average, per day.

In this present work, we have mainly focused on use of Intralipid^{TM} phantom for the design and development for the non-invasive glucose-monitoring device.

1.2.1 Glucose monitoring:

The measurement technique of blood glucose levels can be classified into three well-defined groups depending on the interaction with a human body [Losoya-Leal *et al.* (2012)].

1.2.2 Invasive monitoring:

This method of glucose monitoring consists of a device, which is based on finger-stick investigation. In this method, a little amount of blood is collected by pricking the skin surface of a finger with a lancet. The collected sample is placed over chemically treated disposable strip, which is then inserted into the monitoring device to display the reading of blood glucose concentration [Losoya-Leal *et al.* (2012)].

1.2.3 Semi-invasive monitoring:

This technique of glucose monitoring contains minimally invasive monitoring devices, which are reliable for the analysis of interstitial fluid (IF) by the small implantation of sensor in the sub-dermal region of the body. This embedded sensor changes the glucose level from the patient's interstitial body fluid into an electronic signal [Thennadil *et al.* (2001)].

1.2.4 Noninvasive monitoring:

This technique includes all the glucose-monitoring devices, which utilize non-invasive sensors. The prime purpose and aim of the non-invasive glucose determination research is to reduce the pain and trauma suffered by the diabetic patients due to perforation of the skin in invasive methods of blood glucose monitoring. This non-invasive method of blood glucose monitoring has several advantages over invasive method:

- Less or no pain
- Possibility of real time and continuous measurement
- Lower risks of infections
- Reliable process
- More chances of accuracy

The scientific precision of blood glucose measuring device is the proximity of agreement between the determined value and the reference value of glucose. The reference value is generally acquired from a lab investigation [Losoya-Leal *et al.* (2012)].

By addressing all these issues in this present thesis work, Intralipid^{TM} optical phantom and human blood based *in-vitro* investigations were-performed to design and develop a noninvasive technology using modulated ultrasound and infrared light for blood glucose measurement in the human subjects.

1.3 Research approach:

We have tried to focus on development of optical techniques for health ailment investigations and management purposes. The cause for this development is that optical techniques have the benefit of being innately bloodless and painless in their respective approaches. Various study groups are focusing on the methodologies that permit for the identification of tissue inherent optical characteristics from optical signals that are measured noninvasively from the biological objects. [Pravdin *et al.* (2002)].

The assessment of measurement techniques and investigation based on light transmission in tissues are practically possible in direct experimentations on real bio objects. One encounters broad changes in biochemical and morphological parameters which are away from the routine investigation methods. If the optical based diagnostics device is to be utilized on a usually routine basis, there is a requirement for developing a steady and reproducible calibration techniques. For this reason, constant and reproducible investigation-objects which imitate tissue optical characteristics are required [Pravdin *et al.* (2002)].

Since the time of the late eighties [Karagiannes *et al.* (1989); Flock *et al.* (1987); Linford *et al.* (1986)], different models of the real tissue, which resemble the optical and the structural characteristics of tissue had been develop for different regions of the optical diagnostics. Various researchers have used the tissue phantom models for development of monitoring devices [Kohl *et al.* (1994)] given below: optoacoustics [Esenaliev *et al.* (1997); Oraevsky *et al.* (1997); Quan *et al.* (1993)], tissue fluorescence spectroscopy [Beck *et al.* (1998); Wagnieres *et al.* (1997); Papazoglou *et al.* (1995)], pulsed photo-thermal measurements [Sathyam *et al.* (1997); Prahl *et al.* (1992)], oxygenation monitoring and oximetry [Wolf *et al.* (1999);

Mendelson *et al.* (1989)], and measuring of polarization degree decay [Sankaran *et al.* (2000); Chernova *et al.* (1997)]. Lastly, tissue phantoms have been utilized in the investigation which are associated with the therapeutically execution of the optical emission.

Our research approach represents the effect of amplitude modulated ultrasonic waves in determining the glucose concentration in dextrose or human blood sample mixed in Intralipid^{TM} tissue phantom. The Intralipid^{TM} exhibits biological tissue related light absorption, reflection, scattering properties. The Intralipid^{TM} act as a suitable substitute medium for tissue, in the lab based experimentations. The piezoelectric crystal based ultrasonic transmitter and receiver of 40 kHz were used here. The ultrasound produced here causes series of vibrating pattern on the area of its applications. Molecules usually vibrate specifically based on their structural orientations and physical properties. The vibration patterns of glucose molecule were observed here using infrared light emitting diode (LED) of 940 nm. The resultant output is processed in Fast Fourier Transform Domain for glucose prediction in dextrose or human blood sample mixed Intralipid^{TM} phantoms that has-been-described and discussed throughout this present thesis work. This technique may be utilized for design and develop of noninvasive blood glucose monitoring device. Further, all *in-vitro* experiment has been performed under the controlled conditions of temperature and humidity respectively.

1.4 Aim of the thesis:

The present thesis intends to investigate the usefulness of Intralipid^{TM} phantom mixed human blood samples, in the design and development of the noninvasive blood glucose-monitoring device based on amplitude-modulated ultrasound and infrared light technique.

1.5 Objective of the thesis:

The main objective of the present experimental research work of this thesis is to virtually-pursue the application of amplitude-modulated ultrasound with infrared technique, for measuring glucose concentration in Intralipid^{TM} based tissue phantoms.

- To study the utilization of ultrasonic waves for predicting glucose concentration levels in dextrose mixed Intralipid^{TM} based tissue phantom and to observe the optical clearance effect determination of glucose by near

infrared technique: an experimental study using an Intralipid^{TM} based tissue phantom.

- To examine the *in-vitro* approach based OGTT and fasting-postprandial-random stage based study by modulated ultrasound-infrared light method and GOD/POD (Glucose Oxidase/ Peroxidase) method in blood plasma samples.
- To estimate the measurement of glucose by using modulating ultrasound-infrared light and GOD/POD method in normal healthy and diabetic human blood serum and whole blood samples.
- To assess the *in-vitro* investigation using three different ranges of blood glucose concentration levels in various human whole blood mixed Intralipid^{TM} phantom samples.
- To examine the *in-vitro* determination of glucose (dextrose) concentration in optical phantoms such as water, commercialized milk, chicken breast tissue and human whole blood mediums.

Finally, this thesis will influence the researchers that monitoring of the blood glucose level by using the amplitude-modulated ultrasound with infrared technique is possible.

It will also be revealed that the method provides adequate sensitivity and specificity to measure the glucose concentration in the complex medium containing Intralipid^{TM} based tissue phantom with blood samples.

In this present thesis, the technological challenges for the development of Amplitude Modulated Ultrasound and Infrared Technique for glucose measuring sensor are first to be addressed. The accurate perceptive of the theoretical and designing challenges are essential for the achievement in developing a clinically precise and reliable monitoring system for non-invasive blood glucose measurements. To achieve this, a series of efficient experiments and statistical analysis have been executed.

Mainly the prospective of amplitude-modulated ultrasound with infrared technique unit for *in-vitro* measurements of the glucose levels is to be investigated and developed. The system will include a near infrared light source with their respective detector and piezo crystal (utilize here for ultrasonic source). The software part includes an appropriate spectral processing algorithm. A number of *in-vitro*

experiments involving the utilization of various biological samples as defined in objective were being executed to validate the proposed scheme and approach.

1.6 Research contribution of the present thesis:

- The present investigation establishes that the amplitude modulated ultrasound and infrared light based technique helps in the measurement of blood glucose levels in *in-vitro* phantom mixed biological samples.
- The 40 kHz (kilohertz) ultrasound transmitter produces sufficient excitation inside the *in-vitro* sample mediums. The Infra-Red LED (Light Emitting Diode) of 940 nm helps in the measurement of glucose levels in *in-vitro* sample mediums.
- *In-vitro* investigations validate our indigenously developed amplitude modulated ultrasound and infrared light-based technique. The relationship between the glucose concentration and peak amplitude variations in the Fast Fourier Transform (FFT) domain is established. The conversion table prepared which correlates Peak amplitude in the FFT (Fast Fourier Transform) domain with the *in-vitro* sample blood-glucose-levels.
- *In-vitro* experimental cross-validate blood glucose levels in healthy and diabetic subjects during Fasting, Postprandial, and Random stages respectively.
- *In-vitro* experimental also validate our indigenously developed amplitude modulated ultrasound and infrared light based technique in different types of optical phantoms use to establish glucose measurement.

1.7 Outcome of the present thesis work:

The present thesis work establishes the viability of our indigenously developed amplitude modulated ultrasound and infrared light based technique for glucose concentration measurement in *in-vitro* phantom samples.

The developed prototype measures glucose concentration between 61.0 mg/dl to 299.0 mg/dl for plasma samples, 73.0 mg/dl to 299.0 mg/dl for serum samples, 72.0 mg/dl to 299.2 mg/dl for whole blood samples respectively, with satisfactory medical importance as established by the Error Grid and Statistical analysis.

1.8 Thesis organization:

The present thesis includes seven consequent chapters. The concise frameworks of these chapters are as per the following:

Chapter 1: Introduction

This chapter contains an overview of the thesis and briefly describes the problem and motivation towards the diabetes management. In framework of approaching to the prerequisites for this need, this chapter depicts the aim and objectives, research contributions and outcome of the present thesis work. The thesis organization part briefly highlights of all the chapters presented in this present thesis.

Chapter 2: Literature review

This chapter briefly describes the diabetes mellitus, its major types and three main types of blood glucose monitoring techniques. This part briefly describes tissue optical phantom and its properties, tissue optical phantom basic requirements, phantom utilization as a tissue-simulating process for medical purpose, optical phantom structural orientations and physical properties, selection of tissue optical phantom constituents, tissue optical phantom purposes and their assessment criteria, spectrum of the Intralipid^{TM} scattering coefficients, lipid-based scatterers assessable commercially, biomedical application of Intralipid^{TM} suspension and utilization of Intralipid^{TM} based tissue phantom for developing various non-invasive optical technique in glucose concentration monitoring.

Chapter 3: Prototype design, calibration, methodology and performance analysis

This chapter describes the role of Intralipid^{TM} based tissue phantom, preparation of Intralipid^{TM} based tissue phantom, the significant descriptions regarding the principle applied, fabrication and designing aspects of modulated ultrasound and infrared light-based prototype for *in-vitro* blood glucose measurement. Further, this chapter contains the *in-vitro* examination for calibration aspects along with the brief description of the error grid analysis (Clarke and Parkes) and different statistical parameter used in this present thesis work for estimating the performance based accuracy of our prototype unit in measuring predicted blood glucose levels in *in-vitro* samples.

Chapter 4: Glucose induced optical transmission effect on Intralipid^{TM} based phantom

This chapter describes the results of experimentation, carried out on Intralipid^{TM} phantom to study the optical clearing properties of glucose. Glucose minimizes the refractive index dissimilarity between scatterers and their surrounding media, leading to a smaller scattering coefficient, consequently, a shorter optical path.

Chapter 5: *In-vitro* experimental study based results

This chapter describes the *in-vitro* experimental study performed by various investigational techniques such as standard Oral Glucose Tolerance tests (OGTT), Fasting blood glucose analysis, Postprandial blood glucose analysis and Random blood glucose analysis for measurement of blood glucose by our prototype. During the experimental procedures, our *in-vitro* analysis includes both the normal and diabetic human blood samples such as (a) blood plasma, (b) blood serum and (c) whole blood samples.

Another, significant investigation have been executed by adding different concentration of glucose in different phantom mediums such as water medium, commercialized milk medium, chicken breast tissue medium and human whole blood medium for monitoring the efficiency of our prototype in measuring glucose levels in *in-vitro* samples.

Chapter 6: Discussion

This chapter includes the statistical analysis of our whole *in-vitro* experimental results as provided in chapter 5 of this present thesis. Various statistical parameters applied here includes (a) Deming Regression analysis, (b) CUSUM test for linearity, (c) Paired sample t test based analysis, (d) Mountain Plot analysis, (e) Bland Altman Plot analysis, (f) Rank Correlation analysis, (g) Pearson Correlation analysis, (h) Clarke Error Grid analysis, (i) Parkes Error Grid analysis and (j) Accuracy Measure analysis. Further, the present chapter also includes the comparison between our total *in-vitro* experimental results with various published data, to establish the efficacy of the proposed technique.

Chapter 7: Conclusion and Future work

This chapter represents the conclusion part of the present thesis work and future direction of the research work.

After this, the reference section enlists total research papers referred to pursue this present work.