CHAPTER 2

LITERATURE REVIEW

2.1 Overview:

Diabetes Mellitus is a chronic metabolic disorder characterized by reduced insulin bio-production or physiological inability to utilize circulating insulin effectively. The pancreatic β-cells of islets of Langerhans secretes insulin hormone and plays a key role in glucose transportation inside the body cells. Glucose helps in energy production for proper functioning of the living cells. In diabetics, glucose metabolism dysfunction occurs and surplus amount of glucose circulates within the blood (medical condition called as hyperglycemia). The elevated blood glucose levels injure the body cells in the end. Further, this metabolic anarchy leads to the permanent disability or terminally serious medical complications [ADA (2014); IDF (2013); Chowdhury *et al.* (2013)].

2.2 Classification of Diabetes Mellitus (DM):

As depicted in figure 2.1 there are three main categories of Diabetes Mellitus: Type I Diabetes (an autoimmune disease or lack of insulin), Type II Diabetes (insulin resistance), and Gestational Diabetes [ADA (2014); IDF (2013); Watkins (2003)].

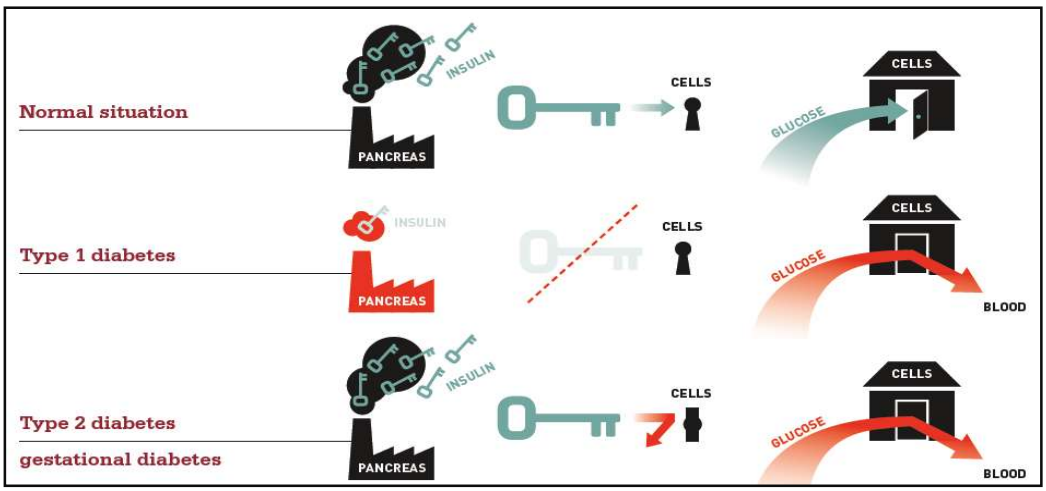

Figure 2.1: Diabetes Mellitus classification [IDF (2013)].

2.2.1 Pre-diabetics with Impaired Glucose Tolerance (IGT) and Impaired Fasting Glucose (IFG) Conditions:

Prediabetic individuals have elevated blood glucose levels but not as much high as compared to the diabetic patients. They suffer generally from the IGT (Impaired Glucose Tolerance) or IFG (Impaired Fasting Glucose) related symptoms. IGT refers to

the medical condition in which blood glucose level exists within the range between (140-199) mg/dl even two hours after food intake.

Similarly, the IFG refers to the medical situation where blood glucose level exists above 110 mg/dl even after overnight fasting. Further, the IGT resembles Type II diabetes symptoms [Chowdhury *et al.* (2015); ADA (2014); IDF (2013)].

2.3 Complications associated with Diabetes Mellitus (DM):

In this contemporary world, Diabetes Mellitus appears to be the main cause behind deaths from terminal medical emergencies arisen due to Diabetic Nephropathy (renal failure), Diabetic Neuropathy, Cerebrovascular, and Coronary heart diseases. Blindness from diabetic retinopathy, effect over Oral health and hygiene, peripheral vascular disease, foot ulcers, limb amputations (diabetic foot) are the severe long-term consequences of this metabolic disorder. Other medical complication includes pregnancy in diabetic women; oral health condition and sleep apnea in diabetic patients [ADA (2014); IDF (2013)].

2.4 Monitoring Blood Glucose Levels:

The blood glucose monitoring principally includes three major different techniques such as (i) Invasive techniques (ii) Semi-invasive techniques (iii) Noninvasive techniques [Tuchin (2009); Watkins (2003)]. This section includes discussion about these three major approaches with the special emphasis over the noninvasive techniques for blood glucose measurement in human subjects.

2.4.1 Invasive technique based approach:

Invasive technique based portable blood glucose measuring devices are widely available nowadays. Invasive techniques follow painful skin puncturing procedures, carry potentiality to cause infections, and impede diabetic subject's everyday life. Varieties of invasive blood glucose monitoring devices are fundamentally similar. A skin tissue-puncturing instrument or sterile lancets obtain a small droplet of blood. The next step involves insertion of this blood droplet into the test strip on one side. While another side into the invasive glucometer for blood glucose analysis. This specially designed test strip contains various patented chemicals that undergo certain chemical reactions or changes with respect to the blood glucose levels. The degree of change correlates with the concentration of blood glucose levels. The invasive glucometers mostly displays blood

glucose levels in mg/dl or in mmol/l. Conventional invasive glucometers generally utilizes electrochemical, colorimetric, or optical methods for blood glucose measurement. Repeated collection of blood causes pain, mental agony, and increases risk of infections (HIV, Hepatitis, etc.). Additionally, the enzymatic reaction based test strips are expensive, that limits the frequency of regular monitoring. Other prominent invasive method includes plasma or serum glucose measurement via laboratory based biochemical analysis. These tests are the most established and trustworthy ones for blood glucose measurements. Usually, the subjects fast for 8 to 10 hours before conduction of such biochemical tests. Contrariwise, this method is not pertinent for diabetic subjects to monitor their blood glucose levels regularly for three to four times per day [Yadav et al. (2015), Hill (2014); Tuchin (2009)].

2.4.1.2 User-Friendly Invasive Blood Glucose Meters:

Various invasive glucometer are available nowadays. The most successful and leading companies in these particular area are (i) Roche Diagnostics GmbH, Mannheim, Germany,(ii) LifeScan,Inc.,California,USA,(iii)Abbott-Diabetes-Care, Inc., California, USA, (iv) Bayer Healthcare Company, Leverkusen, Germany. The figure 2.2 depicts various invasive glucometers of above-mentioned companies. Similarly, the Table 2.1 lists various invasive blood glucometers along with their sample volume and measurement time required [Tuchin (2009)].

Figure 2.2: Invasive blood glucometers of (a) Roche Diagnostics (b) LifeScan (c) Abbott (d) Bayer HealthCare [Tuchin (2009)].

Table 2.1: Invasive blood glucose meters [Tuchin (2009)].

Company Name	**Invasive Glucometer**	**Features**	
		Sample Volume	**Measurement Time**
Roche Diagnostics GmbH, Mannheim, Germany	Accu-Chek Aviva System	0.6 µL	5 sec
	Accu-Chek Active System	1 µL	5 sec
	Accu-Chek Compact plus system	1.5 µL	5 sec
	Accu-Chek Go System	1.5 µL	5 sec
	Accu-Chek Compact System	1.5 µL	8 sec
	Accu-Chek Advantage	4 µL	40 sec
	Accu-Chek Complete System	4 µL	40 sec
LifeScan,Inc., California, USA	OneTouch Ultra 2	1 µL	5 sec
	OneTouch UltraSmart	1 µL	5 sec
	OneTouch UltraMini	1 µL	5 sec
	OneTouch Ultra Easy	1 µL	5 sec
	OneTouch Basic	10 µL	45 sec
	OneTouch SureStep	10 µL	15 sec
Abbott-Diabetes-Care, Inc., California, USA	Freestyle Flash	0.3 µL	5 sec
	FreeStyle Mini	0.3 µL	7 sec
	Precision Xtra	0.6 µL	5 sec
	Optium Xceed with Optium xceed plus test strips	0.6 µL	5 sec
	Optium Xceed w/o Optium Xceed plus test strips	1.5 µL	10 sec
	MediSense Optium	25 µL	20 sec
	MediSense Soft-Sense	25 µL	20 sec
Bayer Healthcare Company, Leverkusen, Germany	Ascenia Contour	0.6 µL	15 sec
	Ascenia Elite	2 µL	30 sec
	Ascenia Elite XL	2 µL	30 sec
	Ascenia Breeze	2.5-3.5 µL	30 sec

2.4.2 Semi-invasive technique based approach:

Semi-invasive approaches are not so much painful as compared to the famous and conventional invasive blood glucose monitoring devices. For blood glucose estimations, these approaches utilize the tiny sharp needles or LASER based sharp pointed pins. Others utilize electrical charges to acquire interstitial or transdermal body fluids through fingers or any other part of the human body. The biochemical reactions between the measuring device and the relevant body fluids provide the blood glucose measurements [Tuchin (2009); Lam (2008); Zhao (2002)].

Very popular and FDA (Food and Drug Administration) approved, blood glucose monitoring semi-invasive devices are GlucoWatch [Hathout *et al.* (2005); Gandrud *et al.* (2004); Pitzer *et al.* (2001)] and Continuous Glucose Monitoring Systems (CGMS) [Jeckelmann *et al.*(2002); Choleau *et al.* (2002); Meiki *et al.*(2001)] respectively. They utilized reverse Iontophoresis and electrochemical-enzymatic sensor techniques for interstitial fluid based glucose monitoring respectively [Tuchin (2009); Lam (2008)]. GlucoWatch emits electrical charges towards the sub dermal portion of the skin and extracts interstitial fluids for measurement of glucose concentration [Leboulanger *et al.* (2004)]. However, skin irritation related issue occurs from the usage of this device [Animas (2005)].

In CGMS for extraction of interstitial fluid, sensor implantation in the abdominal skin has been the primary requisite along with 72 hours for the supervision phase [Tierney *et al.* (2003)]. Both of these devices exist between invasive and noninvasive domains and hence termed as the semi-invasive devices [Lam (2008)].

Interstitial fluid based techniques provide glucose levels of the body fluid available at the particular measurement site. Further, at a given instantaneous point of time, the physiological concentration of blood glucose varies with respect to interstitial fluids [Kulcu *et al.* (2003)]. Similarly, skin sweating may influence the individual BGL measurements [Animas (2005)]. GlucoWatch strives to provide real-time glucose predictions with 10-minute delayed responses. Within 60 minutes, GlucoWatch provides three different BGL readings with large possibility of sweat affected readings. GlucoWatch and CGMS both of them requires 02 hours as machine warm up time and

skin tissue puncturing (finger pricking) based calibration approaches before different personal uses [Animas (2005)].

The clinical trials over transdermal glucose determination versus capillary glucose determination reveals that 15 minutes lag of time exists during rapid glucose level changes [Gebhart et al. (2003)]. Similarly, other same objective based clinical trial describes 17.2±7.2 minutes as time lag [Kulcu *et al.* (2003)]. This delay in response may prove to be fatal for diabetic subjects during severe hypoglycemic attacks [Lam (2008)].

2.4.3 Non-invasive technique based approaches:

Various noninvasive blood glucose measurement approach includes: Near Infrared Spectroscopy, Mid Infrared Spectroscopy, Fluorescent Spectroscopy, Photo-acoustic Techniques, Polarization Techniques, Raman Spectroscopy, Optical Coherence Tomography (OCT), Ocular Spectroscopy.

2.5 Noninvasive optical and other technologies for blood glucose level measurements:

Various researchers, scientists have been attracted towards using optical and other techniques for noninvasive blood glucose monitoring purposes. All those prominent technologies are as follows:

2.5.1 Fluorescent Spectroscopy:

When a beam of incident light from a light or laser source illuminates the exposed human tissues, it generates fluorescence in longer wavelength of the light spectrum [Tura *et al.* (2007); Khalil (2004)]. Visible and UV (Ultra Violet) light present in the surrounding environment may influence the signals and are filtered out from the original fluorescence spectrum. As, for example, when a specific 308 nm based laser beam passes through a glucose solution, the fluorescence is detected within 340 nm to 400 nm ranges with peak amplitude near to 380 nm. Therefore, for proper signal detections, the filtration of interferences from other sources is essential [Chowdhury *et al.* (2013); Tura et al. (2007); Khalil (2004)].

Sierra *et al.* (1997) introduced a Fluorescence-based glucose test; utilizing UV light, glucose oxidase, and biochemical analyzers and reported good result outcomes.

Ballerstadt *et al.* (2000) performed sensor implantation into sub dermal regions for glucose concentration determinations. They utilized 490 nm and 520 nm wavelengths

for study purposes. The result shows promising and potential application of fluorescent light for transdermal glucose determinations.

Some other researchers also applied higher wavelengths like 675 nm and 720 nm for the same experiments and reported good results. However, fluorescence based methods mainly includes in-vitro or semi-invasive glucose measurement approaches [Lam (2008)].

Other approaches include measuring fluorescence based resonance energy transmission involving a donor and an acceptor molecule. To measure the blood glucose concentration, they monitored the glucose concentration induced changes in the fluorescence reagent (enzymes) [Pickup *et al.* (2005); So *et al.* (2012)].

One experimental study reports that glucose concentration in tear molecules correlates with blood glucose concentration. They demonstrated that fluorescent spectroscopy determines blood glucose with a nearly 30 minute time lag, however free from other existing prominent interfering factors [Khalil (2004)]. The visible light diffraction principle based photonic sensing approach can measure various concentrations of glucose molecules. It also provides the estimations based on intensity of fluorescence and decay time calculations [So *et al.* (2012); Khalil (2004)]

Srivastava *et al.* (2011) introduced Smart Tattoo based glucose biosensors including co-encapsulated anti-inflammatory agents. They determined respective glucose levels from the interstitial fluids present in the dermis layer of the human skin through minimally invasive fluorescent technology.

2.5.1.1 Significance:

Fluorescent spectroscopy possesses the potentiality of detecting glucose molecules especially in spectral domain ranging from 340 nm to 400 nm respectively [Tura *et al.* (2007); Khalil (2004)]. Photonic sensing technology is very sensitive. It can safely detect single molecules. Both of these aspects are free from light scattering phenomenon and fluorophore concentration induced losses [So *et al.* (2012); Khalil (2004)].

2.5.1.2 Limitations:

Very strong tissue scattering phenomenon exists especially with Fluorescent spectroscopy based approaches. Other optical and fluorescently active skin tissue

substances may influence glucose specific signals very significantly. Further, the small lifetime of fluorescent molecules and biocompatibility issues are prominent [So *et al.* (2012); Tura *et al.* (2007); Khalil (2004)].

2.5.2 Photo Acoustic (PA) Spectroscopy:

This technique involves optical light beam irradiation over tissue or sample part to generate the heating phenomenon rapidly [So *et al.* (2012); Rosencwaig *et al.* (1996)]. Heating generates acoustic pressure waves. It can be measured utilizing microphone or highly sensitive piezoelectric detectors. The resolution, the sensitivity of Photo Acoustic spectroscopy is higher as compared to traditional spectrometry. However, this technique suffers severe interferences from the environmental factors [Rosencwaig *et al.* (1996); So *et al.* (2012)]. In 1995, the laser diode based in-vitro (glucose solutions) and in vivo (including Oral Glucose Tolerance Tests over nine human volunteers) tests utilizing PA techniques showed promising results. The good correlation exists between predicted (noninvasive) and reference (invasive) blood glucose level values [Duncan *et al.* (1995)].

Mackenzie *et al.* (1999) reported their experimental finding as obtained from the chain of PA tests (both in-vivo and in-vitro tests) for the acquisition of glucose concentrations. They showed that PA response varies with the change in glucose concentration in the test samples (including both the in-vivo and in-vitro tests). Their critical analysis reveals that characteristics of human tissues and complex PA signal responses might influence actual glucose determination processes. They also reported that PA spectroscopy suffers signal acquisition complexity due to tissue scattering noises, presence of unwanted PA active substances, various physiological reasons, etc.

Kinnunen *et al.* (2005) performed PA technique based blood glucose estimations in pig blood models utilizing Nd:YAG (Neodymium doped Yttrium Aluminum Garnet; Nd:$Y_3AL_5O_{12}$) lasers at 1064 nm and 532 nm wavelength respectively. The results showed the influence of many physiological parameters particularly RBC aggregation. It varies the glucose-induced bio-signals. Further, the high scattering index of skin tissues changes the degree of optical energy distribution patterns.

Zhao (2002) reported almost the same inferences stating the effect of physiology, skin composition, and texture factors leading to optical scattering complexity in PA techniques.

2.5.2.1 Significance:

The Photo Acoustic (PA) Technology exhibits better sensitivity towards measurement of glucose than conventional spectroscopy methods. Blood provides better PA responses than water. This phenomenon helps in separate identification of glucose and hydrocarbons respectively. Further, laser light source extending from ultraviolet to near infrared region are effective for PA response measurements [So *et al.* (2012)].

2.5.2.2 Limitations:

Environmental factors, change in temperature and pressures, dense tissue based scattering, other biological compounds exhibits interference towards PA technique sensitiveness. Further, the photo acoustic instruments are highly expensive [So *et al.* (2012)].

2.5.3 Optical Coherence Tomography (OCT):

Optical Coherence Tomography (OCT) involves the determination of coherent backscattered light for generation of particular tissue images. The images undergo reconstruction based on the backscattered lights from different skin tissue layers. The resultant OCT image can attain the good resolution up to 1 mm [Chowdhury *et al.* (2013); Fujimoto (2002); Huang *et al.* (1991)].

Esenaliev *et al.* (2001) performed preliminary trials over hairless Yucatan micro pigs and New Zealand rabbits by utilizing OCT techniques. They showed OCT scattering coefficients connections with corresponding glucose levels.

Larin *et al.* (2002) investigated OCT technique based human clinical studies with 1300 nm wavelength. They conducted eighteen OGTT clinical trials for investigating glucose concentration versus OCT signals in fifteen human healthy volunteers. The left forearm of the human volunteers utilized for capturing OCT images. Similarly, right arm for collecting venous blood samples. The gradient of observed signals measured from the depth of 200-600 μm below the outside skin layer. They acquired a sum of 426 blood samples and 8,437 OCT images respectively. The results showed that gradient of OCT signals varies 2.8% per 10 mg/dl of plasma Blood Glucose Levels. It reveals the promising aspect for utilizing OCT techniques for noninvasive blood glucose estimation.

Kinnunen *et al.* (2006) utilized OCT techniques for observing the glucose-induced variation in in-vitro Intralipid samples and in mouse skin samples. Intralipid

phantom based experiments showed the positive correlation between glucose concentration and OCT signals. Nevertheless, experiment with mouse skin samples reveals poor correlation in the majority of the cases. They indicated the influence of extracellular osmolytes like potassium chloride, urea over the refractive index to alter glucose concentration induced OCT signals.

2.5.3.1 Significance:

Scattering coefficients varies with respect to the refractive index of the tissues that in turn depends on the optical clearing effect of the glucose molecules. Based on this phenomenon OCT signals provides the estimation of glucose concentration for a particular skin tissue. These OCT techniques possess high Signal to Noise ratios (SNR), resolution, and penetration depth respectively [So *et al.* (2012); Tura *et al.* (2007)].

2.5.3.2 Limitations:

During OCT signal acquisition processes movement of reference and sample arm occurs. Motion related artifacts occur here. Density and refractive indexes vary in inhomogeneous skin tissue textures influencing OCT induced signals [So *et al.* (2012); Tura *et al.* (2007)]. The abrupt change in skin temperature produces erroneous results in OCT techniques. However, the degree of error is negligible in small temperature variations [Tuchin (2009); Larin *et al.* (2003)].

2.5.4 Polarization Spectroscopy:

Polarization spectroscopy utilizes the degree of optical rotating characteristics of the chiral molecules like glucose to determine its concentration levels [Koolman *et al.* (2005)]. Concentration of optically active substances directly relates to its optical degree of rotation. In noninvasive technique, the light beam should penetrate the living tissue without the occurrence of depolarization phenomenon. However, a large number of scattering substances in the thick tissues hinders that possibility up to the larger extent. The neighboring environmental factors easily influence this signals [Chowdhury *et al.* (2013); So *et al.* (2012); Tura *et al.* (2007); Koolman *et al.* (2005)].

Cote *et al.* (1992) utilized Helium Neon Laser of 632 nm wavelength for glucose level estimation by polarization techniques. They showed the prediction of D-glucose solution with an error less than 5%. This fact reveals good optical rotating characteristics of D-glucose molecules. However, when tests performed over excised human eye, the

outcome was not promising. Various reasons existed with excised human eye like mechanical strain, little cloudy eye effects. These factors reduced the transparent property of the excised human eye. All this obstacles reveals that numerous factors can depolarize the light, leading to poor glucose concentration induced polarization measurements [Cote *et al.* (1992)].

Cote *et al.* (2004) and Ansari *et al.* (2004) performed experiments with their respective methods using turbid media and aqueous humor part of the human eye. Both the teams reported positive in-vitro results about polarization techniques for glucose concentration measurements. However, these model-based experiments overlooked the effect of the biological factors.

Cote *et al.* (1992) and Wan *et al.* (2005) in their studies showed the use of dual laser wavelength (635 nm and 532 nm) based polarimetry techniques. They utilized aqueous humor of human eye as the measuring site. Their results indicate that this hybrid approach (dual wavelength polarimetry techniques) measures glucose concentration more efficiently. It reduces the birefringence noise arising from corneal motion related artifacts. Polarization measurement performs better when the aqueous humor of human eye acts as the measuring site. However, eye secretion like tears, glucose in eye tissues does not reflect actual blood glucose levels.

Ansari *et al.* (2004) and Boeckle *et al.* (2002) proposed a new idea by introducing polarimetric glucose sensing utilizing Brewster angle off within the ocular lens. Theoretical calculations indicate this approach to be more promising and do not need any external key identical methods or devices. However, clinical trials based on this new approach are under consideration until date [So *et al.* (2012)].

2.5.4.1 Significance:

The absorption and scattering phenomenon occurs less in eye portions. Further, other than glucose, no other similar molecule co-exists in aqueous humor of the eye portion [So *et al.* (2012); Tuchin (2009); Tura *et al.* (2007)].

Polarization spectroscopy utilizes visible light, which is easily available. Additionally, small optical devices for polarization degree measurement are readily available nowadays [Tuchin (2009); Tura *et al.* (2007)].

2.5.4.2 Limitations:

Skin tissue possesses light scattering properties. Scattering influences light depolarization phenomenon. For this reason, skin tissue (stratum corneum) based polarimetry measurements provide erroneous blood glucose level determinations. Eye movements and motion artifacts influence this technique very promptly. Albumin and cholesterol like optically active compounds may influence specificity of this technology [So *et al.* (2012); Tuchin (2009); Tura *et al.* (2007); Khalil (2004)].

2.5.5 Ocular Spectroscopy:

Alexeev *et al.* (2004) developed Ocular Spectroscopy that requires glucose sensitive hydrogels incorporation within the specially designed contact lenses. The Boronic acid derivate finely coats the particular contact lenses utilized. The boronic acid reacts with available glucose molecules in the eye to form the strong covalent bond compound. When illuminated by laser light, the degree of color change correlates with the respective glucose concentration levels. They named this noninvasive glucose sensor as "Photonic Crystal Glucose Sensor" [Tuchin (2009); Tura *et al.* (2007].

2.5.5.1 Significance:

Lower scattering properties of aqueous humor beneath cornea serve as the main measurement site for ocular spectroscopy [So *et al.* (2012); Tuchin (2009)].

2.5.5.2 Limitations:

The physiological glucose level in blood and eye tears varies at a given point of time. Contact lens users suffer from certain unpleasant (irritation, lens drying) issues. Chance of contact lens-induced severe eye infection exists [Chowdhury *et al.* (2013); So *et al.* (2012); Tuchin (2009)].

2.5.6 Raman Spectroscopy:

When a laser light passes through a liquid sample, it induces molecular rotation and vibrational phenomenon inside that particular sample [Nafie (2001); McCheery (2000); Tu (1982)]. The molecular oscillations produce specific molecule based signature signals (scattered light) which are in proportion to its respective concentration. The Raman spectrums are determined with laser lines acquired from the scattered light, which acts on, by the scatter molecular rotation and vibrational phenomenon. Laser light induces the emission from transition line near the excited levels [Tu (1982)]. Raman

techniques are very effective in measuring the molecular vibration of symmetrical dipole strong bonding molecules like C=C stretching [Workman (2001)]. Raman spectra show clear, sharp glucose specific responses within 200 to 1800 cm^{-1} domain [Tuchin (2009); Tura *et al.* (2007)].

Berger *et al.* (1997) utilized Raman Spectroscopy (Laser diode of 830 nm) for whole blood experiments. They reported that Raman spectrum successfully detects the glucose concentration in whole blood samples.

Lyandres *et al.* (2005) and Yonzon *et al.* (2004) performed in-vitro Raman Spectroscopy based glucose measurement experiments utilizing plasma and serum samples of Bovine origin. They reported RMSEP (Root Mean Square Error of Prediction) value obtained as 5.12 mM respectively.

Lambert *et al.* (1998) performed controlled in-vitro experiments with Raman spectroscopy (Laser diode of 785 nm) over Rabbit aqueous humor model. They demonstrated good relevance in glucose concentration determination.

Enejdar *et al.* (2005) performed their Raman spectroscopy based in vivo experiments utilizing 830 nm wavelength. They reported acceptable relationship exist between Raman spectra and Glucose signals but for only two to three hours of glucose measurement processes.

2.5.6.1 Significance:

Raman spectroscopy generates clear, distinct, and less overlapped spectrums as compared to NIR spectrums. The spectral characteristic rigidly correlates with respective molecular entities. Temperature change has less impact over Raman Spectrums. It is less responsive to water and modestly sensitive towards luminescence, fluorescence phenomenon [Chowdhury *et al.* (2013); So *et al.* (2012); Tuchin (2009)].

Single wavelength lasers LEDs are cheaper and widely available nowadays. Recently, various scientists have utilized surface enhanced Raman spectroscopy for noninvasive blood glucose measurements and reported promising results [Chowdhury *et al.* (2013); So *et al.* (2012); Tuchin (2009); Tura *et al.* (2007)].

2.5.6.2 Limitations:

Very frequently, Raman technique based Laser LEDs (Light Emitting Diodes) fluctuates and suffers magnitude variation related issues. Noise effect from other glucose

similar molecules exists effectively in it. Further, Raman spectroscopy requires high acquisition times. It is largely absorbing in nature and produces low signal-to-noise (S/N) ratios. Further, laser light induced skin burn-related issues are prominent [So *et al.* (2012); Tuchin (2009); Tura *et al.* (2007)].

2.5.7 Occlusion Spectroscopy:

Amir *et al.* (2007) introduced a new noninvasive concept termed as Occlusion Spectroscopy. This technique utilizes light source from Red to NIR region of the wavelength spectrum. The brief over-systolic occlusion pressure ceases the blood flow within the finger. The increase in glucose concentration increases light intensity due to less photon absorption phenomenon. This variation in light intensity measured to predict the glucose concentration. They applied this new approach over 21 Type I diabetes subjects in 111 clinical sessions. The Clarke Error Grid Analysis shows that 95.5 % of the readings occupies medically acceptable A (69.7 %) and B (25.7 %) zones respectively. The mean RAD (Relative Absolute Difference) was 17.2 % [Amir *et al.* (2007)].

2.5.7.1 Significance:

Occlusion spectroscopy provides good correlation between predicted and real glucose concentrations even in the existence of complex physiological hindrances [Tuchin (2009); Amir *et al.* (2007)].

2.5.7.2 Limitations:

Orsense Ltd, the firm behind this new technology (Occlusion Spectroscopy) declared that they have introduced this technology for checking market awareness factor, not for its generalized usage [Tuchin (2009); Amir *et al.* (2007)].

2.5.8 Bio-impedance Spectroscopy:

The determinations of bio-impedance have shown promising aspects for noninvasive measurement of body parameters. This technique measures resistance to the flow of electrical current through the living cells. Impedance spectroscopy refers to the determination of the dielectric spectrum of the medium with variation in its respective frequencies [Tao *et al.* (2009)].

Hillier *et al.* (1999) measured glucose within the frequency spectral domain from 0.1 to 100 MHz respectively [So *et al.* (2012)]. Plasma Glucose level variation induces

the reduction in the RBC's sodium ion concentrations and increases potassium ion concentration. It causes the change in membrane potential of RBC. Afterwards, the process involves detection of those changes from cell membrane permittivity and conductivity by applying dielectric spectrums respectively [Caduff *et al.* (2003); Polevaya *et al.* (1999)]. Caduff *et al.* (2009) performed noninvasive glucose estimation over human subjects (Normal and Diabetic) based on this Bio-impedance Spectroscopy.

Narasimham *et al.* (2014) modeled human blood in Ringer's solution and examined utilizing Ag/AgCl (silver/silver-chloride) as the reference electrodes. They showed that the variation in blood glucose concentration affects their respective dielectric property, which varies the blood impedance characteristics correspondingly.

2.5.8.1 Significance:

This spectroscopy technique does not depend upon any statistical model or specific interpretations. It can easily distinguish between extra and intracellular components respectively. This technique is user-friendly and economical [So *et al.* (2012)].

2.5.8.2 Limitations:

This technique requires long equilibration time such as 60 minutes before the acquisition of signals. Other interference factors like temperature, water, moisture, body hydration hinders its measurement processes [So *et al.* (2012)].

2.5.9 Electromagnetic Sensing:

Similar to bio-impedance spectroscopy, this technique measures dielectric factors of the blood. Bio-impedance spectroscopy utilizes electrical currents and electromagnetic sensing utilizes electromagnetic pairing between two inductors [Tura *et al.* (2010); Gourzi *et al.* (2005)]. This technique determines the change in dielectric factors of blood, which correlates with the glucose concentration changes [Moran *et al.* (2000)]. The glucose molecule exhibits frequency response between 2.4 to 2.9 MHz as reported [Moran *et al.* (2000); So *et al.* (2012)].

However, the factor like temperature at the measurement site plays an important role. The efficacy of this technique depends upon the particular glucose specific frequencies. Melikyan *et al.* (2012) documented its optimal operating frequency as 7.77

GHz at 25^0C utilizing pig blood. Similarly, Gourzi *et al.* (2005) recommended the optimal operating frequency as 2.664 MHz at 24^0C respectively.

2.5.9.1 Significance:

Focusing over particular frequency domain can segregate out blood glucose molecules efficiently. It will overcome interferences from other molecules like cholesterol, etc. It is comparatively harmless and molecular ionization does not occur [So *et al.* (2012); Tura *et al.* (2010)].

2.5.9.2 Limitations:

Temperature provides strong effect over its measurement processes and changes the optimal examination frequencies. Moran *et al.* (2000) documented the influence of other molecules over blood dielectric properties. Consequently, exhaustive investigational studies are required for determining potential cofounders, before the reliable advent of this noninvasive technology [So *et al.* (2012); Tura *et al.* (2010)].

2.5.10 Reverse Iontophoresis:

When low electrical current flows between anode and cathode placed on the skin tissue surface, reverse Iontophoresis process takes place. This phenomenon causes migration of sodium and chloride ions from the inner layer of skin towards cathode and anode respectively. The current produced due to sodium ion migration processes [So *et al.* (2012); Sieg *et al.* (2004)]. During this convectional flow, neutral glucose molecules move out from the interstitial fluids. The Glucose molecules accumulate near cathode where conventional glucose sensor positioned to determine respective glucose concentration levels. "GlucoWatch" (Cygnus Inc. Redwood City, California, USA) device used this Reverse Iontophoresis Technology. FDA had approved this noninvasive device. However, poor correlation of this device with actual blood glucose levels causes its effective withdrawal from the market [So *et al.* (2012); Sieg *et al.* (2004)].

2.5.10.1 Significance:

Its fluid extraction process is very simple and easy to use [So *et al.* (2012); Sieg *et al.* (2004)].

2.5.10.2 Limitations:

Firstly, this device fluid extracting electrodes irritates the skin. Secondly, electrodes require at least one hour as the warm-up time that goes beyond the patient

compliances. Thirdly, skin sweating influences the results very badly. Fourthly, instantaneous check over blood glucose level not possible due to extended warm up time. It may be less useful during fatal hypoglycemic or hyperglycemic episodes [So *et al.* (2012].

2.5.11 Mid-Infrared (MIR) Spectroscopy:

Mid-infrared region extends from 2,500-25,000 nm respectively [So *et al.* (2012); Tittel *et al.* (2003)]. MIR shows glucose molecule specific vibrational absorption bands. MIR spectra exhibit higher absorbance due to high wavelength as compared to NIR spectrums [So *et al.* (2012); Tittel *et al.* (2003)]. MIR spectroscopy extensively utilized for biochemical measurements of numerous compounds [So *et al.* (2012); Tuchin (2009); Tura *et al.* (2007)].

Kim *et al.* (2003) performed MIR spectroscopy over in-vitro blood samples to measure glucose levels in it. Similarly, Martin *et al.* (2002) utilized MIR techniques for measuring serum glucose concentration with urea and albumin as local interfering additives. Experimental result reports that acceptable linear correlation exists between predicted (noninvasive) and reference (invasive) values respectively. The error reported was 1.1 mmol/l at 1035 cm^{-1}. They reported that MIR spectroscopy detects blood glucose levels. Heise *et al.* (1989) successfully performed whole blood-related experiments with MIR spectroscopy.

Malchoff *et al.* (2002) showed good results for their noninvasive blood glucose determination for 10 hours utilizing MIR spectroscopy. Tympanic membrane and serum glucose have been utilized here as the measuring site for noninvasive (predicted) and invasive (reference) glucose level detections. Their results represented correlation up to 0.94 respectively.

Heise *et al.* (1998) and Marbach *et al.* (1995) performed human clinical trials applying FT-IR (Fourier Transform-Infra Red) spectroscopy to collect spectrum from human lips with time span of about 4.5 to 09 hours. The finger pricking methods performed for invasive blood glucose determinations, but results correlation was not acceptable. However, researchers still today favor MIR spectroscopy for noninvasive blood glucose measurements [So *et al.* (2012); Tuchin (2009); Lam (2008); Tura *et al.* (2007)].

2.5.11.1 Significance:

The absorption bands of glucose in MIR spectrum are more clear and distinctive as compared to Glucose absorption band in NIR spectrums respectively [Chowdhury *et al.* (2013); So *et al.* (2012); Tuchin (2009)].

2.5.11.2 Limitations:

Tissue penetration is much lower due to the high absorption of MIR spectrums. It can penetrate only up to certain micrometers of the skin layers. For that reason, various investigations involves only the reflected light as no transmission through tissue occurs. Body water content absorption spectrum overlaps with MIR spectrums very prominently [Chowdhury *et al.* (2013); So *et al.* (2012); Lam (2008); Tura *et al.* (2007)].

2.5.12 Near Infrared (NIR) Spectroscopy:

Near Infrared region extends from 730 nm to 2,500 nm respectively [Raghavachari (2001)]. Glucose shows weak NIR absorption patterns per concentration unit of body's major intracellular components. NIR spectra can explore biological tissue within 1 to 100 mm [Raghavachari (2001)]. Generally, the penetration depth decreases with increase in wavelength value.

Large number of biological components and impurities originated from various biochemical processes exhibits absorption and fluorescence properties within 190 nm to 650 nm range. Fluorescent light-based interferences minimize high signal to noise ratios of the NIR signals [Raghavachari (2001)]. Body temperature, room temperature, etc. influences NIR signals [Qu *et al.* (1997)].

NIR spectroscopy resembles same principles of Infrared spectroscopy. NIR spectrum consists of broadband-related overlapping peaks, overtones such as first, second, and combination overtones originated from molecular fundamental vibrations. IR (Infrared) spectrums are more typical than NIR spectrums [Lam (2008); Kelly *et al.* (1995)]. Vibrational spectroscopy can identify functional groups related to water, carbohydrates, proteins, and fats. Stretching bands of C-H, O-H, N-H, C-O, C=O dominates the IR spectra. Only bonds consisting of light hydrogen atoms like C-H, O-H, N-H exhibits intense bands in the NIR spectra. Both IR and NIR spectroscopy principally detects vibration of the particular functional side groups [Lam (2008); Kelly *et al.* (1995)].

The great scientist and main pioneer Heise (1996) investigated various aspects of NIR spectroscopy for noninvasive blood glucose measurement. He described certain limitations for noninvasive techniques also. They are mainly physiological dissimilarities and comparatively minute quantity of glucose presence in the blood. Khalil (2006); Yeh (2005); Khalil *et al.* (2003) reviewed the application of temperature modulated localized reflectance for noninvasive estimation of glucose molecules. They depicted that thermo-optical response of normal subject's skin tissue differs from that of diabetic subjects. Noises from diabetic subjects are dominating over the actual signals. Relationship between thermo-optical signal and blood glucose was acceptable in diabetic female (less than 20 year of diabetic history) subpopulations. Long-term consequences of diabetes like structural and vascular diseases influence the optical estimation of blood glucose levels [Khalil (2006); Yeh (2005); Khalil (1999)].

Maruo *et al.* (2003) utilized fiber optic based NIR diffuse reflectance spectroscopy on the forearm of diabetic subjects. The finding provides acceptable correlation between predicted (noninvasive) and reference (invasive) values. Some in-vitro whole blood or plasma based NIR examinations showed clinically acceptable readings [Heise *et al.* (2000); Heise *et al.* (2000); Youcef *et al.* (2000); Youcef *et al.* (1999); Robinson *et al.* (1992)]. Riley *et al.* (2000) performed NIR spectroscopy based glucose measurement in in-vitro animal cell culture medium. They reported the good correlation of NIR spectroscopy with glucose determination at lower concentrations (0.3 to 0.6) mmol/l also. Further, maximum region of the 'Tissue Optical Window' (700 nm to 1100 nm) exists within the infrared region [Konig (2000)]. For that reason, selecting NIR spectra is the most appropriate option for noninvasive blood glucose measurement through biologically active human skin tissues. Arnold *et al.* (1996) documented that even though measurement errors exist with NIR spectroscopy but a large number of researchers and experimental studies generates potentialities for NIR spectroscopy based noninvasive blood glucose determinations.

2.5.12.1 Significance:

NIR spectroscopy is helpful for skin depth analysis within the range between (1-100) mm. It exhibits high signal to noise ratios, high sensitivity, lower absorbance in skin tissues, non-destructing in nature. NIR spectroscopy can manage large sized and

inhomogeneous samples. Photosensitive detectors exhibit large conductivity towards NIR spectroscopy based techniques. High signal energy exists in NIR samples as compared to MIR samples. NIR spectroscopy is less costly then MIR spectroscopy. Further, wide availability of NIR products makes this technology more popular among various research groups [Chowdhury *et al.* (2013); So *et al.* (2012); Tuchin (2009)].

2.5.12.2 Limitations:

Sensitive to temperature changes and highly scattering in skin tissues. Further, physiological changes, minute quantity of glucose in blood, and hardware related issues influences NIR spectroscopy based glucose measurement processes [Chowdhury *et al.* (2013); So *et al.* (2012); Tuchin (2009)].

2.5.13 Thermal Spectroscopy:

Thermal gradient spectroscopy measures Infrared Emissions from the body due to glucose molecules absorptive effects. The IR absorption effect of glucose molecules were correlated with its respective concentration levels [Tura (2006); Khalil (2004)]. Yeh *et al.* (2003) cooled the skin temperatures up to 8^0C to 10^0C, to minimize such absorptive effects. Measurement site utilized for this approach includes forearm skin, finger and the tympanic membrane in ears respectively [Tura (2006); Yeh *et al.* (2003)].

2.5.13.1 Significance:

Noncontact determination of glucose levels might be possible with this different Thermal Spectroscopy technique [Tura (2006); Yeh *et al.* (2003)].

2.5.13.2 Limitations:

Variation in skin or body temperature affects the glucose-induced IR signals. Even physiologically (for example circadian periodicity) or pathologically (for example fever status) induced body temperature changes influence the results very prominently [Tura (2006)].

2.5.14 Ultrasound Modulated Optical Technique:

Zhu *et al.* (2013) and Zhu *et al.* (2011) demonstrated a new hybridized technique of utilizing Ultrasound Modulated Optical technique for noninvasive blood glucose measurements. They measured the in-vitro glucose concentration in Intralipid phantom and glucose sample solutions. Here modulation depth of ultrasound modulated scattered light has been measured. They also observed the relation between the influence of light

intensity and sample temperature by this new technique. They showed significant in-vitro result correlations with glucose concentration measurements [Chowdhury *et al.* (2015)].

2.5.14.1 Significance:

Hybridized approach of utilizing modulated ultrasound and light together proves to be useful for glucose determinations [Zhu *et al.* (2013) and Zhu *et al.* (2011)].

2.5.14.2 Limitations:

Only preliminary in-vitro results are available, and lack any data about in-vivo clinical study for measurement purposes [Chowdhury *et al.* (2015)].

Table 2.2: Various noninvasive techniques for blood glucose monitoring

Noninvasive Techniques	Advantages	Disadvantages	Remarks	References
Fluorescent Spectroscopy ***Measurement sites:*** Skin, Eyes.	Significant for Biochemical Analysis.	Highly effected by neighboring medium, UV and visual light, Skin pigmentations, redness and thickness influences glucose measurements.	UV and white light with shorter wavelength causes skin burn issues.	Yadav *et al.* (2015); So *et al.* (2012); Vaddiraju *et al.* (2010); Lam (2008); Tura *et al.* (2006); Khalil (2004).
Photo Acoustic Spectroscopy ***Measurement sites:*** Fingers, Skin tissues, Eye sclera.	Good Sensitivity, Not affected by ionic strength or albumins.	Effected mainly by surrounding factors, Laser beams may cause skin burns, High Noisy signals from scattering skin tissues.	Large interference from skin tissues. Refractive index mismatch. Temperature, Pressure changes influences the results, Expensive instruments.	Yadav *et al.* (2015); Chowdhury *et al.* (2013); So *et al.* (2012); Vaddiraju *et al.* (2010) Lam (2008); Tura *et al.* (2006); Khalil (2004).

Optical Coherence Tomography (OCT) ***Measurement sites:*** Forearm skins, Interstitial fluid in upper dermis of skins.	Controlling sampling depths limits the unwanted signals. High signal to noise ratios. Good resolution and penetration depths.	Sensitive to Temperature, Optical in-homogeneity, Refractive index mismatch, motion artifacts effects the signals.	It is free from the influence of urea, ionic strength, temperature, heart rate, hematocrit levels.	Yadav *et al.* (2015); Chowdhury *et al.* (2013); So *et al.* (2012); Vaddiraju *et al.* (2010); Tura *et al.* (2006); Khalil (2004).
Polarization Spectroscopy ***Measurement sites:*** Eye (Aqueous Humor beneath the Cornea).	Uses white light which is abundantly available; Glucose molecule exhibits good optical rotating properties.	Hard to pass through the skin layers; lack of specificity as ascorbic acid, albumin polarizes the light.	Optical rotation is very small as compared to interferences from skins. Scattering, pH, Temperature influences its measurement.	Yadav *et al.* (2015); Chowdhury *et al.* (2013); So *et al.* (2012); Vaddiraju *et al.* (2010); Tura *et al.* (2006).
Ocular Spectroscopy ***Measurement sites:*** Eye (Aqueous Humor beneath the Cornea, tears).	High specificity, Color change phenomenon for glucose detection. Lower scattering from measuring site.	Eyelids blinking, excessive tear secretions, leaching of boronic acid derivatives and motion artifacts influence the results.	Time lag phenomenon. Eye infection from contact lenses. Eye irritations, pH, and ionic strength influence the measurement.	Yadav *et al.* (2015); Chowdhury *et al.* (2013); So *et al.* (2012); Vaddiraju *et al.* (2010); Lam (2008); Lam (2008); Tura *et al.* (2006).
Raman Spectroscopy ***Measurement sites:*** Eyes. Sometimes human skins also.	High glucose specificity, free from luminescence, fluorescence effects; distinct and less overlapped spectrums.	Longer acquisition times; influenced by tissue density, thickness, and hematocrit levels.	Laser LED's fluctuations; Expensive instruments, laser light induced skin burn issues.	Yadav *et al.* (2015); Chowdhury *et al.* (2013); So *et al.* (2012); Vaddiraju *et al.* (2010); Tuchin (2009); Tura *et al.* (2006).

Occlusion Spectroscopy ***Measurement sites:*** Fingers.	Over-systolic occlusion pressures cause artificial kinetics which leads to RBC aggregation, which helps in glucose measurement more efficiently.	Temperature and pressure sensitive.	Signal acquisition issues effect glucose measurements.	Yadav *et al.* (2015); Chowdhury *et al.* (2013); So *et al.* (2012); Vaddiraju et al. (2010); Tuchin (2009); Tura *et al.* (2006).
Bio-impedance Spectroscopy ***Measurement sites:*** Skin, Fingers.	Resistance to the electrical flow of current is measured for glucose measurement.	Long equilibration time. Temperature, water, moisture, body hydration sensitive. Skin irritations.	Time lag phenomenon exists, disease state of the body influences the measurements.	Yadav *et al.* (2015); So *et al.* (2012); Tuchin (2009); Tura *et al.* (2006).
Electro-magnetic Sensing ***Measurement sites:*** Skin Fingers.	Measures dielectric factors.	Body temperature, perspiring, and motion artifacts affects glucose detections.	Influence from other molecules over dielectric properties.	So *et al.* (2012); Vaddiraju *et al.* (2010); Tuchin (2009); Tura *et al.* (2006).
Reverse Iontophoresis ***Measurement sites:*** Wrists. Skin tissues.	Fluid Extracting Technology.	Skin sweating sensitive. Skin irritations.	Time lag phenomenon. Mismatch with interstitial fluid glucose levels with blood glucose levels.	Yadav *et al.* (2015); So *et al.* (2012); Tuchin (2009); Tura *et al.* (2006).
Mid-Infrared (MIR) Spectroscopy ***Measurement sites:*** Skin. Fingers. Oral mucosa.	Best for in-vitro glucose determination.	Water exhibits strong absorption phenomenon.	Less sensitive for in vivo experiments for glucose determinations due to lesser penetrations through skins.	Yadav *et al.* (2015); Chowdhury *et al.* (2013); So *et al.* (2012); Tuchin (2009); Tura *et al.* (2006).

Near Infrared (NIR) Spectroscopy ***Measurement sites:*** Ear lobe, Finger web and finger cuticle. Skin of the forearm. Lip mucosa. Oral mucosa. Tongue, Nasal septum, Cheeks, Arms.	Good signal to noise ratios. Good sensitivity. Less absorbance in skin tissues. Non-destructive in nature. Can manage large sized and in-homogeneous samples.	Sensitive to temperature. Scattering effects.	Large number of researchers pursues their experiments by utilizing NIR techniques.	Yadav *et al.* (2015); Chowdhury *et al.* (2013); So *et al.* (2012); Tuchin (2009); Lam (2008) Tura *et al.* (2006).
Thermal Spectroscopy ***Measurement sites:*** Forearm skin. Finger, Tympanic membrane in ears.	Glucose absorptive effect emits IR radiations from the human body; uses visible light that is abundantly available;	pH, scattering, Body or tissue temperature change influence the results.	Temperature change due physiological (circadian periodicity) or pathological (fever) reasons effects the results.	Yadav *et al.* (2015); Vaddiraju *et al.* (2010); Tuchin (2009); Tura *et al.* (2006).
Ultrasound Modulated Optical Technique ***Measurement sites:*** In-vivo tests not available	Combined approach of utilizing ultrasound and optical technique.	Only in-vitro results available until date.	Large scale In-vitro and In-vivo studies are essential for validating this principle.	Chowdhury *et al.* (2015); Zhu *et al.* (2013); Zhu *et al.* (2011).
Modulated Ultrasound and Infrared light based technique ***Measurement site:*** Human fingers	Good specificity and sensitivity for noninvasive measurement of glucose concentration in human blood.	In-vivo results for extreme hypoglycemic and hyperglycemic ranges needs further explorations.	Large scale In-vivo studies are essential for extensive utilization and validation of this technique.	Chowdhury *et al.* (2015)

2.6 Various developing Noninvasive Glucometers and their respective Approval status:

In this portion, the brief description of recent prototypes of noninvasive glucometer that undergone various clinical trials on normal and diabetic subjects (as documented in peer-reviewed Research Journals and indexed in PubMed) are as follows:

2.6.1. Gluco-Track™ by Integrity Applications Ltd., Ashkelon, Israel:

This instrument utilizes **ultrasonic, electromagnetic, thermal techniques** altogether for measuring noninvasive blood glucose levels. Here, the ear lobes act as the measurement site. The company has been planning for massive clinical trials with this newly developed noninvasive device. In the year 2009, CE (European Conformity) approved this noninvasive device. FDA (Food and Drug Administration) approval has been in pending stage. Caution about its accuracy in real-life scenarios [Chowdhury *et al.* (2014); Ramchandani *et al.* (2012)].

2.6.2 Portable Blood Glucose meter by Glove Instruments, USA:

The new technology named as Optical Bridge Technology combined with Infrared spectroscopy for noninvasive blood glucose measurement. It detects the individual's blood glucose levels within 20 seconds. This device appeared in 2008. At present, the company has been working for miniaturizing the developed device. The CE and FDA approvals not mentioned [Chowdhury *et al.* (2014);Ramchandani *et al.* (2012)].

2.6.3 Noninvasive Glucometer based on Microwave Technology by Baylor University, Texas, USA:

This device is an electronic thumb pad sensor based noninvasive glucometer. It checks for variations in microwave propagation pattern through the skin to predict blood glucose levels. Microwave technology can provide the internal characterization of objects. This working principle measures the noninvasive blood glucose levels. The CE and FDA approvals have been in pre-submission stages [Chowdhury *et al.* (2014); McClung (2008)].

2.6.4 Noninvasive Glucometer based on Near Infrared Optical Spectroscopy and Multivariate Tools by InLight Solutions, Albuquerque, New Mexico, USA:

This device uses distinctive technology by combining Near Infrared Optical Spectroscopy and Multivariate Tools to predict glucose concentration through skin

tissues. This method filters out background noises and concentrates particularly over glucose molecules only. The CE and FDA approvals have been in pending stages [Chowdhury *et al.* (2014); So *et al.* (2012); Ramchandani *et al.* (2012)].

2.6.5 Noninvasive Glucometer by LighTouch Medical Inc., Pennsylvania, USA:

This device works on the principle of Raman Spectroscopy. They have projected fingertip with different colored light wavelength. The re-projected different colored lights were processed and quantified for predicting Blood Glucose Levels. The CE and FDA approvals have been in pending stage [Chowdhury *et al.* (2014); So *et al.* (2012)].

2.6.6 I-Sugar-X Noninvasive Glucometer by Freedom Meditech Inc., California, USA:

This device is a noninvasive, point of care, individual glucometer. Its operation includes positioning this device in front of eyes for shining purposes in less than one second. It is like a mini telescope design. It measures the degree of fluorescence produced particularly by the glucose molecules present in aqueous human eye fluids (tear secretions). This method provided faster and good results. Accurate tear concentration and projection of light is the major concern for its accurate BGL predictions. The CE and FDA approvals have been in pending stage [Chowdhury *et al.* (2014); Ramchandani *et al.* (2012); So *et al.* (2012)].

2.6.7 Contact Lens based Continuous Glucose Monitoring by University of Washington, USA:

This device uses soft hydrogel based contact lens with glucose sensor incorporated in it for tear glucose predictions. They combined this device with wireless technology for signal transmission purposes. Presently, it requires massive clinical trial and more design innovations. The CE and FDA approvals have been in initial submission stages [Chowdhury *et al.* (2014); Ramchandani *et al.* (2012)].

2.6.8 Symphony tCGM by Echo Therapeutics Inc., Philadelphia, USA:

This noninvasive device consists of three different units. Prelude unit removes the stratum corneum layer from the skin. This process increases skin permeability related aspects. The sensor compartment consists of the electrochemical glucose unit and small RF chip built inside it. This sensor part mounts over the previously prepared skin area. The third part acts as signal receiver cum display unit. It shows blood glucose levels

every 60 minutes. Long-term skin infection related side effects are the main concern. The CE and FDA approvals not mentioned [Chowdhury *et al.* (2014); So *et al.* (2012)].

2.6.9 Multisensory Glucose Monitoring System by Biovotion AG, Zürich, Switzerland:

This device utilizes multiple techniques like optical and impedance spectroscopy for measuring information related to blood glucose levels. The CE and FDA approvals not mentioned [Chowdhury *et al.* (2014); So *et al.* (2012); Ramchandani *et al.* (2012)].

2.6.10 TensorTip CoG-Combo Glucometer by Cnoga Medical ltd., Akiva, Israel:

This product contains both the invasive and noninvasive glucometers. This device appeared in 2010. It utilizes invasive glucometer for initial blood glucose level calibration purposes. The invasive glucometer discontinued after self-notification from the device. Noninvasive part of it contains four LED (Light Emitting Diode) in the wavelength range from **600 nm to 1150 nm** respectively [Ramchandani *et al.* (2012)].

When an incident ray from its LEDs passes through the fingertips, the light pattern changes. The camera acting as the detector captured these variation patterns. The inbuilt algorithm separates the output light response signal into three hyper-planes (Red, Green, and Blue) respectively. Acquired light patterns correlated with the vast stored data sets for relative blood glucose measurement. The CE and FDA approvals have been in pending stages [Chowdhury *et al.* (2014); So *et al.* (2012); Ramchandani *et al.* (2012)].

2.6.11 Noninvasive Glucometer by C8 Medisensors by California-based Company, USA:

This device appeared in 2011. It applies Raman Spectroscopy for transdermal glucose predictions. The glucose molecule specific signatures were determined and analyzed. Wireless data transmission facility provided. The CE and FDA approvals not mentioned [Chowdhury *et al.* (2014); So et al. (2012); Ramchandani et al. (2012)].

2.6.12 Easy Check Positive ID (Identification) Noninvasive Glucometer by Positive ID Corporation, the Israel-based Company:

Air inhalation and exhalation occurs during the breathing process. The exhaled breath air consists of various chemicals in the vapor state. One important constituent like acetone targeted for correlating blood glucose levels. Exhaled air reacts with the secret chemical compound present in the instrument. The output from this reaction provides

information about blood glucose levels. This device appeared in 2010. The CE and FDA approvals not mentioned [Chowdhury *et al.* (2014); Ramchandani *et al.* (2012)].

2.6.13 Eye sense Noninvasive Glucometer by EyeSense GmbH, Großostheim, Germany:

This device utilizes fluorescent technology for noninvasive blood glucose estimations. Upper limbs and abdomen portion of the body serve as its measuring site. Pumps for future insulin-delivery option are present with this device. It has been in research and development phase since 2008. As estimated, it will reach the market during 2016. The CE and FDA approvals have been in pending stages [Chowdhury *et al.* (2014); So *et al.* (2012); Ramchandani *et al.* (2012)].

2.6.14 Glucoband Noninvasive Glucometer by Calisto Medical Inc., Texas, USA:

This device applies bio-electromagnetic resonance phenomenon for predicting blood glucose levels. It comes in wristwatch type design. Within that casing, all electronic and necessary components are mounted. This device appeared in 2005 and has been in pilot study phase as of 2011 updates. The CE and FDA approvals not mentioned [Chowdhury *et al.* (2014); So *et al.* (2012); Ramchandani *et al.* (2012)].

2.6.15 Occlusion Spectroscopy based Noninvasive Glucometer by OrSense Ltd., Petah-Tikva, Israel:

This noninvasive device applies over systolic pressures to cause occlusion phenomenon over the measuring site. Its particular measuring site is the human finger. The blood flow cessation generates dynamic signals, which help in predicting blood glucose levels. It is a proprietary technology emerged during the year 2006. OrSense Ltd. Israel, later on, declares that this device have been introduced for checking commercial awareness factor regarding noninvasive glucometer market only. The CE and FDA approvals not mentioned [Chowdhury *et al.* (2014); Amir *et al.* (2007)].

2.6.16 Noninvasive Glucose monitoring device by Biosensor Inc., Newyork, USA:

This device applies Bio-impedance Spectroscopy based proprietary technology for noninvasive blood glucose measurement. This device appeared in 2010 and has been under developmental phase since then. The CE and FDA approvals not mentioned [Chowdhury *et al.* (2014); So *et al.* (2012); Ramchandani *et al.* (2012)].

2.6.17 ClearPath DS-120 by Freedom Meditech, Inc., California, USA:

This device applies fluorescent technology for noninvasive glucose level estimations. This device appeared in 2007. FDA approval has been in pending stage. The CE approval not mentioned [Chowdhury *et al.* (2014); So *et al.* (2012); Ramchandani *et al.* (2012)].

2.6.18 TANGTEST Blood Glucose Meter based on Optical Technology, USA:

The TANGTEST Blood Glucose Meter probably (not clearly documented) utilized NIR technology for noninvasive BGL estimations [Tura (2010)].

Chen *et al.* (2008) claimed that this device measures glucose levels through light intensity change, up to 0.1 Watt based spectrums. The measuring sites are the index or middle finger. They focused mainly on the optical signals of pulsatile microcirculation, to filter out interferences from bone, muscles, body fluid, sweating and other factors. It requires calibration with finger stick based invasive glucometer.

Chen *et al.* (2008) reported clinical trials with this device. Results indicate 113 data sets passed all the clinical criteria and all of them occupies A and B zones in Clarke Error Grid Plot [Clarke *et al.* (1987)] respectively. The CE and FDA approvals not mentioned [Chowdhury *et al.* (2014); Tura (2010)].

2.6.19 Reverse Iontophoresis based Glucose Monitoring Device (RIGMD), Seoul, Korea:

As indicative from its name, this device utilizes Reverse Iontophoresis Technology [Tura (2010)]. The electrochemical enzymatic sensor placed in forearm skin determines the feeble electrical current. It correlates with glucose concentration from the respective body fluids (interstitial fluids). When the current applied between two electrodes due to reverse Iontophoresis process, interstitial fluids extract out from the skin. Now, the extracted glucose molecule in interstitial fluids reacts with glucose oxidase enzyme to yield gluconic acid and hydrogen peroxide (H_2O_2) respectively. The electro-catalytic oxidation reaction occurs to reduce hydrogen peroxide for generating electric current. This electric current variation correlates with respective glucose concentration levels [Tura (2010)]. Rhee *et al.* (2007) reported clinical trials with 19 diabetic subjects (Type I and Type II patients) respectively. As documented, Clarke Error

Grid analysis of the experimental results totally occupies 98.90% of the A and B zones respectively. The CE and FDA approvals not mentioned [Tura (2010)].

2.6.20 Aprise by Glucon Inc., Colorado, USA:

The Aprise noninvasive device uses Photo Acoustic Technology [Tura (2010)]. This device utilizes glucose specific laser light wavelength (short pulses from picoseconds to nanoseconds) for its acoustic response acquisition purposes. Weiss *et al.* (2007) reported clinical trials about this medical device. They performed clinical trials with 62 diabetes subjects (23 Type I and 39 Type II with HbA1c values varies 6% to 12% respectively). In total, it acquired and analyzed 979 data pairs. As reported, Clarke Error Grid Plotting of the experimental data pairs occupies A zone = 66.5 % and B zone = 28.1 % respectively. The CE and FDA approvals not mentioned [Tura (2010); Weiss *et al.* (2007)].

2.6.21 Sentris-100 by GlucoLight Corporation, Pennsylvania, USA:

The Sentris-100 utilized Optical Coherence Tomography for glucose determination purposes [Tura (2010)]. Gabbay *et al.* (2008) reported clinical studies with this Sentris-100 device. In which 12 Type I, and 15 Type II diabetic patients underwent a 50 gm carbohydrate based diet. The process involves measurement of invasive and noninvasive blood glucose readings before meal and after meal every 10-15 minutes over a total period of 2 hours respectively. As reported, it acquired and analyzed 236 data pairs. Here, Clarke Error Grid Plotting of the experimental data pairs occupies A zone = 83% and B zone = 16% respectively. The CE and FDA approvals not mentioned [Tura (2010); Gabbay *et al.* (2008)].

2.7 Noninvasive Glucometer Devices that received Regulatory Approvals:

2.7.1 Diasensor, BICO Inc., Pittsburgh, USA:

Diasensor utilizes NIR spectroscopy based techniques for noninvasive blood glucose measurements. It utilizes 750 to 2500 nm spectra of NIR light for measuring glucose up to the depth of 1 to 100 mm respectively [Tura (2010); Malin *et al.* (1999)]. It provides individual BGL responses within 120 seconds. However, this device does not intend to be alternate for conventional invasive glucometers. Its endeavor is to encourage better observance for self-monitoring. It permits the subject to carry out their maximum blood glucose observation per day by means of a noninvasive glucometer. The Diasensor

commercialized this device at a cost of US$ 9,000 per piece (starting from 1998 to 1999). EuroSurgicals Ltd. of UK distributed this device. However, nowadays the company website does not promote this device. Consequently, stated that it is no longer on sale [Chowdhury *et al.* (2014); Tura (2010); Malin *et al.* (1999)].

2.7.2 Pendra by Pendragon Medical Ltd., Zurich, Switzerland:

Pendra utilized impedance spectroscopy techniques for noninvasive glucose measurement purposes. When a current of known value passes through a tissue, its particular impedances are calculated. Similarly, when alternating currents applied at different wavelengths, it generates the dielectric (impedance) spectrums [Tura (2010)]. Hiller *et al*. (1999) measured the dielectric frequency spectrum within 100 Hz to 100 MHz range respectively. This device resembles a wristwatch design. The circuit produces small currents with particular frequencies and executes glucose determinations. Reported sensitivity of this device was 20-60 mg/dl of glucose/Ohm [Weinzimer *et al.* (2004); Pfutzner *et al.* (2004)]. During the year 2004 (September), the Pendra marketed this device at a cost around 2500 Euros per piece. Within a few months, the product sale stopped due to poor correlation (about 35 %) between Pendra (blood glucose estimations) and conventional invasive glucometer readings respectively. In February 2005, the company faces bankruptcy issues [Tura (2010)]. However, Caduff *et al.* (2009) are still performing investigations with this new technique [Chowdhury *et al.* (2014)].

2.7.3 GlucoWatch by Cygnus Inc., California, USA:

GlucoWatch worked on the principle of Reverse Iontophoresis Techniques. A low current passes through a skin placed between two electrodes (Anode and Cathode). Sodium ions inside the skin start migrating outside and generates current [Tura (2010); Kurnik *et al.* (1999)]. Uncharged molecules like glucose from interstitial fluids migrate out (electro-osmosis) with these sodium ions from the skin [Tura (2010); Pitzer *et al.* (2001)]. The molecule collection occurs in the cathode electrode. A traditional glucometer placed there measures the respective glucose levels [Tura (2010); Pitzer *et al.* (2001)]. When correlated with conventional glucometers 15-minute time lags phenomenon has been reported [Tamada *et al.* (1999)]. After the FDA approval, marketed in USA at a price around US$ 700 per piece [Tura (2010)]. However, in the year 2005, the Cygnus sold all its assets to Animas Corporation. They commercialized

this product in Europe. However, due to some issues they discontinued GlucoWatch G2 Biographer system from 31-July-2007 onwards. However, they provided consumer support for one more year [Chowdhury *et al.* (2014); Tura (2010)].

2.7.4 SCOUT DS system by Vera Light Inc., Manitoba, Canada:

This noninvasive glucometer appeared in 2011 and targets Pre-diabetics and Type-II Diabetic patients. Fluorescent Technology forms the basis for this medical device. It detects the fluorescence sensitive biomarkers called as AGEs (Advanced Glycation End Products). The degree of AGEs presence in human skin tissues directly correlates with the prevailing diabetic conditions. Health Canada and CE (European Commission) approved it for market level distribution only. However, FDA approved it for investigation usage only [Chowdhury *et al.* (2014); Ramchandani *et al.* (2012)].

2.8 Blood glucose:

Blood is a liquid connective tissue, which circulates within the human body. Blood circulates wide range of cell nutrients, gasses and helps in removal of waste products from the human bodies. The figure 2.3 depicts the major compositions of the human blood. Physiologically, in water base of blood plasma, glucose molecule exists chemically in D-glucose form. The physiological and pathological range of blood glucose concentration varies from 18 mg/dl to 450 mg/dl [Zhao (2002)].

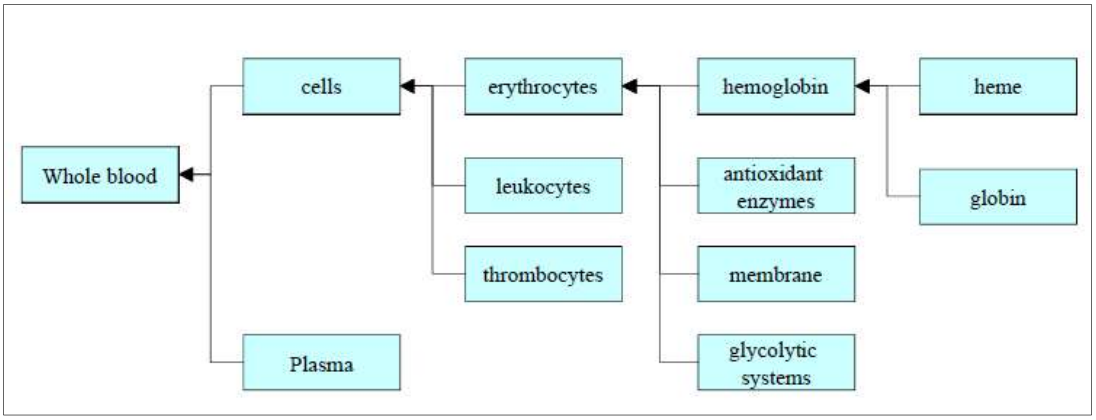

Figure 2.3: Major compositions of the human blood [Zhao (2002)]

2.8.1 Nutritional Carbohydrates:

Glucose exists in carbohydrate forms (like monosaccharide, disaccharides, and polysaccharides) in a large number of natural food products. Glucose acts as the main

source of energy supplier to the living body cells. Ingested in complex polymer form (starch) and digested to convert it into simpler form called as glucose. Further, depending upon the body's need glucose is stored or utilized. The shortage or surplus of glucose is harmful. Certain NIR spectroscopy sensitive functional groups present in glucose molecule are **-CH_2, -CH** and **-OH** respectively [Lam (2008); Smith *et al.* (2005); Benyon (2003)].

2.8.2 Physiological regulation of blood glucose levels:

When the blood glucose level decreases, the pancreas secretes glucagon hormone in the blood system. Glucagon release initiates the process of liver glycogen breakdown to yield glucose molecules. These newly formed glucose molecules released into the main blood stream, increases the blood glucose levels. Similarly, when blood glucose level increases, the pancreatic hormone insulin secretion occurs. The release of insulin initiates the process of glucose conversion to glycogen form in the liver. Consequently, the blood glucose level decreases. Hence, in this way, our body maintains the blood glucose homeostasis [IDF (2013); Lam (2008); Koolman (2005); Smith (2005)].

2.8.3 Glucose:

Molecular formula and weight of the glucose molecule is $C_6H_{12}O_6$ and 180.157 respectively. Physiologically glucose (D-Glucose) molecule exists in two isomeric forms like alpha-D-Glucose and beta-D-Glucose respectively [Lam (2008)].

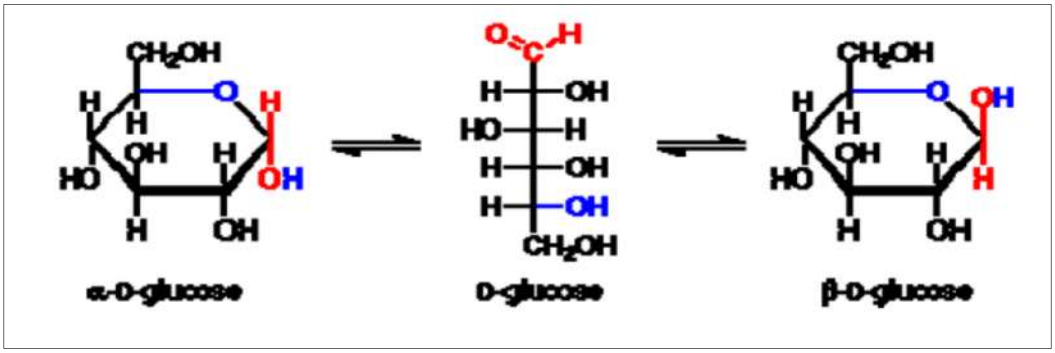

Figure 2.4: The D-Glucose transformation phenomenon [Lam (2008)].

As shown in the figure 2.4 the inter-conversion between two different isomers may occur physiologically [Lam (2008); Zhao (2002); Collins (1987)]. NIR spectroscopy technique stimulates the side functional groups (-CH2, -CH and -OH) of the glucose

molecule to excite vibrations in the carbon bonds. This vibration causes overtone formation by the release of energy. NIR absorption spectrum shows four overtones bands [Lam (2008); Arnold *et al.* (2002)]. This phenomenon directs towards the presence and concentration of side functional groups **(-CH_2, -CH and -OH)** of the respective glucose molecule. Classification of overtones based regional groups are as follows [Lam (2008); Arnold *et al.* (2002)]:

- Band region extending from 1950 nm to 2500 nm respectively
- Overtone region between1475 nm to 2050 nm respectively
- Overtone region between 1050 nm to 1650 nm respectively
- Overtone region between 700 nm to 1150 nm respectively

2.9 Human skin tissue:

The structural property of human skin varies significantly with different parts of the body.

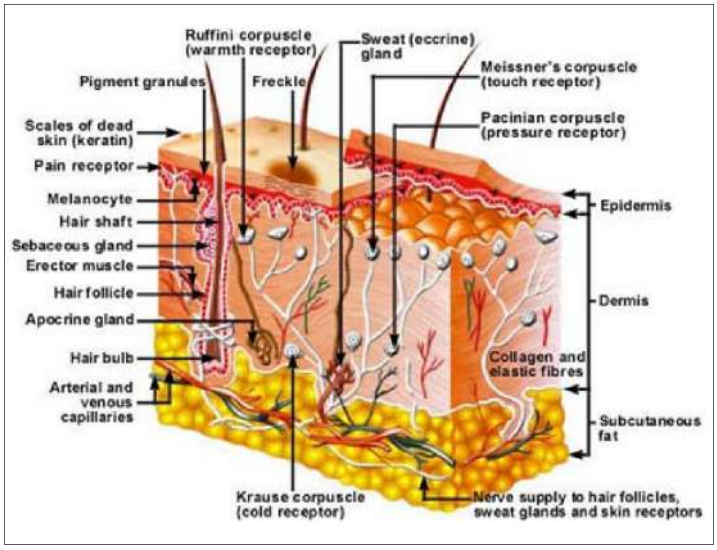

Figure 2.5: Structural morphology of the human skin [Zhao (2002); Duck (1990)].

The figure 2.5 depicts the typical structural morphology of the human skin. The human skin is composed of three different layers such as epidermis, dermis, and subcutaneous fat layer with their respective sub-layers also. Total skin thickness is about 3-4 mm respectively [Zhao (2002); Goldsmith (1991); Duck (1990)].

2.10 Human fingertip:

The human fingertip is hairless, thin, and composed of tissues, blood capillaries, nails, and bones respectively. Easily accessible and vascular structure of human finger provides an optimal situation for blood glucose measurement. Further, nails can bear the shock and protect the fingertips from any structural deformation during object touching. The rigid characteristic of nails also provides appropriate measuring condition for noninvasive glucose determination purposes. Further, the degree of deformation occurrence will be much lower in the human finger and fingertips, thus boosting NIR spectroscopy signal acquisition. Further, the fingertips show good correlation with actual blood glucose levels and it is independent from time lag phenomenon. Hence, selection of fingertip for blood glucose measurement is essential for respective calibration and measurement purposes [Mukaibo *et al.* (2005); Chen *et al.* (2005)].

2.11 Optical clearing effect related studies:

Kohl *et al.* (1994) and Kohl *et al.* (1995) studied the effect of glucose concentration upon the transport of light in in-vitro tissue simulating phantoms. By investigating the physical background of this effect, they showed that glucose molecule affects the refractive index, exhibiting influence over the respective molecular scattering phenomenon present in that aqueous medium. Sudheendran *et al.* (2010) assessed the tissue optical clearing as a function of glucose concentration utilizing OCT techniques and reported glucose concentration induced increase in optical clearing of per tissue thickness for **10%, 30%,** and **50%** glucose solution has been **4.7 ± 1.6 % mm^{-1} (n = 6), 10.6 ± 2.0% mm^{-1} (n = 7) and 21.8 ± 2.2 % mm^{-1} (n =5)** respectively.

2.12 In-vitro experiments:

Researchers regularly examine the NIR glucose concentration in in-vitro samples for preliminary investigations before proceeding with in-vivo determinations. In this aspect, Intralipid^{TM} based optical phantoms utilized to validate various investigational objectives. Intralipid suspension main constituents are **soya bean oil, lecithin, glycerin, and water** [Flock *et al.* (1992); Staveren *et al.* (1991)]. Commercially, available as Intralipid^{TM}-10% (**10% lipid indicates 10 g of lipid per 100 ml of suspension**) and Intralipid^{TM}-20%. The table 2.3 depicts the constituents of 10% Intralipid^{TM} in a 500 ml bottle according to the manufacturer are:

Table 2.3: 10 % Intralipid^{TM} suspension constituents [Flock *et al.* (1992); Staveren *et al.* (1991)].

10% Intralipid^{TM} suspension constituents		
Soybean oil	50 g	53.94 ml
Lecithin from egg yolk	6 g	5.82 ml
Glycerin ($C_3H_8O_3$)	11.25 g	8.92 ml
Water (H_2O)	430.5 g	431.33 ml
Total Volume	497.75 g	500 ml

2.13 In-vivo experiments:

In-vivo refers to living beings, which include human subjects (Healthy normal non-diabetic, pre-diabetic and, diabetic). Usually, OGTT and fasting, postprandial and random stages tests [Ruchti *et al.* (2006)] were performed in-vivo to measure glucose tolerance loads in respective human subjects within a given time period.

2.13.1 OGTT (Oral Glucose Tolerance Test):

NIR technique based human studies commonly includes oral glucose tolerance tests. All these subjects used to drink a known glucose concentration solution after overnight fasting. After that, individual responses towards glucose level monitored over two, three hours or for more time intervals. The blood glucose levels normally exist between the 3.9 mM (70.2 mg/dl) to 11 mM (199 mg/dl) respectively [Lam *et al.* (2008)].

Li-Na *et al.* (2009); Maruo *et al.* (2002 and 2003), Heise *et al.* (1996), Kayashima *et al.* (1991), Enejder *et al.* (2005), Larin *et al.* (2002), Chen *et al.* (2005) Li-Na *et al.* (2009) and various other prominent scientist preferred OGTT based analysis for measuring the performance index of the developed noninvasive technique with respect to the invasive techniques. In this present work, we have followed the same OGTT based analysis for cross checking our prototype performances.

2.13.2 HbA1c (Glycated Hemoglobin) levels impact over the blood glucose levels:

Huisman *et al.* (1958) first isolated HbA1c from other structural entities of hemoglobin utilizing column chromatography techniques [Huisman *et al.* (1958)} and categorized under glycoprotein groups [Bookchin *et al.* (1968)]. Various scientists have noticed that HbA1c levels increase in concentration with subjects suffering from Diabetes Mellitus [Rahbar *et al.* (1969)]. Bunn and his research group have first proposed its specific mechanism of action leading to its formation [Bunn *et al.* (1975)]. Nowadays,

HbA1c levels provide the degree of blood glucose management in the diabetic subjects [Koenig *et al.* (1976); Tuchin (2009)].

One Indian study including 20,554 Type II Diabetic volunteers reports that their mean Glycated hemoglobin (HbA1c) biomarker level was around 9.2 % [Mohan *et al.* (2013)]. This marks towards the poor and weak glycemic management in our country. The consequences of this poor glycemic control in India counts for 23.6 % more macro and micro vascular neuropathic patients over the heart disease patients [Mohan *et al.* (2013); Perry *et al.* (2001)].

During elevated blood glucose levels, glucose molecules bind with the hemoglobin moiety present inside the RBC cells. The longer prevailing of high blood glucose levels, higher the binding of hemoglobin to the glucose molecules and so produces the larger amount of Glycated hemoglobin moieties. Once the Glycation process takes place, it continues to stay in that form only. This Glycated product starts to accumulate within that respective RBC cells. Hence, this phenomenon represents the total exposure of the RBC cells in its full life span to the average of blood glucose levels. Further, the impact from the subjects current food diet regimen, his/her psychological status provides negligible effect on the HbA1c levels. The HbA1c levels indicate the homeostasis of the individual's plasma blood glucose for past 60 to 90 days [Syed *et al.* (2011); Kumar *et al.* (2010); Chandalia *et al.* (2002)].

Ediger *et al.* (2009) performed the direct comparison of their noninvasive technique versus Fasting Plasma Glucose (FPG) and Glycated Hemoglobin (HbA1c) with 2-hour 75 gm glucose dose based OGTT as a screening examination. They reported 17 % coefficient variations from their observations respectively. Based on these aspects, we have performed our clinical study and documented results in Chapter 4 of this present thesis.

2.13.3 Blood glucose level and blood pressure related studies:

Several categories of literature on these prospects are as follows: Filipovsky *et al.* (1996) investigated the blood pressure relation with carbohydrate metabolism in 6424 subjects. Their observation reveals that mean blood glucose levels were elevated in all the diabetic men subjects. Boer *et al.* (2008) investigated hypertension and hyperglycemia relationship in 1441 Type I Diabetic subjects. Their study shows that increase in blood

glucose levels is a serious threat for developing episodes of high blood pressure in Type I diabetic subjects. Holman *et al.* (2008) examined Type II diabetic subjects after long-term blood pressure control in them. Their study indicates that healthy blood pressure levels help in reducing long-term complications in both the Type II diabetic and Hypertensive subjects respectively. However, the medical significance disappears when blood pressure not controlled.

UK Prospective Diabetes Study Group (1999) performed one exhaustive examination by strict blood pressure management to prevent and reduce the threat of micro and macro vascular complications in Type II diabetic subjects. Various reports depict that good control over blood pressure helps in reducing the diabetes-related mortality and various medical complications. Monitoring blood pressure impact is essential in hyperglycemic control regimen [IDF (2013); Danaei *et al.* (2011); Wild *et al.* (2004)]. Based on these aspects, we have performed our clinical study and documented results in Chapter 4 of this present thesis.

2.14 Additional effects:

While utilizing NIR spectroscopy techniques for blood glucose determination purposes various additional factors present are as follows:

2.14.1 Machine and Time drift effects:

During longer signal acquisition processes, the machine and time drift phenomenon hinders the experimental results. The trouble may arise from the instrument or from the surrounding environments [Liu *et al.* (2005); Blank *et al.* (2002)]. In this present work, various effective measures such as averaging [lam (2008)] of five consecutive noninvasive signals adopted for reducing the time and machine drift effects.

2.14.2 Temperature effect:

Temperature factor plays a vital role over NIR techniques based glucose determination experiments. Many scientists reports that temperature change in measurement site influences NIR measurement readings [So *et al.* (2014); Yeh *et al.* (2003); Tarumi *et al.* (2002); Burmeister *et al.* (1998)].

The significant influence of temperature exists with the noninvasive blood glucose measurement based clinical studies. In this present work, constant controlling of

the temperature and humidity in the air-conditioned room performed during conduction of the respective clinical studies [Tarumi *et al.* (2002); Burmeister *et al.* (1998)].

2.14.3 Contact interface:

Stability of the NIR measurement position may assist in the noise reduction due to the physiological and physical changes of different body portions. Various types of forces or even similar forces act over the contact surface to influence the spectral acquisition of signals like tissue deformation reactions, as skin tension may vary now and then. This factor depends on the body responses to external stimuli and generates irrational optical measurements. Various optically uneven tissue characteristics provide spectral shifts and scattering phenomenon respectively [Lam *et al.* (2008)]. Chen *et al.* (2005) reported that the NIR spectral signals are more constant after 30 seconds of the probe and measurement site contact. Hence, for this reason, in our clinical correlation based studies we have selected fingertip as the measurement site due to its uniform, even and steady physiological structure. Further, we performed our experiments after 30 seconds contact time to reduce the contact interface related issues.

2.14.4 Location of the body:

Time lag phenomenon dependent glucose concentration determination on different body part locations is an essential issue. MaGarraugh *et al.* (2001) reported 5 to 20-minute time lag exists between finger glucose values and arm glucose values. As large depositions of fat cells in thick arm skin layers interfere with the NIR measurement techniques [Burmeister *et al.* (1999)].

Burmeister *et al.* (1999) reported tongue appears to be the most suitable site as it is free from fat based interferences. Again, Burmeister *et al.* (2000) performed experiments over 05 Type I diabetic human subjects and reported saliva with its other constituents, and food intake before measurements, influence the results. Further, Oral mucosa site appears to be easier location than tongue for better acquisition of NIR signals. However, the measurement site usually suffers from 10-minute time lag when compared with capillary blood glucose levels [Marbach *et al.* (1993)].

Eyes act as the challenging measurement site for glucose estimation by NIR techniques. Laser light application is difficult and eye tears, eyeball movements alter results. The eyes glucose level does not resemble actual physiological blood glucose

levels. It can co-exist as an alternative indicator of the glucose levels. Further, the eye flickering effect and the low reflectance of eye lenses influences the results [Schrader *et al.* (2005)]. Bina *et al.* (2003) documented that glucose reading in palm and fingers are identical. This represents that the time drift effect might be minimal in finger and palm regions respectively. Human fingertips are the most suitable location of the body for noninvasive glucose measurement purposes [Lam (2008); Mukaibo *et al.* (2005); Chen *et al.* (2005)].

The human fingertips are hairless, physiologically rich in blood supply due to dense vascular networks. It is anatomically rigid in structure and free from deformation issues due to toughness of fingernails and underlying bones. Human fingertips have simple physiological structure, easily accessible and are device friendly. Further, it is independent from time lag phenomenon and less physiological interferences will aid to reflect actual physiological glucose concentration levels. [Lam (2008); Mukaibo *et al.* (2005); Chen *et al.* (2005)]. For all these reasons, we have selected fingertip portion of the human body for our clinical correlation based studies to measure invasive and noninvasive blood glucose levels.

2.15 Conclusion:

Noninvasive blood glucose measurement techniques mainly includes Fluorescent spectroscopy, Photo-Acoustic spectroscopy, Optical Coherence Tomography, Polarization spectroscopy, Ocular spectroscopy, Raman spectroscopy, Occlusion spectroscopy, Bio-impedance spectroscopy, Electro-magnetic sensing technique, Reverse Iontophoresis, Mid-Infrared spectroscopy, Near Infrared spectroscopy, Ultrasound Thermal Infrared spectroscopy and, Ultrasound Modulated Optical technique, [Yadav *et al.* (2015); Chowdhury *et al.* (2013), Tuchin (2009)]. However, Near Infrared technique is among such methods with promising features and results [Yadav *et al.* (2015); So *et al.* (2012); Chowdhury *et al.* (2013); Tuchin (2009); Lam (2008)].

Hence, for this reason, we have also amalgamated near infrared technique along with amplitude modulated ultrasound principles for noninvasive blood glucose monitoring over normal, pre-diabetic, and diabetic human subjects and obtained promising results. The next coming chapters of this present thesis work represent those various clinical investigation based results incorporating aforementioned technology.