CHAPTER 5

IN-VITRO EXPERIMENTAL STUDY BASED RESULTS

5.1 *In-vitro* approach based OGTT study by modulated ultrasound-infrared light method and GOD/POD (Glucose Oxidase/ Peroxidase) method:

5.1.1 Introduction-Intralipid^{TM} phantom and blood plasma mixed *in-vitro* study:

The present work describes the critical investigation about the practicability of Intralipid^{TM} optical tissue resembling phantoms for developing noninvasive blood glucometer.

For achieving this objective, preliminary standard Oral Glucose Tolerance Test (OGTT) were performed over 03 normal human subjects by our indigenously developed MUS-IR (Modulated Ultra Sound-Infra Red) unit for estimation of Blood Glucose Levels (BGL). Further, for cross validation of the results obtained, readings have-been-compared with the findings of the GOD/POD method as performed on Digital spectrophotometer respectively. The blood plasma samples have been-collected from all the study subjects during the fasting stage of the subjects and at each 30 minutes after 75 gm glucose intake up to total time of 2 hours and 30 minutes respectively.

Blood plasma samples (1 ml) have-been-mixed with Intralipid^{TM} phantom (3 ml) and processed through indigenously developed MUS-IR unit for respective samples glucose concentration-determinations. Outcome of the results indicates that Intralipid^{TM} phantom samples serves as a feasible option in *in-vitro* experimentations for designing and developing noninvasive glucometer.

5.1.2 Study subjects:

In total three adult subjects (two males and one females) participated in this OGTT (Oral Glucose Tolerance Test) based *in-vitro* clinical study. Here, the study subjects are healthy normal (age = 26.5±2.5 years, height = 162±3.5 cm, weight = 74±4.0 kg human beings. The clinical studies reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of Institute of Medical Sciences-Banaras Hindu University, Varanasi approved the clinical study.

5.1.3 Experimental procedure:

5.1.3.1 Preparation of human blood plasma samples:

The 05 ml of blood sample has been collected from the right/left hand of each human subject in vacuum-based collecting vials containing EDTA (Ethylene Di-amine Tetra Acetic acid) inside it as an anti-clotting agent. Vials containing the blood

samples were centrifuged for 10 minutes. Afterwards, the supernatant part of the fluid (03 ml) called as blood plasma had been collected after the centrifugation process.

After that, 01 ml of the prepared blood plasma sample has been utilized for blood glucose concentration measurement by MUS-IR unit. Simultaneously, for performing GOD/POD method for the measurement glucose concentration 02 ml of the prepared blood plasma sample has been utilized here.

5.1.4 Result and Discussion:

To evaluate the working of the indigenously developed MUS-IR experimental setup, the standard protocol of the Oral Glucose Tolerance Test (OGTT) [IDF (2009); Watkins (2003)] has been conducted over 03 healthy subjects. The OGTT conducted as given below:

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

Step A. Fasting blood glucose samples of the subjects were-obtained at 00 minute for our indigenously developed MUS-IR unit and for GOD/POD method performed by Digital spectrometer based measurements.

Step B. 75 gm of glucose in 100 ml of water [Li *et al.* (2009)] has-been-provided to the subjects for drinking in a time span of 5 minutes after the step A.

Step C. This part contains postprandial sample collections for glucose concentration measurements by MUS-IR prototype and GOD/POD method based units respectively.

The data obtained from the OGTT (Oral Glucose Tolerance Test) experimentations at different time intervals using MUS-IR unit and GOD/POD method has-been-shown in figure 5.1 respectively.

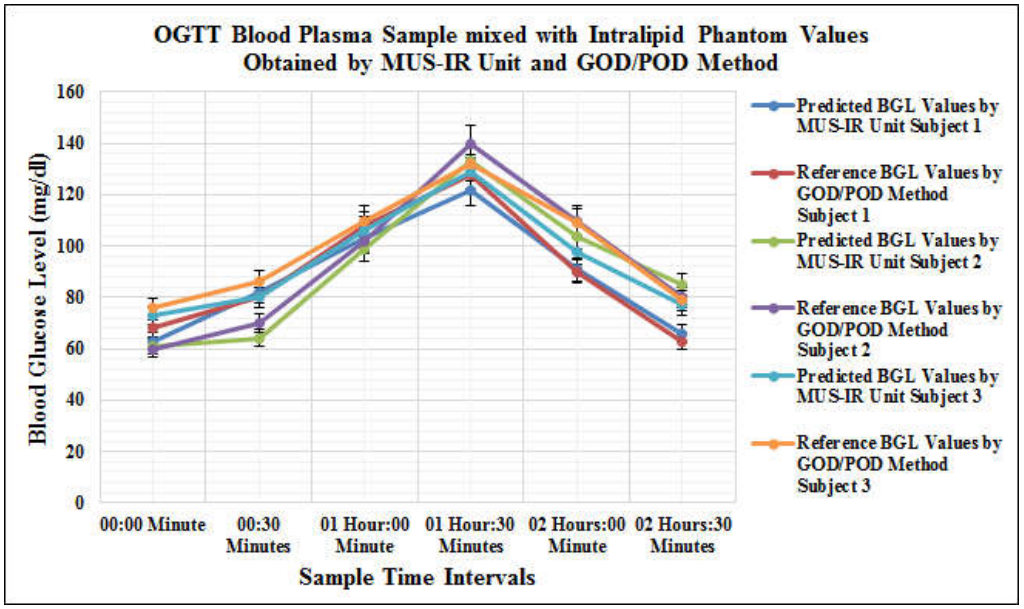

Figure 5.1: OGTT response curve of the study subjects (1 to 3); error bars indicate ±5 percentage error.

Further, the Error Grid (Clarke and Parkes) and statistical analysis is used here to measure the performance metrics of our *in-vitro* technique based prototype unit in measuring blood glucose levels in human blood plasma mixed with Intralipid™ phantom samples. The figure 5.2 to figure 5.3 and Table 5.1 to Table 5.3 represent the Error Grid (Clarke and Parkes) and statistical analysis respectively.

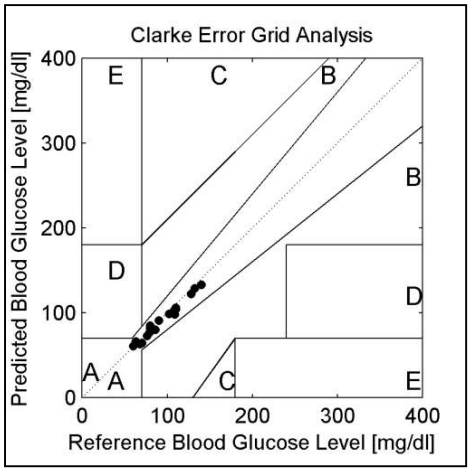

Figure 5.2: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 03 human subject's blood plasma mixed with Intralipid™ phantom samples.

Table 5.1: Clarke error grid analysis of reference and predicted blood glucose levels as acquired during OGTT over 03 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	18	100.00%
B Zone	**Medically acceptable**	00	00.00%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

The figure 5.2 illustrates Clarke Error Grid analysis of all the reference and predicted blood glucose data pair sets as acquired during OGTT based *in-vitro* examination over three healthy normal subjects. In Table 5.1, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (18) falling in the zones A, B, C, D, and E are 100% (18 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Subsequently, all the 18 data pairs occupy the medically significant A zones. Further, none of the data pair sets occupies medically acceptable B and medically insignificant and potentially dangerous C to E zones respectively.

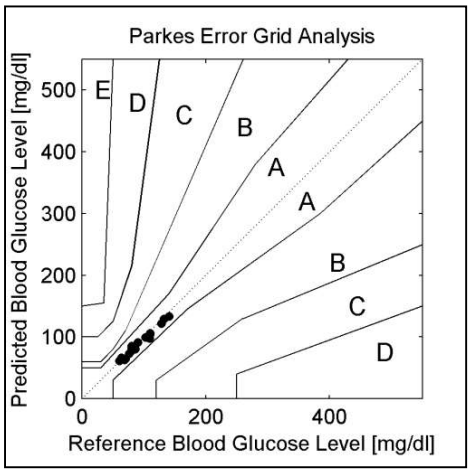

Figure 5.3: Parkes error grid analysis based plot for reference and predicted blood glucose measurement as obtained from 03 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Table 5.2: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during OGTT over 03 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	18	100.00%
B Zone	**Slight**	00	00.00%
C Zone	**Moderate**	00	00.00%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 5.3 and Table 5.2 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired during OGTT based *in-vitro* examination over three healthy normal subjects.

The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (18) falling in zones A, B, C, D, and E are 100% (18 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively.

Henceforth, the Parkes Error Grid analysis illustrates that 100% (18 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 00.00% (00 data pairs) of the *in-vitro* estimations is in slight risk B zone (clinically acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

The Table 5.3 illustrates our performance assessment values as obtained through OGTT study over three healthy normal study subjects and the results were-compared with the published data ranges of other developing glucose monitoring techniques. The performance metrics based errors such as Pearson's Correlation Coefficient (r) values and SEP (Standard Error of Prediction) were 00.98 and 03.56 mg/dl respectively. The MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 04.37 mg/dl, 04.50 mg/dl, and 04.99 mg/dl respectively. Additionally, performance metrics based percentage errors for example Percentage-MARE (Percentage of Mean Absolute Relative Error),

and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 04.67%, and 04.65% respectively.

Table 5.3: Statistical parameters utilized for accuracy assessment and the results comparison with the published data ranges of other developing glucose monitoring techniques.

Statistical Parameters	Assessment Values	Published Data Ranges of other Developing Glucose Monitoring Techniques	References
Pearson Correlation Coefficient (R-Value)	00.98	00.49 to 00.95	Vaddiraju *et al.* (2010); Tuchin (2009); Oliver *et al.* (2009)
Standard Error of Prediction (SEP)	03.56 mg/dl	07.10 to 35.30 mg/dl	Ozaki *et al.* (2009); Yoon (2009); Tuchin (2009); Heise *et al.* (1998)
Mean Absolute Error (MAE)	04.37 mg/dl	07.00 to 30.00 mg/dl	Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Myllyla *et al.* (2009); Tuchin (2009); Enejder *et al.* (2005); Bockle *et al.* (2002); Zhao (2002); Heise *et al.* (1998); Robinson *et al.* (1992)
Median Absolute Error (MdAE)	04.50 mg/dl	10.40 to 19.10 mg/dl	Valgimigli *et al.* (2010)
Root Mean Squared Error (RMSE)	04.99 mg/dl	25.00 to 46.00 mg/dl	Guevara *et al.* (2010); Ozaki *et al.* (2009); Tuchin (2009)
Percentage of Mean Absolute Relative Error (% MARE)	04.67 mg/dl	08.60 to 40.80%	Pai *et al.* (2015); Mohammadi *et al.* (2014); Vashist (2012); Ramchandani *et al.* (2012); Caduff *et al.* (2011); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Caduff *et al.* (2009); Lipson *et al.* (2009); Gabbay *et al.* (2008); Amir *et al.* (2007); Weiss *et al.* (2007); Bockle *et al.* (2002); Malchoff *et al.* (2002); Tamada *et al.* (1999)
Percentage of Median Absolute Relative Error (% MdARE)	04.65 mg/dl	07.70 to 30.00%	Harman-Boehm *et al.* (2010); Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2009); Gabbay *et al.* (2008); Lipson *et al.* (2009); Weiss *et al.* (2007); Zhao (2002); Bockle *et al.* (2002); Zilberman *et al.* (2009)

Further, as illustrated from Table 5.3, the output results acquired by our MUS-IR unit based technique is better than or comparable with other developing blood glucose measuring techniques for noninvasive blood glucose monitoring. Further, its accuracy levels are comparative with other commercially existing Continuous Glucose Monitoring System. Consequently, all these overlaid accuracy measures based statistical analysis illustrated the strong promising aspect for developing noninvasive procedure for blood glucose estimation in *in-vitro* samples as obtained from the human subjects.

5.1.5 Conclusion:

Our MUS-IR unit has been successful in determining blood glucose levels in blood plasma mixed Intralipid^{TM} phantom samples. The Error Grid and Statistical analysis depicts the acceptable efficiency of the developed prototype unit.

5.2 Extended *in-vitro* approach based OGTT and fasting-postprandial-random stage based study by modulated ultrasound-infrared light method and GOD/POD method:

5.2.1 Introduction:

In this present work, the blood samples of the thirty study subjects during oral glucose tolerance test and fasting, postprandial and random stages were-investigated respectively. The OGTT and fasting-postprandial-random stage based investigations has been performed in two different phases. Phase I for OGTT study and Phase II for fasting-postprandial-random stage based study respectively.

The result as obtained from oral glucose tolerance tests and fasting, postprandial and random stages blood glucose tests showed peak amplitude values in Fast Fourier Transform domain varies in corresponding to blood glucose levels in *in-vitro* samples. The Error Grid and statistical analysis represent the potentiality and feasibility of our technique respectively for developing non-invasive blood glucose measurement technique.

5.2.2 Study subjects:

In first phase, total thirty adult subjects (eighteen males and twelve females) participated in this OGTT (Oral Glucose Tolerance Test) based clinical study. Here, the study subjects are healthy normal (age = 27.3±4.6 years, height = 163±3.5 cm, weight = 75±4.0 kg human beings. The experimentation were performed for six consecutive days.

Further, in second phase, thirty more adults inclusive of healthy and diabetic subjects (twenty-one males and nine females) are participated for measuring blood glucose levels during their fasting stage, postprandial stage and random stage respectively. Here, the study subjects includes healthy normal and diabetic subjects of age = 43±15 years, height = 170±5.5 cm, and weight = 76±5.5 kg. These experimentations were performed for two days. The clinical studies reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of Institute of Medical Sciences-Banaras Hindu University, Varanasi approved the clinical study.

5.2.3 Preparation of human blood plasma samples:

For preparing human blood plasma samples, we have collected 5 ml of whole blood samples from the right/left hands of the study subjects in EDTA (Ethylene Di-amine Tetra Acetic Acid) treated blood collecting vials.

Afterwards, all the collected samples undergo centrifugation process for 10 to 15 minutes. The centrifugation process produces supernatant fluid portion (3 ml), which is termed as blood plasma [Raghu (2003)]. In our proposed work, we have added 1 ml of blood plasma sample with the 3 ml of 10% Intralipid^{TM} tissue phantom solution to resemble blood-tissue-phantom medium complex. Further, the 2 ml of the blood plasma has-been-used for performing GOD/POD method to measure blood glucose concentration in *in-vitro* samples.

5.2.4 Experimental Protocol:

In this present work, we have performed our clinical study in two phases that includes oral glucose tolerance test and fasting, postprandial, random stage based blood glucose test respectively to validate the clinical relationship between the established invasive methodology and our proposed *in-vitro* methodology. Further, all the blood glucose measurements reported in this present work was-performed under the controlled conditions of temperature and humidity respectively.

5.2.4.1 Phase I: *In-vitro* Oral Glucose Tolerance Tests (OGTT) investigation:

In first phase, the oral glucose tolerance tests were-conducted in the morning over 30 healthy subjects after 10-12 hours overnight fasting period. The period of each test has been 2 hours and 30 minutes inclusive of baseline monitoring after 75-gm glucose solution intake. Both the reference and predicted blood glucose levels were-recorded every 30 minutes from blood samples of right and left hand

respectively. For reference and predicted results, we have performed blood glucose measurement using established GOD/POD (Glucose Oxidase/ Peroxidase) [Trinder (1969)] method and by our proposed technique based MUS-IR unit respectively. During the course of the tests, all the study subjects were-instructed to remain static to reduce the influence of motion artifacts. Further, any kind of food or liquid intake has been-prohibited. The experiment conducted at six consecutive days. The data as obtained from the OGTT (Oral Glucose Tolerance Test) experimentations at different time intervals for six consecutive days using MUS-IR unit and GOD/POD method has-been-shown in figure 5.4 to figure 5.9 respectively.

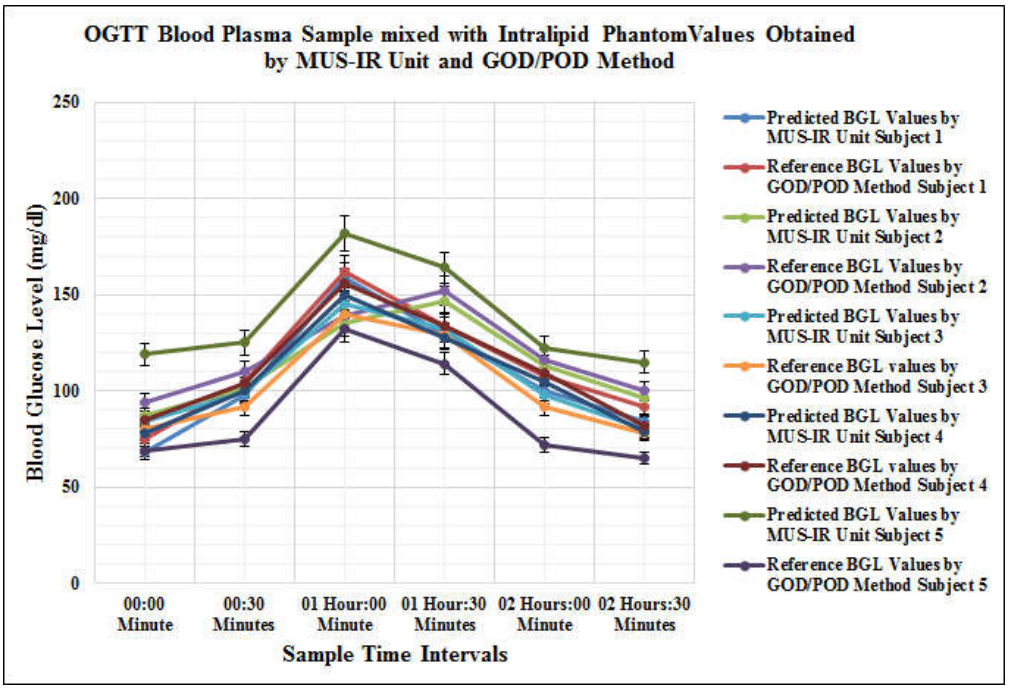

Figure 5.4: OGTT response curve of the study subjects (1 to 5) on 1st day; error bars indicate ±5 percentage error.

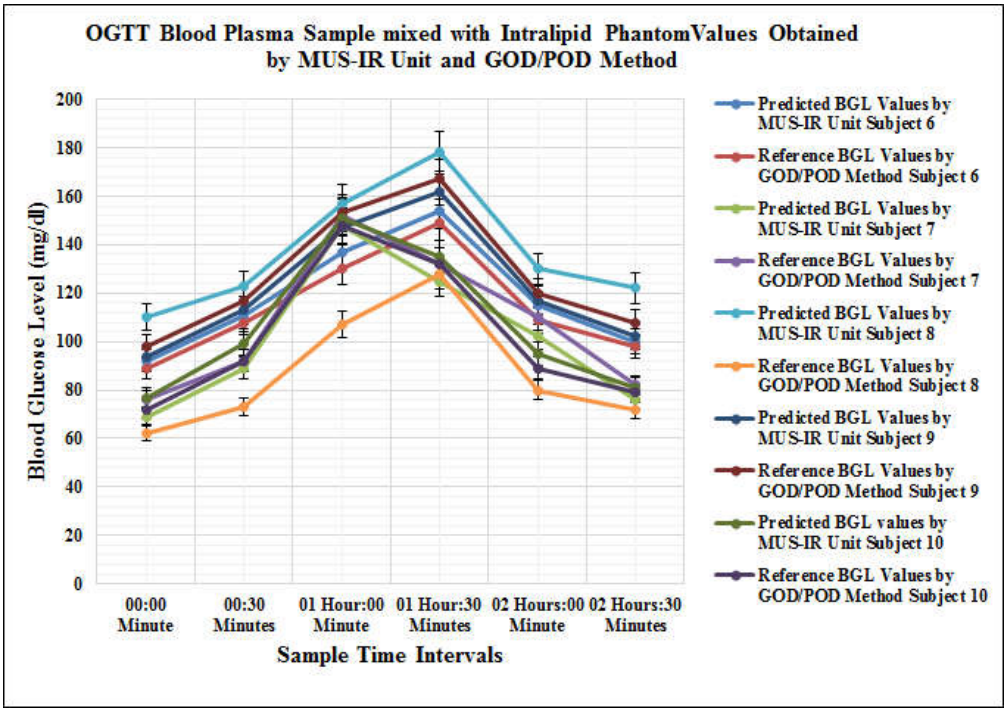

Figure 5.5: OGTT response curve of the study subjects (6 to 10) on 2nd day; error bars indicate ±5 percentage error.

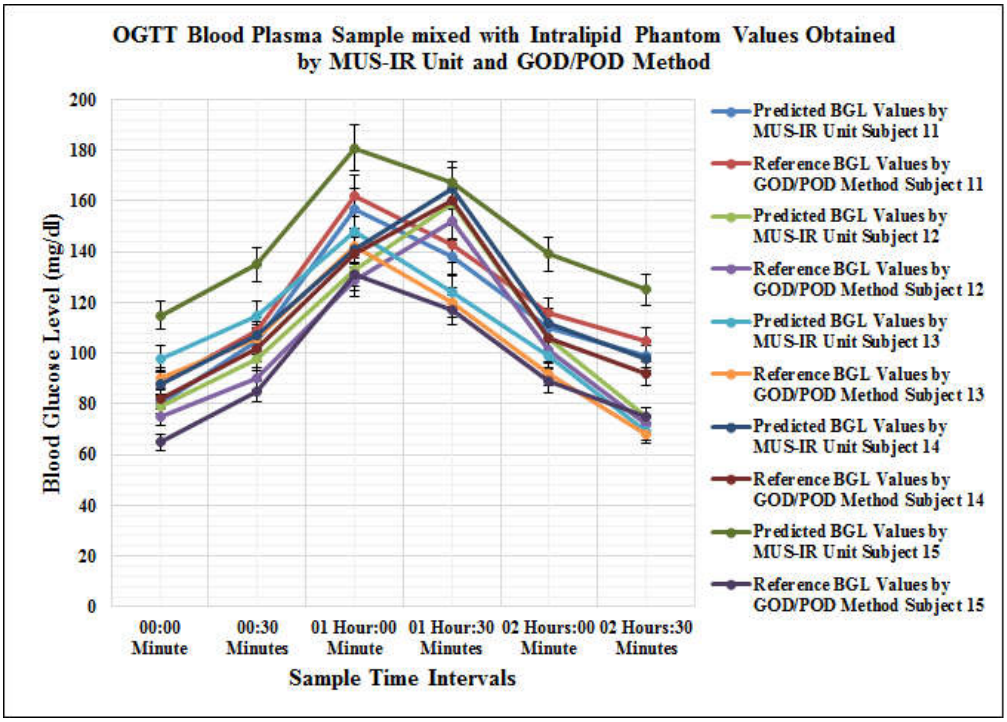

Figure 5.6: OGTT response curve of the study subjects (11 to 15) on 3rd day; error bars indicate ±5 percentage error.

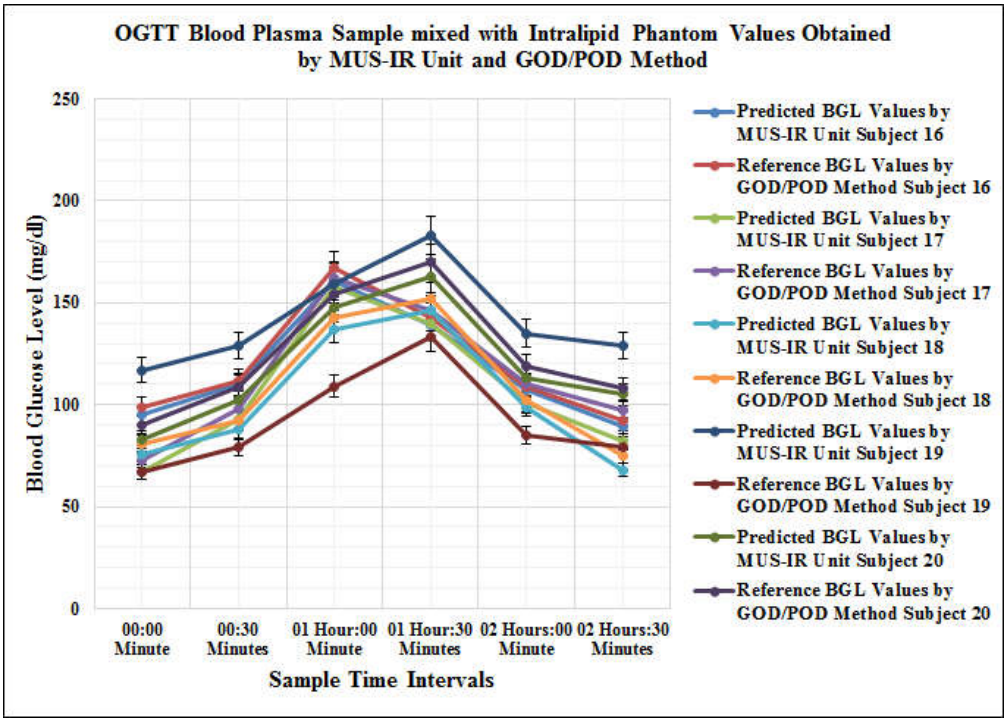

Figure 5.7: OGTT response curve of the study subjects (16 to 20) on 4th day; error bars indicate ±5 percentage error.

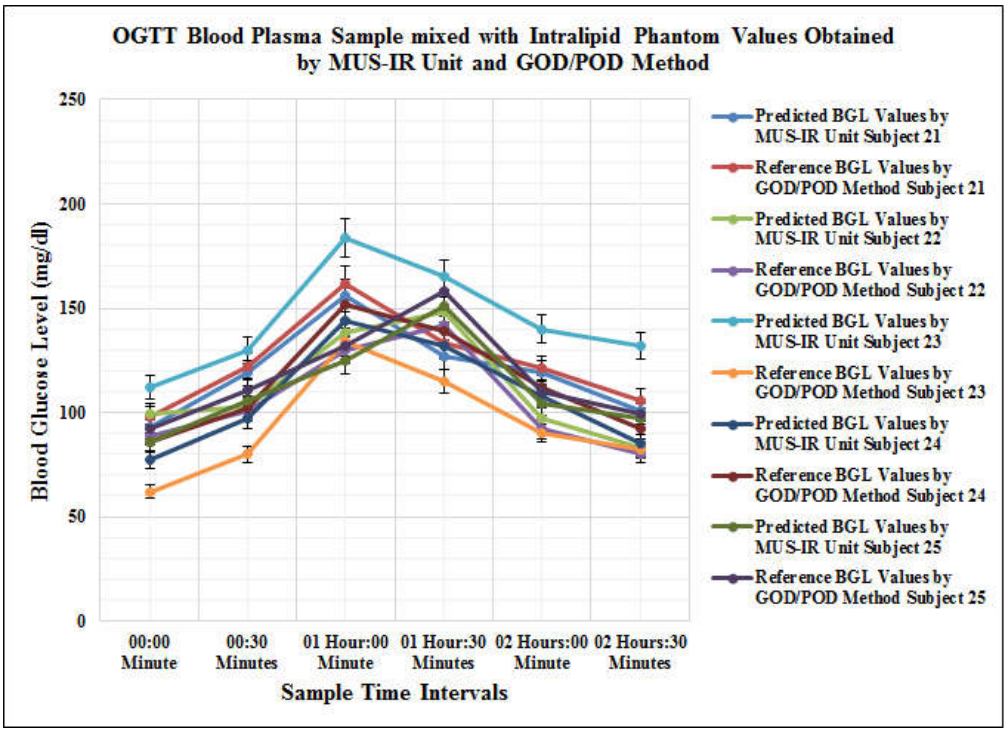

Figure 5.8: OGTT response curve of the study subjects (21 to 25) on 5th day; error bars indicate ±5 percentage error.

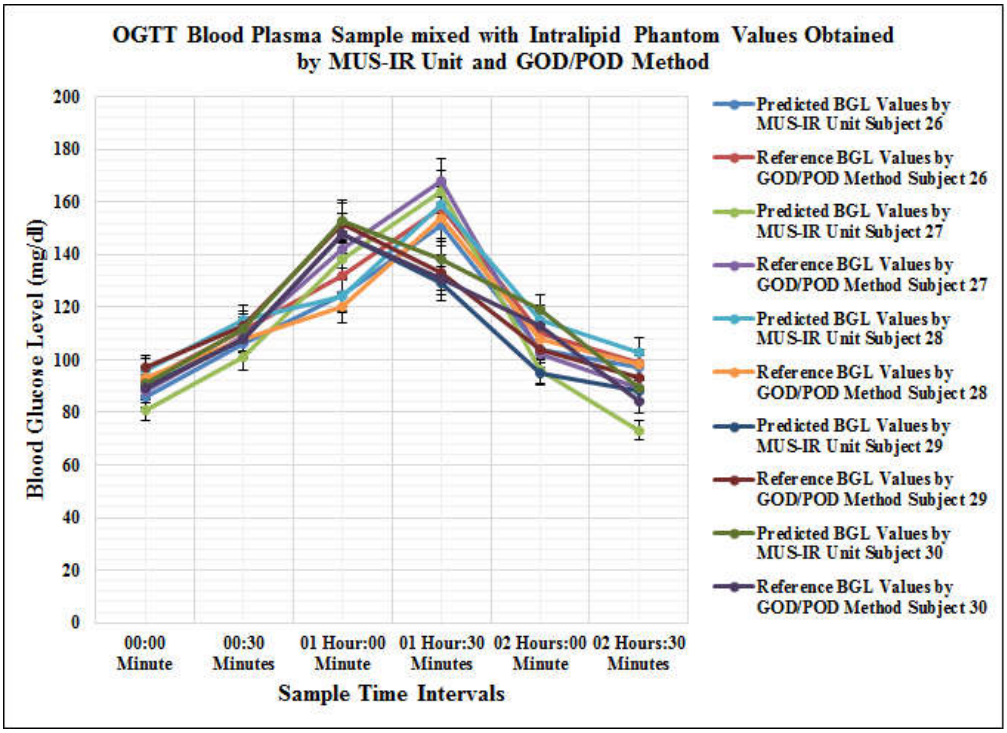

Figure 5.9: OGTT response curve of the study subjects (26 to 30) on 6th day; error bars indicate ±5 percentage error.

5.2.4.2 Phase II: *In-vitro* investigation during fasting, postprandial and random stages:

During the second phase, we have performed Blood glucose level tests over 30 healthy normal and diabetic subjects during fasting stage, postprandial stage and random stage respectively. The experiment conducted at two consecutive days. The data obtained from the OGTT (Oral Glucose Tolerance Test) experimentations at different time intervals using MUS-IR unit and GOD/POD method has-been-shown in figure 5.10 to figure 5.11 respectively.

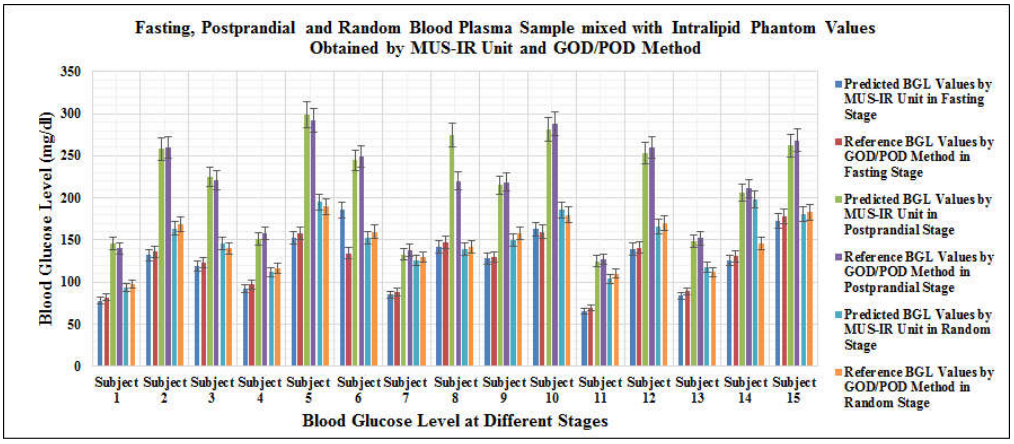

Figure 5.10: Fasting, postprandial and random stage response bars of the study subjects (01 to 15) on 1st day; error bars indicate ±5 percentage error.

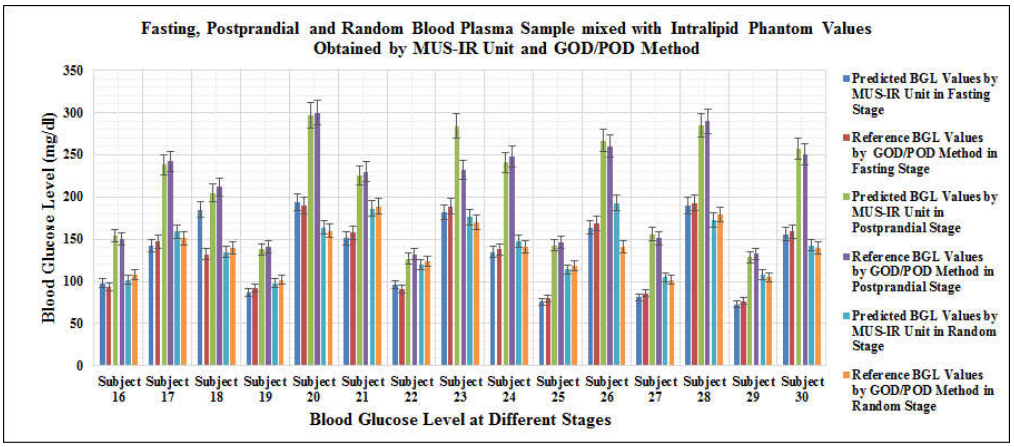

Figure 5.11: Fasting, postprandial and random stage response bars of the study subjects (16 to 30) on 2nd day; error bars indicate ±5 percentage error.

5.2.5 Result and Discussion:

In this section, we have performed Error Grid analysis and statistical analysis for the result assessment purposes.

5.2.5.1 Result of Phase I:

The figure 5.12 illustrates the Clarke Error Grid analysis of all the reference and predicted blood data pair sets as acquired during OGTT based *in-vitro* examination over thirty healthy study subjects.

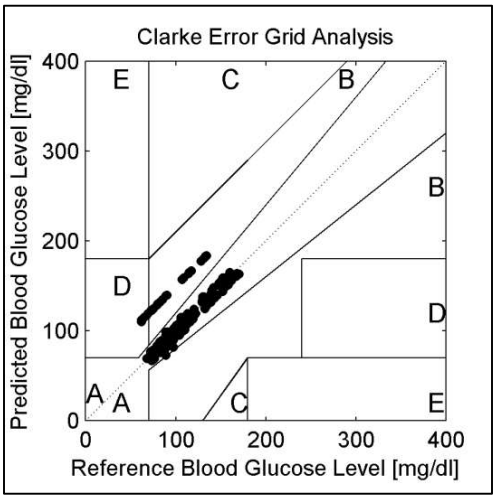

Figure 5.12: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 30 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Table 5.4: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired during OGTT over 30 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	150	83.34%
B Zone	**Medically acceptable**	24	13.33%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		06	03.33%
E Zone		00	00.00%

In Table 5.4, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (180) falling in the zones A, B, C, D, and E are 83.34% (150 data pairs), 13.33% (24 data pairs), 00.00% (00 data pairs), 03.33% (06 data pairs), and 00.00% (00 data pairs) respectively. Henceforth, all the 174 data pairs occupy the medically significant A and B zones respectively. Further, the 06 data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

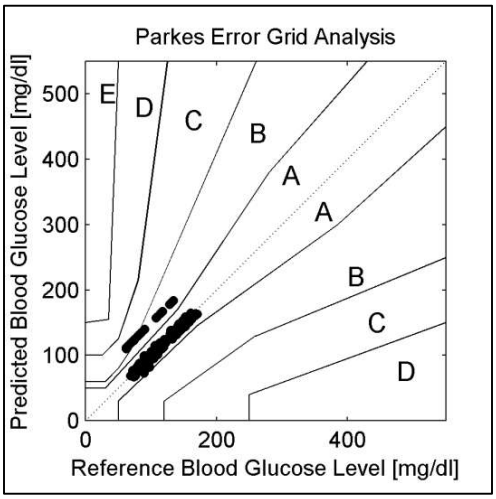

Figure 5.13: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 30 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Table 5.5: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during OGTT over 30 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	150	83.34%
B Zone	**Slight**	25	13.89%
C Zone	**Moderate**	05	02.77%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 5.13 and Table 5.5 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired during OGTT based *in-vitro* examination over thirty healthy study subjects. The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (180) falling in zones A, B, C, D, and E are 83.34% (150 data pairs), 13.89% (25 data pairs), 02.77% (05 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Subsequently, the Parkes Error Grid analysis illustrates that 83.34% (150

data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 13.89% (25 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). Further, 02.77% (05 data pairs) readings occupy C (moderate risk zone). None of the readings involves D (significant risk zone) and E (dangerous risk zone) zones respectively.

The Table 5.6 illustrates our performance assessment values as procured during OGTT over thirty healthy study subjects and the results were-compared with published data of other developing glucose monitoring techniques. The performance metrics based errors such as Pearson's Correlation Coefficient (r) values and SEP (Standard Error of Prediction) were 00.73 and 18.87 mg/dl respectively.

The MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 12.77 mg/dl, 06.00 mg/dl, and 21.06 mg/dl respectively. Additionally, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 13.89% and 05.14% respectively.

Further, as illustrates from Table 5.6, the output results acquired by our MUS-IR system is better than or comparable with other developing blood glucose measuring techniques for noninvasive blood glucose monitoring.

Table 5.6: Statistical parameters utilized for accuracy assessment and the results comparison with the published data ranges of other developing glucose monitoring techniques.

Statistical Parameters	Assessment Values	Published Data Ranges of other Developing Glucose Monitoring Techniques	References
Pearson Correlation Coefficient (R-Value)	00.73	00.49 to 00.95	Vaddiraju *et al.* (2010); Tuchin (2009); Oliver *et al.* (2009)
Standard Error of Prediction (SEP)	18.87 mg/dl	07.10 to 35.30 mg/dl	Ozaki *et al.* (2009); Yoon (2009); Tuchin (2009); Heise *et al.* (1998)
Mean Absolute Error (MAE)	12.77 mg/dl	07.00 to 30.00 mg/dl	Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Myllyla *et al.* (2009); Tuchin (2009); Enejder *et al.* (2005); Bockle *et al.* (2002); Zhao (2002); Heise *et al.* (1998); Robinson *et al.* (1992)
Median Absolute Error (MdAE)	06.00 mg/dl	10.40 to 19.10 mg/dl	Valgimigli *et al.* (2010)
Root Mean Squared Error (RMSE)	21.06 mg/dl	25.00 to 46.00 mg/dl	Guevara *et al.* (2010); Ozaki *et al.* (2009); Tuchin (2009)
Percentage of Mean Absolute Relative Error (% MARE)	13.89%	08.60 to 40.80%	Pai *et al.* (2015); Mohammadi *et al.* (2014); Vashist (2012); Ramchandani *et al.* (2012); Caduff *et al.* (2011); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Caduff *et al.* (2009); Lipson *et al.* (2009); Gabbay *et al.* (2008); Amir *et al.* (2007); Weiss *et al.* (2007); Bockle *et al.* (2002); Malchoff *et al.* (2002); Tamada *et al.* (1999)
Percentage of Median Absolute Relative Error (% MdARE)	05.14%	07.70 to 30.00%	Harman-Boehm *et al.* (2010); Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2009); Gabbay *et al.* (2008); Lipson *et al.* (2009); Weiss *et al.* (2007); Zhao (2002); Bockle *et al.* (2002); Zilberman *et al.* (2009)

Further, its accuracy levels are comparable with other commercially existing Continuous Glucose Monitoring System. Subsequently, all these overlaid accuracy measures based statistical analysis illustrates the strong promising aspect for developing noninvasive procedure for blood glucose estimation in *in-vitro* samples as obtained from the human subjects.

5.2.5.2 Result of Phase II:

This section describes the Clarke and Parkes Error Grid analysis of fasting, postprandial and random stage as obtained. Further, the statistical parameters were-utilized for accuracy assessment and the results comparison with published data ranges of other developing glucose monitoring techniques.

The figure 5.14 illustrates Clarke Error Grid analysis of all the reference and predicted blood glucose data pair sets as acquired during Fasting, Postprandial and Random stage based clinical examination over eleven healthy and nineteen diabetic study subjects.

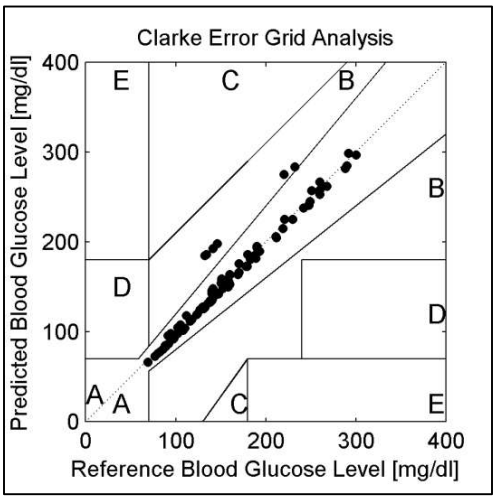

Figure 5.14: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 30 human subject's blood plasma mixed with Intralipid^{TM} samples.

Table 5.7: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired during fasting, postprandial and random stage examination over 30 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	84	93.33%
B Zone	**Medically acceptable**	06	06.67%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

In Table 5.7, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (90) falling in the zones A, B, C, D, and E are 93.33% (84 data pairs), 06.67% (06 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Consequently, all the 90 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

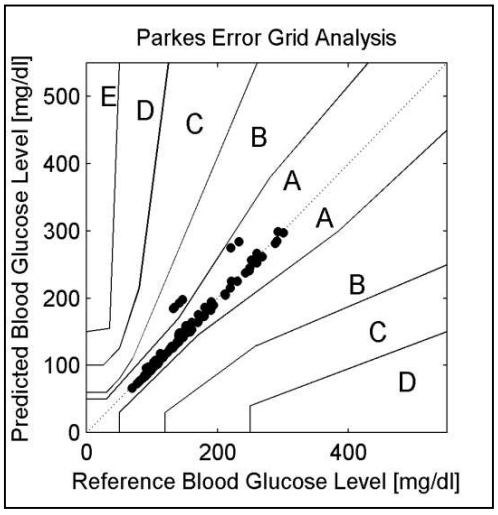

Figure 5.15: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 30 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Table 5.8: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during fasting, postprandial and random stage examination over 30 human subject's blood plasma mixed with Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	86	95.56%
B Zone	**Slight**	04	04.44%
C Zone	**Moderate**	00	00.00%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 5.15 and Table 5.8 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired during fasting, postprandial and random stage based clinical examination over eleven healthy and nineteen diabetic study subjects. The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (90) falling in zones A, B, C, D, and E are 95.56% (86 data pairs), 04.44% (04 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Consequently, the Parkes Error Grid analysis illustrates that 95.56% (86 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 04.44% (04 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

The Table 5.9 illustrates our performance assessment values as procured during fasting, postprandial and random stage based results obtained from eleven normal and nineteen diabetic study subjects. Further, results were-compared with published data ranges of other developing glucose monitoring techniques. The performance metrics based errors such as Pearson's Correlation Coefficient (r) values and SEP (Standard Error of Prediction) were 00.97 and 14.46 mg/dl respectively. The MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 07.88 mg/dl, 05.00 mg/dl, and 14.40 mg/dl respectively.

Table 5.9: Statistical parameters utilized for accuracy assessment and the results comparison with the published data ranges of other developing glucose monitoring techniques.

Statistical Parameters	Assessment Values	Published Data Ranges of other Developing Glucose Monitoring Techniques	References
Pearson Correlation Coefficient (R-Value)	00.97	00.49 to 00.95	Vaddiraju *et al.* (2010); Tuchin (2009); Oliver *et al.* (2009)
Standard Error of Prediction (SEP)	14.46 mg/dl	07.10 to 35.30 mg/dl	Ozaki *et al.* (2009); Yoon (2009); Tuchin (2009); Heise *et al.* (1998)
Mean Absolute Error (MAE)	07.88 mg/dl	07.00 to 30.00 mg/dl	Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Myllyla *et al.* (2009); Tuchin (2009); Enejder *et al.* (2005); Bockle *et al.* (2002); Zhao (2002); Heise *et al.* (1998); Robinson *et al.* (1992)
Median Absolute Error (MdAE)	05.00 mg/dl	10.40 to 19.10 mg/dl	Valgimigli *et al.* (2010)
Root Mean Squared Error (RMSE)	14.40 mg/dl	25.00 to 46.00 mg/dl	Guevara *et al.* (2010); Ozaki *et al.* (2009); Tuchin (2009)
Percentage of Mean Absolute Relative Error (% MARE)	05.20%	08.60 to 40.80%	Pai *et al.* (2015); Mohammadi *et al.* (2014); Vashist (2012); Ramchandani *et al.* (2012); Caduff *et al.* (2011); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Caduff *et al.* (2009); Lipson *et al.* (2009); Gabbay *et al.* (2008); Amir *et al.* (2007); Weiss *et al.* (2007); Bockle *et al.* (2002); Malchoff *et al.* (2002); Tamada *et al.* (1999)
Percentage of Median Absolute Relative Error (% MdARE)	03.24%	07.70 to 30.00%	Harman-Boehm *et al.* (2010); Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2009); Gabbay *et al.* (2008); Lipson *et al.* (2009); Weiss *et al.* (2007); Zhao (2002); Bockle *et al.* (2002); Zilberman *et al.* (2009)

Further, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE

(Percentage of Median Absolute Relative Error) values were 05.20%, and 03.24% respectively. Further, as illustrated from Table 5.9, the output results acquired by our MUS-IR unit is better than or comparable with other developing blood glucose measuring techniques for noninvasive blood glucose monitoring. Further, its accuracy levels are comparable with other commercially existing Continuous Glucose Monitoring System. Consequently, all these overlaid accuracy measures based statistical analysis illustrates the strong promising aspect for developing noninvasive procedure for blood glucose estimation in *in-vitro* samples as obtained from the human subjects.

5.2.6 Conclusion:

In this present work, we illustrated the feasibility of the modulated ultrasound and infrared light based procedure for blood glucose estimation *in-vitro* Intralipid^{TM} mixed blood samples. The *in-vitro* results produce promising results. In addition, the Error grid analysis and statistical analysis illustrates the acceptable and effectiveness of the proposed method in measuring blood glucose levels *in-vitro* samples as acquired during OGTT and fasting, postprandial and random stages respectively. Our proposed method has been clinically safe and simple to use as reflected from the overall study subject's compliances.

5.3 Measurement of glucose by using modulating ultrasound-infrared light and GOD/POD method in normal and diabetic human blood serum:

5.3.1 Introduction:

In near future our country will have highest number of diabetic patients as compared to global prevalence [Danaei *et al.* (2011); Wild *et al.* (2004); IDF (2009)]. The invention of noninvasive blood glucometer would serve as blessing for the suffering diabetic community [Tura *et al.* (2007); Khalil (2004)]. Encouraged by this aspect, we have performed *in-vitro* experimentations by mixing blood serum and Intralipid^{TM} phantom samples to mimic real life scenarios.

The experimental studies for blood glucose detection in human blood serum mixed Intralipid^{TM} samples were conducted to evaluate the working of amplitude modulated ultrasound and Infrared light techniques. This present work consists of two phases:

(i) The effect of various glucose concentrations in *in-vitro* normal and diabetic human blood serum mixed with Intralipid^{TM} phantom samples.

(ii) To examine the glucose concentration levels in *in-vitro* samples of different stages such as fasting, postprandial and random stage respectively.

5.3.2 Study subjects:

In total thirty subjects inclusive of normal and diabetic subjects (twenty-three males and seven females) participated in this first phase of this clinical study. Here, the study subjects includes normal and diabetic subjects of age = 28±3 years, height = 170±5.5 cm, and weight = 60±10 kg. The experimentation were performed for three consecutive days.

Further, in second phase, thirty-one more adults inclusive of normal and diabetic subjects (twenty-three males and eight females) are participated for measuring blood glucose levels during their fasting stage, postprandial stage and random stage respectively. Here, the study subjects includes normal and diabetic subjects of age = 46±15 years, height = 171±5.5 cm, and weight = 74±5.5 kg. The experimentation were-performed for two consecutive days.

The clinical studies reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of Institute of Medical Sciences-Banaras Hindu University, Varanasi approved the clinical study.

5.3.3 Experimental procedure:

5.3.3.1 Preparation of human blood serum samples:

Whole blood sample of 5 ml is withdrawn from the veins of the study subjects. Total thirty subjects (normal and diabetic) selected for phase I (random test) investigation and thirty-one subjects for phase II (fasting, postprandial and random stage based tests) were-selected for this experimental purpose. The whole blood samples collected were allowed to clot at normal room temperature for 15-30 minutes. Herein, centrifugation process applied for breaking the formation of clot and serum respectively. After transferring the serum into the safe place through pasteur pipette. All the samples as above mentioned were stored at 2-8^{0}C [Raghu (2003)] for examining the random samples of phase one and fasting-postprandial-random stage of phase two-based samples as obtained from the normal and diabetic subjects for *in-vitro* glucose concentration measurements. After that, 01 ml of the prepared blood serum sample has-been-utilized for blood glucose concentration-measurement by

MUS-IR unit. Simultaneously, for performing GOD/POD method to measure glucose concentration 02 ml of the prepared blood serum sample has been utilized here.

5.3.4 Result and Discussion:

5.3.4.1 Phase I: *In-vitro* investigation on normal and diabetic human blood serum mixed Intralipid™ samples:

This phase depicts the *in-vitro* investigation on normal and diabetic human blood serum mixed Intralipid™ samples. Further, phase I is divided into two-parts (i) *in-vitro* study of normal human blood serum mixed with Intralipid™ samples and (ii) *in-vitro* study of diabetic human blood serum mixed with Intralipid™ samples. The experimental study conducted for three consecutive days.

5.3.4.2 Part I: *In-vitro* study of normal human blood serum mixed with Intralipid™ samples:

In this part, the blood serum samples of fifteen normal subjects with Intralipid™ tissue phantom are-checked for their respective glucose levels in indigenously designed Modulated Ultrasound Infrared (MUS-IR) unit and by the established GOD/POD method performed through the digital spectrophotometer. The results of the samples in the figure 5.16, figure 5.18 and figure 5.20 shows that the glucose levels were in normal ranges respectively.

5.3.4.3 Part II: *In-vitro* study of diabetic human blood serum mixed with Intralipid™ samples:

This part of the experiment deals with fifteen diabetic subjects blood serum with Intralipid™ as tissue phantom mixed samples. Here, the glucose levels in all samples were-obtained after processing through our Modulated Ultrasound Infrared (MUS-IR) unit and the established GOD/POD method performed by digital spectrophotometer. The data as seen in figure 5.17, figure 5.19 and figure 5.21 indicates that the glucose concentration of diabetic patients occupies the higher level as compared to the normal subject's glucose levels respectively.

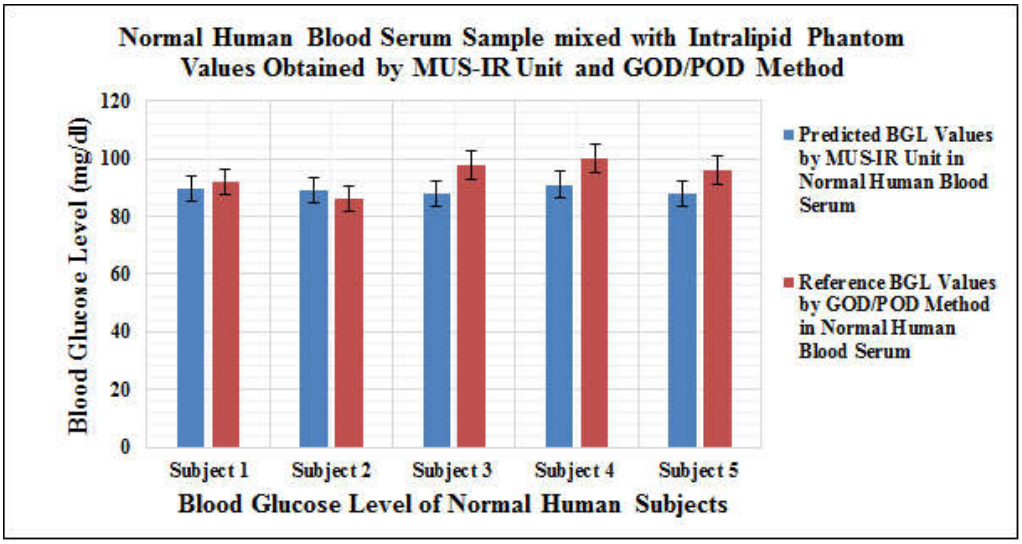

Figure 5.16: Normal human blood serum mixed with Intralipid^{TM} phantom samples response bars of the study subjects (1 to 5) on 1st day; error bars indicate ±5 percentage error.

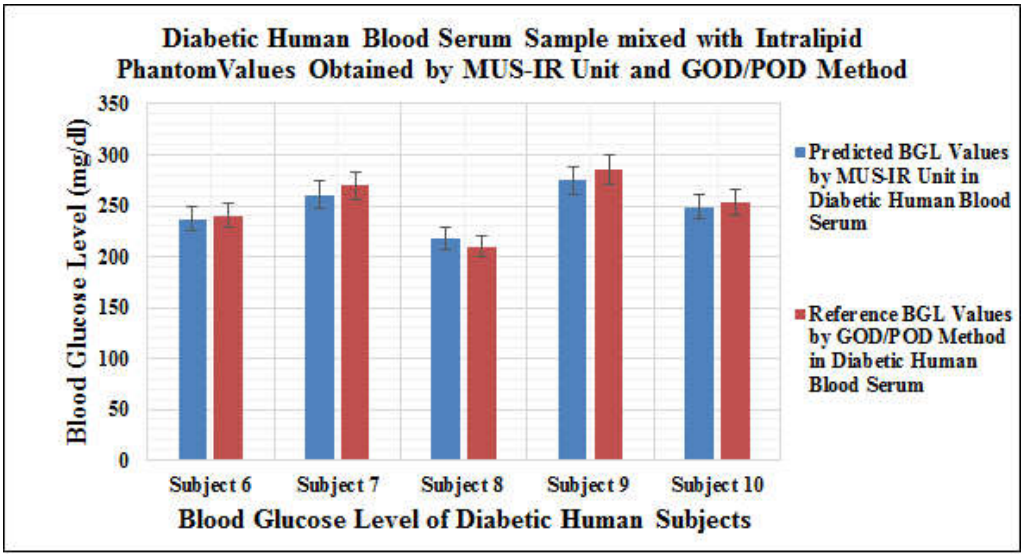

Figure 5.17: Diabetic human blood serum mixed with Intralipid^{TM} phantom samples response bars of the study subjects (6 to 10) on 1st day; error bars indicate ±5 percentage error.

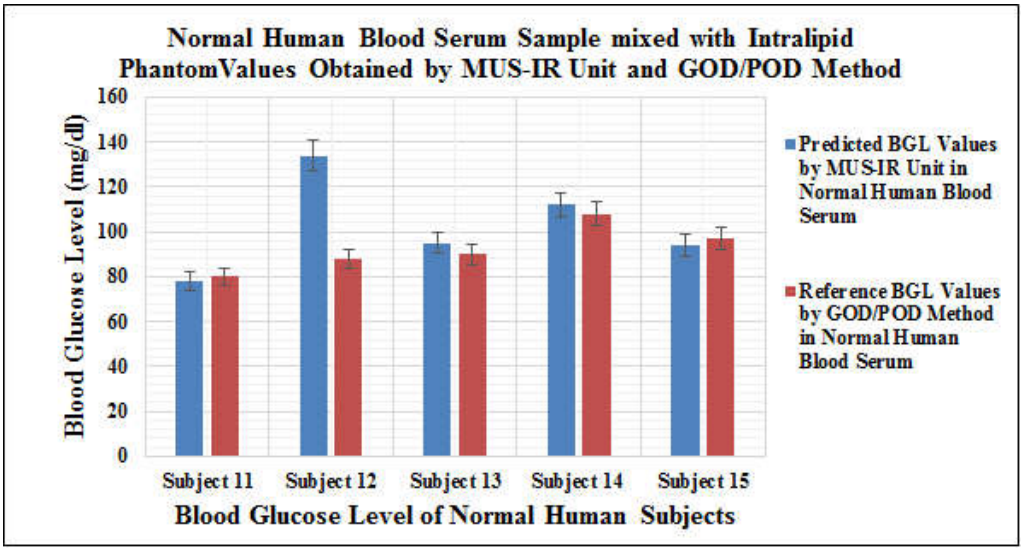

Figure 5.18: Normal human blood serum mixed with Intralipid^{TM} phantom samples response bars of the study subjects (11 to 15) on 2nd day; error bars indicate ±5 percentage error.

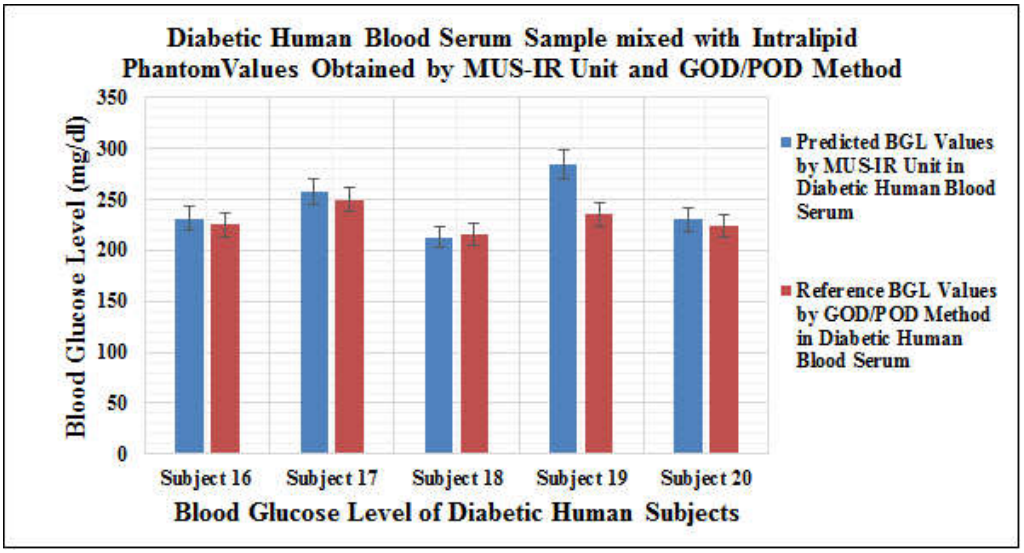

Figure 5.19: Diabetic human blood serum mixed with Intralipid^{TM} phantom samples response bars of the study subjects (16 to 20) on 2nd day; error bars indicate ±5 percentage error.

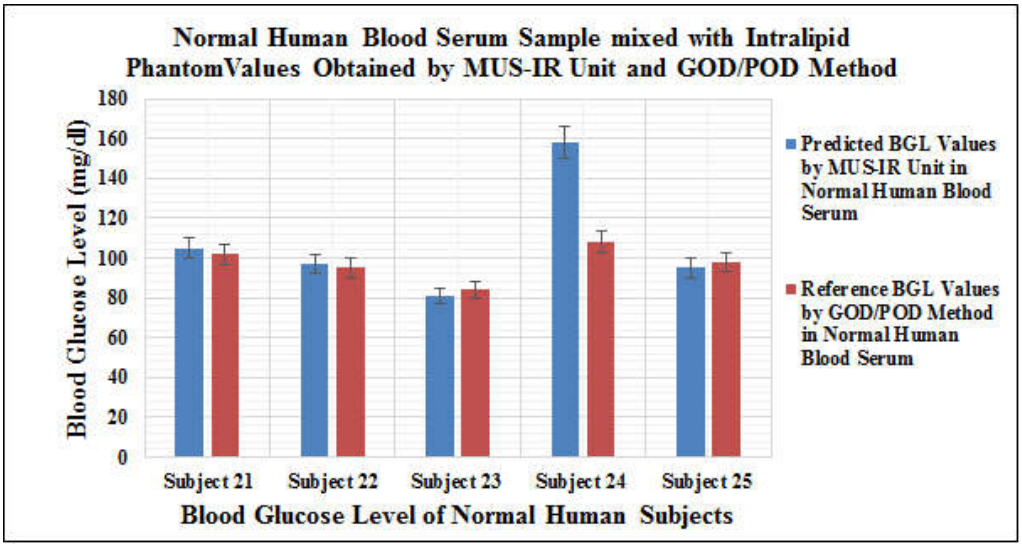

Figure 5.20: Normal human blood serum mixed with Intralipid^{TM} phantom samples response bars of the study subjects (21 to 25) on 3rd day; error bars indicate ±5 percentage error.

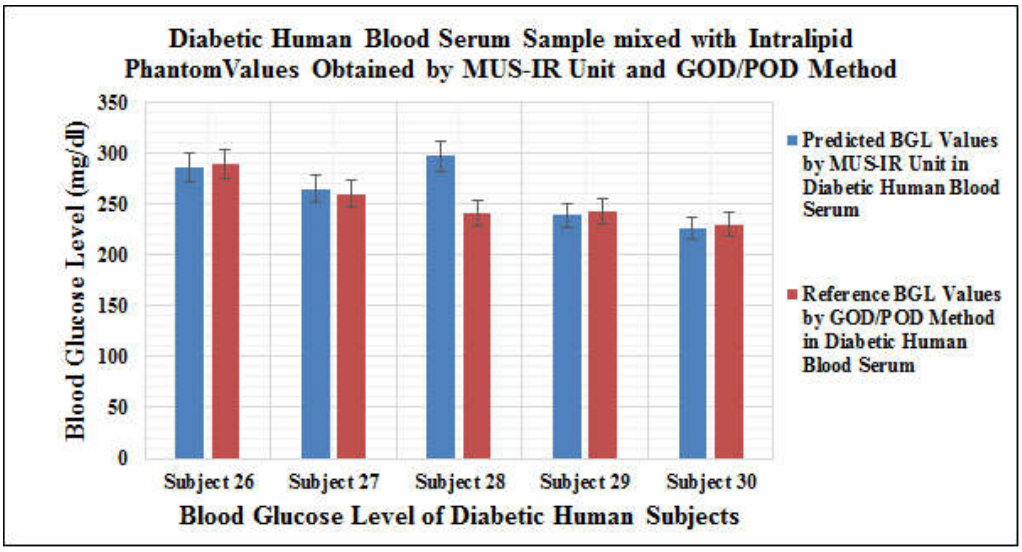

Figure 5.21: Diabetic human blood serum mixed with Intralipid^{TM} phantom samples response bars of the study subjects (26 to 30) on 3rd day; error bars indicate ±5 percentage error.

5.3.4.4 Phase I: Error Grid (Clarke and Parkes) and statistical analysis on normal and diabetic human blood serum mixed with Intralipid^{TM} phantom samples.

The Error Grid (Clarke and Parkes) and statistical analysis is used here to measure the performance metrics of our *in-vitro* technique based prototype unit in measuring blood glucose levels in human blood serum mixed with Intralipid^{TM} phantom samples. The figure 5.22 to figure 5.23 and Table 5.10 to Table 5.12 represent the error grid and statistical analysis respectively. The figure 5.22 illustrates Clarke Error Grid analysis of all the reference and predicted blood glucose data pair sets as acquired during normal and diabetic stage based clinical examination over fifteen normal and fifteen diabetic study subjects.

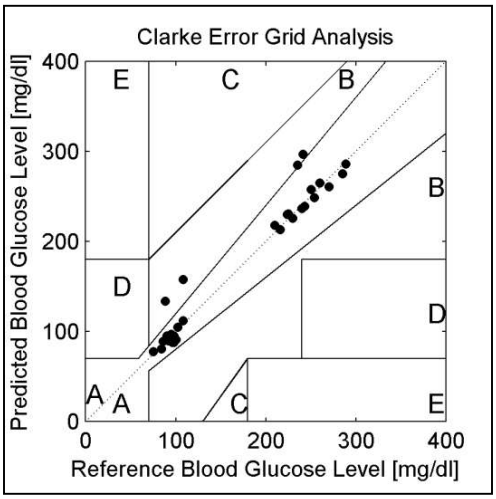

Figure 5.22: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 15 normal and 15 diabetic human subject's blood serum mixed with Intralipid^{TM} phantom samples.

Table 5.10: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired from 15 normal and 15 diabetic human subject's blood serum mixed with Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	26	86.67%
B Zone	**Medically acceptable**	04	13.33%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

In Table 5.10, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (30) falling in the zones A, B, C, D, and E are 86.67% (26 data pairs), 13.33% (04 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Consequently, all the 30 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

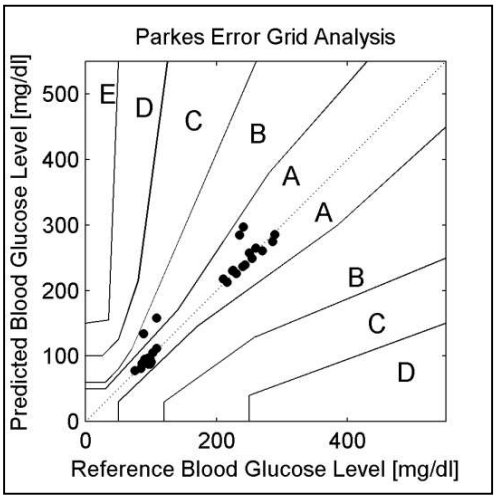

Figure 5.23: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 15 normal and 15 diabetic human subject's blood serum mixed with Intralipid^{TM} phantom samples.

Table 5.11: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired from 15 normal and 15 diabetic human subject's blood serum mixed with Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	28	93.33%
B Zone	**Slight**	02	06.67%
C Zone	**Moderate**	00	00.00%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 5.23 and Table 5.11 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired during Normal and Diabetic stage based clinical examination over fifteen normal and fifteen diabetic study subjects. The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (30) falling in zones A, B, C, D, and E are 93.33% (28 data pairs), 06.67% (02 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Consequently, the Parkes Error Grid analysis illustrates that 93.33% (28 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 06.67% (02 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically satisfactory acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

The Table 5.12 illustrates our performance assessment values as obtained during Normal and Diabetic stage based clinical examination over fifteen normal and fifteen diabetic study subjects. The results are compared with published data ranges of other developing glucose monitoring techniques. The performance metrics based errors such as Pearson's Correlation Coefficient (r) values and SEP (Standard Error of Prediction) were 00.97 and 18.96 mg/dl respectively. The MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 11.14 mg/dl, 05.00 mg/dl, and 19.23 mg/dl respectively. Henceforth, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 07.83%, and 03.27% respectively.

Further, as illustrated from Table 5.12, the output results obtained by our MUS- IR technique is better than or comparable with other developing blood glucose measuring techniques for noninvasive blood glucose monitoring.

Table 5.12: Statistical parameters utilize for accuracy assessment and the results comparing with published data ranges of other developing glucose monitoring techniques.

Statistical Parameters	Assessment Values	Published Data Ranges of other Developing Glucose Monitoring Techniques	References
Pearson Correlation Coefficient (R-Value)	00.97	00.49 to 00.95	Vaddiraju *et al.* (2010); Tuchin (2009); Oliver *et al.* (2009)
Standard Error of Prediction (SEP)	18.96 mg/dl	07.10 to 35.30 mg/dl	Ozaki *et al.* (2009); Yoon (2009); Tuchin (2009); Heise *et al.* (1998)
Mean Absolute Error (MAE)	11.14 mg/dl	07.00 to 30.00 mg/dl	Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Myllyla *et al.* (2009); Tuchin (2009); Enejder *et al.* (2005); Bockle *et al.* (2002); Zhao (2002); Heise *et al.* (1998); Robinson *et al.* (1992)
Median Absolute Error (MdAE)	05.00 mg/dl	10.40 to 19.10 mg/dl	Valgimigli *et al.* (2010)
Root Mean Squared Error (RMSE)	19.23 mg/dl	25.00 to 46.00 mg/dl	Guevara *et al.* (2010); Ozaki *et al.* (2009); Tuchin (2009)
Percentage of Mean Absolute Relative Error (% MARE)	07.83%	08.60 to 40.80%	Pai *et al.* (2015); Mohammadi *et al.* (2014); Vashist (2012); Ramchandani *et al.* (2012); Caduff *et al.* (2011); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Caduff *et al.* (2009); Lipson *et al.* (2009); Gabbay *et al.* (2008); Amir *et al.* (2007); Weiss *et al.* (2007); Bockle *et al.* (2002); Malchoff *et al.* (2002); Tamada *et al.* (1999)
Percentage of Median Absolute Relative Error (% MdARE)	03.27%	07.70 to 30.00%	Harman-Boehm *et al.* (2010); Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2009); Gabbay *et al.* (2008); Lipson *et al.* (2009); Weiss *et al.* (2007); Zhao (2002); Bockle *et al.* (2002); Zilberman *et al.* (2009)

Further, its accuracy levels are additionally comparative with other commercially existing Continuous Glucose Monitoring System. Henceforth, all these overlaid accuracy measures based statistical analysis depicts the strong promising

aspect for developing noninvasive procedure for blood glucose estimation in *in-vitro* samples as obtained from the human subjects.

5.3.4.5 Phase II: *In-vitro* investigation at fasting, postprandial and random stage in human blood serum mixed with Intralipid^{TM} phantom samples.

The *in-vitro* investigation on normal and diabetic human blood serum mixed Intralipid^{TM} samples at different investigational stage such as fasting, postprandial and random respectively was conducted. The experimental study conducted on two consecutive days.

This portion of the experiment deals with thirty-one normal and diabetic subject's blood serum samples which is mixed with the Intralipid^{TM} tissue phantom. Here, the glucose levels in all the samples were obtained after processing through our Modulated Ultrasound Infrared (MUS-IR) unit and the established GOD/POD method performed by digital spectrophotometer. The data as seen in figure 5.24 to figure 5.25 indicates that the glucose concentration varies according the different investigational stage such as fasting, postprandial and random stage based *in-vitro* study. This accurate, stable working of our prototype for glucose detection would be helpful for developing noninvasive optical glucometer.

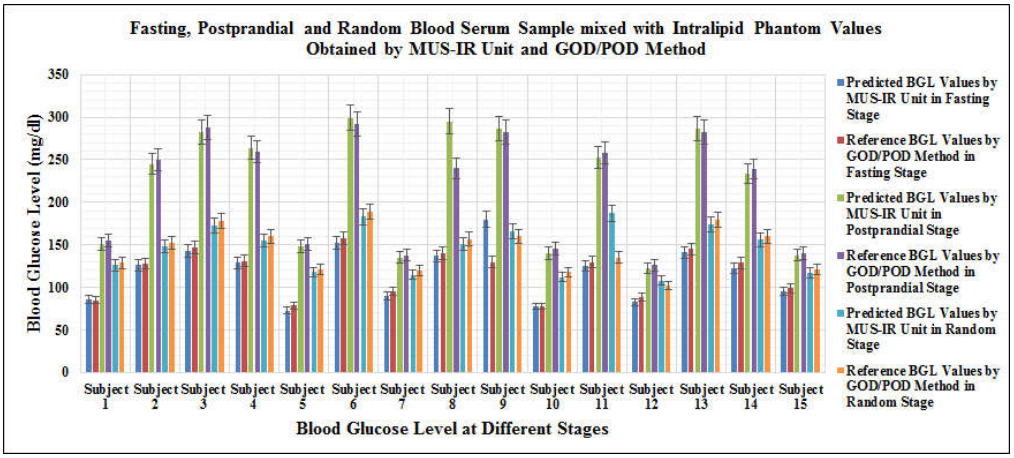

Figure 5.24: Fasting, postprandial and random stage response bars of the study subjects (01 to 15) on 1st day; error bars indicate ±5 percentage error.

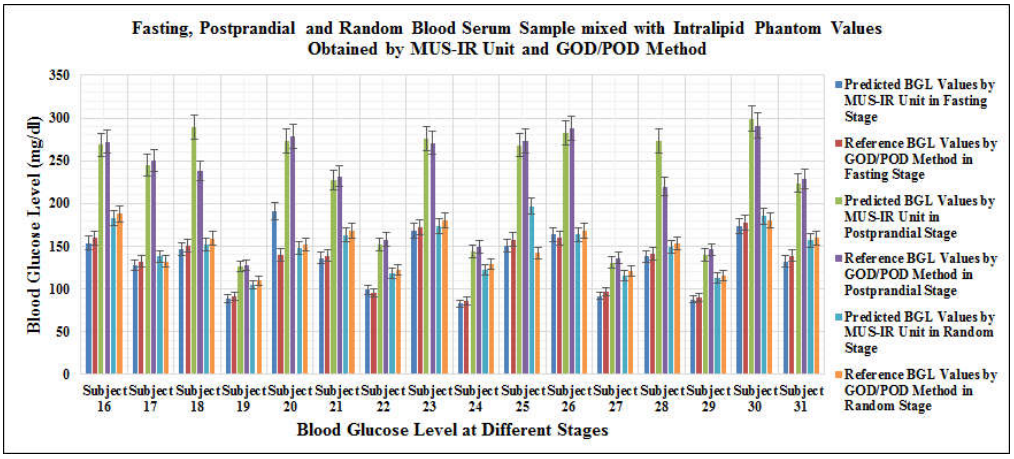

Figure 5.25: Fasting, postprandial and random stage response bars of the study subjects (16 to 31) on 2nd day; error bars indicate ±5 percentage error.

5.3.4.6 Phase II:

The Error Grid (Clarke and Parkes) and statistical analysis were-used here to measure the performance metrics of our *in-vitro* technique based prototype unit in measuring blood glucose levels in human blood serum mixed with Intralipid™ phantom samples. The figure 5.26 to figure 5.27 and Table 5.13 to Table 5.15 represent the error grid and statistical analysis respectively.

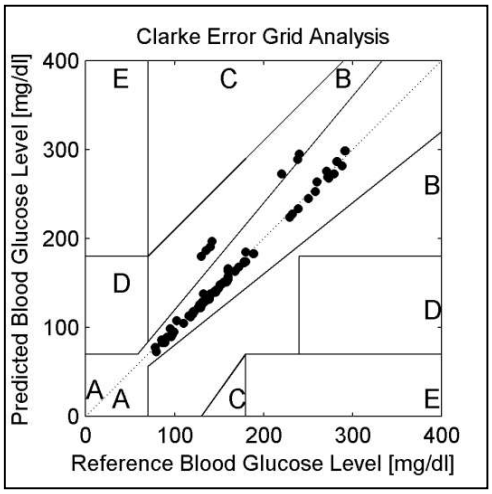

Figure 5.26: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 31 human subject's blood serum mixed with Intralipid™ phantom samples.

The figure 5.26 illustrates Clarke Error Grid analysis of all the reference and predicted blood glucose data pair sets as acquired during Fasting, Postprandial and

Random stage based clinical examination over eleven normal and twenty diabetic study subjects.

Table 5.13: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired during fasting, postprandial and random stage examination over 31 human subject's blood serum mixed with Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	86	92.47%
B Zone	**Medically acceptable**	07	07.53%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

In Table 5.13, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (93) falling in the zones A, B, C, D, and E are 92.47% (86 data pairs), 07.53% (07 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Subsequently, all the 93 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

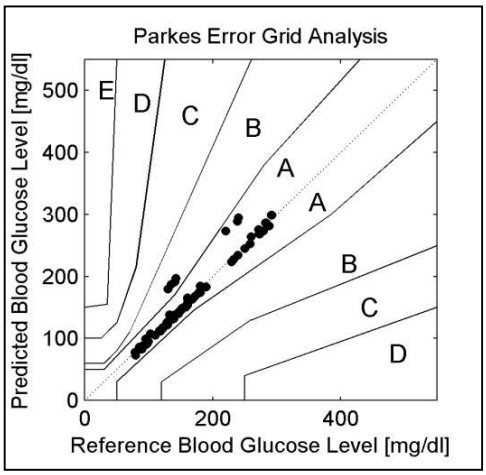

Figure 5.27: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 31 human subject's blood serum mixed with Intralipid^{TM} phantom samples.

Table 5.14: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during fasting, postprandial and random stage examination over 31 human subject's blood serum mixed with Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	89	95.70%
B Zone	**Slight**	04	04.30%
C Zone	**Moderate**	00	00.00%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 5.27 and Table 5.14 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired during fasting, postprandial and random stage based clinical examination over eleven normal and twenty diabetic study subjects.

The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (93) falling in zones A, B, C, D, and E are 95.70% (89 data pairs), 04.30% (04 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Subsequently, the Parkes Error Grid analysis illustrates that 95.70% (89 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 04.30% (04 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

The Table 5.15 illustrates our performance assessment values as obtained during Fasting, Postprandial, Random stage based clinical examination over eleven normal, and twenty diabetic study subjects and the results were-compared with published data ranges of other developing glucose monitoring technique. The performance metrics based errors such as Pearson's Correlation Coefficient (r) values and SEP (Standard Error of Prediction) were 00.97 and 15.14 mg/dl respectively. The MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 08.16 mg/dl, 05.00 mg/dl, and 15.12 mg/dl respectively.

Table 5.15: Statistical parameters utilized for accuracy assessment and the results comparison with the published data ranges of other developing glucose monitoring techniques.

Statistical Parameters	Assessment Values	Published Data Ranges of other Developing Glucose Monitoring Techniques	References
Pearson Correlation Coefficient (R-Value)	00.97	00.49 to 00.95	Vaddiraju *et al.* (2010); Tuchin (2009); Oliver *et al.* (2009)
Standard Error of Prediction (SEP)	15.14 mg/dl	07.10 to 35.30 mg/dl	Ozaki *et al.* (2009); Yoon (2009); Tuchin (2009); Heise *et al.* (1998)
Mean Absolute Error (MAE)	08.16 mg/dl	07.00 to 30.00 mg/dl	Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Myllyla *et al.* (2009); Tuchin (2009); Enejder *et al.* (2005); Bockle *et al.* (2002); Zhao (2002); Heise *et al.* (1998); Robinson *et al.* (1992)
Median Absolute Error (MdAE)	05.00 mg/dl	10.40 to 19.10 mg/dl	Valgimigli *et al.* (2010)
Root Mean Squared Error (RMSE)	15.12 mg/dl	25.00 to 46.00 mg/dl	Guevara *et al.* (2010); Ozaki *et al.* (2009); Tuchin (2009)
Percentage of Mean Absolute Relative Error (% MARE)	05.16 mg/dl	08.60 to 40.80%	Pai *et al.* (2015); Mohammadi *et al.* (2014); Vashist (2012); Ramchandani *et al.* (2012); Caduff *et al.* (2011); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Caduff *et al.* (2009); Lipson *et al.* (2009); Gabbay *et al.* (2008); Amir *et al.* (2007); Weiss *et al.* (2007); Bockle *et al.* (2002); Malchoff *et al.* (2002); Tamada *et al.* (1999)
Percentage of Median Absolute Relative Error (% MdARE)	02.76 mg/dl	07.70 to 30.00%	Harman-Boehm *et al.* (2010); Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2009); Gabbay *et al.* (2008); Lipson *et al.* (2009); Weiss *et al.* (2007); Zhao (2002); Bockle *et al.* (2002); Zilberman *et al.* (2009)

Further, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 05.16%, and 02.76% respectively. Further, as illustrated from Table 5.15, the output results acquired by our MUS-IR method is better than or comparable with other developing blood glucose measuring techniques for noninvasive blood glucose monitoring. Further, its accuracy levels are likewise comparable with other commercially existing Continuous Glucose Monitoring System. Henceforth, all these overlaid accuracy measures based statistical analysis illustrates the strong promising aspect for developing noninvasive procedure for blood glucose estimation in *in-vitro* samples as obtained from the human subjects.

5.3.5 Conclusion:

The amplitude modulated ultrasounds with infrared techniques were used here to measure glucose concentration in human blood serum mixed with Intralipid^{TM} as tissue phantom. Results confirm the accurate working of our developed prototype. In near future, this technique would be applied for various experiments to design and develop noninvasive blood glucometer.

5.4 Measurement of glucose concentration in human whole blood mixed Intralipid^{TM} phantom samples of healthy and diabetic subjects:

5.4.1 Introduction:

This present work consists of two phases:

(i) The effect of various glucose concentrations in healthy and diabetic human whole blood samples mixed with Intralipid^{TM} phantom *in-vitro* based study.

(ii) Examination of the glucose concentration level in different stages such as fasting, postprandial and random stage respectively.

5.4.2 Study subjects:

In total ten subjects were five non-diabetic subjects (normal healthy) adults (three male and two female). The aged 33.8±4.2 years, of height 164.8±8.8 cm, weight 56.6±13.1 kg and five diabetic subjects (three type-I male and two type-II male). The aged 42.3±11:9 years, of height 159.6±7.9 cm, weight 50.1± 14:5 kg participated in this healthy and diabetic based first phase of clinical study. Further, in second phase, twenty-three more adults inclusive of healthy and diabetic subjects (eighteen males and five females) are participated for measuring blood glucose levels during their fasting stage, postprandial stage and random stages respectively. Here,

the study subjects includes healthy and diabetic subjects of age = 48±15 years, height = 170±5.5 cm and weight = 75±5.5 kg. The experimentation were performed for two consecutive days. The clinical studies reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of Institute of Medical Sciences-Banaras Hindu University, Varanasi approved the clinical study.

5.4.3 Preparation of whole blood samples-experimental steps:

The Intralipid^{TM} phantom had been designed to mimic the scattering and absorption properties of the human finger in the near-infrared domain. Whereas absorption is accounted for the direct use of Intralipid^{TM} mixed whole blood samples [Amir *et al.* (2007)].

Both components are mixed to mimic the blood–tissue compound and are inserted into a sample holder. During the experimentation, following three physiological parameters have been explored:

(i) Whole blood (1 ml) from healthy and diabetic subjects were collected in vacuum-based blood collecting vials where K_2 EDTA is present as anticlotting agent.

(ii) Deoxygenation is induced through nitrogen bubbling of the whole blood samples for 45 minutes. The variability of the hematocrit and oxygen concentrations hinders the glucose-induced effect in the output data. This property indicates the impact of physiological changes on the acquisition of the glucose signature based signals.

(iii) Phosphate buffer solution (PBS) has been used to maintain the pH level of the whole blood samples during the experimental procedures. Afterwards, 3 ml of Intralipid^{TM} suspension has been added to it for the experimental purposes.

5.4.4 Result and Discussion:

5.4.4.1 The experiments were-performed in two phases:

The experimental studies for blood glucose detection in human whole blood mixed Intralipid^{TM} samples were conducted to evaluate the working of amplitude modulated ultrasound and Infrared techniques.

5.4.4.2 Phase I: *In-vitro* investigation of the blood glucose levels in healthy and diabetic subject's whole blood mixed Intralipid^{TM} samples:

Phase I consists of two parts. Part I includes *in-vitro* study of healthy human whole blood mixed Intralipid^{TM} phantom samples and part II includes *in-vitro* study of diabetic human whole blood mixed Intralipid^{TM} phantom samples.

5.4.4.3 Part I: *In-vitro* study of healthy human whole blood mixed Intralipid^{TM} phantom samples.

In this part, the whole blood samples of five healthy subjects were mixed with the Intralipid^{TM} tissue phantoms. Afterwards prepared samples were processed for their respective glucose concentration determination by the indigenously designed Modulated Ultrasound Infrared (MUS-IR) unit and the established GOD/POD method. The results of the samples in the figure 5.28 shows that the glucose levels in the normal ranges.

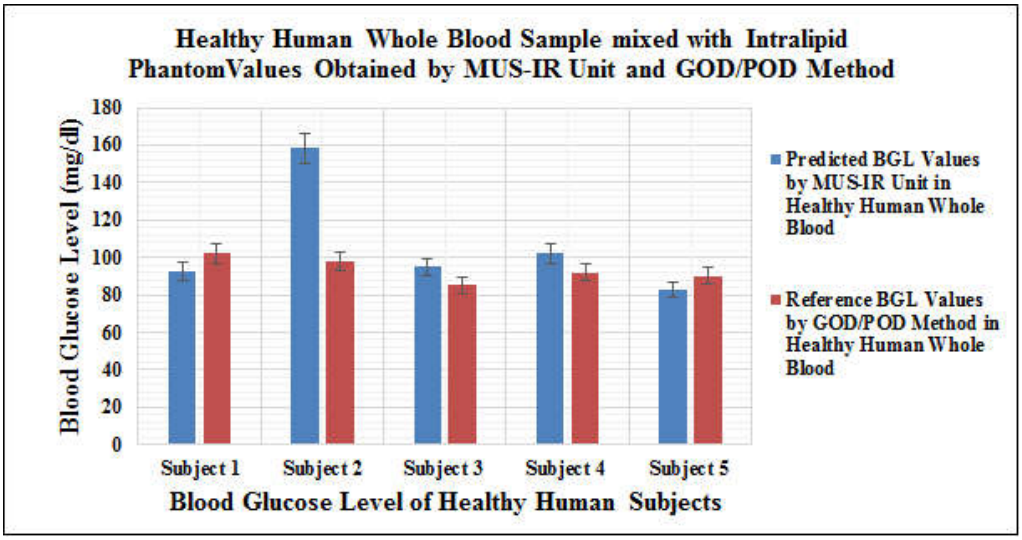

Figure 5.28: Healthy human whole blood mixed with Intralipid^{TM} phantom samples response bars of the study subjects (1 to 5); error bars indicate ±5 percentage error.

5.4.4.4 Part II: *In-vitro* study of diabetic human whole blood mixed with Intralipid^{TM} phantom samples.

This part of the experiment deals with five Diabetic subject's whole blood mixed with Intralipid^{TM} tissue phantom samples. Here, both the glucose levels in those samples were obtained after processing through our Modulated Ultrasound Infrared (MUS-IR) unit and the established GOD/POD method performed by digital spectrophotometer. The data as depicts in figure 5.29 indicates that the glucose concentration of diabetic patients occupies the higher concentrations as compared to

the normal subject's glucose levels. This accurate, stable working of our prototype detection would be helpful for developing noninvasive optical glucometer.

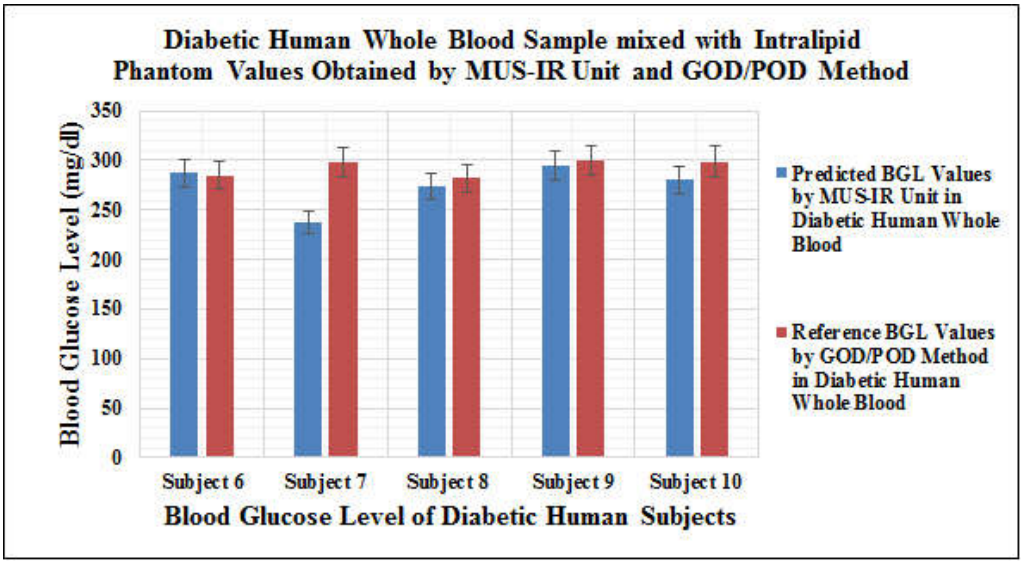

Figure 5.29: Diabetic human whole blood mixed with Intralipid^{TM} phantom samples response bars of the study subjects (6 to 10); error bars indicate ±5 percentage error.

5.4.4.5 Phase I result analysis: Error Grid (Clarke and Parkes) and statistical analysis were performed over the *in-vitro* whole blood mixed with Intralipid^{TM} phantom samples of healthy and diabetic study subjects.

The Error Grid (Clarke and Parkes) and statistical analysis were-used here to measure the performance metrics of our *in-vitro* technique based prototype unit in measuring blood glucose levels in human whole blood mixed with Intralipid^{TM} phantom samples. The figure 5.30 to figure 5.31 and Table 5.16 to Table 5.18 represents the error grid analysis and statistical analysis respectively.

The figure 5.30 illustrates Clarke Error Grid analysis of all the reference and predicted blood glucose data pair sets as acquired from the five healthy and five diabetic subjects during clinical investigations.

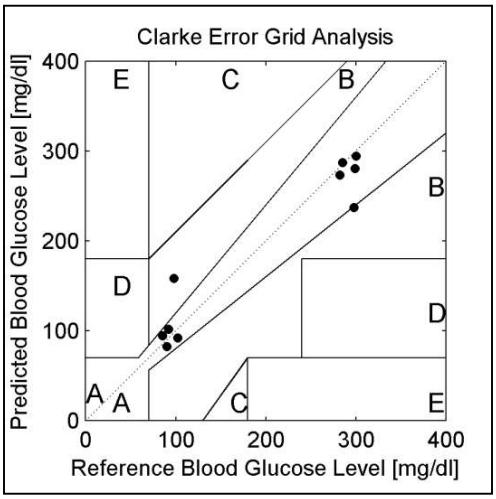

Figure 5.30: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 10 human subject's whole blood mixed Intralipid^{TM} phantom samples.

Table 5.16: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired from 10 human subject's whole blood mixed Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	08	80.00%
B Zone	**Medically acceptable**	02	20.00%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

In Table 5.16, the Clarke Error Grid analysis demonstrates the percentage of the total data sets (10) falling in the zones A, B, C, D, and E are 80.00% (08 data pairs), 20.00% (02 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Consequently, all the 10 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

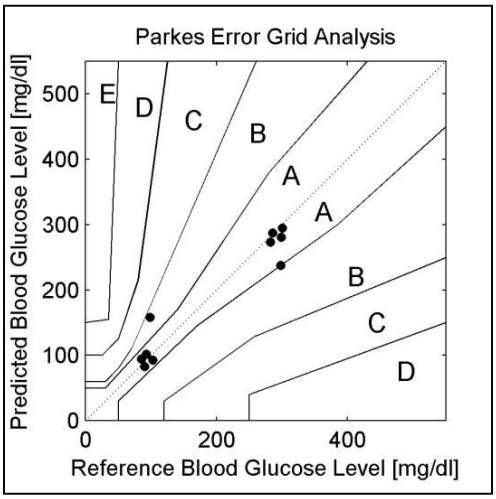

Figure 5.31: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 10 human subject's whole blood mixed Intralipid^{TM} phantom samples.

Table 5.17: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired from 10 human subject's whole blood mixed Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	09	90.00 %
B Zone	**Slight**	01	10.00 %
C Zone	**Moderate**	00	00.00 %
D Zone	**Significant**	00	00.00 %
E Zone	**Dangerous**	00	00.00 %

The figure 5.31 and Table 5.17 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired during clinical examination over five healthy and five diabetic study subjects respectively. The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (10) falling in zones A, B, C, D, and E are 90.00% (09 data pairs), 10.00% (01 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Consequently, the Parkes Error Grid analysis represents

that 90.00% (09 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 10.00% (01 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

Table 5.18: Statistical parameters utilized for accuracy assessment and the results comparison with the published data ranges of other developing glucose monitoring techniques.

Statistical Parameters	Assessment Values	Published Data Ranges of other Developing Glucose Monitoring Techniques	References
Pearson Correlation Coefficient (R-Value)	00.96	00.49 to 00.95	Vaddiraju *et al.* (2010); Tuchin (2009); Oliver *et al.* (2009)
Standard Error of Prediction (SEP)	26.45 mg/dl	07.10 to 35.30 mg/dl	Ozaki *et al.* (2009); Yoon (2009); Tuchin (2009); Heise *et al.* (1998)
Mean Absolute Error (MAE)	19.21 mg/dl	07.00 to 30.00 mg/dl	Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Myllyla *et al.* (2009); Tuchin (2009); Enejder *et al.* (2005); Bockle *et al.* (2002); Zhao (2002); Heise *et al.* (1998); Robinson *et al.* (1992)
Median Absolute Error (MdAE)	09.70 mg/dl	10.40 to 19.10 mg/dl	Valgimigli *et al.* (2010)
Root Mean Squared Error (RMSE)	28.45 mg/dl	25.00 to 46.00 mg/dl	Guevara *et al.* (2010); Ozaki *et al.* (2009); Tuchin (2009)
Percentage of Mean Absolute Relative Error (% MARE)	13.37%	08.60 to 40.80%	Pai *et al.* (2015); Mohammadi *et al.* (2014); Vashist (2012); Ramchandani *et al.* (2012); Caduff *et al.* (2011); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Caduff *et al.* (2009); Lipson *et al.* (2009); Gabbay *et al.* (2008); Amir *et al.* (2007); Weiss *et al.* (2007); Bockle *et al.* (2002); Malchoff *et al.* (2002); Tamada *et al.* (1999)
Percentage of Median Absolute Relative Error (% MdARE)	08.87%	07.70 to 30.00%	Harman-Boehm *et al.* (2010); Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2009); Gabbay *et al.* (2008); Lipson *et al.* (2009); Weiss *et al.* (2007); Zhao (2002); Bockle *et al.* (2002); Zilberman *et al.* (2009)

The Table 5.18 illustrates our performance assessment values as procured during clinical examination over five healthy and five diabetic study subjects. Further the results are-compared with published data ranges of other developing glucose monitoring techniques. The performance metrics based errors such as Pearson's Correlation Coefficient (r) values and SEP (Standard Error of Prediction) were 00.96 and 26.45 mg/dl respectively. The MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 19.21 mg/dl, 09.70 mg/dl, and 28.45 mg/dl respectively. Also, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 13.37%, and 08.87% respectively. Further, as illustrated from Table 5.18, the output results acquired by our MUS-IR unit based technique is better than or comparable with other developing blood glucose measuring techniques for noninvasive blood glucose monitoring. Further, its accuracy levels are likewise comparable with other commercially existing Continuous Glucose Monitoring System. Consequently, all these overlaid accuracy measures based statistical analysis illustrates the strong promising aspect for developing noninvasive procedure for blood glucose estimation in *in-vitro* samples as obtained from the human subjects.

5.4.4.6 Phase II: *In-vitro* investigation using fasting, postprandial and random stage based human whole blood samples mixed with Intralipid^{TM} phantom:

This part depicts the *in-vitro* investigation on healthy and diabetic human whole blood samples mixed with Intralipid^{TM} phantom samples at different investigational stages such as fasting, postprandial and random stage respectively. The experimental study has been conducted for two consecutive days.

The experiment deals with twenty-three healthy and diabetic subject's whole blood samples mixed with Intralipid^{TM} tissue phantom. Here, both the glucose levels in these samples were obtained, through our Modulated Ultrasound Infrared (MUS-IR) unit and by using established GOD/POD method performed by digital spectrophotometer. The data as seen in figure 5.32 and figure 5.33 indicates that the glucose concentration varies according the different investigational stages such as fasting, postprandial and random stage based *in-vitro* study. This accurate, stable working of our prototype for glucose detection would be helpful for developing noninvasive optical glucometer.

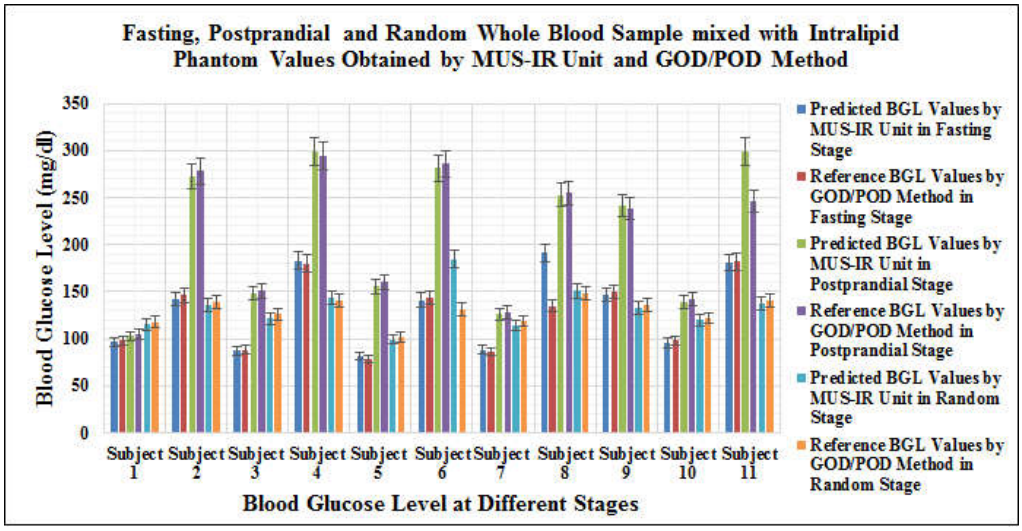

Figure 5.32: Fasting, postprandial and random stage response bars of the study subjects (1 to 11) on 1st day; error bars indicate ±5 percentage error.

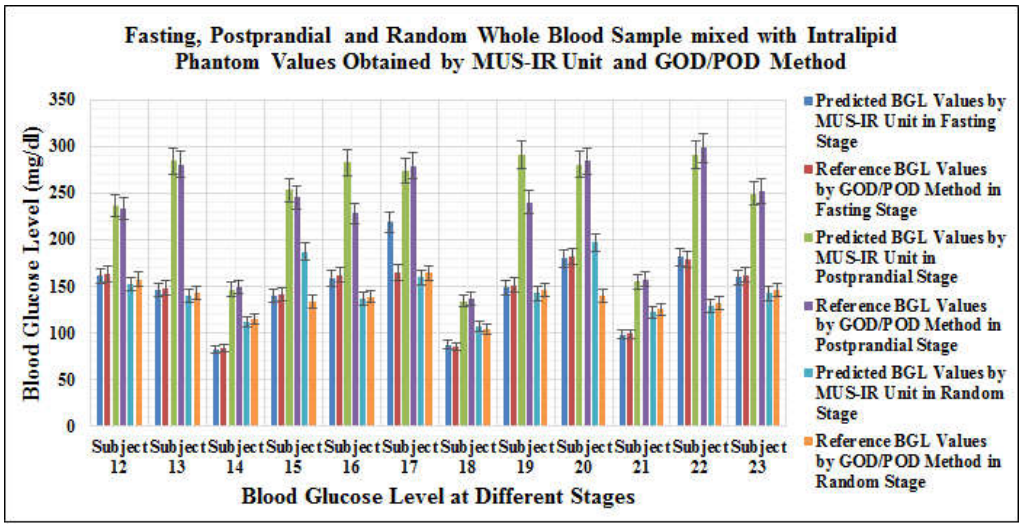

Figure 5.33: Fasting, postprandial and random stage response bars of the study subjects (12 to 23) on 2nd day; error bars indicate ±5 percentage error.

5.4.4.7 Phase II result analysis:

The Error Grid (Clarke and Parkes) and statistical analysis were-performed over the *in-vitro* whole blood mixed with Intralipid™ phantom samples as acquired during fasting, postprandial and random stages of healthy and diabetic study subjects. The Error Grid (Clarke and Parkes) and statistical analysis used here to measure the performance metrics of our *in-vitro* technique based prototype unit in measuring

blood glucose levels *in-vitro* whole blood mixed with Intralipid^{TM} phantom samples respectively. The figure 5.34 to figure 5.35 and Table 5.19 to Table 5.21 represents the error grid and statistical analysis respectively.

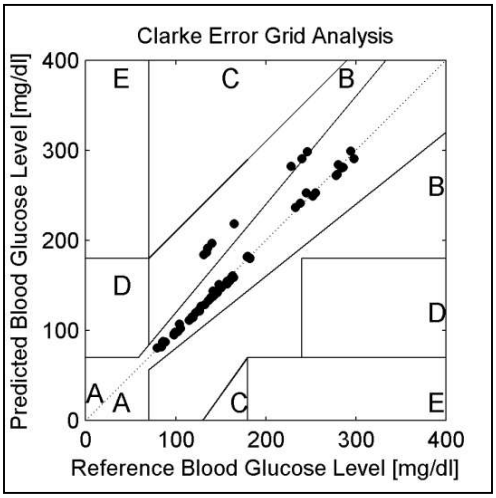

Figure 5.34: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 23 human subject's whole blood mixed with Intralipid^{TM} phantom samples.

Table 5.19: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired from 23 human subject's whole blood mixed with Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	61	88.40%
B Zone	**Medically acceptable**	08	11.60%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

The figure 5.34 illustrates Clarke Error Grid analysis of all the reference and predicted blood glucose data pair sets as acquired during fasting, postprandial and random stage based clinical examination over eight healthy and fifteen diabetic study subjects. In Table 5.19, the Clarke Error Grid analysis demonstrates the percentage of

the total data pairs (69) falling in the zones A, B, C, D, and E are 88.40% (61 data pairs), 11.60% (08 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Henceforth, all the 69 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

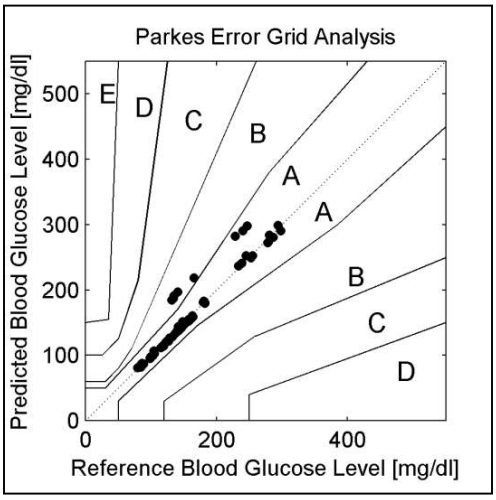

Figure 5.35: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 23 human subject's whole blood mixed Intralipid^{TM} phantom samples.

Table 5.20: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during fasting, postprandial and random stage examination over 23 human subject's whole blood mixed Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	65	94.20%
B Zone	**Slight**	04	05.80%
C Zone	**Moderate**	00	00.00%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 5.35 and Table 5.20 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired

during fasting, postprandial and random stages based clinical examinations over eight healthy and fifteen diabetic study subjects. The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (69) falling in zones A, B, C, D, and E are 94.20% (65 data pairs), 05.80% (04 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Henceforth, the Parkes Error Grid analysis illustrates that 94.20% (65 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 05.80% (04 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

The Table 5.21 illustrates our performance assessment values as procured during Fasting, Postprandial, Random stages based clinical examination over eight healthy, and fifteen diabetic study subjects and the results are-compared with published data ranges of other developing glucose monitoring technique. The performance metrics based errors such as Pearson's Correlation Coefficient (r) values and SEP (Standard Error of Prediction) were 00.96 and 18.21 mg/dl respectively. The MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 09.00 mg/dl, 03.00 mg/dl, and 18.65 mg/dl respectively. Additionally, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 05.57%, and 02.13% respectively. Further, as illustrated from Table 5.21, the output results acquired by our MUS-IR unit based technique is better than or comparable with other developing blood glucose measuring techniques for noninvasive blood glucose monitoring.

Table 5.21: Statistical parameters utilized for accuracy assessment and the results comparison with the published data ranges of other developing glucose monitoring techniques.

Statistical Parameters	Assessment Values	Published Data Ranges of other Developing Glucose Monitoring Techniques	References
Pearson Correlation Coefficient (R-Value)	00.96	00.49 to 00.95	Vaddiraju *et al.* (2010); Tuchin (2009); Oliver *et al.* (2009)
Standard Error of Prediction (SEP)	18.21 mg/dl	07.10 to 35.30 mg/dl	Ozaki *et al.* (2009); Yoon (2009); Tuchin (2009); Heise *et al.* (1998)
Mean Absolute Error (MAE)	09.00 mg/dl	07.00 to 30.00 mg/dl	Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Myllyla *et al.* (2009); Tuchin (2009); Enejder *et al.* (2005); Bockle *et al.* (2002); Zhao (2002); Heise *et al.* (1998); Robinson *et al.* (1992)
Median Absolute Error (MdAE)	03.00 mg/dl	10.40 to 19.10 mg/dl	Valgimigli *et al.* (2010)
Root Mean Squared Error (RMSE)	18.65 mg/dl	25.00 to 46.00 mg/dl	Guevara *et al.* (2010); Ozaki *et al.* (2009); Tuchin (2009)
Percentage of Mean Absolute Relative Error (% MARE)	05.57 mg/dl	08.60 to 40.80%	Pai *et al.* (2015); Mohammadi *et al.* (2014); Vashist (2012); Ramchandani *et al.* (2012); Caduff *et al.* (2011); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Caduff *et al.* (2009); Lipson *et al.* (2009); Gabbay *et al.* (2008); Amir *et al.* (2007); Weiss *et al.* (2007); Bockle *et al.* (2002); Malchoff *et al.* (2002); Tamada *et al.* (1999)
Percentage of Median Absolute Relative Error (% MdARE)	02.13 mg/dl	07.70 to 30.00%	Harman-Boehm *et al.* (2010); Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2009); Gabbay *et al.* (2008); Lipson *et al.* (2009); Weiss *et al.* (2007); Zhao (2002); Bockle *et al.* (2002); Zilberman *et al.* (2009)

Further, its accuracy levels are additionally comparable with other commercially existing Continuous Glucose Monitoring System. Subsequently, all

these overlaid accuracy measures based statistical analysis represents the strong promising aspect for developing noninvasive procedure for blood glucose estimation in *in-vitro* samples as obtained from the human subjects.

5.4.5 Conclusion:

Glucose measurements using amplitude modulated ultrasound and infrared light based technique were conducted in Intralipid™ and human whole blood mixed phantom samples to ascertain how glucose affects the optical properties of Intralipid™ and human whole blood samples at 940 nm. It had been found that the glucose-induced change in the peak-to-peak values of the signal was significant in Intralipid™ and human whole blood samples. The results demonstrate the capability of the amplitude-modulated ultrasound and infrared light based technique unit used in studying the human whole blood mixed Intralipid™ phantom samples. Henceforth, this technique proved to be potential methodology for noninvasive blood glucose monitoring in the near future.

5.5 *In-vitro* investigation using three different ranges of blood glucose concentration levels in various human whole blood mixed with Intralipid™ phantom samples:

5.5.1 Introduction:

The present section represents the outcome of blood glucose determination tests performed over various human whole blood mixed Intralipid™ samples. Here, the indigenously developed MUS-IR unit based results including three different ranges of blood glucose concentration in the phantom samples has been crosschecked by the established invasive glucometer. Moreover, its performance evaluations were measured by applying Clarke and Parkes Error Grid and Statistical Analysis respectively.

5.5.2 Study subjects:

The group of thirty adult volunteers (twenty five males and five females of height 171±6.3 cm, weight 78±5.1 kg) has been chosen for this pilot study. Out of which ten adults volunteers were selected with Random BGL (Blood Glucose Level) ranging between (70-140) mg/dl. Furthermore, next ten adults were selected with Random BGL (Blood Glucose Level) ranging between (141-199) mg/dl. Similarly, remaining ten adults were selected with Random BGL (Blood Glucose Level) ranging between (200-260) mg/dl. The clinical studies reported here are in accordance with

the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of Institute of Medical Sciences-Banaras Hindu University, Varanasi approved the clinical study.

5.5.3 Experimental procedures:

During the laboratory experimental works, the following steps were followed:

- The whole blood samples of all the adult volunteers were collected in vacuum-based blood collecting vials where K_2 EDTA is present as an anticlotting agent.
- Change in the hematocrit and oxygen concentration level varies the glucose-induced signals [Amir *et al.* (2007)]. Therefore, Nitrogen bubbling has-been-applied to induce de-oxygenation in the whole blood samples for 45 minutes.
- The pH level of the blood samples were maintained by the Phosphate Buffer Solution (PBS).
- 1 ml of whole blood sample obtained from every adult volunteer had been mixed with 1 ml of Intralipid^{TM} phantom samples respectively as test preparation for the experimental purposes.
- This whole blood mixed Intralipid^{TM} samples were placed in the indigenously developed instrumental setup (MUS-IR unit) for its respective glucose concentration measurements.

The blood glucose concentration obtained from the indigenously developed instrumental setup had been crosschecked for its accuracy with the results as obtained by the established invasive glucometer (Accu-Chek Active of Roche Diagnostics). Here, the invasive glucometer based measurements were preferred over the GOD/POD method, in order to cope with (i) overall study subject's compliances, (ii) faster selection of our self-assigned categories (I, II, III), (iii) ease of sample handling and (iv) time constraints respectively.

Further, both the BGL readings were-processed through the Error Grid and statistical analysis for indigenously developed prototype setup performance-evaluation purposes.

We had obtained blood samples from the respective study subjects with Random BGL levels ranging for 70 mg/dl to 260 mg/dl for our experimental purposes. Consequently, we had divided those samples into three categories depending upon their Random blood glucose levels.

Category I- includes samples with Random blood glucose levels ranging from 70 mg/dl to 140 mg/dl.

Category II- includes samples with Random blood glucose levels ranging from 141 mg/dl to 199 mg/dl.

Category III- includes samples with Random blood glucose levels ranging from 200 mg/dl to 260 mg/dl.

The samples from each category (I, II, III) have been prepared by adding 1 ml of blood samples from each category to the 1 ml of Intralipid^{TM} phantom suspension respectively. This procedure ha -been-followed for preparing all the human whole blood mixed Intralipid^{TM} phantom samples. The final prepared samples have been placed inside the sample holder section of the prototype setup (MUS-IR unit) for its glucose concentration determinations by our proposed technique. Simultaneously, invasive glucometer has been applied for glucose concentration measurement of the study subjects during respective sampling periods.

All final readings acquired by our proposed technique has been crosschecked by the invasive glucometer based readings. Consequently, its performance evaluation has-been-done with help of Error Grid analysis for all the three categories of Random blood glucose level mixed with Intralipid^{TM} phantom samples respectively.

5.5.4 Result and Discussion:

This portion of the experiment deals with thirty study subjects with different category I, II, and III whole blood mixed with Intralipid^{TM} phantom samples respectively. Here both the glucose levels in these samples were obtained through our Modulated Ultrasound Infrared (MUS-IR) unit and the established invasive method. The data as seen in figure 5.36 to figure 5.38 indicate that the glucose concentration increases *in-vitro* samples from category I to category III respectively. This accurate, stable working of our prototype for glucose detection would be helpful for developing noninvasive optical glucometer.

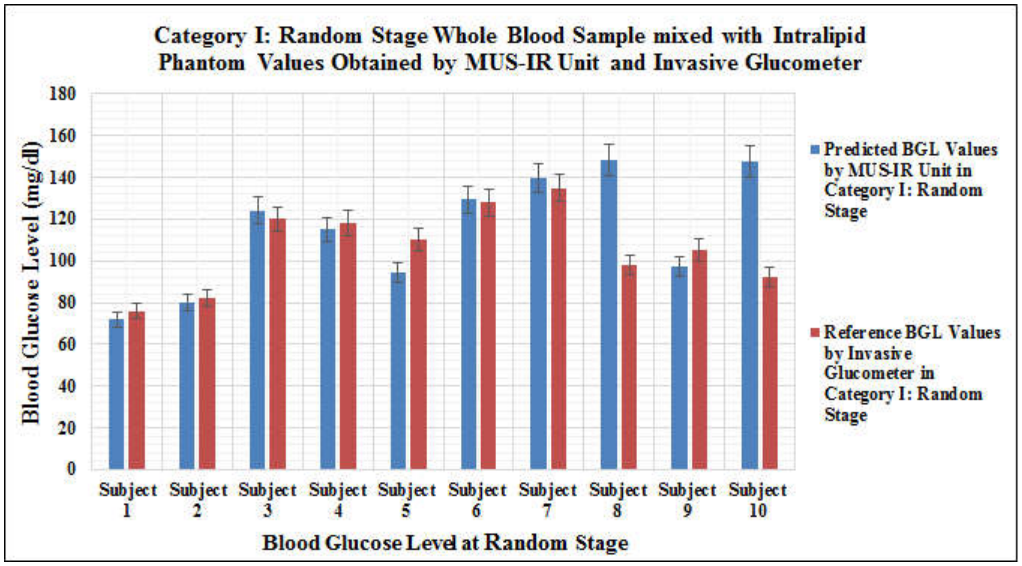

Figure 5.36: Category I: Random stage based whole blood mixed with Intralipid™ phantom samples response bars of the study subjects (1 to 10); error bars indicate ±5 percentage error.

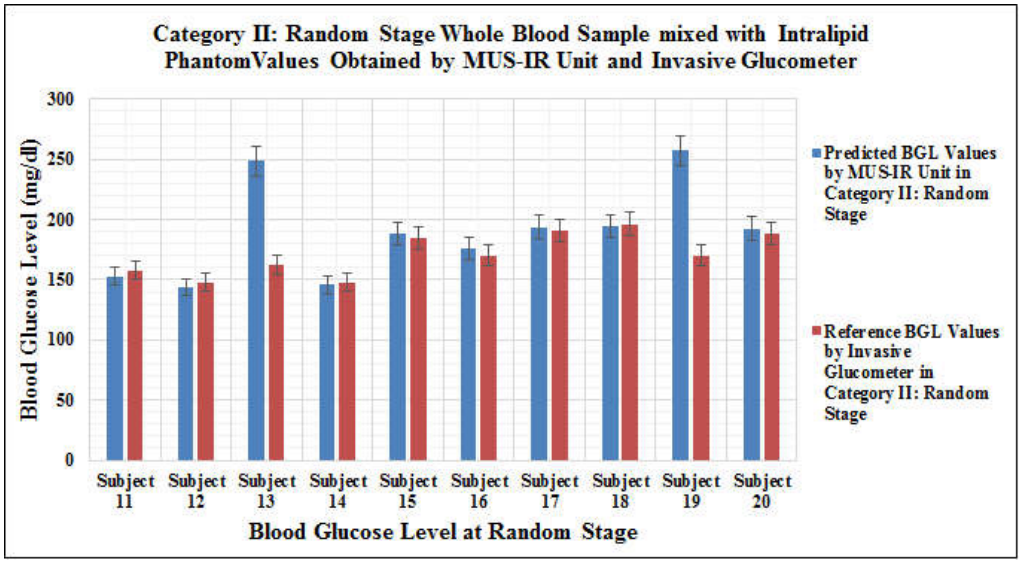

Figure 5.37: Category II: Random stage based whole blood mixed with Intralipid™ phantom samples response bars of the study subjects (11 to 20); error bars indicate ±5 percentage error.

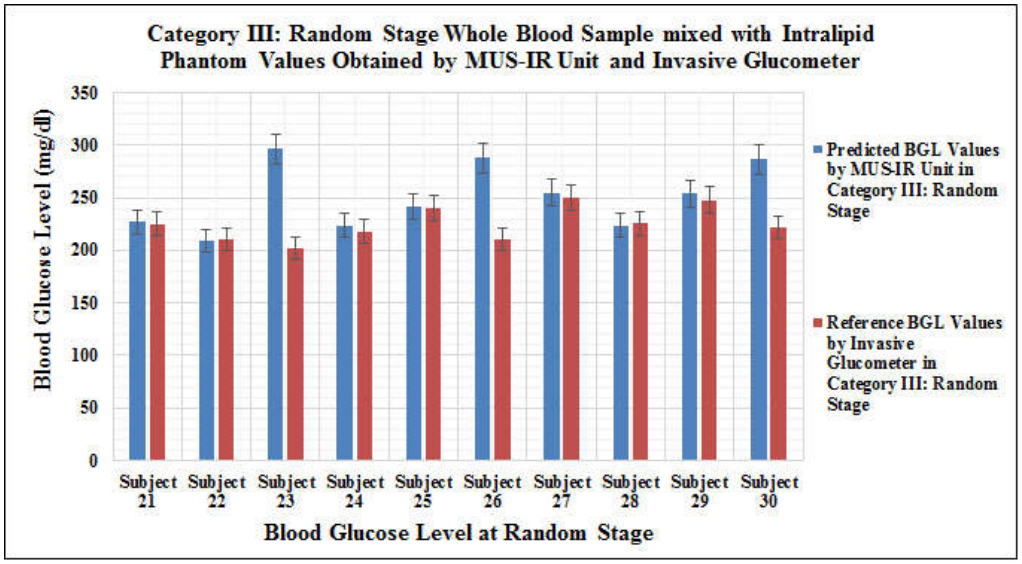

Figure 5.38: Category III: Random stage based whole blood mixed with Intralipid^{TM} phantom samples response bars of the study subjects (21 to 30); error bars indicate ±5 percentage error.

5.5.4.1 Category I: Error Grid (Clarke and Parkes) analysis at random stage: study on human whole blood mixed with Intralipid^{TM} phantom samples.

The Error Grid (Clarke and Parkes) analysis used here to measure the performance metrics of our *in-vitro* technique based prototype unit in measuring blood glucose levels in human whole blood mixed with Intralipid^{TM} phantom samples. The figure 5.39 to figure 5.40 and Table 5.22 to Table 5.23 represent the error grid analysis respectively.

The figure 5.39 illustrates Clarke Error Grid analysis of all the reference and predicted blood glucose data pair sets as acquired during Category I: Random stage based clinical examination over ten study subjects.

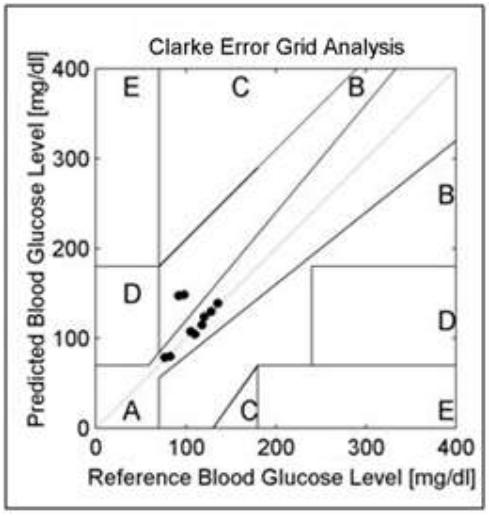

Figure 5.39: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples in Category I: random stage examination.

Table 5.22: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired in Category I: random stage based examination over 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	08	80.00%
B Zone	**Medically acceptable**	02	20.00%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

In Table 5.22, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (10) falling in the zones A, B, C, D, and E are 80.00% (08 data pairs), 20.00% (02 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Henceforth, all the 10 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

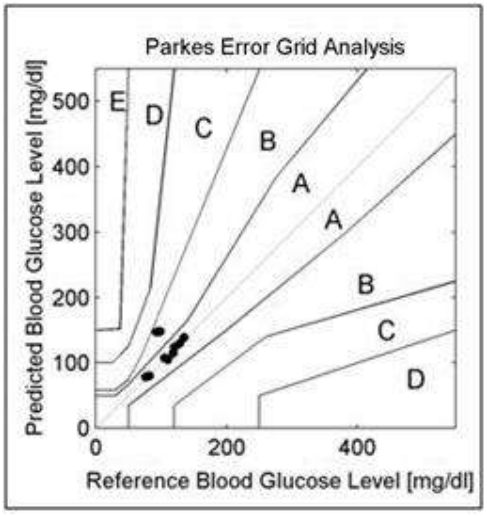

Figure 5.40: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples in Category I: random stage examination.

Table 5.23: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during Category I: random stage based examination over 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	08	80.00%
B Zone	**Slight**	02	20.00%
C Zone	**Moderate**	00	00.00%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 5.40 and Table 5.23 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired in Category I: Random stage based clinical examination over ten study subjects. The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (10) falling in zones A, B, C, D, and E are 80.00% (08 data pairs), 20.00% (02 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Consequently, the Parkes Error Grid analysis delineates that 80.00% (08

data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 20.00% (02 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

5.5.4.2 Category II: Error Grid (Clarke and Parkes) analysis at random stage: study on human whole blood mixed with Intralipid^{TM} phantom samples.

The Error Grid (Clarke and Parkes) analysis used here to measure the performance metrics of our *in-vitro* technique based prototype unit in measuring blood glucose levels in human whole blood mixed with Intralipid^{TM} phantom samples. The figure 5.41 to figure 5.42 and Table 5.24 to Table 5.25 represent the error grid analysis respectively.

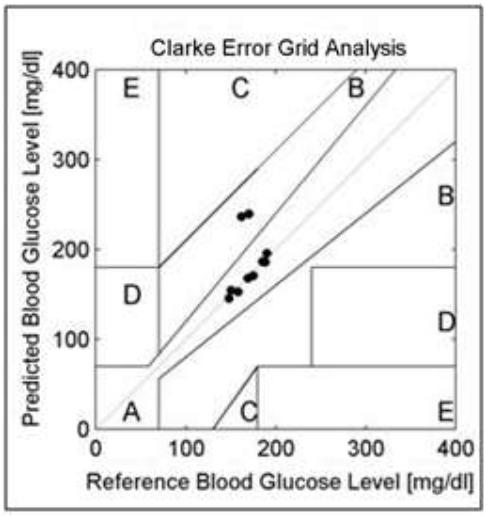

Figure 5.41: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples in Category II: random stage examination.

The figure 5.41 illustrates Clarke Error Grid analysis of all the reference and predicted blood glucose data pair sets as acquired within Category II: Random stage based clinical examination over ten study subjects.

Table 5.24: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired during Category II: random stage based examination over 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	08	80.00%
B Zone	**Medically acceptable**	02	20.00%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

In Table 5.24, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (10) falling in the zones A, B, C, D, and E are 80.00% (08 data pairs), 20.00% (02 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Subsequently, all the 10 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

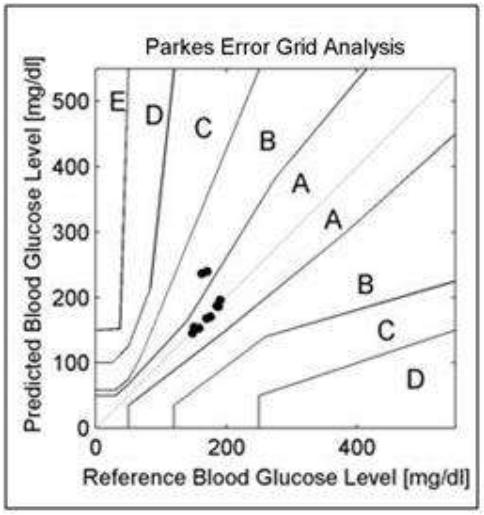

Figure 5.42: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples in Category II: random stage examination.

Table 5.25: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during Category II: random stage based examination over 10 human subject's whole blood mixed with Intralipid™ phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	08	80.00%
B Zone	**Slight**	02	20.00%
C Zone	**Moderate**	00	00.00%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 5.42 and Table 5.25 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired during Category II: Random stage based clinical examination over ten study subjects. The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (10) falling in zones A, B, C, D, and E are 80.00% (08 data pairs), 20.00% (02 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Henceforth, the Parkes Error Grid analysis illustrates that 80.00% (08 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 20.00% (02 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

5.5.4.3 Category III: Error Grid (Clarke and Parkes) analysis at random stage: study on human whole blood mixed with Intralipid™ phantom samples.

The Error Grid (Clarke and Parkes) analysis used here to measure the performance metrics of our *in-vitro* technique based prototype unit in measuring blood glucose levels in human whole blood mixed with Intralipid™ phantom samples. The figure 5.43 to figure 5.44 and Table 5.26 to Table 5.27 represent the error grid analysis respectively.

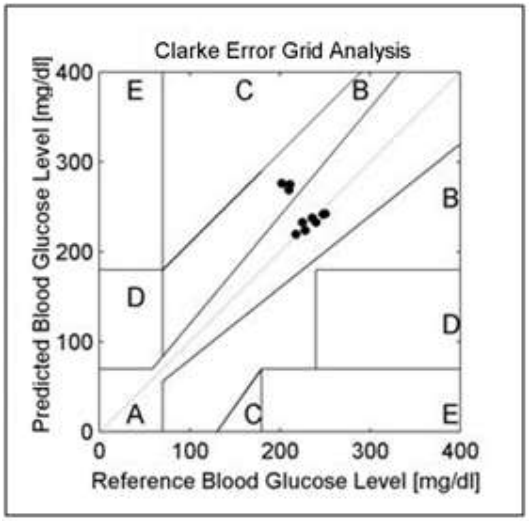

Figure 5.43: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples in Category III: random stage examination.

Table 5.26: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired during Category III: random stage based examination over 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	07	70.00%
B Zone	**Medically acceptable**	03	30.00%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

The figure 5.43 illustrates Clarke Error Grid analysis of all the reference and predicted blood glucose data pair sets as acquired during Category III: Random stage based clinical examination over ten study subjects. In Table 5.26, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (10) falling in the zones A, B, C, D, and E are 70.00% (07 data pairs), 30.00% (03 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Consequently, all the 10 data pairs occupy the medically significant A and B zones

respectively. Further, none of the data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

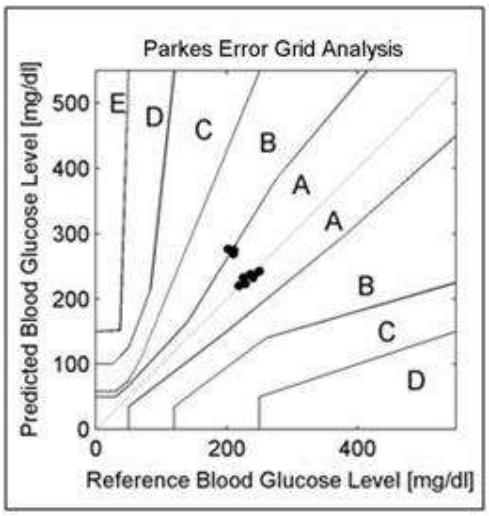

Figure 5.44: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples in Category III: random stage examination.

Table 5.27: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during Category III: random stage based examination over 10 human subject's whole blood mixed with Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	07	70.00%
B Zone	**Slight**	03	30.00%
C Zone	**Moderate**	00	00.00%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 5.44 and Table 5.27 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired during Category III: Random stage based clinical examination over ten study subjects. The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs

(10) falling in zones A, B, C, D, and E are 70.00% (07 data pairs), 30.00% (03 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Henceforth, the Parkes Error Grid analysis illustrates that 70.00% (07 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 30.00% (03 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

5.5.4.4 Total category (I, II, III) results: Error Grid (Clarke and Parkes) and statistical analysis at random stage study on human whole blood mixed with Intralipid^{TM} samples.

The Error Grid (Clarke and Parkes) and statistical analysis used here to measure the performance metrics of our *in-vitro* technique based prototype unit in measuring blood glucose levels in human whole blood mixed with Intralipid^{TM} phantom samples. The figure 5.45 to figure 5.46 and Table 5.28 to Table 5.30 represent the error grid and statistical analysis respectively.

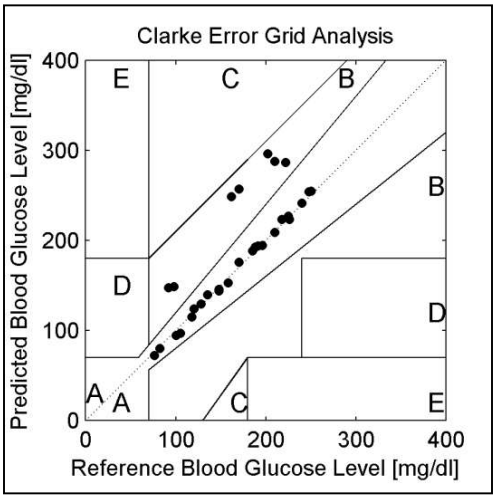

Figure 5.45: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 30 human subject's whole blood mixed with Intralipid^{TM} phantom samples in Category I, II and III: random stage examination.

The figure 5.45 illustrates Clarke Error Grid Analysis of all the reference and predicted blood glucose data pair sets as acquired amid Categories: I, II and III Random stage based clinical examination over thirty study subjects.

Table 5.28: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired during Category I, II and III: random stage based examination over 30 human subject's whole blood mixed with Intralipid™ phantom samples.

Clarke Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**Medically accurate**	23	76.67%
B Zone	**Medically acceptable**	07	23.33%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		00	00.00%
E Zone		00	00.00%

In Table 5.28, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (30) falling in the zones A, B, C, D, and E are 76.67% (23 data pairs), 23.33% (07 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Subsequently, all the 30 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair set occupies medically insignificant and potentially dangerous C to E zones respectively.

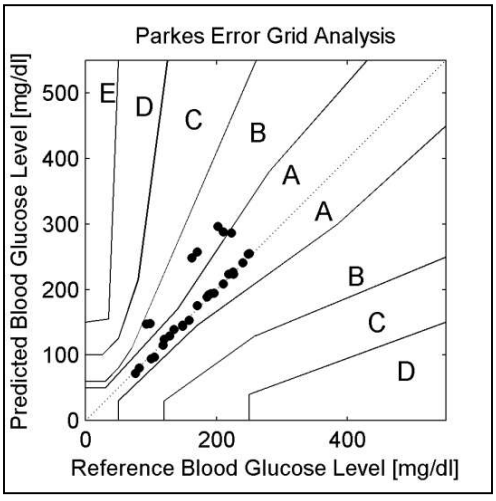

Figure 5.46: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from 30 human subject's whole blood mixed with Intralipid™ phantom samples in Category I, II and III: Random stage examination.

Table 5.29: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during Category I, II and III: random stage based examination over 30 human subject's whole blood mixed with Intralipid^{TM} phantom samples.

Parkes Error Grid Analysis			
Zones	**Medical Risk Assessment**	**Total number of data pairs occupying A to E zones**	**Percentage of total data pairs occupying A to E zones**
A Zone	**None**	23	76.66%
B Zone	**Slight**	07	23.34%
C Zone	**Moderate**	00	00.00%
D Zone	**Significant**	00	00. 0%
E Zone	**Dangerous**	00	00.00%

The figure 5.46 and Table 5.29 illustrates Parkes Error Grid analysis of all blood glucose data pair sets including reference and predicted readings as acquired during Category: I, II and III Random stage based clinical examination over thirty study subjects.

The Parkes Error Grid analysis demonstrates that the percentage of the total data pairs (30) falling in zones A, B, C, D, and E are 76.66% (23 data pairs), 23.34% (07 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Henceforth, the Parkes Error Grid analysis illustrates that 76.66 % (23 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 23.34% (07 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). None of the readings occupies C (moderate risk zone), D (significant risk zone) and E (dangerous risk zone) zones respectively.

The Table 5.30 illustrates our statistical performance assessment values as obtained Category: I, II and III Random stage based clinical examination over thirty study subjects and the comparison of results with published data ranges of other developing glucose monitoring techniques. The performance metrics based errors such as Pearson's Correlation Coefficient (r) values and SEP (Standard Error of Prediction) were 00.86 and 33.12 mg/dl respectively. The MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 20.07 mg/dl, 04.50 mg/dl, and 36.63 mg/dl respectively. Henceforth, the performance metrics based percentage errors such as Percentage-MARE (Percentage

of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 12.94% and 02.64% respectively.

Table 5.30: Statistical parameters utilized for accuracy assessment and the results comparison with the published data ranges of other developing glucose monitoring techniques.

Statistical Parameters	**Assessment Values**	**Published Data Ranges of other Developing Glucose Monitoring Techniques**	**References**
Pearson Correlation Coefficient (R-Value)	00.86	00.49 to 00.95	Vaddiraju *et al.* (2010); Tuchin (2009); Oliver *et al.* (2009)
Standard Error of Prediction (SEP)	33.12 mg/dl	07.10 to 35.30 mg/dl	Ozaki *et al.* (2009); Yoon (2009); Tuchin (2009); Heise *et al.* (1998)
Mean Absolute Error (MAE)	20.07 mg/dl	07.00 to 30.00 mg/dl	Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Myllyla *et al.* (2009); Tuchin (2009); Enejder *et al.* (2005); Bockle *et al.* (2002); Zhao (2002); Heise *et al.* (1998); Robinson *et al.* (1992)
Median Absolute Error (MdAE)	04.50 mg/dl	10.40 to 19.10 mg/dl	Valgimigli *et al.* (2010)
Root Mean Squared Error (RMSE)	36.63 mg/dl	25.00 to 46.00 mg/dl	Guevara *et al.* (2010); Ozaki *et al.* (2009); Tuchin (2009)
Percentage of Mean Absolute Relative Error (% MARE)	12.94 mg/dl	08.60 to 40.80%	Pai *et al.* (2015); Mohammadi *et al.* (2014); Vashist (2012); Ramchandani *et al.* (2012); Caduff *et al.* (2011); Harman-Boehm *et al.* (2010); Harman-Boehm *et al.* (2009); Caduff *et al.* (2009); Lipson *et al.* (2009); Gabbay *et al.* (2008); Amir *et al.* (2007); Weiss *et al.* (2007); Bockle *et al.* (2002); Malchoff *et al.* (2002); Tamada *et al.* (1999)
Percentage of Median Absolute Relative Error (% MdARE)	02.64mg/dl	07.70 to 30.00%	Harman-Boehm *et al.* (2010); Valgimigli *et al.* (2010); Harman-Boehm *et al.* (2009); Gabbay *et al.* (2008); Lipson *et al.* (2009); Weiss *et al.* (2007); Zhao (2002); Bockle *et al.* (2002); Zilberman *et al.* (2009)

Further, as illustrates from Table 5.30, the output results acquired by our MUS- IR technique is better than or comparable with other developing blood glucose measuring techniques for noninvasive blood glucose monitoring. Further, its accuracy levels are likewise comparable with other commercially existing Continuous Glucose Monitoring System. Henceforth, all these overlaid accuracy measures based statistical analysis illustrates the strong promising aspect for developing noninvasive procedure for blood glucose estimation in *in-vitro* samples as obtained from the human subjects.

5.5.5 Conclusion:

All the outcomes occupy the medical significant A and B Zones in Clarke and Parkes Error Grid analysis. Henceforth, it demonstrates that our indigenously developed prototype setup was fruitful in distinguishing different blood glucose levels in the range extending from 70 mg/dl to 260 mg/dl through human whole blood mixed Intralipid^{TM} phantom samples respectively.

Determination of blood glucose content in human whole blood mixe Intralipid^{TM} phantom samples has been conducted in this section with the help of amplitude modulated ultrasound and infrared unit respectively. Prototype performances were critically verified through Error Grid analysis. Outcome of Clarke and Parkes Error Grid analysis depicts that all the experimental output readings occupy medically acceptable A and B zones respectively. These results direct towards the successful detection of respective blood glucose concentrations in sample preparations. In turn, it proves the potentiality of our indigenously developed MUS-IR prototype setup for blood glucose concentration determinations in phantom samples.

5.6 *In-vitro* determination of glucose concentration in various optical phantoms:

5.6.1 Introduction:

This present work explains the impact of the glucose concentration determinations in different types of optical tissue property resembling phantoms. In this present work we have utilized various samples like distilled water, commercialized milk, chicken breast tissue, human whole blood as phantom medium. Further, dextrose (glucose) solutions were added at an increasing rate to all of them. The effects of dextrose (glucose) addition to the phantom samples are detected and critically analyzed here. Concentrations of the glucose molecules were varied in phantom samples, its effects were observed through indigenously developed modulated ultrasound and infrared technique based unit.

The result signifies the effective functioning of our indigenously developed MUS-IR (Modulated Ultrasound-Infrared) unit for measuring Dextrose (glucose) concentrations in particular optical phantoms.

5.6.2 Result and Discussion:

5.6.2.1 Dextrose water solution:

Dextrose anhydrous purified powder is white in color with brilliant hydrophilic characteristics. When mixed with distilled water, micro-air bubbles were released forming the clear and colorless dextrose (glucose) solution. In the light wavelength band between 1550 nm and 905 nm, the absorption coefficients of dextrose (glucose) solution changed by ±20% (0.98-0.007) mm^{-1} correspondingly [Ya *et al.* (2014); Zhao (2002)]. When IR-light source of 940 nm and 40 kHz ultrasonic wave have been applied to the glucose solution, the output signal have been produced due to this phenomenon.

Table 5.31: *In-vitro* experiments performed and measured by the MUS-IR Unit on Distilled Water treated as a phantom by adding different concentration of dextrose (glucose) solutions.

S.N.	Phantom Medium	Different Concentration of Samples	Obtained Voltage Amplitude Values (mV)
1.	1 ml Distilled Water	1 ml Distilled Water as Blank	00.0
2.	1 ml Distilled Water	1 ml Dextrose (Glucose) solution from prepared 500 mg in 10 ml Distilled Water	30.6
3.	1 ml Distilled Water	1 ml Dextrose (Glucose) solution from prepared 1000 mg in 10 ml Distilled Water	45.0
4.	1 ml Distilled Water	1 ml Dextrose (Glucose) solution from prepared 1500 mg in 10 ml Distilled Water	51.3

Different samples like 00 mg (blank), 500 mg, 1000 mg and 1500 mg of dextrose (glucose) powder per 10 ml of distilled water were prepared. Usually, 1 ml from each and every concentration of dextrose (glucose) sample solutions as stated before have been mixed with 1 ml of the distilled water sample respectively for different experimental purposes. Experiments started with lower concentrations followed by increasing trends towards the higher concentrations levels as described in the Table 5.31. The correlation between the dextrose (glucose) solution concentration and the relative change in the voltage amplitude (mV) values in FFT (Fast Fourier

Transform) domain are shown in figure 5.47 and Table 5.31 respectively. It can be noted that when dextrose (glucose) concentration increases in the distilled water solution, the peak amplitude in the FFT domain increases accordingly.

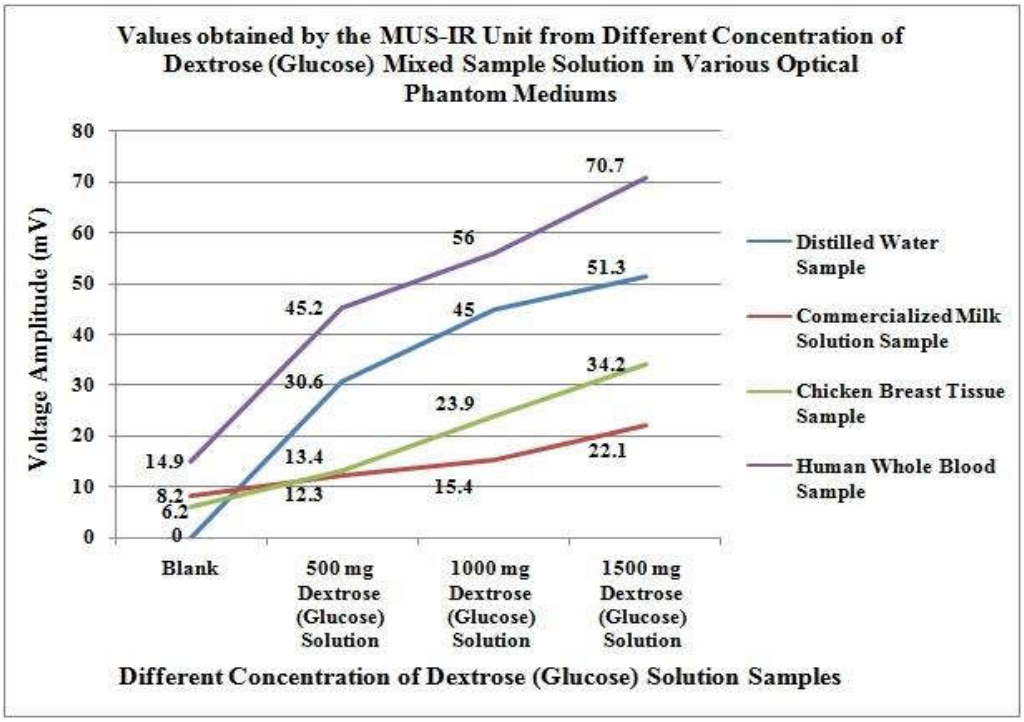

Figure 5.47: Variations in voltage amplitude values with increase in concentration of dextrose (glucose) in different types of tissue optical phantom mediums.

5.6.2.2 Commercialized milk solution:

General ingredients of the commercialized milk solution include water (87.1%), fat (3.94%), protein (3.27%), lactose (4.93%), ash (0.76%), etc. It reveals that dry or waterless content ranges from 12% to 13% in it [Jacques (1997); Duck (1990); Antila (1973)]. Fatty globules or substances dimensions range from 0.1 μm to 10 μm respectively. On the other hand, the diameter of casein micelles extends from 0.01 μm to 0.3 μm respectively. As compared with the skin of humans [Zhao (2002); Jacques (1997)] the waterless contents in milk is low except lactose contents. The absorption coefficient of water and milk in the range between 905 nm to 1000 nm is more or less equal in nature. Moreover, in the tissue optical window range (700 nm to 1100 nm), scattering particles with diameter greater than 50 nm disperse light in correlation with the Mie scattering phenomenon. For that reason, milk, the natural

emulsion acts as a good scattering agent as compared with the absorbing properties of it [Jacques (1997); Duck (1990); Antila (1973)]. Different samples like 00 mg (blank), 500 mg, 1000 mg and 1500 mg of dextrose (glucose) powder per 10 ml of distilled water were prepared. Usually 1 ml from each concentration of dextrose (glucose) sample solutions as stated before has-been-mixed with 1 ml of the milk sample respectively for different experimental purposes. Experiments started with lower concentrations followed by increasing trends towards the higher concentrations levels as described in the Table 5.32.

Table 5.32: *In-vitro* experiments performed and measured by the MUS-IR Unit on Commercialized Milk solution treated as an optical tissue phantom by adding different concentration of dextrose (glucose) solutions.

S.N.	Phantom Medium	Different Concentration of Samples	Obtained Voltage Amplitude Values (mV)
1.	1 ml Commercialized Milk Solution	1 ml Distilled Water as Blank	8.2
2.	1 ml Commercialized Milk Solution	1 ml Dextrose (Glucose) solution from prepared 500 mg in 10 ml Distilled Water	12.3
3.	1 ml Commercialized Milk Solution	1 ml Dextrose (Glucose) solution from prepared 1000 mg in 10 ml Distilled Water	15.4
4.	1 ml Commercialized Milk Solution	1 ml Dextrose (Glucose) solution from prepared 1500 mg in 10 ml Distilled Water	22.1

The correlation between the change in dextrose (glucose) solution concentration and the relative change in the voltage amplitude (mV) values in FFT domain are shown in figure 5.47 and Table 5.32 respectively. It can be noted that when dextrose (glucose) concentration increases in the commercialized milk solution, the peak amplitude in the FFT domain increases accordingly.

5.6.2.3 Chicken breast tissue sample:

This portion describes the dextrose (glucose) measurement in chicken breast tissue samples. The shape and size of the tissue were selected after careful observations. As larger is the sample size, larger is the dextrose (glucose) diffusion time. Similarly, very smaller sample size distorts the measurement by changing the output signal due to glucose in liquid portions, not due to glucose in tissue portions. Moreover, other biological fluids like blood, interstitial fluid tend to ooze out from the

tissue portions to the dextrose (glucose) medium. To avoid such phenomenon, the selected chicken breast tissue portion must be free from blood and other fluids before performing the experimentations [Zhao (2002)]. Blood itself has a great impact over the absorption profile of the chicken breast tissue. In this experimental protocol, the chicken breast tissue sample has been engrossed within phosphate buffer solution and cleaned for few occasions in a total time period of 48 hours. This cleaning process continues till capillary blood and interstitial fluids were completely drained out and stored at 4°C temperature in a refrigerator for its further experimental uses. It has-been-immersed in the phosphate buffer solution during the experimental procedures to lessen the effect of ultrasonic wave reflections at the boundaries between the chicken breast tissue, sensor and wall of the sample test tube [Zhao (2002)].

Table 5.33: *In-vitro* experiments performed and measured by the MUS-IR Unit on Chicken Breast Tissue sample treated as a phantom by adding different concentration of dextrose (glucose) solutions.

S. N.	Phantom Medium	Different Concentration of Samples	Obtained Voltage Amplitude Values (mV)
1.	Prepared Bit of Chicken Breast Tissue Sample	1 ml Distilled Water as Blank	6.2
2.	Prepared Bit of Chicken Breast Tissue Sample	1 ml Dextrose (Glucose) solution from prepared 500 mg in 10 ml Distilled Water	13.4
3.	Prepared Bit of Chicken Breast Tissue Sample	1 ml Dextrose (Glucose) solution from prepared 1000 mg in 10 ml Distilled Water	23.9
4.	Prepared Bit of Chicken Breast Tissue Sample	1 ml Dextrose (Glucose) solution from prepared 1500 mg in 10 ml Distilled Water	34.2

Different samples like 00 mg (blank), 500 mg, 1000 mg and 1500 mg of dextrose (glucose) powder per 10 ml of distilled water were prepared. Usually 1 ml from each and every concentration of dextrose (glucose) sample solutions as stated before have been mixed with the prepared chicken breast tissue samples separately for different experimental purposes. Experiments started with lower concentrations followed by increasing trends towards the higher concentrations levels as described in Table 5.33. The correlation between the dextrose (glucose) solution concentration and

the relative change in the voltage amplitude (mV) values in FFT domain is shown in figure 5.47 and Table 5.33 respectively. It can be noted that when dextrose (glucose) concentration increases in the chicken breast tissue samples, the peak amplitude in the FFT domain increases accordingly.

5.6.2.4 Human whole blood sample:

Human blood comprises generally active ingredients with continuing biophysical and biochemical variations within it [Zhao (2002)]. For this phenomenon, the signal characterization in blood samples have been very complicated in nature. The coefficient of absorption, scattering and the anisotropy factor of whole blood samples are robustly influenced by various parameters like hematocrit levels, velocity of blood flow, fluid osmolarity, blood oxygen saturations and haemolysis of blood [Friebel *et al.* (1999)]. In this experiment, human blood has been drawn from the left arm vein of the volunteer and collected within the vacutainer with EDTA (Ethylene Di-amine Tetra Acetic Acid) in it as an anti-caking agent. To avoid changes due to hematocrit level variations, only single blood sample have been utilized here. The room temperature has been kept constant during the experimental procedures to avoid blood sample destabilization due to temperature changes [Zhao (2002)].

Table 5.34: *In-vitro* experiments performed and measured by the MUS-IR Unit on Human Whole Blood sample treated as a phantom by adding different concentration of dextrose (glucose) solutions.

S. N.	Phantom Medium	Different Concentration of Samples	Obtained Voltage Amplitude Values (mV)
1.	1 ml Prepared Human Whole Blood Sample	1 ml Distilled Water as Blank	14.9
2.	1 ml Prepared Human Whole Blood Sample	1 ml Dextrose (Glucose) solution from prepared 500 mg in 10 ml Distilled Water	45.2
3.	1 ml Prepared Human Whole Blood Sample	1 ml Dextrose (Glucose) solution from prepared 1000 mg in 10 ml Distilled Water	56.0
4.	1 ml Prepared Human Whole Blood Sample	1 ml Dextrose (Glucose) solution from prepared 1500 mg in 10 ml Distilled Water	70.7

In order to maintain the osmolarity of the sample, the Dextrose (glucose) solution has been prepared in phosphate buffer solutions with same human blood isotonicity index [Zhao (2002)]. Usually 1 ml from each concentration of dextrose (glucose) sample solutions as stated above has been mixed with 1 ml of the prepared human whole blood samples separately for different experimental purposes. Experiments started with lower concentrations followed by increasing trends towards the higher concentrations levels as described in Table 5.34.

The correlation between the dextrose (glucose) solution concentration and the relative change in the voltage amplitude (mV) values in FFT domain is shown in figure 5.47 and Table 5.34 respectively. It can be noted that when dextrose (glucose) concentration increases in the human whole blood samples, the peak amplitude in the FFT domain increases accordingly.

5.6.3 Conclusion:

Dextrose (glucose) concentration measurement utilizing amplitude modulated ultrasound and infrared techniques has been conducted in various types of medium like distilled water, commercialized milk, chicken breast tissues and human whole blood samples. The peak to peak voltage amplitude responses in the Fast Fourier Domain (FFT) with respect to the change in dextrose concentrations in various samples were significant. The result indicates the efficient working of our indigenously developed MUS-IR (Modulated Ultrasound-Infrared) unit for detecting Dextrose (glucose) concentrations in various optical phantoms samples.

Overall, in all the *in-vitro* experiments using (i) human blood plasma, (ii) human blood serum and (iii) human whole blood has-been-mixed with Intralipid^{TM} phantom samples. Afterwards, experiments were-conducted to measure the glucose concentration in it.

Our results indicate that our MUS-IR unit based technique has been efficient in measuring blood glucose levels in *in-vitro* samples. Further, the prototype efficiently and effectively measures glucose concentration in different phantom mediums respectively.