CHAPTER 6

DISCUSSION

6.1 Introduction:

The noninvasive supervision of blood glucose levels have attracted significant researchers and undergone prodigious innovations in the last three decades. As Diabetes Mellitus appears to be the chief endemic in this contemporary world, research, and development of noninvasive blood-glucose measurement technique is essential as well as demanding [So *et al.* (2012); Khalil (2004); Khalil (1999)].

Noninvasive measurement of blood glucose levels will reduce the skin puncturing, infection liabilities, pain, mental agony, and expenses linked with the invasive technology. Freedom from skin prick procedures will provide regular and repeated monitoring of blood glucose-levels. This positive act of the diabetics will deliver smooth, firm, balanced and medically significant blood glucose management. Further, the noninvasive approach will enhance patient awareness and care. It will postpone the onset of terminal medical complications and emergencies related to Diabetes Mellitus and associated expenditures [Yadav *et al.* (2015); Chowdhury *et al.* (2013)].

Further, the essential requirement of (i) medical attention, (ii) care for diabetic patients and (iii) promising commercial aspect of the noninvasive approach for blood glucose measurement has provided the firm flurry for innovative research, patenting activities in this particular biomedical field of exploration. Furthermore, the advent of numerous minimally invasive methodologies, alternate sample monitoring and requirement of minute blood samples (especially from the fingertips that are rich in vascular networks and nerve endings) by new semi invasive devices, has raised hope for the successful noninvasive technology in near future [Yadav *et al.* (2015); So *et al.* (2012); Khalil (2004); Khalil (1999)].

Various prototype design, calibration aspects, signal transmission, and data analysis have undergone substantial transformations and frequent publication about new clinical investigations unfolding every innovative modifications proves the underlying impulse for successful noninvasive blood glucose-measurement technique. As predictable, that noninvasive technique will flourish intensely in near future and possibly, it will unlock the newfangled therapeutic procedures. However, failure to make use these new noninvasive techniques will hinder their application and usage [Clarke *et al.* (2007)].

In perspective of addressing the requirement for this necessity, the present thesis work represents our indigenously developed noninvasive technique for blood glucose measurement in the human subjects.

6.2 Overall result comparison and evaluation:

Establishing the medically accurate and acceptable noninvasive blood glucose measurement is a difficult assignment. In order to validate, we gathered important data from the available English language based research literatures for method comparing and applied various statistical evaluation methods to judge the performance of our noninvasive technique based overall blood glucose measurements.

The statistical evaluation methods include (i) Clarke Error Grid Analysis (ii) Parkes Error Grid Analysis (iii) Accuracy Measure Analysis (iv) Pearson Correlation Analysis (v) Rank Correlation Analysis (vi) Bland Altman plot analysis (vii) Mountain plot analysis (viii) Independent sample t test based analysis (ix) CUSUM test for linearity and (x) Deming Regression analysis.

For all the statistical analysis, the significance level has been 0.05 or except stated else. All hypothesis are examined utilizing two-sided tests except stated else.

In this present work, the Statistical analyses were performed using MedCalc for Windows, version 15.11 (MedCalc Software, Ostend, Belgium). The comparison and statistical analysis based our overall result evaluations are as follows:

6.2.1 Clarke Error Grid Analysis:

The paired (Reference and Predicted) blood glucose measurement based readings that usually undergo Clarke Error Grid (CEG) analysis that has five different zones. These zones signify the medical importance ranging from no action to potentially unsafe or contradictory management. Hence, this analysis method determines the accuracy of glucose estimation to its medical significance, established in between 1987-89 [Wentholt *et al.* (2008); Clarke *et al.* (1987)].

Our overall clinical study as reported in chapter 5 of this present thesis includes investigation over 151 (male = 105; female = 46; in which normal non-diabetic healthy subjects = 84; pre-diabetic subjects = 15, diabetic subjects = 52) adult study subjects, that yields total 627 data pairs of reference (invasive) and predicted (noninvasive) blood

glucose levels. Further, in paired data set of 627, the corresponding reference blood-glucose range has been 71-302 mg/dl.

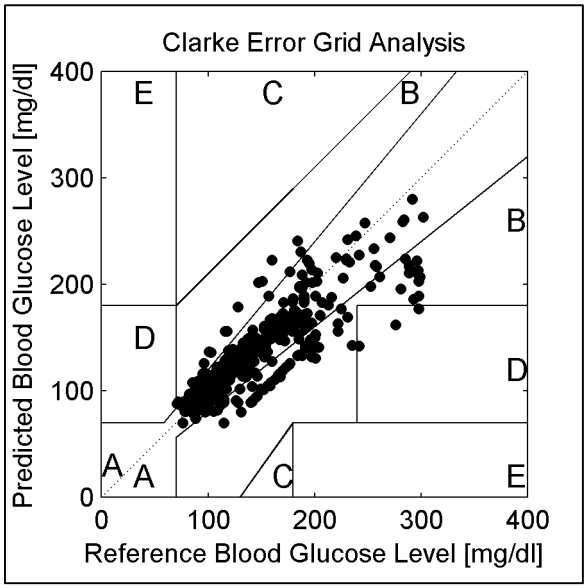

Figure 6.1: Clarke Error Grid Analysis of overall Reference (Invasive) and Predicted (Noninvasive) blood glucose measurement.

Table 6.1: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	527	97	00	03	00
Percentage of total data pairs occupying A to E zones	84.05%	15.47%	00.00%	0.48%	00.00%

Table 6.2: Performance comparison of non-invasive blood glucose measurement-techniques and electrochemical CGMS utilizing Clarke Error Grid Analysis

Clarke Error Grid Analysis						
Technique	**A to E Zones**					**References**
	A ZONE	**B ZONE**	**C ZONE**	**D ZONE**	**E ZONE**	
	Medically accurate	Medically acceptable	Medically insignificant and potentially harmful			
Near Infrared Diffuse Spectroscopy	71.30%	21.30%	00.00 %	07.40 %	00.00 %	Ozaki *et al.* (2009)
Joint Optical-Electrical Technique	77.86%	22.14%	00.00 %	00.00 %	00.00 %	Guevara *et al.* (2010)
NIR Spectroscopy	87.50%	08.30%	00.00 %	04.20 %	00.00 %	Maruo *et al.* (2003)
Raman Spectroscopy	53.00%	39.00%	Not Mentioned			Lipson *et al.* (2009)
Instantaneous Differential Near Infrared Spectrophotometry (For two sets of experiments)	90.05%	09.95%	00.00 %	00.00 %	00.00 %	Yamakoshi *et al.* (2006)
	92.20%	07.80%	00.00 %	00.00 %	00.00 %	
Pulse Glucometry (For three sets of experiments)	84.00%	16.00%	00.00 %	00.00 %	00.00 %	Yamakoshi *et al.* (2009)
	78.00%	22.00%	00.00 %	00.00 %	00.00 %	
	80.00%	19.00%	01.00%			
Pulse Glucometry	73.00%	17.00%	00.00 %	00.00 %	00.00 %	Ogawa *et al.* (2013)
Occlusion Spectroscopy	69.70%	25.70%	Not Mentioned			Amir *et al.* (2007)
Impedance Spectroscopy	56.00%	37.00%	07.00%			Caduff *et al.* (2011)
Dielectric Spectroscopy (For two sets of experiments)	39.20%	49.80%	04.50 %	04.60 %	01.90 %	Caduff *et al.* (2006)
	37.80%	49.40%	04.50 %	06.30 %	02.00 %	
Thermal Infrared Spectroscopy	90.00%	10.00%	00.00 %	00.00 %	00.00 %	Malchoff *et al*. (2002)
Impedance Spectroscopy	78.40%		06.50 %	10.80 %	04.30 %	Wentholf *et al.* (2005)
Electrochemical-CGMS	90.00% to 98.30%		01.70% to 10.00%			Pai *et al.* (2015)
Proposed Technique	**84.05%**	**15.47%**	**00.00%**	**00.47%**	**00.00%**	**--**

The figure 6.1 and Table 6.1 depicts Clarke Error Grid Analysis of our overall blood glucose data pair sets inclusive of reference (invasive) and predicted (noninvasive) readings as obtained during our overall clinical studies. The Clarke Error Grid analysis shows that the percentage of the total data pairs (627) falling in zones A, B, C, D, and E are 84.05% (527 data pairs), 15.47% (97 data pairs), 00.00% (00 data pairs), 0.48% (03 data pairs), and 00.00% (00 data pairs), respectively. Hence, the Clarke Error Grid Analysis depicts that maximum (624 data pairs) of the noninvasive estimations are in medically significant and acceptable A and B zones respectively. Very few results (03 data pairs) occupy medically insignificant D zone respectively.

In this present section, the performance of our proposed noninvasive technique based prototype unit compared to various other prominent noninvasive blood glucose measurement-techniques and electrochemical-CGMS(s) respectively. The Table 6.2 represents the Clarke Error Grid Analysis based comparison of our proposed noninvasive technique performance with other noninvasive techniques based published data that mainly consists of Near Infrared spectroscopy, Near Infrared Diffuse spectroscopy, Joint optical electrical technique, Raman spectroscopy, Instantaneous Differential Near-Infrared spectrophotometry, Pulse Glucometry, Occlusion spectroscopy, Impedance spectroscopy, Dielectric spectroscopy, and Thermal Infrared spectroscopy. Comparisons with the Electrochemical-Continuous Glucose Monitoring System(s) are also included.

The last row in the Table 6.2 depicts our overall noninvasive technique based results. Hence, the Clarke Error Grid Analysis based comparisons in the Table 6.2 shows that our noninvasive technique based overall results are better than or comparable with other noninvasive techniques along with electrochemical-CGMS(s) also. Further, future investigations in advance directions will improve its performances.

6.2.2 Parkes Error Grid Analysis:

Parkes *et al.* (2000) re-entered the concept of error grid zones and designed a new set of innovative error grids, based on the proficiency of big group of medical experts. These new Error Grids were designed differentiating for Type I and Type II diabetic subjects. Parkes Error Grids are classified into five zones such as Zone A to Zone E respectively. Zone A signifies medically correct determinations, with no consequence

over medical supervision. Zone B signifies changed medical action, minute, or no consequences over medical treatment. Zone C signifies changed medical action, probable to influence medical treatment. Zone D signifies changed medical action, might comprise imperative medical jeopardy. Zone E signifies changed medical action, might comprise unsafe effects [Pfutzner *et al.* (2013)].

In contrast to CEG, the PEG does not possess risk boundaries that bounce categories. However, the PEG also contains five boundaries and marginally differs from CEG. The A and B zones have larger areas in PEG, thus the consensus error grid is more lenient as compared to CEG based analysis. However, no borders of all the zones are free from the arbitrariness [Pfutzner *et al.* (2013); Wentholt *et al.* (2008)].

Further, Pfutzner *et al.* (2013) recommended using Type I Diabetes version of Parkes Error Grid for any system clinical accuracy measurements. As Type I diabetes version of Parkes Error Grid analysis have more stringent borders as compared to the Type II diabetes version of the Parkes Error Grid analysis [Pfutzner *et al.* (2013)].

In this present work, the figure 6.2 and Table 6.3 depicts the Parkes Error Grid Analysis (Type I diabetes version of Parkes Error Grid Analysis-commonly known as Parkes Error Grid Analysis) of our overall clinical study based invasive and noninvasive blood glucose measurement.

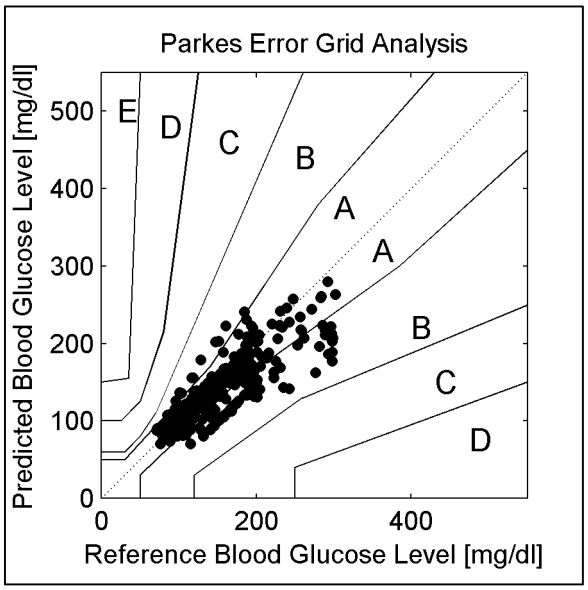

Figure 6.2: Parkes Error Grid Analysis of overall Reference (Invasive) and Predicted (Noninvasive) blood glucose measurement.

Table 6.3: Parkes Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Parkes Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
Risk assigned	**none**	**slight**	**moderate**	**significant**	**dangerous**
Total number of data pairs occupying A to E zones	536	91	00	00	00
Percentage of total data pairs occupying A to E zones	85.49%	14.51%	00.00%	00.00%	00.00%

The figure 6.2 and Table 6.3 depicts Parkes Error Grid Analysis of our overall blood glucose data pair sets inclusive of reference (invasive) and predicted (noninvasive) readings as obtained during our overall clinical studies. The Parkes Error Grid analysis shows that the percentage of the total data pairs (627) falling in zones A, B, C, D, and E are 85.49% (536 data pairs), 14.51% (91 data pairs), 00.00% (00 data pairs), 00.00% (00) data pairs), and 00.00% (00 data pairs), respectively. Hence, the Parkes Error Grid Analysis depicts that 85.49% (536 data pairs) of the noninvasive estimations are in risk free A zone (clinically accurate). Further, 14.51% (91 data pairs) of the noninvasive estimations are in slight risk B zone (clinically acceptable). More importantly, none of the readings occupies C (moderate risk zone), D (significant risk zone), and E (dangerous risk zone) zones respectively.

6.2.3 Accuracy Measure based analysis:

In this present work, the statistical measures of error for accuracy assessment includes (i) Mean Absolute Error (MAE); (ii) Median Absolute Error (MdAE); (iii) Percentage of Mean Absolute Relative Error (%MARE); (iv) Percentage of Median Absolute Relative Error (%MdARE); (v) Root Mean Squared Error (RMSE); (vi) Standard Error of Prediction (SEP).

The accuracy measure methods utilizing paired glucose values comprises the Mean Absolute Error (MAE), Median Absolute Error (MdAE), Percentage of Mean Absolute Relative Error (percentage-MARE), Percentage of Median Absolute Relative

Error (percentage-MdARE) to meet the requirements per standard limits as documented in published literatures or ISO standards. The MAE (mean of the predicted sensor values minus the reference sensor values) and MdAE (Median of the predicted sensor values minus the reference sensor values) expresses the systemic under or over estimation of one method in comparison to other. However, the negative and positive errors counterbalance each other, and the over-estimation and under-estimation of blood glucose values flattens out. Hence, this method estimates constant absolute or relative bias of one technique relative to the other. The percentage-MARE and Percentage-MdARE represents the mean and median absolute errors, correspondingly, between both the methods (reference and predicted), divided by the reference method and multiplied by 100 to convert the proportion into a percentage. It shows that by how much percentage the predicted method differs from that reference method including either under or over estimations. The measurements of all these parameters are simple, and the outcomes are easier to understand. The percentage-MARE and Percentage-MdARE provides information about bias and variation. When larger bias either or both variation within both the predicted and reference method occurs, it produces high values of percentage-MARE and Percentage-MdARE. In general, the percentage-MdARE values for noninvasive and CGMS systems are lower as compared to percentage-MARE values. Even though, the percentage-MdARE appears to be more statistically significant as compared to percentage-MARE, the published literatures reports largely about percentage-MARE based values [Wentholt *et al.* (2008)].

In this section, the accuracy measure based statistical parameters as mentioned above are applied to judge the accuracy of our overall predicted (noninvasive) blood glucose measurements in comparison to all the reference (invasive) measurements. The Tables 6.4 to 6.9 depicts accuracy measure based performance comparison of our proposed technique overall results with other noninvasive technique based published data that mainly consist of Near Infrared Diffuse Reflectance Spectroscopy, Near Infrared Reflection Spectroscopy, Raman Spectroscopy, Polarimetry, Photo Acoustic Spectroscopy, Thermal Emission Spectroscopy, Optical Coherence Tomography, Occlusion Spectroscopy, and Electrical Impedance Spectroscopy. Comparisons with the Electrochemical-Continuous Glucose Monitoring System(s) are also included. The last

row in all the Tables 6.4 to 6.9 depicts our respective noninvasive technique based results.

Table 6.4: Accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques, and Electrochemical CGMS(s) utilizing Mean Absolute Error (MAE).

Technique	**MAE (mg/dl)**	**References**
Near Infrared Diffuse Reflectance Spectroscopy	19.8 mg/dl	Robinson *et al.* (1992)
Near Infrared Reflection Spectroscopy	30.0 mg/dl	Tuchin (2009)
Raman Spectroscopy	07.80 mg/dl	Enejder *et al.* (2005)
Polarimetry	07.00 mg/dl	Boeckle *et al.* (2002)
Photo Acoustic Spectroscopy	(14.9 to 25.0) mg/dl	Zhao *et al.* (2002)
	(15.27 to 23.75) mg/dl	Pai *et al.* (2015)
	(14.90 to 25.00) mg/dl	Myllyla *et al.* (2009); Tuchin (2009)
Electrochemical-CGMS	(11.90 to 22.30) mg/dl	Valgimigli *et al.* (2010)
Proposed Technique	**15.61 mg/dl**	**--**

The Table 6.4 depicts accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques, and Electrochemical CGMS(s) utilizing Mean Absolute Error based statistical function.

Our overall blood glucose measurement based clinical study indicates that the Mean Absolute Error (MAE) has been 15.61 mg/dl. As depicted in Table 6.4, our clinical study based MAE value is better than or comparable with other techniques based published values that range in-between 07.00 mg/dl to 30.00 mg/dl respectively.

Table 6.5: Accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques and Electrochemical-CGMS(s) utilizing Percentage of Mean Absolute Relative Error (%MARE)

Technique	%MARE	References
Near Infrared-CGMS	13.80%	Mohammadi *et al.* (2014)
Raman Spectroscopy	38.00%	Lipson *et al.* (2009)
Thermal Emission Spectroscopy	08.60% to 11.60%	Malchoff *et al.* (2002)
OCT-Sentris-100, GlucoLight, Bethlehem, Pennsylvania, USA	11.50%	Gabbay *et al.*(2008)
Photo Acoustic Spectroscopy	11.78%	Pai *et al.* (2015)
Ultrasonic, Electromagnetic, Thermal techniques based Multi-sensor-GlucoTrack® (Integrity Applications Ltd., Ashkelon, Israel)	22.40% to 29.9%	Boehm *et al.* (2010); Boehm *et al.* (2009);
Multi-Sensor-MGMS (Multi-Sensor Glucose Monitoring System)	18.00% to 40.80%	Caduff *et al.* (2011); Caduff *et al.* (2009);
Occlusion Spectroscopy	17.20%	Amir *et al.* (2007)
Electrochemical-CGMS	11.80% to 20.00%	Vashist (2013)
Photo Acoustic Spectroscopy [CGMS: Aprise Sensor (Glucon Inc., Boulder, Colorado, USA)]	19.90%	Weiss *et al.*(2007)
Reverse Iontophoresis [GlucoWatch Automatic Glucose Biographer (Cygnus Inc.,USA)]; Minimally Invasive	15.60%	Tamada *et al.*(1999)
Symphony Transdermal-CGMS (Echo Therapeutics Inc., Philadelphia, USA)	12.60%	Ramchandani *et al.* (2012)
Proposed Technique	**10.30%**	**--**

The Table 6.5 depicts accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques, and Electrochemical CGMS(s) utilizing Percentage of Mean Absolute Relative Error (percentage-MARE) based statistical function. Our overall blood glucose measurement based clinical study indicates that the Percentage of Mean Absolute Relative Error has been 10.30%. As depicted in Table 6.5, our clinical study based percentage-MARE value is better than or comparable with other techniques based published values that range in-between 08.60% to 40.80% respectively.

Table 6.6: Accuracy measure based performance comparison of our non-invasive blood glucose Technique to Electrochemical/Micro-dialysis based CGMS(s) utilizing Median Absolute Error (MdAE).

Technique	MdAE (mg/dl)	References
Electrochemical-CGMS Guardian® (Medtronic Diabetes, California, USA)	14.80 mg/dl	Valgimigli *et al.* (2010)
Electrochemical-CGMS DexCom STS® (DexCom Inc., California, USA)	19.10 mg/dl	
Electrochemical-CGMS Navigator (Abbott Diabetes Care, Inc., California, USA)	15.30 mg/dl	
Microdialysis-CGMS GlucoDay®S (A. Menarini Diagnostics, Florence, Italy)	15.60 mg/dl	
Microdialysis-CGMS GlucoMen® Day (A. Menarini Diagnostics, Florence, Italy)	10.40 mg/dl	
Proposed Technique	**08.00 mg/dl**	**--**

The Table 6.6 depicts accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques, and Electrochemical CGMS(s) utilizing Median Absolute Error (MdARE) based statistical function. Our overall blood glucose measurement based clinical study indicates that the Percentage of Median Absolute Error has been 08.00 mg/dl. As depicted in Table 6.6, our clinical study based percentage-

MdARE value is better than or comparable with other techniques based published values that range in-between 10.40 mg/dl to 19.10 mg/dl respectively.

Table 6.7: Accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques and Electrochemical-CGMS(s) utilizing Percentage of Median Absolute Relative Error (%MdARE)

Technique	%MdARE	References
Raman Spectroscopy	30.00%	Lipson *et al.* (2009)
Occlusion Spectroscopy	11.20%	Zilberman *et al*.(2009)
Optical Coherence Tomography	08.20%	Gabbay *et al.* (2008)
Photo Acoustic Spectroscopy	13.20%	Zhao *et al.* (2002)
Multi-sensor-GlucoTrack® (Integrity Applications Ltd., Ashkelon, Israel)	19.90%	Boehm *et al.* (2009)
	15.90%	Boehm *et al.* (2010)
Electrochemical-CGMS	07.70% to 18.40%	Valgimigli *et al.* (2010)
Symphony Transdermal-CGMS (Echo Therapeutics Inc., Philadelphia, USA)	11.80%	Ramchandani *et al*. (2012)
Photo Acoustic Spectroscopy [CGMS: Aprise Sensor (Glucon Inc., Boulder, Colorado, USA)]	13.20%	Weiss *et al.* (2007)
Proposed Technique	**06.29%**	**--**

The Table 6.7 depicts accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques, and Electrochemical CGMS(s) utilizing Percentage of Median Absolute Relative Error (percentage-MdARE) based statistical function. Our overall blood glucose measurement based clinical study indicates that the Percentage of Median Absolute Relative Error has been 06.29%. As depicted in Table 6.7, our clinical study based percentage-MdARE value is better than or comparable with

other techniques based published values that range in-between 07.70% to 30.00% respectively.

Table 6.8: Accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques utilizing Root Mean Squared Error (RMSE).

Technique	RMSE	References
NIR Spectroscopy	25 mg/dl to 46 mg/dl	Yadav *et al.* (2015); Guevara *et al.* (2010); Guevara *et al.* (2008).
Raman Spectroscopy		
Electrical Impedance Spectroscopy		
Photo Acoustic Spectroscopy		
Near Infrared Diffuse Reflectance Spectroscopy	36.40 mg/dl	Marbach *et al.* (1995)
Proposed Technique	**24.72 mg/dl**	**--**

The Table 6.8 depicts accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques utilizing Root Mean Squared Error (RMSE) based statistical function.

Our overall blood glucose measurement based clinical study indicates that the Root Mean Squared Error has been 24.72 mg/dl.

As depicted in Table 6.8, our clinical study based RMSE value (24.72 mg/dl) is better than or comparable with other noninvasive techniques based published values that range in-between 25.00 mg/dl to 46.00 mg/dl respectively.

The lower value of RMSE (Root Mean Squared Error) indicates better quality of results [Vaddiraju *et al.* (2010); Yadav *et al.* (2015].

Table 6.9: Accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques utilizing Standard Error of Prediction (SEP)

Technique	SEP	References
Near Infrared Diffuse Reflectance Spectroscopy	23.70 mg/dl	Ozaki *et al.*(2009); Tuchin (2009)
Mid-Infrared Spectroscopy	10.00 mg/dl to 24.90 mg/dl	Yoon (2009); Tuchin (2009)
Near Infrared Spectroscopy	21.5 mg/dl to 33.10 mg/dl	
Near Infrared Spectroscopy (Diffuse Reflectance, Transmission Spectroscopy, Reflectance)	13.14 mg/dl to 54.00 mg/dl	Yadav *et al.*(2015)
Proposed Technique	**18.45 mg/dl**	--

The Table 6.9 depicts accuracy measure based performance comparison of non-invasive blood glucose measurement-techniques utilizing Standard Error Prediction (SEP) based statistical function. Our overall blood glucose measurement based clinical study indicates that the Standard Error Prediction (SEP) has been 18.45 mg/dl. As depicted in Table 6.9, our clinical study based SEP value is better than or comparable with other techniques based published values that range in-between 10.00 mg/dl to 54.00 mg/dl respectively. Further, the lower value of SEP (Standard Error of Prediction) indicates better quality of results [Yadav *et al.* (2015].

6.2.4 Pearson correlation coefficient analysis:

In general, the Pearson Correlation Coefficient (parametric analysis) function evaluates the degree of association among the two measurement based results. Hence, it is a measure of precision and measures how far each observation deviates from the best-fit line [Bland M (2000); Altman DG (1991)].

In this present work, the two-measurement result includes Reference Blood Glucose Level (RBGL) in mg/dl and Predicted Blood Glucose Level (PBGL) in mg/dl of

our overall clinical studies. In the paired data set of 627, the reference blood-glucose range has been 71-302 mg/dl. The scatter plot figure 6.3 depicts the graphical relationship between the measurement of Reference (RBGL in mg/dl) and Predicted blood glucose levels (PBGL in mg/dl). As depicted from figure 6.3, both the blood glucose measurement results yield the points in the scatterplot graph. The Reference Blood Glucose Level (RBGL in mg/dl) represents the horizontal axis and the other Predicted Blood Glucose Level (PBGL in mg/dl) represents the vertical axis. Here in, the red dotted line in the scatter plot represents the line of equality (Y=X).

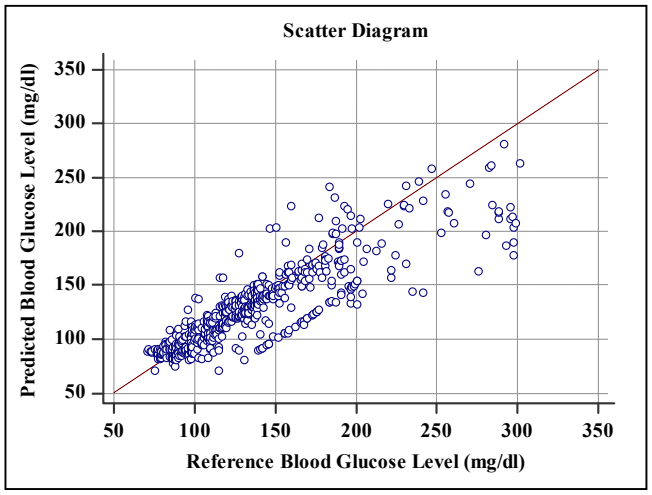

Figure 6.3: The scatter diagram of Reference and Predicted Blood Glucose Levels.

Table 6.10: Pearson correlation coefficient (r) analysis

Pearson correlation coefficient (r value) analysis	
Variable Y	PBGL (mg/dl)
Variable X	RBGL (mg/dl)
Sample Size (n number of data pairs)	627
Correlation coefficient (r)	0.86
Significance level	**P<0.0001**
95% Confidence interval for r	0.84 to 0.88

The Table 6.10 depicts Pearson correlation coefficient (r) Analysis**.** The Table 6.10 represents:

- **Sample size**: the total number of BGL (RBGL and PBGL) data pairs
- **Correlation coefficient with significance level**: Pearson correlation coefficient value of 0.8691, with significance level of P-value (<0.0001) depicts the correlation is statistically significant.
- **95% CI (Confidence Interval) for r**: the 95% confidence interval for the correlation coefficient, specifically states the range value from 0.84 to 0.88 that embraces the true correlation coefficient with probability of 95%.

Table 6.11: Performance measures of different blood glucose measuring techniques classified based on their degree of invasiveness

Degree of Invasiveness	Technique	r value	Reference
Noninvasive	Photo Acoustic Spectroscopy	0.71	Oliver *et al.* (2008); Vaddiraju *et al.* (2010)
	Optical Coherence Tomography	0.80-0.95	
	Polarimetry (ex-vivo)	0.99	
	Thermal Infrared Spectroscopy	0.87	
	Raman Spectroscopy	0.83-0.91	
	Impedance Spectroscopy	0.49-0.59	
	Occlusion Spectroscopy	0.75	
	NIR Spectroscopy	0.50-0.90	Tuchin (2009)
	Proposed Technique	**0.86**	**--**
Minimally Invasive	Iontophoresis	0.90	Oliver *et al.* (2008); Vaddiraju *et al.*(2010)
	Sonophoresis	0.70	
	Micropores	0.94-0.95	
Invasive	Subcutaneous	0.85-0.88	
	Intravenous	0.83-0.93	
	Microdialysis	0.90	

The Table 6.11 depicts precision measure based performance comparison of non-invasive blood glucose measurement-techniques utilizing Pearson's Correlation Coefficient (r-value) based statistical function. Our overall blood glucose measurement based clinical study indicates that the r-value has been 0.86. Hence, as depicted in Table 6.11, our clinical study based r-value (Pearson's Correlation Coefficient) is better than or comparable with other techniques based values that range in-between 00.49 to 00.95 respectively.

6.2.5 Rank Correlation Coefficients analysis:

The Rank Correlation (nonparametric analysis) measures the degree of association between the two variable and the data ranking occurs in order of their sizes, as well as the measurements depending on the ranks of equivalent values in X and Y variables [Armitage *et al.* (2002)]. In this present work, we have performed Spearman's coefficient of rank correlation (rho) and Kendall's tau coefficient of rank correlation based analysis to examine the degree of association between Reference Blood Glucose Level (RBGL) and Predicted Blood Glucose Level (PBGL).

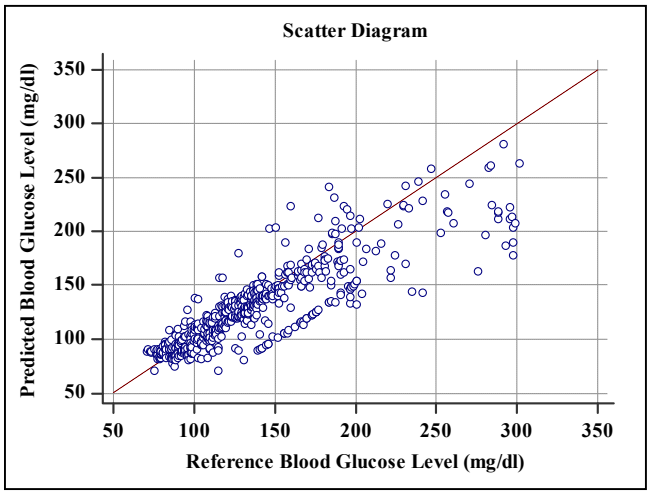

Figure 6.4: The scatter diagram of Reference and Predicted Blood Glucose Levels.

As depicted from figure 6.4, both the blood glucose measurement results yield the points in the scatterplot graph. The Reference Blood Glucose Level (RBGL in mg/dl) represents the horizontal axis and the other Predicted Blood Glucose Level (PBGL in mg/dl) represents the vertical axis. Here in, the red dotted line in the scatter plot represents the line of equality (Y = X).

Table 6.12: Rank Correlation coefficients Analysis

Rank Correlation coefficients Analysis	
Variable Y	PBGL (mg/dl)
Variable X	RBGL (mg/dl)
Sample size (n number of data pairs)	627
Spearman's coefficient of rank correlation (rho)	0.88
Significance level	**P<0.0001**
95% Confidence Interval for rho	0.86 to 0.90
Kendall's Tau	0.72
Significance level	**P<0.0001**
95% Confidence Interval for Tau[a]	0.69 to 0.75

[a] *BC_a bootstrap confidence interval (500 iterations; random number seed: 978).*

The Table 6.12 depicts Rank Correlation coefficient Analysis**.** The Table 6.12 represents:

- **Sample size**: the total number of BGL (RBGL and PBGL) data pairs.
- **Spearman's** coefficient of rank correlation (rho) value (0.88) with significance level of P<0.0001, indicates that the correlation is statistically significant. The 95% confidence interval for the correlation coefficient, specifically states the range value from 0.86 to 0.90 that embraces the true correlation coefficient with probability of 95%.
- **Kendall's tau** coefficient of rank correlation value (0.72) with significance level of P<0.0001, indicates that the correlation is statistically significant. The 95% confidence interval for the correlation coefficient, specifically states the range value from 0.69 to 0.75 that embraces the true correlation coefficient with probability of 95%.

Hence, the Rank Correlation coefficient analysis depicts statistical significance of our overall blood glucose measurement during clinical studies. This phenomenon directs towards the acceptable and statistically significant capability of our noninvasive technique based prototype to perform noninvasive blood glucose measurement in human subjects.

6.2.6 Bland-Altman Plot:

The Bland-Altman plot (difference plot) represents the graph-based approach to equate two measurement methods. This graphical approach depicts the plotting of the differences between the two methods versus the mean of the two methods. The Bland-Altman plot (parametric method) signifies the connection between the differences and the mean of two methods to find systemic biases [Bland *et al.* (1986); Bland *et al.* (1999)].

Here, the two measurement methods indicates the results as obtained from Reference and Predicted Blood Glucose Levels.

As per Bland *et al.* (1999) and Bland *et al.* (1986), in this present work, the Bland-Altman Plot with the mean of the same two methods on x-axis and the differences of the two methods on y-axis has been plotted.

For validating successful clinical study, the bias had to be ≤15 mg/dl (null hypothesis) [Klonoff *et al*. (2014); Amir *et al.* (2007)]. In this present work, the testing of this hypothesis performed at overall blood glucose levels as obtained during our overall clinical studies. In the paired data set of 627, the overall reference blood-glucose range has been 71-302 mg/dl.

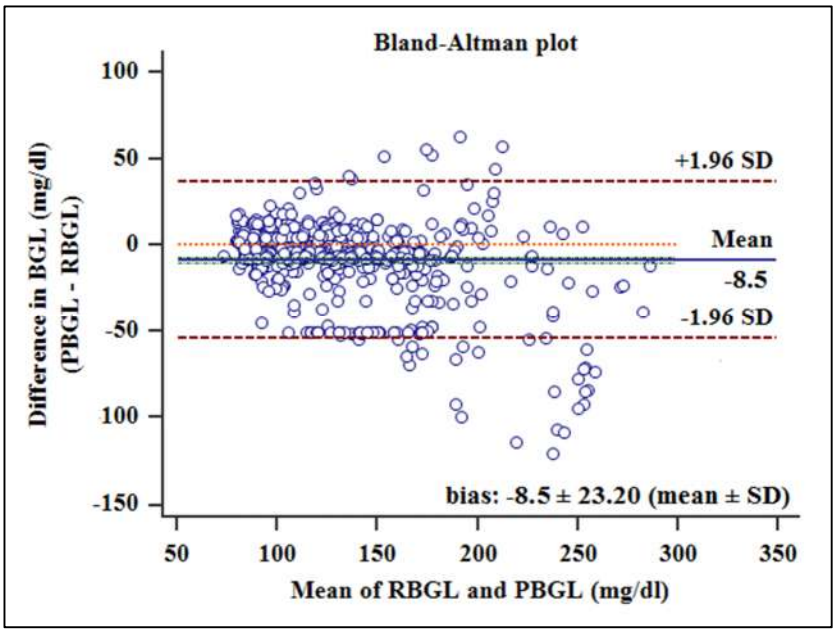

Figure 6.5: Bland-Altman Plot based analysis.

In figure 6.5, the dotted red line represents the line of equality (difference = 0). The horizontal line (dash blue line) and horizontal dotted green lines in figure 6.5 depicts

the mean difference line and 95% confidence interval of mean differences line respectively. This horizontal dotted green lines show the magnitude of systemic difference. If the line of equality does not present in this interval, it indicates statistically significant systemic difference exists.

Table 6.13: Bland-Altman Plot based analysis

Bland-Altman Plot based analysis	
Method A	PBGL (mg/dl)
Method B	RBGL (mg/dl)
Differences in mg/dl	
Sample size (n number of data pairs)	627 data pairs
Bias	-8.5 mg/dl
95% CI	-10.40 to -6.76 mg/dl
P-value	**<0.0001**
Standard deviation	±23.20 mg/dl

The limits of agreements (dotted brown lines), that states the mean difference ± 1.96 times the SD (Standard Deviation) of the differences respectively.

The Table 6.13 depicts the result of the Bland-Altman analysis for 627-paired data, corresponding to Predicted and Reference blood glucose levels. In order to compare the Predicted BGL method with the standard Reference BGL method, a Bland-Altman plot based analysis performed on all paired glucose values and utilized to measure bias of glucose overall the range of values. The measured bias in mg/dl at the overall glucose levels was found to be (95% Confidence Interval) -8.5 (-10.40 to -6.76). Based on these outcome, the null hypothesis (bias >15 mg/dl) has been rejected and both sided P-value (<0.0001) less than the conventional 0.05 significance level implies that the bias of the overall blood glucose measurement has been statistically significant. The Standard Deviation (SD) in mg/dl of the overall blood glucose-measurement differences as per Bland-Altman plot based analysis has been ±23.20 mg/dl. Hence, using modulated ultrasound and infrared light based method; we found the bias and precision of -8.5 ± 23.20 (mean ± SD) mg/dl as obtained from the 627-paired data corresponding to the range in Reference Blood Glucose Levels from 71 to 302 mg/dl.

As per Clarke *et al.* (1987) and Wentholt *et al.* (2008), the positive and negative bias signifies overestimation and underestimation of actual blood glucose levels respectively. In this present work, negative bias signifies underestimation of Reference Blood Glucose Levels by our noninvasive technique based Predicted Blood Glucose Levels. Hence, the Bland-Altman plot analysis depicts statistical significance of our overall blood glucose-measurement during clinical studies. This phenomenon directs towards the capability of our noninvasive technique based prototype unit to perform noninvasive blood glucose measurement in human subjects.

6.2.7 Mountain Plot:

The mountain plot represents "*bias at peak in percentile graph folded at median*" [Kost *et al.* (2008)]. The 'folded empirical cumulative distribution plot' often referred as 'Mountain Plot' permits evaluation between the two methods (Predicted and Reference). A nonparametric method modestly orders differences between a predicted and reference method to reach at the 2.5^{th} and 97.5^{th} percentiles (limits that cover 95% of data) [Krouwer *et al*. (1995)].

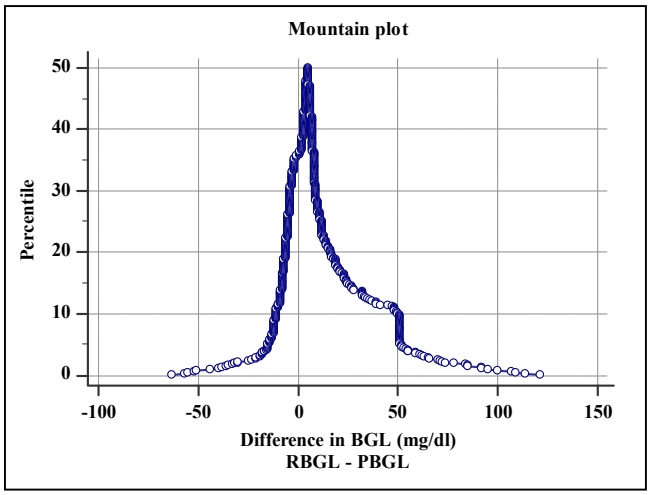

Figure 6.6: Mountain Plot based analysis

This type of plot represents percentile computation for each ranked difference between the two methods (Reference and Predicted). To obtain the folded plot, subsequent transformation executed for entire percentiles beyond 50: percentile = 100 - percentile. The next step includes plotting of all these percentiles versus the differences between the two methods [Krouwer *et al*. (1995)].

The mountain plot often utilized as the complementary plot for Bland and Altman plot. The significance of the Mountain Plot includes: (a) it is simple to find the central 95% of the data, and even applicable in case of irregular data distributions, (b) Comparison between different distribution is simple [Krouwer *et al.* (1995)].

Table 6.14: Mountain Plot based analysis

Mountain Plot based analysis	
First method	RBGL (mg/dl)
Second method	PBGL (mg/dl)
First - second method: (mg/dl)	
Sample size (n number data pairs)	627 (data pairs)
Lowest value (mg/dl)	-63.00
Highest value (mg/dl)	121.00
Median (mg/dl)	5.00
Percentiles	
2.5^{th}	-22.65
5^{th}	-15.00
10^{th}	-11.00
25^{th}	-5.00
75^{th}	12.00
90^{th}	50.00
95^{th}	51.15

The figure 6.6 depicts the Mountain Plot that offers the pattern of distribution of the differences between reference blood glucose and predicted blood glucose measurement methods. The Table 6.14 depicts precise statistics on the best significant distribution in percentiles. Herein, the mountain plot center differ from the zero point (median = 5 mg/dl) in the x-axis scale in a statistically significant way. This indicates that the biasness between the two methods is statistically significant and within the prescribed

limits (≤15 mg/dl) as per Klonoff *et al.* (2014) and Amir *et al.* (2007) references. Even, the short tails in the plot reveals statistically significant measurement differences between the two-methods.

6.2.8 Linear model validity:

The CUSUM (**CU**MULATIVE **SUM**) test for linearity examines how well the linear model fits the overall data provided. The CUSUM test for linearity checks the applicability of the method under evaluation (our noninvasive technique for blood glucose measurement) with the reference method [Passing H and Bablok W (1983)].

Table 6.15: Linear model validity

Linear model validity		
Variable X	RBGL (mg/dl)	
Variable Y	PBGL (mg/dl)	
Sample Size (n number of data pairs)	627 (blood glucose data pairs)	
Statistics name	**Variable X (mg/dl)**	**Variable Y (mg/dl)**
Lowest value	71.00	70.00
Highest value	302.00	280.00
Arithmetic mean	136.62	128.04
Median	129.00	123.00
Standard deviation	46.48	37.27
Standard error of the mean	1.85	1.48
Regression Equation	y = 10.410256 + 0.871795 x	
Linear model validity		
CUSUM test for linearity	**No significant deviation from linearity (P=0.27)**	

The Table 6.15 depicts the outcomes obtained from linear model validity as per Passing-Bablok Regression analysis [Passing H and Bablok W (1983)]. The Table 6.15 represents:

- **Sample Size:** The total sample size of 627 implies our overall blood glucose data pairs (reference and predicted blood glucose level) as obtained from our clinical studies.
- **Summary Statistics:** This portion shows the lowest value, highest value, Arithmetic mean, Median, Standard Deviation, and Standard error of the mean of both the our overall Reference and Predicted Blood Glucose Level data as obtained from our clinical studies respectively. The regression equation also provided here.
- **CUSUM test for linearity:** In general, this method is not applicable when P value is smaller than the significance level ($P<0.05$). Then, the CUSUM test for linearity implies that linear relationship does not exists between the two methods (Reference and Predicted methods) [Passing H and Bablok W (1983)].

In Table 6.15, CUSUM test for linearity shows that the $P = 0.27$, which signifies that no significant deviation from linearity. Hence, our noninvasive method for blood glucose measurement passes the CUSUM test for linearity.

6.2.9 Independent sample t-tests:

The Independent sample t-tests perform the mean comparison of the two independent samples (Reference and Predicted Blood Glucose samples). The independent sample t-tests evaluate the null hypothesis that the difference between the means of the two samples is equal to zero (null hypothesis) Armitage *et al.* (2002). The Table 6.16 depicts the X (RBGL) and Y (PBGL) variables, summary statistics, F-test for equal variances and welch test (t-test with a correction for unequal variances) respectively.

Initially, F-test has been performed and the P-value as obtained is statistically significant ($P<0.05$), which depicts that the variances of the two samples are not equal to be zero. Hence, the next step includes utilization of this phenomenon to perform the t-test with the correction of unequal variances (Welch test). Now, the Welch-test based results in Table 6.16 shows two the Difference, along with the 95% Confidence Interval of this Difference. It also includes the Test statistic t, the Degrees of Freedom (DF) and the Two-tailed probability P. Herein, as per Welch-test, the Difference, Standard Error, and 95% CI (Confidence Interval) of difference between Reference and Predicted Blood Glucose Levels has been -8.58 mg/dl, 2.37 mg/dl and (-13.24 to -3.91) mg/dl

respectively. Further, all these values are within the prescribed limit (≤15 mg/dl) as per Klonoff *et al.* (2014) and Amir *et al.* (2007) references. Further, the P-value ($P < 0.0003$) is less than the conventional 0.05, and hence the null hypothesis is rejected and the inference is that the two means differ in a statistically significant way.

Table 6.16: Independent samples t-test and Welch-test

Independent samples t-test and Welch-test		
Variable (Sample 1)	RBGL (Reference Blood Glucose Level) mg/dl	
Variable (Sample 2)	PBGL (Predicted Blood Glucose Level) mg/dl	
Statistics summary	Sample 1 (mg/dl)	Sample 2 (mg/dl)
Sample size (n number of data pairs)	627	627
Arithmetic mean (mg/dl)	136.62	128.04
95% CI for the mean (mg/dl)	132.98 to 140.27	125.12 to 130.96
Variance (mg/dl)	2161.22	1389.31
Standard deviation (mg/dl)	46.48	37.27
Standard error of the mean (mg/dl)	1.85	1.48
F-test for equal variances	**P < 0.001**	
t-test with a correction for unequal variances (Welch-test)		
Difference	-8.58 mg/dl	
Standard Error	2.37 mg/dl	
95% CI of difference	(-13.24 to -3.91) mg/dl	
Test statistic t(d)	-3.60	
Degrees of Freedom (DF)	1195.5	
Two-tailed probability	**P < 0.0003**	

Hence, this phenomenon directs towards the acceptable and statistically significant capability of our noninvasive technique based prototype to perform noninvasive blood glucose measurement in human subjects.

6.2.10 Deming Regression:

The Deming Regression accounts for error measurement in both the methods (Reference and Predicted) applied. However, conventional linear regression method undertakes simply that the Y (predicted) method accompanying arbitrary measurement errors. The measurement of slope B and intercept A performed including Standard Error and 95% confidence intervals. The confidence intervals help in measuring, if chance difference exists between B and 1 and between A and 0 [Amir *et al.* (2007); Armitage *et al.* (2002); Cornbleet *et al.* (1979)].

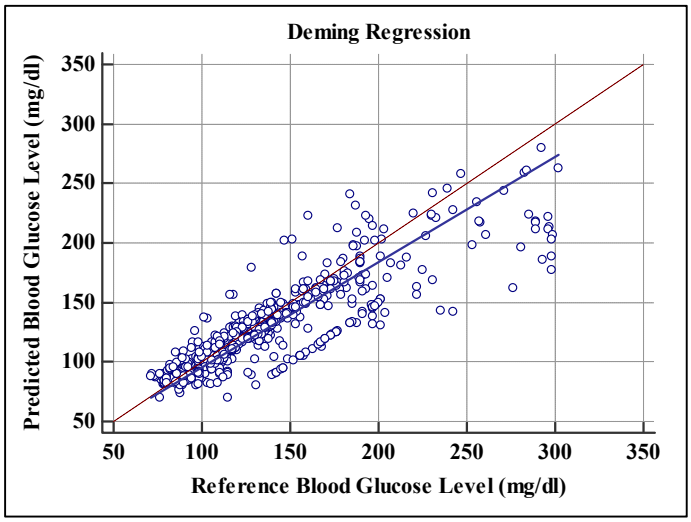

Figure 6.7: Deming Regression based analysis

Table 6.17: Deming Regression Analysis

Deming Regression Analysis			
Method	Mean	Coefficient of Variation (%)	
X (RBGL)	136.62 mg/dl	1.00	
Y (PBGL)	128.04 mg/dl	1.00	
Sample size (n number of data pairs)	627 data pairs		
Significance level	**P < 0.0001**		
Regression Equation	y= 7.2126 + 0.8844 x		
Parameter	Coefficient	Standard Error	95% CI
Intercept (mg/dl)	7.21	3.61	0.10 to 14.32
Slope	0.88	0.029	0.82 to 0.94

The Coefficient of Variance (%) used in this Deming Regression has been used as 1% for Predicted Blood Glucose Measuring method (our noninvasive technique) and as per Wentholt *et al.* (2008) 1.00% for Reference Blood Glucose Measuring method (Accu-Chek Active of Roche Diagnostics, GmbH, Mannheim, Germany).

In the paired data set of 627, the reference blood-glucose range has been 71-302 mg/dl. The figure 6.7 and Table 6.17 depicts the result of the Deming Regression analysis for 627-paired data, corresponding to Predicted and Reference blood glucose levels. In figure 6.7 the red dotted line indicates the line of equality (Y=X) and the blue dash line indicates the regression line that helps in determining slope and intercept for measuring 95% confidence intervals range, useful to assess the accuracy of the respective measurement. The Table 6.17 depicts the mean and Coefficient of Variation (%) for the reference and predicted method was 136.62 mg/dl, 1.00% and 128.04 mg/dl, 1.00% respectively.

The regression analysis provides intercept and slope with 95% confidence intervals. The Deming Regression analysis provides the slope with 95% confidence interval range from 0.82 to 0.94. This range is very close to 1.0 reflecting acceptable correlation between reference and predicted blood glucose measuring methods.

The intercept values with 95% confidence intervals range from 0.10 mg/dl to 14.32 mg/dl, which are within the prescribed limit (≤15 mg/dl) as per Klonoff *et al.* (2014) and Amir *et al.* (2007) references.

Further, the P-value ($P < 0.0001$) is less than the conventional 0.05, and hence the null hypothesis is rejected and the inference is that the two means differ in a statistically significant way.

6.2.11 ISO compliance:

As per ISO (International Organization for Standardization) 15197-2003 accuracy means “closeness of agreement between a test result and the accepted reference values” and the accuracy “involves a combination of random error components and common systemic error or bias component.” Henceforth, all these are classified as “Total Error Limits.” [Krouwer *et al.* (2008)].

In this present work, the accuracy signifies important benchmarks utilized to judge the performance of our noninvasive technique for blood glucose measurement. The

accuracy aspect comprises both the analytical performance and its medical necessity [Wentholt *et al.* (2008)]. Here, the accuracy measure evaluates our overall clinical study based blood glucose data pairs that belong to various blood glucose ranges.

In this present work, the overall results were tested for compliance with ISO 15197-2013, which specifies that "*95% of the data pairs should be within ±15 mg/dl from reference for reference glucose levels < 100 mg/dl, or within ±15% from reference for reference glucose levels ≥100 mg/dl"* [ISO 15197-2013; Klonoff *et al.* (2014)].

Hence, the ISO standard signifies the usage of both the absolute and relative errors between the predicted and reference values [Krouwer *et al.* (2008)], and our results are dichotomized here, as to examine either satisfying these criterions or not.

Table 6.18: Total Error Limits: ISO 15197-2013 [Klonoff *et al.* (2014)]

Total Error Limits		
Blood glucose levels and Total Error limits	95% of the data pairs should be within ±15 mg/dl from reference for reference glucose levels < 100 mg/dl	95% of the data pairs should be within ±15% mg/dl from reference for reference glucose levels ≥100 mg/dl
Proposed Technique based results	**+7.61 mg/dl (MAE)**	**10.77% (%MARE)**
	+6.00 mg/dl (MdAE)	**6.15% (%MdARE)**

The Table 6.18 depicts Total Error Limits as per ISO 15197-2013 recommendations. Further, our proposed noninvasive technique based Mean Absolute Error (MAE) = +7.61 mg/dl and Median Absolute Error (MdAE) = +6.00 mg/dl corresponding to the Reference blood glucose levels <100 mg/dl shows that both the values are within the prescribed limits of ±15 mg/dl respectively. Further, Percentage of Mean Absolute Relative Error (%MARE) = 10.77% and Percentage of Median Absolute Relative Error (%MdARE) = 6.15% by our proposed noninvasive technique corresponding to the Reference blood glucose levels ≥100 mg/dl shows that both the

values are within the prescribed limits of ±15% respectively. Hence, from here we can accomplish that our clinical study based results (MAE, MdAE, %MARE, %MdARE) are in compliance with the ISO 15197-2013 standard based accuracy limits. Further, our results are better or comparable with the published results of other developing noninvasive techniques and electrochemical-CGMS systems respectively.

6.3 Conclusion:

The overall results of Clarke Error Grid analysis, Mean Absolute Error, percentage-Mean Absolute Relative Error, Median Absolute Error, percentage-Median Absolute Relative Error, Root Mean Squared Error, and Standard Error of Prediction are compared with the published data in English language based research literatures available. The comparison shows that our noninvasive method based results are better or comparable with other developing noninvasive methods and Electrochemical or Microdialysis based Continuous Glucose Monitoring system(s). Further, various statistical evaluation methods including Clarke Error Grid, Parkes Error Analysis, Accuracy measure parameters, Bland-Altman plot, Mountain plot, Correlation coefficients, CUSUM test for linearity, Independent sample t tests based analysis, Deming regression analysis over our overall blood glucose measurement data yields statistically significant results.

Henceforth, all the blood glucose values reported in this present investigation was acquired by correlating the noninvasive (predicted) blood glucose values with the invasive (reference) blood glucose values as measured by the invasive glucometer (Accu-Chek Active of Roche Diagnostics, GmbH, Mannheim, Germany) respectively.

Further, the Roche Diagnostics GmbH of Mannheim-Germany assessed the accuracy of the Accu-Chek Active system as per the ISO (International Organization for Standardization) 15197 and the Accu-Chek Active system meets the entire accuracy requirement for the ISO 15197 standards [ISO_Active_EN, (2006); Lam (2008)].

All these facts indicate reliable and sensitive performance by our noninvasive technique based prototype unit. The vital factor driving this noninvasive technique comprises:

(i) Amplitude Modulated Ultrasonic wave utilizations for exciting specific molecules (glucose) present within the blood tissue complex.

(ii) Specific and useful extraction of amplitude-modulated ultrasound induced blood glucose concentration related information based embedded signals from the transmitted infrared light signal.

The combined use of ultrasound and infrared light provides a new dimension for noninvasive detection of blood glucose levels in human subjects.

Further, the signal processing toolbox of MATLAB performs observed signal analysis in Fast Fourier Transform (FFT) domain to extract blood glucose level related embedded information. The peak amplitude in FFT domain serves as the functional indicator for measuring actual blood glucose level in study subjects. Hence, this principle aspect forms the basis of our noninvasive blood glucose measurement. This is the main driving factor that distinguishes our technique from others and plays an important role for glucose measurement throughout our in-vitro and in-vivo sample based clinical studies.

In this present work, we achieve the benefit of beam forming at the ultrasonic frequency for localizing the radiating force energy towards the particular measurement site (human finger). It initiates the vibration phenomenon at the lower frequency such that the molecular displacements are large enough for measurement with infrared technique respectively [Urban *et al.* (2010)]. Steady and coherent observation of the ultrasound modulated output light signals provides an added advantage. Our combined approach performs noninvasive blood glucose measurement with medical significance and accuracy. Further, lower interference from other optically active components like oxyhemoglobin, deoxyhemoglobin, melanin, water, etc., in the tissue optical window domain provides significant advantage in acquiring blood glucose concentration based bio-signals [Konig (2000); Tenhunen *et al.* (1998)].

However, observation of certain error-induced bio-signals occurred due to multiple superfluous causes. It includes finger placement, finger shape and size, motion artifacts, time and machine drift issues, melanin induced skin pigmentations, variation in multiple physiological parameters (blood pressure, heart rate, skin sweating, body temperature), environmental changes, etc. which changes blood tissue optical characteristics and induce variations in the signal acquisition processes. Future research considering all this hurdles, will concrete the way for successful realization of this noninvasive blood glucose monitoring technique.