CHAPTER 6

DISCUSSION

6.1 Introduction:

Worldwide plenty of researchers, scientists have been utilizing numerous scientific and technological ideas to pave the successful invention of non-invasive blood glucose monitoring devices. The essential criteria about the noninvasive glucometer device are that:

(i) It must be stable clinically

(ii) User friendly

(iii) Easily portable

(iv) Cost efficient

The noninvasive blood glucose determining technology is in developing stage at present. The weak blood glucose signals, over lapping with water absorption spectra, weak signal to noise ratios are the main hurdles in the path of realization for noninvasive blood glucose detection technology. Large number of diabetic population worldwide had anticipated the steep rise in demand for noninvasive technology [So *et al.* (2012); Ramchandani *et al.* (2012)].

Various quantitative analytical tools like multivariate approaches provide results based on the data available. For blood glucose predictions, subject related specificity; individual result calibration approach needed use for successful results [So *et al.* (2012); Ramchandani *et al.* (2012)]. Factors like blood glucose level variation in different parts of the body at a time, temperature fluctuations, skin pigmentations, contact area pressure related glucose variation issues, physiological status of the subject concern, metabolic rate, body fluid circulations, must be considered in optical technology based noninvasive blood glucose monitoring devices. Due to large variation factors, universal approach for noninvasive blood glucose suffers real life setbacks. The noninvasive technology might be successful when subject based individual calibration would be targeted [So *et al.* (2012); Ramchandani *et al.* (2012)].

6.2 Phantom properties:

The biological tissue resembling phantoms generally contains identical absorption and scattering profiles of living human tissue characteristics including light wavelength selection properties [Burmeister *et al.* (1998); Beck *et al.* (1998)]. Moreover, the refractive index of phantoms must comply with the refractive index of the living biological tissues. The practical efficiency of the phantoms to mimic living tissue optical properties must be independent of various primary factors such as day

and night temperature changes, atmospheric moistures, photosensitive dyes, different climatic changes [Pravdin *et al.* (2002); Beck *et al.* (1998)]. The tissue phantom with small production cost, trouble free dispensation, better constancy, good tissue optical correlations, ease to transport are famous, demanding factors in the research and industry domains [Pravdin *et al.* (2002); Royston *et al.* (1996)]. To predict the glucose induced optical properties inside the human physiological body, the above-mentioned properties and characteristics were needed in ideal tissue phantoms [Tuchin *et al.* (2007); Burmeister *et al.* (1998)]. This feature also drives the essential need for noninvasive blood glucose determining factors. The glucose molecules exhibit very low and feeble signals. This complex and difficult phenomenon hinders the design and development of clinically acceptable noninvasive blood glucometer. The essential and predictive features required in the tissue phantom for noninvasive blood glucose experimentation purposes were as follows: (a) functional modeling of physiological phenomenon's which incorporates both the geometrical and tissue optical properties for light propagation through it. (b) The components of tissue phantoms must be chemically firm and steady to yield constant results for light spectroscopy. (c) The experimental samples results must be constant, distinctive and repetitive in nature. (d) The static and dynamic properties of tissue phantoms must be inactive towards vaporizations, diffusion and time related factors. (e) Advanced molding methods, phantom slab stacking techniques for homogeneous and inhomogeneous tissue phantom preparations must be followed as per standard guidelines. (f) trouble free, harmless, versatile and quick sample preparation procedures are welcome nowadays [Tuchin *et al.* (2007); Royston *et al.* (1996)].

6.3 Assessment and comparison of our total results:

Developing noninvasive blood glucose estimation-technique is a challenging endeavor. Further, its medical acceptance as well as recognition is essential and mandatory. For our *in-vitro* result verification purposes, the various published data from the English language based journals were-compared with our *in-vitro* experimental results respectively. Various statistical approaches were applied here, as follows: (i) Deming Regression analysis, (ii) CUSUM test for linearity, (iii) Paired sample t test based analysis, (iv) Mountain Plot analysis, (v) Bland Altman Plot analysis, (vi) Pearson Correlation analysis, (vii) Rank Correlation analysis, (viii)

Clarke Error Grid analysis, (ix) Parkes Error Grid analysis, (x) Accuracy Measure analysis.

All the statistical parameters for the result analysis used here are-based on two-sided test or as reported if necessary. In general, the statistical significance level of 0.05 has-been-followed or else mentioned as required.

In this present thesis, the MedCalc for Windows, version 15.11 (MedCalc Software, Ostend, Belgium) software has been used for the statistical analysis of our *in-vitro* experimental result analysis. The correlation and statistical analysis based on our total *in-vitro* result assessments are as per following:

6.3.1 Deming Regression based analysis:

In general, the Deming Regression performs error estimations in both the techniques (Reference and Predicted) which have been utilized for performing and assessing the experimental works. Nonetheless, it is common in conventional linear regression technique, to assume that the Y (predicted) method contains random measurement errors within it. The intercept A and slope B determinations has been executed along with the estimations of Standard Errors and 95% confidence intervals respectively. The confidence intervals assist in determining chance difference within B and 1, and within A and 0 respectively, only when this difference exists [Amir *et al.* (2007); Armitage *et al.* (2002); Cornbleet *et al.* (1979)].

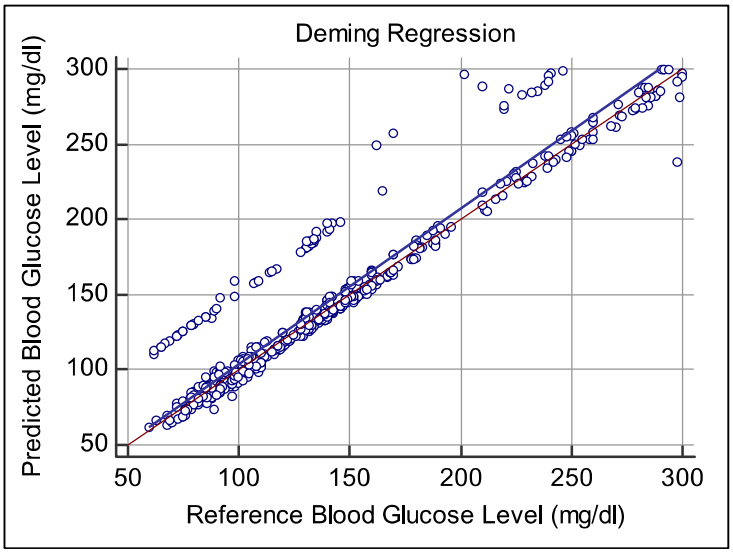

Figure 6.1: Deming Regression based analysis.

Table 6.1: Deming Regression based analysis.

Deming Regression Analysis			
Method	Mean	Coefficient of Variation (%)	
X (RBGL)	143.08 mg/dl	1.00	
Y (PBGL)	147.86 mg/dl	1.00	
Sample size (n number of data pairs)	520 data pairs		
Variance Ratio	0.94		
Regression Equation	y = -0.05935 + 1.0338 x		
Parameter	Coefficient	Standard Error	95% CI
Intercept (mg/dl)	-0.05	2.38	-4.74 to 4.62
Slope	1.03	0.017	0.99 to 1.06

In Deming Regression, the Coefficient of Variance as obtained has been 1% for Predicted Blood Glucose estimation technique (our developed measuring technique) and for GOD/POD and Accu-Chek Active invasive glucometer method the percentage of Coefficient of Variance value has been 1% for Reference Blood Glucose estimation technique [www.nzytech.com (2015); Wentholt *et al.* (2008)].

In our total 520-paired data sets, the reference blood glucose level ranges from 60 mg/dl to 300 mg/dl respectively. The figure 6.1 and Table 6.1 represents the Deming Regression analysis of the 520-paired data set of Predicted and Reference blood glucose levels as obtained during our *in-vitro* investigations.

In figure 6.1, the line of equality (Y=X) is shown as red dotted line and the regression line in blue dashed line which helps in determining slope and intercept for estimating 95% confidence intervals range. This is valuable to evaluate the accuracy of the particular estimations. The Table 6.1 represents the mean and Coefficient of Variation (%) for the reference and predicted technique which are 143.08 mg/dl, 1.00% and 147.86 mg/dl, 1.00% respectively.

In this regression analysis, intercept and slope were-calculated with a confidence interval of 95%. The range of slope values within 95% confidence interval is from 0.99 to 1.06 that is very near to 1 (one). Further as per Deming Regression analysis, the variance ratio of our total data based results as obtained has been 0.94 (very near to 01.00). Hence, these entire phenomenon's indicate a strong correlation between the reference and predicted blood glucose measuring techniques.

The range from -4.74 mg/dl to +4.62 mg/dl of intercept values lies within 95% confidence intervals as obtained during Deming Regression analysis. Further, it is within the recommended limit (≤15 mg/dl) as per the limits provided in references such as Klonoff *et al.* (2014) and Amir *et al.* (2007).

6.3.2 Linear model validity:

To observe how effectively the linear model fits the total data provided are examined in the CUSUM (CUMULATIVE SUM) test for linearity. According Passing H and Bablok W (1983), CUSUM test for linearity checks the appropriateness of the technique under assessment (our technique for *in-vitro* blood glucose estimation) with the reference technique.

Table 6.2: Linear model validity.

Linear model validity		
Variable X	RBGL (mg/dl)	
Variable Y	PBGL (mg/dl)	
Sample Size (n number of data pairs)	520 (blood glucose data pairs)	
Statistics name	**Variable X (mg/dl)**	**Variable Y (mg/dl)**
Lowest value	60.00	61.00
Highest value	300.00	299.00
Arithmetic mean	143.08	147.86
Median	132.00	135.45
Standard deviation	57.64	59.59
Standard error of the mean	2.52	2.61
Regression Equation	y = -3.160714 + 1.001786 x	
Linear model validity		
CUSUM test for linearity	No significant deviation from linearity (P=0.55)	

The Table 6.2 represents the results of our total data (Reference and Predicted blood glucose levels) as acquired using linear model validity based on Passing Bablok

Regression analysis. Further, it contains the descriptions about the sample size, summary statistics and CUSUM test for linearity.

- **Sample Size:** There are 520 data pairs as acquired from our *in-vitro* investigations. Here, each data-pair contains reference blood-glucose levels (mg/dl) and its corresponding predicted blood glucose levels (mg/dl).
- **Summary Statistics:** The acquired data from our *in-vitro* investigations, the results demonstrates the (i) lowest value, (ii) highest value, (iii) Arithmetic mean, (iv) Median, (v) Standard Deviation and (vi) Standard error of the mean of the total 520 data pairs. Further, the Table 6.2 contains the expression for the regression equation applied here.
- **CUSUM test for linearity:** As on the basis of significance level this technique is not appropriate if the P value smaller in comparison of significance level ($P<0.05$). At that point, the CUSUM test for linearity signifies that linear relationship does not exists within the two techniques (Reference and Predicted techniques) [Passing H and Bablok W (1983)].

The analysis of CUSUM test for linearity demonstrates that the $P = 0.55$, as illustrated in Table 6.2. Henceforth, our *in-vitro* technique for blood glucose estimation passes the CUSUM test for linearity.

6.3.3 Paired sample t-tests:

The paired sample t-tests performs the mean comparison of the two-paired samples (Reference and Predicted Blood Glucose samples). The paired sample t-tests evaluates the null hypothesis that the difference between the means of the two samples is equal to zero (null hypothesis) Altman (1991). The Table 6.3 depicts the X Reference Blood Glucose Level (RBGL) and Y Predicted Blood Glucose Level (PBGL) summary statistics respectively.

Initially, the P-value as obtained is statistically significant ($P<0.05$), which depicts that the average variances of the two samples are not equal to be zero. Hence, the next step includes utilization of this phenomenon to perform the Paired samples t-test. Now, the Paired samples t-test based results in Table 6.3 shows two Differences (i) Mean and (ii) Standard error of mean respectively. It also includes the 95% Confidence Interval, Test statistic t, the Degrees of Freedom (DF) and the Two-tailed probability. Herein, as per Paired samples t-test, the Mean difference, Standard error of the mean difference and 95% confidence interval between Reference and Predicted

Blood Glucose Levels has been 4.78 mg/dl, 0.84 mg/dl and 3.12 mg/dl to 6.43 mg/dl respectively. Further, all these values are within the prescribed limit (≤15 mg/dl) as per 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. The inference is that the difference between two arithmetic means is statistically significant.

Table 6.3: Paired samples t-test.

Paired samples t-test		
Sample 1	RBGL (Reference Blood Glucose Level) mg/dl	
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)	520	520
Arithmetic mean (mg/dl)	143.08	147.86
95% CI for the mean (mg/dl)	138.12 to 148.05	142.73 to 153.00
Variance (mg/dl)	3323.41	3551.86
Standard deviation (mg/dl)	57.64	59.59
Standard error of the mean (mg/dl)	2.52	2.61
Paired samples t-test		
Mean difference		4.78 mg/dl
Standard error of mean difference		0.84 mg/dl
95% CI		3.1243 to 6.4357 mg/dl
Test statistic t		5.67
Degrees of Freedom (DF)		519
Two-tailed probability		P < 0.0001

Henceforth, our technique-based prototype has been efficient to perform *in-vitro* blood glucose estimation in human blood mixed with Intralipid^{TM} phantom samples. Further, the outcomes shows the acceptable and statistical significant results.

6.3.4 Mountain Plot analysis:

Kost *et al.* (2008) represents the mountain plot "*bias at peak in percentile graph folded at median*". The 'Mountain Plot' it is also introduced as 'folded empirical cumulative distribution plot'. As a nonparametric method, this analysis method permits assessment between the two techniques (Predicted and Reference). In this technique the percentiles values reach at the 2.5^{th} and 97.5^{th} (the limit range covers 95% of data) [Krouwer *et al.* (1995)].

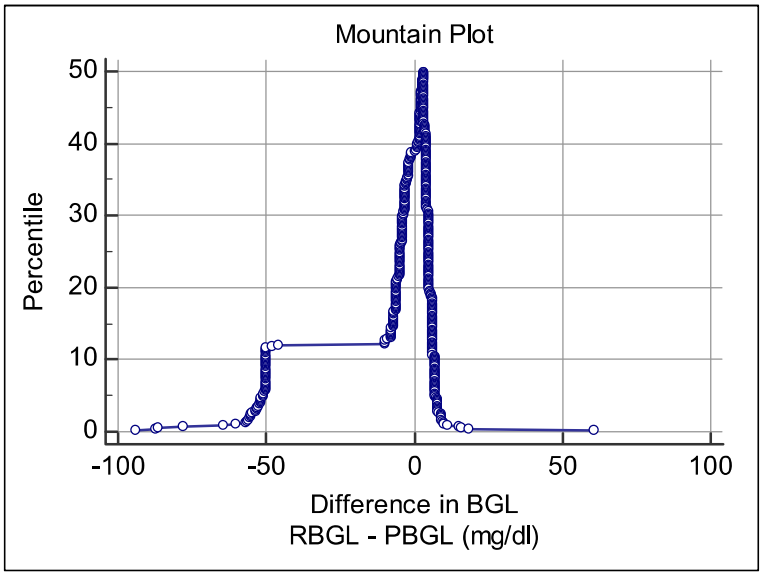

Figure 6.2: Mountain Plot based analysis.

For each ranked difference between the two techniques (Reference and Predicted) this plot signifies a percentile measurement. Consequently, a transformation is performed for percentiles beyond 50: percentile = 100 - percentile to acquire the folded plot. In the continuation of step, it plots of all these percentiles versus the differences between the two techniques [Krouwer *et al.* (1995)].

The mountain plot is usually utilized for Bland and Altman plot as the complementary plot. The importance of the Mountain Plot includes:

(i) In this technique the central 95% of the data is easily found, and it is applicable in the case of asymmetrical data distributions

(ii) Easy comparisons between various types of distributions [Krouwer *et al.* (1995)].

Table 6.4: 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)	520 (data pairs)
Lowest value (mg/dl)	-94.20
Highest value (mg/dl)	60.50
Median (mg/dl)	3.00
Percentiles	
2.5^{th}	-54.85
5^{th}	-51.00
10^{th}	-50.00
25^{th}	-5.00
75^{th}	5.00
90^{th}	7.00
95^{th}	7.00

The pattern of distribution of the differences within the reference blood glucose and predicted blood glucose estimation techniques, provided by the Mountain Plot analysis, is illustrated in figure 6.2. The optimum significant distribution in percentiles based on accurate statistics is represented in Table 6.4. In a statistically significant perspective, the mountain plot center differ from the zero point (middle = 3 mg/dl) in the x-axis scale. According to Klonoff *et al.* (2014) and Amir *et al.* (2007) the biasness between the two techniques which is within the recommended limits (≤15 mg/dl) and most importantly it is statistical significant. The short tails present in Mountain Plot shows the statistically significant estimation differences between the two-techniques.

6.3.5 Bland-Altman Plot analysis:

Bland-Altman plot also known as difference plot, it is the graph-based method used to equate two estimation techniques. The difference within the two techniques

versus the mean of the two techniques has-been-represented by plotting the graphical method. Hence, to find systemic biases between the differences and the mean of two techniques Bland-Altman plot based analysis is significant [Bland *et al.* (1999); Bland *et al.* (1986)].

Here, two estimation techniques demonstrate the outcomes as acquired from Reference and Predicted Blood Glucose Levels.

According to Bland *et al.* (1999) and Bland *et al.* (1986), in this experimental work, the Bland-Altman Plot with the mean of the same two techniques on x-axis and the differences of the two techniques on y-axis has been plotted.

For verifying successful *in-vitro* investigation, the bias must be ≤15 mg/dl (null hypothesis) [Klonoff *et al.* (2014); Amir *et al.* (2007)]. In this experimental work, the testing of this hypothesis executed for total blood glucose levels as acquired throughout our total clinical examinations. In the paired data set of 520, the total reference blood-glucose range has been 60-300 mg/dl.

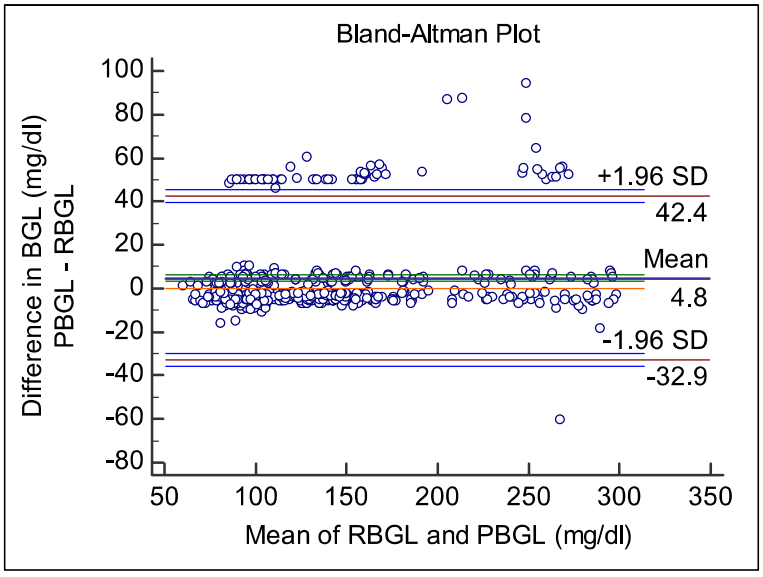

Figure 6.3: Bland-Altman Plot based analysis.

The line of equality (difference = 0) as shown the (red dotted line) in figure 6.3. In figure 6.3, the mean difference line and 95% confidence interval of mean differences line are represented as horizontal line (dash blue line) and the horizontal (dotted green lines) respectively. The magnitude of systemic difference is shown by

the horizontal dotted green lines. It demonstrates that statistically significant systemic difference exists when the line of equality is not present in the interval.

Table 6.5: 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)	520 data pairs
Bias	4.78 mg/dl
95% CI	3.12 to 6.43 mg/dl
P-value	**<0.0001**
Standard deviation	±19.21 mg/dl

The dotted brown lines represents the limits of agreements, the SD (Standard Deviation) of the differences that expresses the mean difference ± 19.21 times respectively.

The outcome of 520 paired data during the Bland-Altman analysis respective to Predicted and Reference blood glucose levels is shown in Table 6.5. The Bland-Altman plot predicated investigation is performed on total paired glucose values. It is used to evaluate bias of glucose to compare the Predicted BGL technique and the standard Reference BGL technique. The assessed bias in mg/dl at the total glucose levels was found to be (95% Confidence Interval) 4.78 (3.21 to 6.43).

On the basis of these result the P-value of both sided (<0.0001) are less compared to the conventional 0.05 significance level, that signifies that the bias of the total blood glucose estimation is statistically significant and the null hypothesis (bias>15 mg/dl) has been rejected. In total *in-vitro* blood glucose estimation the differences according to Bland-Altman plot based examination, the Standard Deviation (SD) has been ±19.21 mg/dl. Subsequently, the bias 4.78, as acquired from the 520 paired data respective to the Reference Blood Glucose Levels from 60 to 300 mg/dl.

The positive and negative bias means overestimation and underestimation of real blood glucose levels according to the Clarke *et al.* (1987) and Wentholt *et al.* (2008) respectively. In this present work, the positive bias indicates overestimation of

Reference Blood Glucose Levels by our *in-vitro* technique based Predicted Blood Glucose Levels.

Henceforth, our total blood glucose estimation during *in-vitro* based investigation the Bland-Altman plot analysis represents statistical significant results. This phenomenon directs towards the ability of our technique based prototype unit to execute *in-vitro* blood glucose estimation in human blood mixed with Intralipid^{TM} phantom samples.

6.3.6 Pearson Correlation Coefficient analysis:

The degree of association between the two estimation-based results is known as Pearson Correlation Coefficient (parametric analysis). According to the Bland M *et al.* (2000) and Altman D G (1991) it is an estimation of exactness and assesses how far each observation diverges from the best-fit line respectively.

In this present work the Reference Blood Glucose Level (RBGL) in mg/dl and Predicted Blood Glucose Level (PBGL) in mg/dl it contains two-estimation outcomes for our total *in-vitro* investigations. In our 520 paired data set, which includes the reference blood glucose range from 60-300 mg/dl. The graphical relationship between the estimation of Reference (RBGL in mg/dl) and Predicted blood glucose levels (PBGL in mg/dl) represented in scatter plot figure 6.4. The outcomes yields the points in the scatter plot graph, that contains both the blood glucose estimations as represented in figure 6.4. The horizontal axis represents Reference Blood Glucose Level (RBGL in mg/dl) and another vertical axis represents Predicted Blood Glucose Level (PBGL in mg/dl). The line of equality (Y=X) represented as red dotted line in the scatter plot.

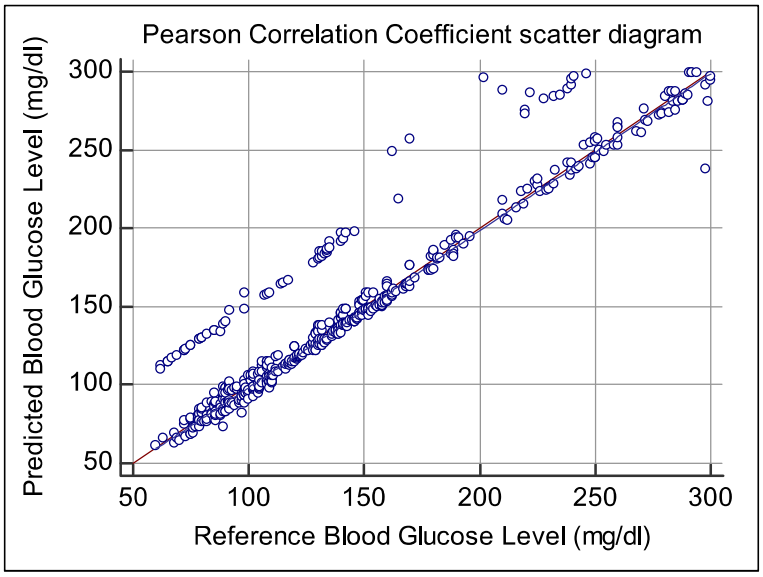

Figure 6.4: The scatter diagram of Reference and Predicted Blood Glucose Levels.

Table 6.6: 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)	520
Correlation coefficient (r)	0.94
Significance level	**P<0.0001**
95% Confidence interval for r	0.93 to 0.95

The Table 6.6 illustrates Pearson correlation coefficient (r) analysis. The Table 6.6 represents:

- **Sample size:** the (RBGL and PBGL) data pairs of overall number of blood glucose levels.
- **Correlation coefficient with significance level:** The significance level of P-value (<0.0001) represents that the relationship is statistical significant. Our Pearson correlation coefficient value is 0.94.

- **95% CI (Confidence Interval) for r:** the 95% confidence interval for the correlation coefficient, particularly expresses the value ranges from 0.93 to 0.95 that embraces the real correlation coefficient with probability of 95%.

6.3.7 Rank Correlation Coefficients analysis:

According to Armitage *et al.* (2002) the degree of relationship among the two variable and the data ranking take place in direction of their sizes, and also the estimations with respect to the ranks of equivalent values in X and Y variables are known as Rank Correlation (nonparametric analysis). In this present work, to study the degree of relationship between Reference Blood Glucose Level (RBGL) and Predicted Blood Glucose Level (PBGL) we have executed Spearman's coefficient of rank correlation (rho) and Kendall's tau coefficient of rank correlation predicated examination.

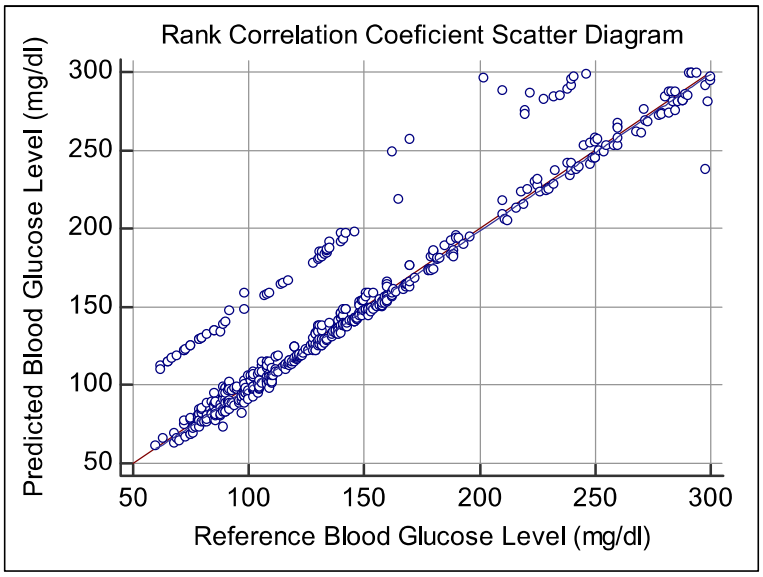

Figure 6.5: The scatter diagram of Reference and Predicted Blood Glucose Levels.

The points in the scatter plot graph of both the blood glucose estimation results output as represented in figure 6.5. The horizontal axis represents Reference Blood Glucose Level (RBGL in mg/dl) and another vertical axis represents Predicted Blood Glucose Level (PBGL in mg/dl). Here, the line of equality (Y = X) is presented as the red dotted line in the scatter plot.

Table 6.7: 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)	520
Spearman's coefficient of rank correlation (rho)	0.92
Significance level	P<0.0001
95% Confidence Interval for rho	0.90 to 0.93
Kendall's Tau	0.81
Significance level	P<0.0001
95% Confidence Interval for Tau[a]	0.78 to 0.84

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

The Table 6.7 illustrates Rank Correlation coefficient analysis. The Table 6.7 represents:

- **Sample size:** the (RBGL and PBGL) data pairs of overall number of blood glucose levels.
- **Spearman's** coefficient of rank correlation (rho) value is (0.92) along with the significance level of P<0.0001, it shows that the relationship is statistical significant. The 95% confidence interval for the correlation coefficient, particularly expresses the value ranges from 0.90 to 0.93, which embraces the real correlation coefficient with probability of 95%.
- **Kendall's tau** coefficient of rank correlation value is (0.81) along with the significance level of P<0.0001, it demonstrates that the relationship is statistical significant. The 95% confidence interval for the correlation coefficient, particularly expresses the values ranging from 0.78 to 0.84, which embraces the real correlation coefficient with possibility of 95%.

Subsequently, our total *in-vitro* blood glucose estimation during clinical examinations represents the statistical significance Rank Correlation Coefficient analysis. This phenomenon indicates the acceptable and statistical significant ability of our technique-based prototype to execute *in-vitro* blood glucose estimation in human blood mixed with Intralipid^{TM} phantom samples.

6.3.8 ISO compliance analysis:

According to ISO (International Organization for Standardization) 15197-2003 accuracy signifies "*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".* Hereafter, all these are categorized as "Total Error Limits." [Krouwer (2008)].

In this present work, to judge the performance of our develop technique for *in-vitro* blood glucose estimation the accuracy implies significant benchmarks. According to Wentholt *et al.* (2008) the accuracy features contains both the experimental significance and its medical usefulness. Here, the accuracy based measure assesses our total *in-vitro* experimental study based on blood glucose data pairs that belong to different blood glucose ranges.

In this present work, the total results were examined for compliance with ISO 15197-2013, which identifies 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"* [Klonoff *et al.* (2014); ISO 15197-2013].

Henceforth, the ISO standard means the use of both the absolute and relative errors between the predicted and reference values [Krouwer (2008)], and our outcomes are divided here, as to study either we are satisfying these criterions or not.

Table 6.8: 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	**+12.91 mg/dl (MAE)**	**+10.01% (%MARE)**
	+5.00 mg/dl (MdAE)	**+3.00% (%MdARE)**

According to ISO 15197-2013 recommendations the Total Error Limits represents in Table 6.8. Further, for our proposed measuring technique Mean Absolute Error (MAE) = +12.91 mg/dl and Median Absolute Error (MdAE) = +5.00 mg/dl relating to the Reference blood glucose levels <100 mg/dl demonstrates that both the values are within the recommended limits of ±15 mg/dl respectively.

Further, Percentage of Mean Absolute Relative Error (%MARE) = +10.01% and Percentage of Median Absolute Relative Error (%MdARE) = +3.00% by our proposed measuring technique respective to the Reference blood glucose levels ≥100 mg/dl demonstrates that both the values are within the recommended limits of ±15% respectively. Thus, from here we can conclude that our *in-vitro* experimental study based results (MAE, MdAE, %MARE, %MdARE) are in compliance with the ISO 15197-2013 standard based accuracy limits. Further, our *in-vitro* study based outcomes are better or comparable with the published results of other developing glucose monitoring techniques.

6.3.9 Clarke Error Grid analysis:

The Clarke Error Grid analysis is based on paired readings of the (Reference and Predicted) blood glucose measurement. The Clarke Error Grid analysis contains five different zones. These zones indicate the medical significance ranging from no action to conceivably risky or opposing management. According Wentholt *et al.* (2008) and Clarke *et al.* (1987) recognized in the year of 1987-89 this analysis technique determines the accuracy of glucose measurement to its medical significance.

Our total *in-vitro* examination as reported in previous chapter 5 of this present thesis contains examination over 187 (male = 138 and female = 49; in which normal healthy subjects are = 93 and diabetic subjects = 94) all the study subjects are adult, that outputs total 520 data pairs of reference and predicted blood glucose levels. Further, in 520-paired data set, the equivalent reference blood-glucose range has been 60-300 mg/dl.

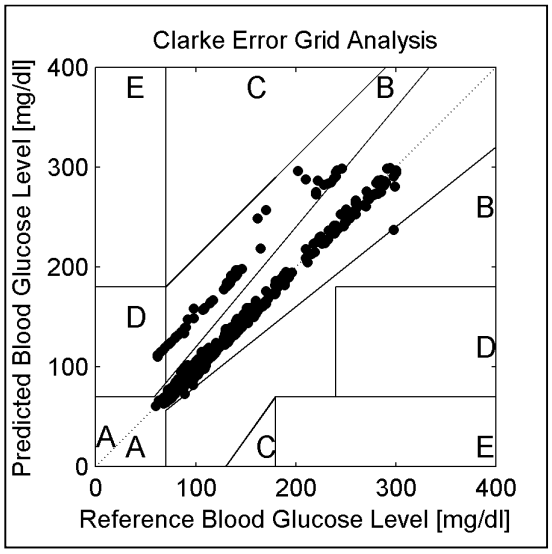

Figure 6.6: Clarke Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from overall human subject's blood samples mixed with Intralipid™ phantom.

Table 6.9: Clarke Error Grid analysis of reference and predicted blood glucose levels as acquired during *in-vitro* examination of overall human subject's blood samples mixed with Intralipid™ phantom.

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**	456	87.69%
B Zone	**Medically acceptable**	58	11.16%
C Zone	**Medically insignificant and potentially harmful**	00	00.00%
D Zone		06	01.15%
E Zone		00	00.00%

The figure 6.6 illustrated Clarke Error Grid analysis of all the reference and predicted blood data pair sets as acquired during *in-vitro* examination. In Table 6.9, the Clarke Error Grid analysis demonstrates the percentage of the total data pairs (520) falling in the zones A, B, C, D, and E are 87.69% (456 data pairs), 11.16% (58 data pairs), 00.00% (00 data pairs), 01.15% (06 data pairs) and 00.00% (00 data pairs) respectively. Consequently, all the 514 data pairs possess the medically significant A

and B zones respectively. Further, the 06 data pair set possesses medically insignificant and potentially dangerous C to E zones respectively.

6.3.10 Parkes Error Grid analysis:

Based on the ability of enormous group of medical specialists the Parkes *et al.* (2000) turn back into the idea of error grid zones and developed a new pair of innovative error grids. The distinguishing feature between the Type I and Type II diabetic subjects in this new Error Grids were ideally performed for identification. Category wise the Park Error Grids divided into five zones for example Zone A to Zone E respectively [Pfutzner *et al.* (2013)].

(i) Zone A indicates medically correct determinations, with no significance over medical supervision.

(ii) Zone B indicates changed medical action, minute, or no significance over medical treatment.

(iii) Zone C indicates changed medical action, possible to effect medical treatment.

(iv) Zone D indicates changed medical action, may include essential medical risk.

(v) Zone E indicates changed medical action, may include risky effects.

The Park Error Grids have not risk borders that bounce the categories in the comparison to Clarke Error Grid and Park Error Grids marginally varies from the Clarke Error Grid zone wise. In the Park Error Grids the zone A and B bigger in area in this way the consensus error grid is more permissive when compared with Clarke Error Grid based investigation. According to the Pfutzner *et al.* (2013) and Wentholt *et al.* (2008), none of the borders of all the zones are free from the arbitrariness.

The Parkes Error Grid is utilized for the clinical accuracy estimations of evaluating any method to examine the Type I Diabetes version. Compared between Type I and Type II diabetes version in Parkes Error Grid analysis the Type I have more stringent borders [Pfutzner *et al.* (2013)].

In this present work, our total clinical examination based *in-vitro* blood glucose estimation performed by Parkes Error Grid analysis represented in figure 6.7 and Table 6.10.

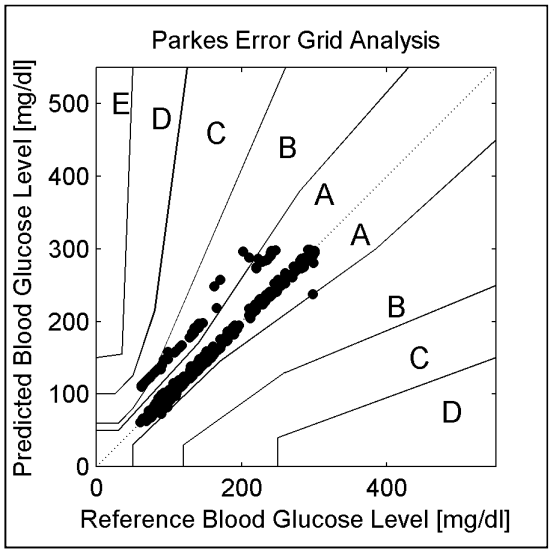

Figure 6.7: Parkes Error Grid analysis based plot for reference and predicted blood glucose measurement as obtained from overall human subject's blood samples mixed with Intralipid^{TM} phantom.

Table 6.10: Parkes Error Grid analysis of reference and predicted blood glucose levels as acquired during *In-vitro* examination of overall human subject's blood samples mixed with Intralipid^{TM} phantom.

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**	466	89.66%
B Zone	**Slight**	50	09.61%
C Zone	**Moderate**	04	00.73%
D Zone	**Significant**	00	00.00%
E Zone	**Dangerous**	00	00.00%

The figure 6.7 and Table 6.10 illustrates Parkes Error Grid analysis of our overall blood glucose data pair sets including reference and predicted readings as acquired during our overall *in-vitro* examinations. The Parkes Error Grid Analysis demonstrates that the percentage of the total data pairs (520) falling in zones A, B, C, D, and E are 89.66% (466 data pairs), 09.61% (50 data pairs), 0.73% (04 data pairs), 00.00% (00 data pairs) and 00.00% (00 data pairs) respectively. Subsequently, the

Parkes Error Grid analysis illustrates that 89.66% (466 data pairs) of the *in-vitro* estimations are in risk free A zone (clinically accurate). Further, 09.61% (50 data pairs) of the *in-vitro* estimations are in slight risk B zone (clinically acceptable). Further, 00.73% (04 data pairs) readings occupies C (moderate risk zone). None of the readings occupies D (significant risk zone), and E (dangerous risk zone) zones respectively.

6.3.11 Accuracy measure analysis:

In this present work, the Accuracy measures of error for accuracy evaluation incorporates (i) Standard Error of Prediction (SEP), (ii) Mean Absolute Error (MAE), (iii) Median Absolute Error (MdAE), (iv) Root Mean Squared Error, (v) Percentage of Mean Absolute Relative Error (%MARE), (vi) Percentage of Median Absolute Relative Error (%MdARE) [Srivastava *et al.* (2016); Chowdhury (2015)].

The paired glucose values using for accuracy assessment techniques includes (i) Mean Absolute Error (MAE), (ii) Median Absolute Error (MdAE), (iii) Percentage of Mean Absolute Relative Error (rate MARE), (iv) Percentage of Median Absolute Relative Error (rate MdARE) to meet the necessities per standard limits as archived in published works or ISO standards [Wentholt *et al.* (2008)].

The Mean Absolute Error (MAE) and Median Absolute Error (MdAE) describes the systemic under or over estimation of one technique in comparison to other. The MAE (mean of the predicted sensing technique values minus the reference sensing technique values) and MdAE (Median of the predicted sensing technique values minus the reference sensing technique values).

However, the overestimation and underestimation of blood glucose values straightens out the negative and positive errors counter balances one other. Henceforth, this technique measures the stable absolute or relative bias of one method with respect to the other.

The percentage MARE and Percentage MdARE presents the mean and median absolute errors within (reference and predicted) both the techniques respectively. Further percentage is also calculated. It also demonstrates percentage difference between predicted technique and reference technique, it also contains an under or over estimation respectively.

The estimations of all these parameters are easy, and the results are simpler to understand. The bias and variation information are obtained by the percentage MARE

and Percentage MdARE. At the point higher bias either or both variation between both the predicted and reference technique dose occurs, it generates high values of percentage MARE and Percentage MdARE.

Generally, the percentage MdARE values for Continuous Glucose Monitoring Systems are lower than the percentage MARE values. Despite the fact that, the percentage MdARE seems to be more statistical significant than percentage MARE, the published literatures reports largely about percentage MARE predicated values [Wentholt *et al.* (2008)].

The Table 6.11 illustrates our performance assessment values as procured during overall subjects and the results comparing with published data ranges of other developing glucose monitoring technique. The performance metrics based errors the SEP (Standard Error of Prediction) value were 19.19 mg/dl. The MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 10.76 mg/dl, 05.00 mg/dl, and 19.79 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 08.99%, and 03.40% respectively.

Further, as illustrates from Table 6.11, the yield results acquired by our MUS IR system is better than or comparable with other developing blood glucose measuring techniques for noninvasive blood glucose monitoring. Its precision levels are additionally comparable with other commercially existing Continuous Glucose Monitoring System.

Table 6.11: Statistical parameters utilized for accuracy assessment and the results comparison with published data ranges of other developing glucose monitoring techniques.

Statistical Parameters	Assessment Values	Published Data Ranges of other Developing Glucose Monitoring Techniques	References
Standard Error of Prediction (SEP)	19.19 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)	10.76 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)	19.79 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)	08.99 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)	03.40 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 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.

6.4 Conclusion:

The various statistical evaluation methods includes (i) Deming Regression analysis, (ii) CUSUM test for linearity, (iii) Paired sample t test based analysis, (iv) Mountain Plot analysis, (v) Bland Altman Plot analysis, (vi) Pearson Correlation analysis, (vii) Rank Correlation analysis, (viii) Clarke Error Grid analysis, (ix) Parkes Error Grid analysis had demonstrated the statistically significant results, of our proposed glucose measurement technique.

Further, the total results of (i) Standard Error of Prediction, (ii) Mean Absolute Error, (iii) Median Absolute Error, (iv) Root Mean Squared Error, (v) Percentage Mean Absolute Relative Error, (vi) Percentage Median Absolute Relative Error, are has been compared with the published results. The comparison shows that our measuring technique based results are better or comparable with other developing glucose monitoring techniques.

The total blood glucose values reported in this present examination was obtained by correlating the our technique based (predicted) blood glucose values with the established technique based (reference) blood glucose values as measured by GOD/POD method and one experiment performed by invasive glucometer (Accu-Chek Active of Roche Diagnostics, GmbH, Mannheim, Germany).

Each one of these realities shows reliable and good performance by our technique based prototype unit. The significant factor driving this *in-vitro* technique based prototype unit contains the Amplitude Modulated Ultrasonic wave utilization for exciting particular particles (especially glucose) present inside of the blood mixed Intralipid^{TM} phantom samples as well as in different phantom mediums like water, commercialized milk, chicken breast tissue and whole blood respectively. Extraction of the embedded specific signal information related to glucose concentration is obtained by modulated ultrasound and infrared light based technique.

Additionally, to extract the blood glucose level related encoded information in Fast Fourier Transform (FFT) domain signal it is executed by the processing of signal analysis toolbox of MATLAB. The peak amplitude in FFT domain provides as the functional indicator for estimating real blood glucose level in human blood mixed with Intralipid^{TM} phantom samples. Henceforth, this principle perspective forms the

basis of our *in-vitro* examination based study for blood glucose estimation. On basis of differentiations, this main operating factor differentiates our technique from the others. It plays a significant role for glucose estimation all through our *in-vitro* based experimental studies. The advantage of ultrasonic frequency for focusing the specific estimation site and its sufficiently significant molecular displacement estimation with infrared light technique is additional advantage for consistent and coherent estimation technique for blood glucose monitoring.

Our combined method for *in-vitro* examination based study performs blood glucose estimation is medically significant and acceptable. Further, lower interferences from other optical active constitutes like water, oxyhemoglobin, deoxyhemoglobin, melanin, and so forth, in the tissue optical window region provides important advantage in obtaining blood glucose concentration based bio-signals [Konig (2000); Tenhunen *et al.* (1998)]. Hence, the proposed prototype unit provides a new direction for developing noninvasive estimation of blood glucose levels.

However, observation of certain error-induced bio-signals occurred due to multiple superfluous causes. It includes characteristic variations in Intralipid^{TM} sample constituents, *in-vitro* sample handling complexities, storage issues, difficulties in samples collection procedures, environmental changes, contaminations, egg yolk from different species, sample tube positioning in the sample holder of the MUS-IR unit. These factors provide impact over the phantom medium optical characteristics. Our future *in-vitro* investigations considering all this challenges, will concrete the route for more fruitful realization of this *in-vitro* blood-glucose measurement technique for developing the non-invasive glucometer.