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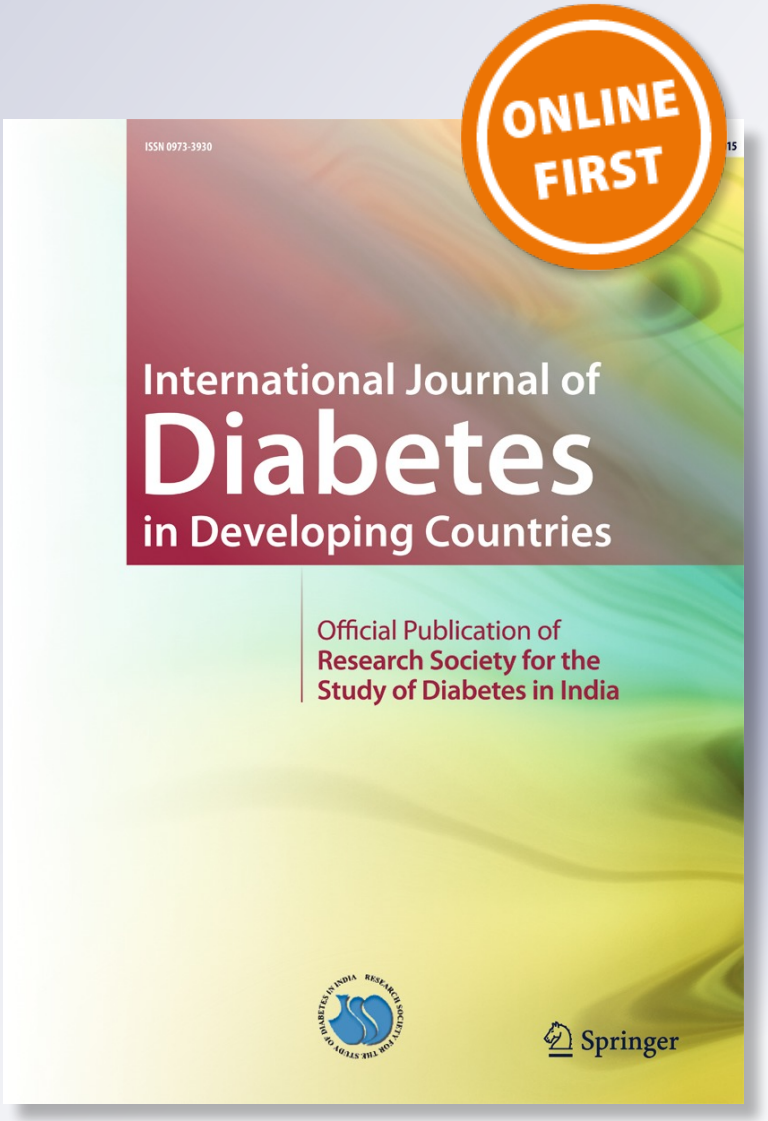

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ORIGINAL ARTICLE

Noninvasive blood glucose measurement utilizing a newly designed system based on modulated ultrasound and infrared light

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Abstract The approach of a new noninvasive innovation for blood glucose measurement will reform management of diabetes alongside expanded patient compliance, decline load on therapeutic crisis, and diabetes associated complications. In this present work, we represent a modulated ultrasound and infrared technique-based noninvasive system for blood glucose measurement over human subjects. The oral glucose tolerance test- and random blood glucose level test-based clinical study was performed over human subjects to measure the performance of our indigenously designed system. The accuracy was examined by pairing and comparing our noninvasive predicted data with the invasive reference data. The oral glucose tolerance test and random blood glucose test produced a total of 180 and 30 paired glucose values (noninvasive vs. invasive), respectively. The root mean squared error between the noninvasive and invasive glucose value for oral glucose tolerance test and random blood glucose measurement was 23.76 mg/dl and 28.20 mg/dl, respectively. The Pearson correlation coefficient between the noninvasive and invasive glucose value for both the tests was 0.76 and 0.85, respectively. Similarly, the mean absolute error for both the tests was 15.92 mg/dl and 17.76 mg/dl, respectively. Further, the Clarke Error Grid analysis depicts that both the tests result occupy medically accurate and acceptable zones A and B, respectively (oral glucose tolerance test: zone A=78.33 %; zone B=21.66 % and random blood glucose measurement: zone A=83.33 %; zone B=16.66 %). Hence, the present study direct towards the potentiality of our technique for noninvasive blood glucose measurement. The instrument was medically safe and well tolerated.

Keywords Oral glucose tolerance test · Random blood glucose level · Ultrasound · Infrared light · Noninvasive technique

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Introduction

Diabetes has progressed as an important healthcare endemic of the contemporary world. Globally, the aggregate number of individuals with diabetes ascends from 382 million in 2013 to 592 million in 2030. Similarly, in our country (India), the total number of diabetic population increase from 65.1 million in 2013 to 109 million in 2030. Overall, the numbers of diabetics are expanding at an enormous alarming pace [1].

The diabetic subject needs to monitor blood glucose level (BGL) regularly for four to five times per day for circumventing the terminal stage medical complexities and expenses. The invasive device-based blood glucose monitoring comprises painful procedures, mental agony, and infection liabilities. For all these reasons, at present, a new noninvasive technique for blood glucose measurement is extremely demanding [2–6].

In perspective of addressing the requirement for this necessity, the present work's objective is to validate our indigenously developed noninvasive technique for blood glucose measurement in the human subjects.

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Literature review and our concept for noninvasive blood glucose measurement

Zhu et al. (2013) and Zhu et al. (2010) demonstrated a new hybridized technique of utilizing an ultrasound-modulated optical technique for noninvasive blood glucose measurements. Measurement of modulation depth through ultrasound-modulated scattered light produces significant in vitro results. However, their technique lacks in in vivo experiments [7, 8].

They had applied ultrasound with light modulation techniques [7, 8], but in our application, the modulated ultrasound produces vibration and the infrared light quantity, those molecule specific vibrations for noninvasive measurement of blood glucose levels in the human subjects.

When the amplitude modulating ultrasonic waves enters inside the measurement site (human finger), the blood constituent molecules undergoes vibration due to the influence of the amplitude-modulated ultrasonic waves. The pressure of the ultrasound waves generates those changes. The intrinsic properties of these molecules present in the blood medium influences the compressibility factor. The shape and size of the molecules present in the blood medium (segment) play an important role. The magnitude of influence over larger molecules is more as compared to the smaller ones [9–11].

Hence, the vibration produced in the blood medium (segment) depends on (i) its spatial arrangement, (ii) intrinsic property of the molecules and medium, and (iii) strength and frequency of the amplitude-modulated ultrasonic waves, respectively [9–11].

The pressure amplitude of the ultrasonic standing wave has maximum and minimum values twofold above the distance of a unit wavelength. Within the propagating blood tissue medium (segment), discontinuities such as blood molecules (glucose), cells achieve location-specific ultrasonic potential energy due to their presence in the respective ultrasonic zone. The suspended blood molecules (glucose) start to move and accumulate near to the zone of least ultrasonic potential energy. For blood molecules (glucose), these concentrated zones are commonly near to the pressure nodes, distanced from each other by half a wavelength space [9–11].

Now, when the molecular diameter is smaller than the wavelength of propagating ultrasound inside the measurement site, the main force of radiation (F_r) acting on the molecular volume (V_c), positioned by path of distance (z) from the pressure node, is obtained from the gradient of the molecular ultrasonic potential energy [9–11], and mathematically expressed as:

$$F_r = -\left[\frac{\pi p_0^2 V_c \beta_w}{(2\lambda)}\right].\Phi(\beta,\rho).\sin(4\pi z/\lambda) \quad (1)$$

In this present work, P_o stands for peak amplitude of the ultrasonic pressure. (λ) denotes ultrasound wavelength in the suspending segment. β_w signifies compressibility of the suspending segment [9–11] and mathematically expressed as:

$$\Phi(\beta,\rho) = \left[\frac{5_{\rho_c} - 2_{\rho_w}}{2_{\rho_c} + \rho_w} - \left(\frac{\beta_c}{\beta_w}\right)\right] \quad (2)$$

where β_c stands for compressibility of the molecules. Notations ρ_c and ρ_w represent the respective densities of the molecules and the suspending segments, respectively [9–11].

The well-known Lambert-Beer was law applied here to measure the specific absorption (A) property of the glucose molecule at a definite light wave number (v) and mathematically expressed as:

$$A(v) = -\log I(v)/I_0(v) \quad (3)$$

In this present work, the I_o denotes the background intensity and I represents the specific light intensity at the particular wave number (v) of actual measurements [11].

Hence, as per Urban et al. (2010) and Silva et al. (2007), we achieve the benefit of beam forming at ultrasonic frequency for localizing the radiating force energy towards the particular measurement site (human finger). It initiates the vibration phenomenon at lower frequency such that the displacements are large enough for quantification with the infrared technique, respectively [12–14].

In this present work, 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 voltage amplitude variations 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.

Our noninvasive technique-based system descriptions

Initially, it includes the description regarding ultrasound transducer and light wavelength selection criterions followed by the explanation of the indigenously designed system.

Ultrasound frequency selection

Ultrasonic transducers have found numerous applications in household appliances, medical fields, industries, and oceanography [15, 16]. In the biomedical field, the 40-kHz piezoelectric-based ultrasonic transducers aids in enhancing fibrinolysis [17], tissue penetration [17, 18], clot dissolution [18, 19], thrombolysis [19], and wound healing therapy [20]. In this present work, we have selected 40-kHz central frequency-based ultrasonic transmitters for providing modulated ultrasonic waves to the measurement site (fingertip). Further, (i)

easy availability, (ii) medically safe and tolerable [15–20], (iii) less tissue heating [16–20], (iv) high skin tissue penetration [15–20], and (v) low costs of 40-kHz ultrasonic transducers have boosted our motivation for its selection.

Light wavelength selection

The composition of human blood is complex and constitutes numerous components in it. Further, glucose molecules exhibit very weak signals. The wavelength selection needs careful and well-judged approaches to overcome this particular phenomenon [21].

Kulkarni et al. [2010] and Konig [2004] described that the region extending from 600 to 1100 nm is the "Tissue Optical Window" of the living biological tissues.

The absorption spectrum of oxyhemoglobin and deoxyhemoglobin is comparatively low within the spectral wavelength band 900 to 1000 nm, respectively. The blood oxygenations vary blood optical absorption properties [22–27].

Khalil [2004] and Khalil [1999] presented that glucose molecules exhibit absorption peaks at the 939-nm (very near to 940 nm) NIR spectral band. So, considering wavelength bands within the "Tissue Optical Window" where water, oxyhemoglobin, deoxyhemoglobin exhibit low absorption profiles and good absorption properties from glucose molecules will be better to avoid any particular noise interferences [26, 27].

Further, for significant wavelength selection, we have performed glucose specificity and sensitivity analysis. The specificity refers here to the particular light wavelength where glucose exhibits maximum absorption peak characteristics. Similarly, the sensitivity refers here to the ability of the particular light wavelength to respond in accordance with the effective variations in the glucose concentration levels.

Glucose specificity analysis

To determine the maximum wavelength specificity of glucose molecule in distilled water between the 900 and 980 nm wavelength domain, we have prepared a stock solution of 2500 mg dextrose (glucose) per 10 ml of distilled water. From that prepared concentration (*w*/*v*) stock solution, 2 ml of glucose in distilled water solution has been pipetted out, poured inside the cuvette. The Digital Spectrometer Model 305 of M.S Electronics (India) is used in this present work to measure absorbance (in arbitrary unit=au) of glucose in distilled water solution. Figure 1 depicts the absorption spectra of the glucose in distilled water. Figure 1 reveals that at 940 nm, the glucose in distilled water exhibits maximum absorption peak characteristics within the infrared spectral domain of 900 to 980 nm, respectively. Further, glucose molecule exhibits absorption peak characteristics at 939 nm (very close to 940 nm) due to possible stretch and vibration of the second O-H overtone band in its molecular structure [25, 26].

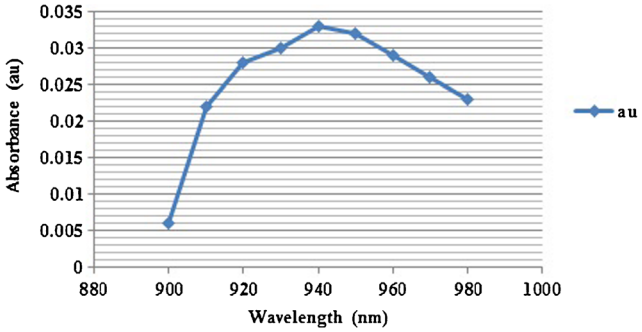

Fig. 1 Absorption spectra of glucose in distill water between 900 and 980 nm

Glucose sensitivity analysis at 940 nm

To determine the 940-nm wavelength degree of sensitivity for respective change in glucose concentration, we have prepared three sample concentration (*w*/*v*) solutions (dextrose in distilled water) like 2500 mg/10 ml, 5000 mg/10 ml, and 7500 mg/10 ml, respectively.

The Digital Spectrometer Model 305 of M.S Electronics (India) performs the measurement analysis here. Table 1 depicts the output data from those three prepared sample concentration (*w*/*v*) solutions for glucose sensitivity analysis at a 940-nm wavelength. The output data in absorbance (au) and concentration (parts per million=ppm) presents that the 940-nm wavelength has been sensitive and linearly detects the respective variations of glucose concentration (*w*/*v*) in the prepared sample solutions.

Hence, various factors like (i) "Tissue Optical Window" range (600 to 1100 nm) [21, 22], (ii) typical peak characteristic of the glucose molecule at 940 nm [21–27], (iii) acceptable specificity cum sensitivity for glucose at 940 nm [21–27], (iv) easy commercial availability [15], and (v) several literatures [28–35] favoring 940 nm for noninvasive blood glucose measurement strongly influenced us for this particular wavelength selection.

Experimental setup

In this pilot study, we have utilized the modulated ultrasound and infrared light technique-based system. The ultrasonic transmitter and receiver operate at the frequency of 40.0± 1.0 kHz. It can resist utmost input voltage of 20 V_{rms} and

Table 1 Glucose sensitivity analysis at 940 nm wavelength

Wavelength (nm)	2 ml from the prepared sample solutions (*w*/*v*)	Absorbance (au)	Concentration (ppm)
940	2500 mg/10 ml	0.033	071
	5000 mg/10 ml	0.045	113
	7500 mg/10 ml	0.067	150

produces a sound pressure output of 110±5 dB at 10 V and 30 cm. In this present work, we have utilized the 940-nm infrared light emitting diode (B5B-940-8) of Roithner LaserTechnik, Vienna, Austria, for irradiating the fingertip of the study subjects. It is a round type with a 5-mm diameter, works with GaAlAs/GaAs technology, and possesses radiated output power (P_o) of 32 to 48 mW. Similarly, we have utilized EPD-1300-5.3, InGaAs selective photodiode of Roithner LaserTechnik, Vienna, Austria, for capturing transmitted light from the infrared light (940 nm)-irradiated human finger to measure noninvasive blood glucose levels. This photodiode is perfect for detection of pulsed light with sensitivity starting from 800 to 1750 nm. Figure 2 represents the block diagram of our experimental system utilized in this pilot study.

In this system as depicted in Fig. 2, the carrier wave and the modulating signal unit provides two types of typical sine wave inputs for producing the amplitude-modulated signal waves. This modulated signal serves as an input to the ultrasonic transmitter to provide the amplitude-modulated ultrasonic signal waves to the finger (measurement site) through the finger holder. Here, the ultrasonic receiver unit monitors the characteristics of the generated modulated ultrasonic waves. The modulated ultrasonic wave direction of propagation and the light source point of focus are geometrically perpendicular to the finger-positioning angle. The infrared light focused to the ultrasonic zone of impact produces output as the amplitude-modulated ultrasound light signals. The infrared detector sensitive in the infrared region acquires those specific output signals. The signal amplifier block amplifies the acquired signals. Afterwards, acquired signals were stored in the computer for further processing through the signal processing toolbox of MATLAB to predict and display the noninvasive blood glucose levels.

Calibration

The calibration of our experimental system depends upon the peak voltage amplitude (mV) in the FFT domain corresponding to the blood glucose concentration. The calibration factor multiplies with the peak voltage amplitude (mV) in the FFT domain to provide predicted (noninvasive) blood glucose levels in milligram/deciliter and mathematically expressed as:

$$V_{\text{peak}} \times \text{CF} = \text{PBGL} \tag{4}$$

where V_{peak} stands for peak voltage amplitude (mV) in the FFT domain, CF stands for calibration factor, and PBGL refers to predicted blood glucose level in milligram/deciliter.

In vitro experiment

In order to establish a noninvasive blood glucose measurement technique, in vitro quasi-finger system (optical tissue phantoms)-based experiments are highly significant. Optical phantoms resemble tissue optical properties, in which various elementary principles and scientific concepts are tested [36–38].

In this present work, our initial approach includes preparing the quasi-finger system to mimic finger absorption and scattering characteristics in the infrared spectral domain. The

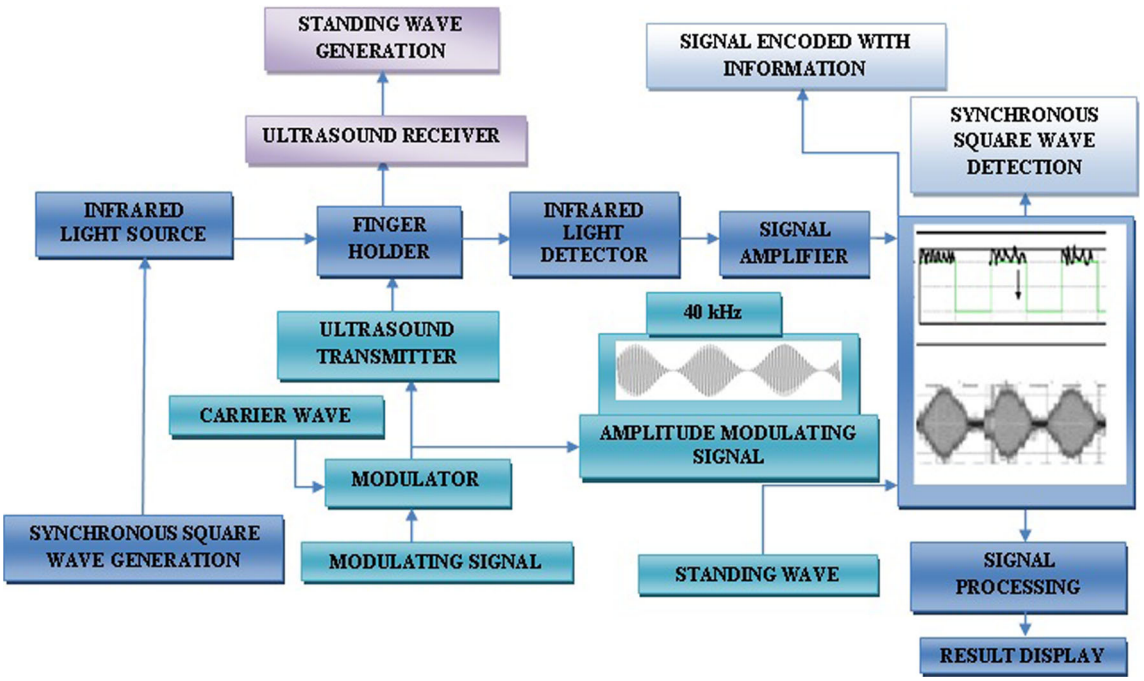

Fig. 2 Noninvasive technique-based blood glucose measuring system

intralipid phantom resembles tissue scattering aspects, while absorption is accounted here by direct addition of the whole blood samples (fasting and postprandial) to it to form the quasi-finger system [36–38].

Mixture of both of these constituents resembles blood tissue medium. The constant level of oxygen in prepared samples is essential to obtain glucose concentration-derived results [36–38].

Herein, in vitro (with this prepared quasi-finger system) phantom sample analysis was performed, to explore the glucose sensitivity of our noninvasive technique-based prototype unit.

Study subjects

In total, 12 adult subjects participated in this pilot study. Two subjects are healthy normal, three subjects had prediabetes, and seven subjects had diabetes. The mean±standard deviations of age is 41±5 years old and mean±standard deviations of body mass index is 27.3±3 kg/m^2. Overall, ten subjects were male and two subjects were female. The pilot study reported here is in accordance with the standard ethical procedures and performed with the informed consent of all the respective subjects. The pilot study was approved by the Ethical Committee Board of Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Sample preparation

The sample preparation steps include:

Step 1: Collection of whole blood samples in vacuum-based blood collecting vials, where K_2 EDTA (ethylene diamine tetra acetic acid) is present as an anticlotting agent. Addition of phosphate buffer solution (PBS) to maintain the pH levels in the samples

Step 2: De-oxygenation of the whole blood samples by nitrogen gas bubbling for 45 min to reduce oxygen influence over glucose measurement

Step 3: One milliliter of the intralipid suspension mixed with 1 ml of whole blood sample to form a quasi-finger system resembling blood tissue optical properties

Step 4: Placing each prepared phantom samples inside the sample holder of our prototype unit for its respective glucose concentration measurement

The invasive measurements were performed to measure and compare the blood glucose levels with the FFT domain-based peak voltage amplitude (mV).

Table 2 represents the voltage amplitude in the FFT domain during fasting stage and its effective correlation with the invasive blood glucose levels. Similarly, Table 3 represents the voltage amplitude in the FFT domain during postprandial stage and its effective correlation with the invasive blood glucose levels.

Table 2 In vitro glucose sensitivity in phantom samples of fasting stage

Study subject	Invasive fasting blood glucose level (mg/dl)	Peak voltage amplitude (mV) in the FFT domain during fasting stage
Healthy subject 1	87	8.9
Healthy subject 2	89	9.2
Prediabetic subject 3	124	12.9
Prediabetic subject 4	113	11.6
Prediabetic subject 5	119	12.3
Diabetic subject 6	179	18.3
Diabetic subject 7	221	23.8
Diabetic subject 8	168	17.8
Diabetic subject 9	171	16.6
Diabetic subject 10	164	17.2
Diabetic subject 11	201	19.8
Diabetic subject 12	233	22.7

In both Tables 2 and 3, the voltage amplitude in the FFT domain changes in correlation with the variation of invasive blood glucose levels.

These correlated changes indicate towards the sensitivity (at 940 nm) of our prototype unit in detecting glucose concentration variations in respective quasi-finger system based in vitro samples.

Further, voltage amplitude in the FFT domain increases with increase in blood glucose levels, this phenomenon indicates towards the glucose concentration-induced light clearing

Table 3 In vitro glucose sensitivity in phantom samples of postprandial stage

Study subject	Invasive postprandial blood glucose level (mg/dl)	Peak voltage amplitude (mV) in the FFT domain during postprandial stage
Healthy subject 1	103	10.5
Healthy subject 2	109	11.3
Prediabetic subject 3	159	16.5
Prediabetic subject 4	164	17.2
Prediabetic subject 5	153	15.8
Diabetic subject 6	261	25.9
Diabetic subject 7	311	31.9
Diabetic subject 8	307	32.2
Diabetic subject 9	272	28.9
Diabetic subject 10	291	30.8
Diabetic subject 11	288	31.5
Diabetic subject 12	301	33.7

effects. As increase in glucose concentration in vitro samples causes reduction of the scattering effects, which minimizes the mismatch of the refractive index acquiescently, increase in light transmission occurs [36–38].

The effective correlation in vitro experiment forms the benchmark as well as foundation for performing in vivo experiments to establish the performance and efficacy of our technique in blood glucose measurement.

In vivo experiment

Study subjects

In total, 60 adult subjects participated in this pilot study, out of which, 30 healthy adult subjects participated for OGTT analysis. Next, 30 adult subjects participated for random blood glucose level tests. Eighteen subjects are healthy normal, seven subjects had prediabetes, and five subjects had diabetes. All the prediabetic and diabetic subjects followed their normal routine of meal intake and medications. The mean±standard deviations of age is 40±4 years old and mean±standard deviations of body mass index is 26.2±2 kg/m^2. Overall, 44 subjects were male and 16 subjects were female. The pilot study reported here is in accordance with the standard ethical procedures and performed with the informed consent of all the respective subjects. The pilot study was approved by the Ethical Committee Board of Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University; Varanasi, India.

Experimental protocol

In this present work, the pilot study consists of two phases (oral glucose tolerance test and random test) to validate the clinical correlation between the invasive technique- and noninvasive technique-based blood glucose levels.

During the first phase, we have performed oral glucose tolerance tests over healthy subjects after their overnight fasting. The duration of each experiment was 2 h and 45 min (10 to 15 min for baseline observation before intake of 75-g glucose solution). The invasive and noninvasive data were recorded every 30 min from the right and left-hand fingers, respectively.

The Accu Chek Active of Roche Diagnostics, Germany [39] and our technique-based system performed reference (invasive) and predicted (noninvasive) blood glucose measurement here, respectively.

Throughout the investigation, the study subjects remain static to reduce motion artifacts and intake of any food or liquids were restricted.

During the second phase, we have performed random blood glucose level analysis (both invasive and noninvasive) over normal, prediabetic, and diabetic subjects, respectively.

Result and discussion

In this present work, for result analysis, we have performed Clarke error analysis and statistical analysis.

The Clarke error grid analysis critically evaluates the medical importance of the differences between noninvasive (predicted) and invasive (reference) blood glucose measurements.

The Clarke Error Grid analysis has been the universal approach for evaluating medical significance of the developing glucose sensor (mostly noninvasive)-based techniques for blood glucose determination.

The Clarke Error Grid consist of five different zones in the XY-Cartesian graph with the following interpretations: Zone A: medically accurate, Zone B: medically significant and tolerable, and Zone C to E: medically insignificant and dangerous. Further, the diagonal line where $X=Y$, expresses the ideal measurements. The data points below and above the diagonal line represent the overestimation and underestimation of the real blood glucose values, respectively. When any data pair points fall over the borderline of any zones, the nearness of its (X, Y) coordinate towards either zone determines its zone of occupancy [40–46].

Figure 3 depicts the Clarke Error Grid analysis of the oral glucose tolerance test (180 data pairs)-based BGL data measured by our noninvasive system in comparison to the BGL data measured by invasive device (Accu Chek Active of Roche Diagnostics, Germany).

The Clarke Error Grid analysis presents as follows: A zone=78.33 % and B zone=21.66 %, and none of the values in C to E zones. Hence, oral glucose tolerance test yields that all the noninvasive blood glucose readings occupy A and B zones which are medically important and acceptable.

Table 4 represents invasive and noninvasive blood glucose levels as measured during random blood glucose tests in over 30 study subjects. Figure 4 represents the Clarke Error Grid

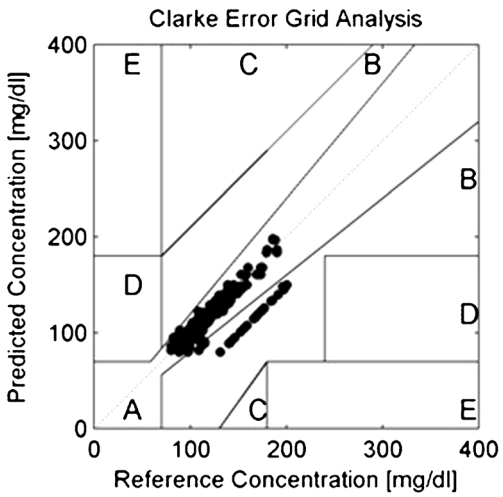

Fig. 3 Clarke Error Analysis of blood glucose data as measured during oral glucose tolerance tests

analysis of the random blood glucose measurement. The Clarke Error Grid Analysis depicts that all the measurements occupy A and B zones (Zone A=83.33 %, Zone B=16.66 %).

Hence, the random blood glucose measurement represents that all the noninvasive readings are medically significant and acceptable.

Now, the statistical analysis applied here evaluates the blood glucose levels measuring accuracy of our noninvasive technique-based prototype unit during oral glucose tolerance test and random blood glucose measurements.

Table 5 represents the mathematical expressions of the accuracy measure parameters utilized in this present work over the N number of total samples. All these accuracy measure evaluates the correctness of predicted noninvasive blood glucose levels with respect to the reference invasive blood glucose levels.

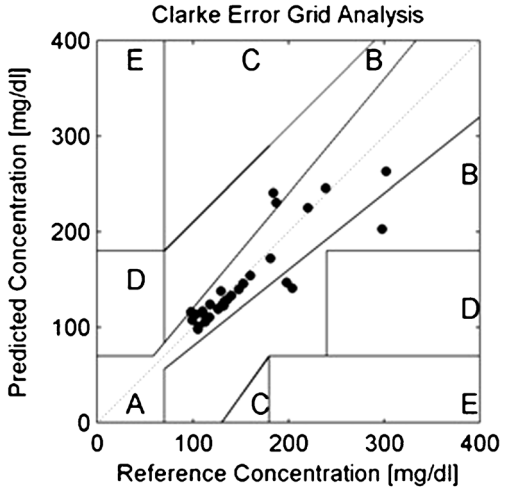

Fig. 4 Clarke Error Analysis of blood glucose data as measured during random blood glucose measurements

Table 4 Invasive and noninvasive blood glucose levels measured during random blood glucose tests

Subjects	Reference (invasive) BGL (mg/dl)	Predicted (noninvasive) BGL (mg/dl)
01	105	98
02	153	146
03	98	116
04	130	122
05	302	263
06	118	124
07	181	172
08	126	119
09	148	140
10	133	127
11	136	129
12	184	241
13	110	117
14	113	106
15	198	147
16	132	123
17	140	133
18	220	225
19	110	115
20	204	141
21	106	101
22	130	122
23	239	246
24	117	111
25	298	203
26	160	154
27	103	114
28	129	138
29	187	231
30	99	108

Table 6 depicts the statistical analysis-based summary of invasive vs. noninvasive accuracy measures obtained during the pilot study over study subjects. The performance metrics-based errors MAE, MSE, and RMSE values range from 15.92 mg/dl to 17.76 mg/dl, 564.78 mg^2/dl^2 to 795.76 mg^2/dl^2, and 23.76 mg/dl to 28.20 mg/dl, respectively. The performance metrics-based relative errors MARE, MSRE, and RMSRE values range from 0.11 mg/dl/min to 0.10 mg/dl/min, 0.02 $mg^2/dl^2/min^2$ to 0.01 $mg^2/dl^2/min^2$, and 0.15 mg/dl/min to 0.13 mg/dl/min, respectively. Similarly, the performance metrics-based percentage errors MAPE, MSPE, and RMSPE values range from 11.09 % to 10.10 %, 232.48 % squared to 181.40 % squared, and 15.24 % to 13.46 %, respectively. Further, the correlation coefficient (R value) value ranges from 0.76 to 0.85 respectively.

Table 6 depicts that our root mean square error (RMSE) for oral glucose tolerance test and random blood glucose measurement was 23.76 mg/dl and 28.20 mg/dl, respectively. These values are significantly comparable with other noninvasive technique-based ranges that extend from 25 to 46 mg/dl [46, 51, 52].

Further, Table 6 depicts the R value (Pearson correlation coefficient) for oral glucose tolerance test and random blood glucose measurement was 0.76 and 0.85, respectively. These values are important and comparable with the R value (correlation coefficient) of various other in vivo noninvasive techniques, which extends from 0.49 to 0.95 [51, 52], respectively.

From the perspective of the Clarke and Error Grid analysis, all the noninvasive readings occupy medically significant zones (A and B) and none of the measurements occupy medically insignificant zones (C–E).

Hence, all the overlaid findings including in vitro analysis, Clarke error grid analysis, and performance metrics-based

Table 5 Accuracy measure parameters

Accuracy measure	Mathematical expression	Symbol notations	References
MAE (mean absolute error)	$\frac{\sum_{i=1}^{N}\lvert y_i-\hat{y}_i\rvert}{N}$	Where y_i is the reference blood glucose level (RBGL), $\hat{y}_i$ is the predicted blood glucose level (PBGL), and N represents the total number of samples	[47–49]
MSE (mean squared error)	$\frac{\sum_{i=1}^{N}(y_i-\hat{y}_i)^2}{N}$		
RMSE (root mean squared error)	$\sqrt{\frac{\sum_{i=1}^{N}(y_i-\hat{y}_i)^2}{N}}$		
MARE (mean absolute relative error)	$\frac{\sum_{i=1}^{N}\left\lvert\frac{y_i-\hat{y}_i}{y_i}\right\rvert}{N}$		
MSRE (mean squared relative error)	$\frac{\sum_{i=1}^{N}\left(\frac{y_i-\hat{y}_i}{y_i}\right)^2}{N}$		
RMSRE (root mean squared relative error)	$\sqrt{\frac{\sum_{i=1}^{N}\left(\frac{y_i-\hat{y}_i}{y_i}\right)^2}{N}}$		
MAPE (mean absolute percentage error)	$\frac{\sum_{i=1}^{N}\left\lvert\frac{y_i-\hat{y}_i}{y_i}\right\rvert\times 100}{N}$		
MSPE (mean squared percentage error)	$\frac{\sum_{i=1}^{N}\left[\left(\frac{y_i-\hat{y}_i}{y_i}\right)\times 100\right]^2}{N}$		
RMSPE (root mean squared percentage error)	$\sqrt{\frac{\sum_{i=1}^{N}\left[\left(\frac{y_i-\hat{y}_i}{y_i}\right)\times 100\right]^2}{N}}$		
Pearson correlation coefficient (R value)	$\frac{N(\sum xy)-(\sum x)(\sum y)}{\sqrt{\left[N\sum x^2-(\sum x^2)\right]\left[N\sum y^2-(\sum y^2)\right]}}$	Where x represents RBGL value, y represents PBGL value, and N represents total number of samples.	[50]

Table 6 The accuracy measurement summary of OGTT and random blood glucose measurement

Statistic function (unit)	Accuracy measurement	
	Oral glucose tolerance test	Random blood glucose measurement
MAE (mg/dl)	15.92	17.76
MSE (mg^2/dl^2)	564.78	795.76
RMSE (mg/dl)	23.76	28.20
MARE (mg/dl/min)	0.11	0.10
MSRE ($mg^2/dl^2/min^2$)	0.02	0.01
RMSRE (mg/dl/min)	0.15	0.13
MAPE (%)	11.09	10.10
MSPE (% squared)	232.48	181.40
RMSPE (%)	15.24	13.46
R value	0.76	0.85

accuracy analysis depict the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

However, certain error-induced bio-signals were observed due to multiple superfluous causes such as 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), and environmental changes, which changes blood tissue optical characteristics and induce variations in the signal acquisition processes. In future works, acquiring preventive measures is essential to reduce the abovementioned interferences.

Conclusion

We have represented the indigenously designed modulated ultrasound and infrared technique-based system for

noninvasive blood glucose measurement on human subjects. Both the in vitro and in vivo results showed good correlation in glucose measurements.

Further, the result of the Clarke error grid analysis and statistical evaluations values validates that our noninvasive system is potentially capable in performing noninvasive blood glucose measurement over human subjects.

Our noninvasive system was medically safe, easy, and acceptable, as reflected by the overall study subject's well-tolerated compliances.

Therefore, a new technique for noninvasive blood glucose measurement using modulated ultrasound and infrared technique is developed and the observation validates the supposition of the new concept. Positively, our technique will show its functional usage in the future for noninvasive estimations of blood glucose levels in human subjects.

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Authors' contribution Md. Koushik Chowdhury, the Doctoral student, wrote the manuscript and is the corresponding author of the manuscript. Md. Koushik Chowdhury and Anuj Srivastava performed experimentations and data collection during the studies. Dr. Neeraj Sharma (associate professor) and Dr. Shiru Sharma (assistant professor) helped in overall supervision for the experimentations, final editing of the manuscript, and getting the necessary formal applications required for the experimental purposes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Research involving human participants and/or animals-ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Error Grid Analysis of Reference and Predicted Blood Glucose Level Values as Obtained from the Normal and Prediabetic Human Volunteers

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Abstract **Background:** In this research paper, we represent a new noninvasive blood glucose level determining technology based on Amplitude Modulated Ultrasound and Infrared techniques. The successful advent of a noninvasive blood glucose determining technology will be helpful for patients with abnormal episodes of elevated Blood Glucose Levels. Noninvasive device will increase patient's compliances along with firm control over elevated Blood Glucose Levels (BGL). Moreover, it will reduce diabetes related medical emergency and burden from the shoulders of healthcare professionals. **Research Design:** A total of 10 adult human volunteers (02 Normal and 08 Prediabetic) had been engaged in this experimental pilot study. Main objective of these experiments are to analyze and compare the blood glucose levels as obtained from the established invasive (Accu-chek Active invasive blood glucose monitoring system from Roche Diagnostics) and indigenously developed noninvasive BGL determining technology (Amplitude Modulated Ultrasound and infrared Unit) respectively. The blood glucose levels after overnight fasting and 02 hour after meal had been observed in Normal and Prediabetic volunteers. Again following the next day, blood glucose level at fasting stage and 02 hour after 75gm/100ml glucose solution consumption had been monitored in those Normal and Prediabetic volunteers. Moreover, the invasive (reference) and noninvasive (predicted) blood glucose levels as obtained had been plotted over Clarke and Parkes Error Grids for evaluating indigenously developed technique performances. **Results:** The experimental findings reveal that Normal volunteers fasting blood glucose level exists more or less between (80-110) mg/dl. Similarly in separate pilot studies, their Blood Glucose Level varies more or less between (130-140) mg/dl after 02 hour of meal consumption and 75gm/100ml of glucose solution consumption respectively. But in case of Prediabetic volunteers, their fasting blood glucose level exists more or less above 110 mg/dl. Likewise in separate experimental studies, their Blood Glucose Level ranges more or less between (140-199) mg/dl after 02 hour of meal consumption and 02 hour after 75gm/100ml glucose solution consumption respectively. Moreover, the invasive (reference) and noninvasive (predicted) blood glucose levels of all the volunteers (normal and prediabetics) occupies medically significant and acceptable A and B zones in Clarke and Parkes Error Grids Analysis respectively. **Conclusions:** Experimental observations indicates the potential and prospective capability of our indigenously developed noninvasive Blood Glucose Level determining technique (Amplitude Modulated Ultrasound and Infrared unit) as revealed from the pilot studies over Normal and Prediabetic volunteers.

Keywords Clarke and Parkes Error Grid Analysis, Invasive, Noninvasive, Blood Glucose, Amplitude Modulated Ultrasound, Infrared Techniques

1. Introduction

Individual with elevated blood glucose level but not as much high as compared with diabetic patients are generally suffering from the IGT (Impaired Glucose Tolerance) or IFG (Impaired Fasting Glucose) related symptoms [1-3]. IGT refers to the medical condition in which blood glucose level elevates within the range between (140-199) mg/dl even two hours after food consumption [1-3]. Similarly, the IFG refers to the medical situation where blood glucose level elevates above 110mg/dl even after overnight fasting time period [1-3]. The global populations resembling such typical symptoms are termed as prediabetics [1-3]. Consequently; the individuals with Prediabetic symptoms are severely prone to develop Type II Diabetes in near future [1-3]. Moreover, the IGT resembles Type II Diabetes symptoms adjunct with obesity, age progression, incapability of the human bodies to utilize insulin secreted form beta cells of the pancreas [1-3].

Complete change in lifestyle pattern, increased physical activity, controlled diet with low glycemic index food

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consumption checks the progression of IGT towards Type II Diabetes Mellitus [1-3]. Globally 31.6 crores of people suffer from IGT related symptoms [1]. These numbers globally shares 6.9% of the present total adult population [1]. Moreover, large proportion of these population resides in lower as well as middle income territories. Worldwide 15.3 crores of the adult population suffer from these IGT symptoms within 50 year span of their life [1]. They also possess increased possibility to extend it towards Type II Diabetes Mellitus in later part of their life [1]. The global dominance of IGT resembles diabetes pervasiveness, with enormous proportion in African and European countries as compared to South-East Asian countries [1]. As per future estimations during the year 2035 A.D, 47.1 crores of peoples from the world population will suffer from IGT related symptoms or nearly 8.0% from the then total adult world population [1].

The crucial points of care for robust supervision of elevated blood glucose level include its 3 to 4 times regular monitoring on a daily basis [1-5]. Invasive glucometers rigorously perform such measurements each and every time with respective tissue puncturing procedures. Consequently the diabetic patients generally experiences mental agony for regular tissue puncturing and patient incompliance occurs [3-5]. All this factors drives the urge for a nascent, novel noninvasive blood glucose determining technology with successful clinical applications [4, 5, 20-22]. In recent years, several optical techniques based noninvasive blood glucose level determining approaches had arrived with good potentiality and promising aspects [4-6, 20-22]. Such novel approaches mainly consists of Infrared Spectroscopy [7, 8, 20-22], Raman Spectroscopy [9, 20-22], Scattering Spectroscopy [10], Fluorescent Spectroscopy [11, 20-22], Polarimetry [12, 13, 20-22], Thermal Gradient Spectroscopy [14, 20-22], Photo-Acoustic Technology [15, 20-22], OCT (Optical Coherence Tomography) [16, 20-22], Occlusion Spectroscopy [17, 20-22], Photo-Thermal Technology [18], Ultrasound-Modulated Optical Techniques [19], etc.

The blood glucose exhibit weak signals and overlapped by numerous interferences from the surrounding optically active similar molecules [20-22]. To override such signal interferences we had applied Amplitude Modulated Ultrasound and Infrared Techniques altogether here. Moreover, this research paper focuses on the performance evaluation of indigenously developed noninvasive technique for determination of blood glucose levels in Normal and Prediabetic human volunteers.

Rest of Research Paper organizations are as follows: Section II describes the principle and working methodology. Section III illustrates the Instrumental block diagram, light wavelength and ultrasound band selection criteria. Section IV contains the experimental results and discussion portions. Section V provides the conclusive part of the research paper followed by acknowledgment and references.

2. Principle and Working Methodology

Noninvasive technique includes mainly amplitude modulated ultrasonic waves, infrared light beam and its respective IR (Infra Red) detector for determining blood glucose levels in Normal and Prediabetic Human Volunteers.

When standing ultrasonic waves propagates through the finger based blood tissue complex medium of human volunteers, it initiates the process of vibration throughout that respective medium. The molecules vibrate depending upon their respective mass, physical, chemical properties [23-25]. Moreover, the effect of radiation forces applied over the molecules had been derived from the gradient of molecular acoustic potential energy [23-32] and expressed as follows:

$$\mathbf{F_r} = -\left[\frac{\pi \mathbf{p_o^2 V_c \beta_w}}{(2\lambda)}\right].\phi(\boldsymbol{\beta}, \boldsymbol{\rho}).\sin(4\pi \mathbf{z}/\boldsymbol{\lambda}) \quad (1)$$

The symbols like **(F_r), (V_c), (z), (P_0),** and **(λ)** stands for radiation force characteristics, volume of the respective molecules, space form the node of pressure, ultrasonic wave peak amplitude and wavelength of ultrasound respectively [23-32].

When compressibility aspects ($\boldsymbol{\beta_w}$) of the suspending (blood tissue complex) segment present in human volunteers finger are considered, the mathematical expression had been represented as given below:

$$\phi(\boldsymbol{\beta}, \boldsymbol{\rho}) = \left[\frac{5\rho_c - 2\rho_w}{2\rho_c + \rho_w} - \left(\frac{\beta_c}{\beta_w}\right)\right] \quad (2)$$

The symbols like ($\boldsymbol{\beta_c}$) stands for compressibility of the molecules and ($\boldsymbol{\rho_c}$), ($\boldsymbol{\rho_w}$) signifies molecular density of the suspending molecules and suspending segment respectively [23-32].

When infrared light beam propagates through these ultrasound (amplitude modulated ultrasonic waves) excited (blood tissue complex) optical medium, the glucose molecule vibration specific signatures are captured by the respective IR sensitive detectors. This light interaction phenomenon had been represented by Beer-Lambert Law [23-32] as follows:

$$\mathbf{A(v)} = -\mathbf{log\, I(v)/I_0(v)} \quad (3)$$

The symbols like **(A), (v), (I_0)** and **(I)** signifies Absorption patterns, wave number, light intensity from the surrounding medium and light intensity after transmission through the path length of measurement sample respectively [23-32].

3. MUS-IR Experimental Setup and Its Functional Part Depictions

Main functional parts of the MUS-IR Experimental setup had been illustrated as follows:

Synchronous square wave generator:

This functional part produces square wave based pulses to the IR LED (Infra Red Light Emitting Diode) light source.

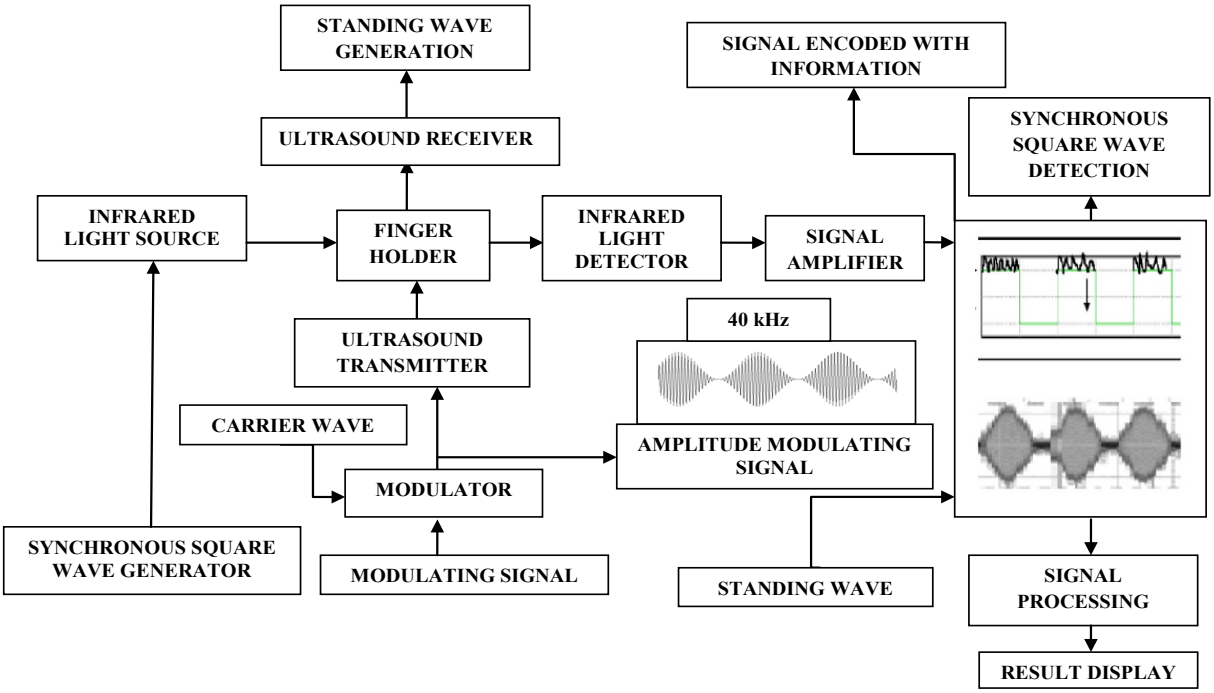


Figure 1. Block diagram of the MUS-IR (Modulated Ultra Sound-Infrared) Experimental Setup

Infrared light source:

IR LED of specific 940nm spectral wavelength had been selected and applied here. Actually 940nm occupies the position between "tissue optical window range" extending from 700nm to 1100nm [4, 33]. Within this zone the unwanted influence of other optically active molecules such as water, oxyhemoglobin, deoxyhemoglobin, etc. are reasonably negligible as depicted from Figure No.2, 4 respectively [4, 34-36]. Moreover, from Figure No.3 it can be revealed that glucose molecule exhibits absorption peaks near to 940nm wavelength [4, 34-36]

Modulating unit:

The modulating unit produces amplitude modulating standing wave pulses to UST (Ultra Sound Transmitter) unit. This functional component had been connected with two other primary parts such as carrier wave and modulating signal units.

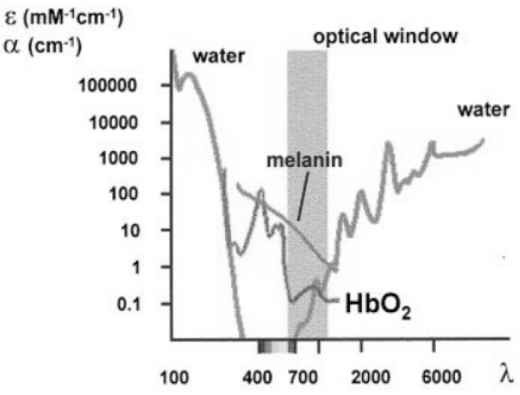


Figure 2. Absorption characteristics of chief intracellular components within the light spectral domain extending from 100nm to 6000nm [4, 33]

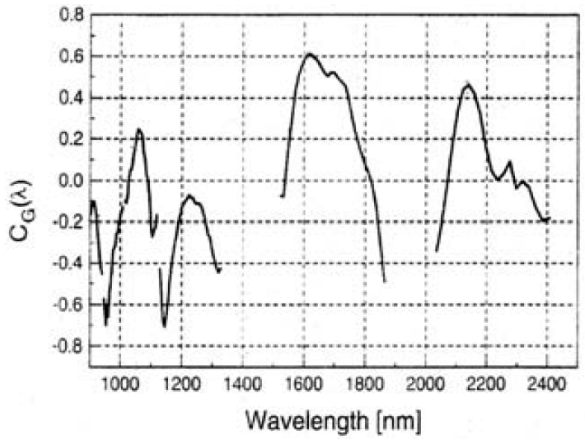


Figure 3. Absorption coefficient characteristics of Glucose within the light spectral domain extending from 900nm to 2400nm [4, 34-36]

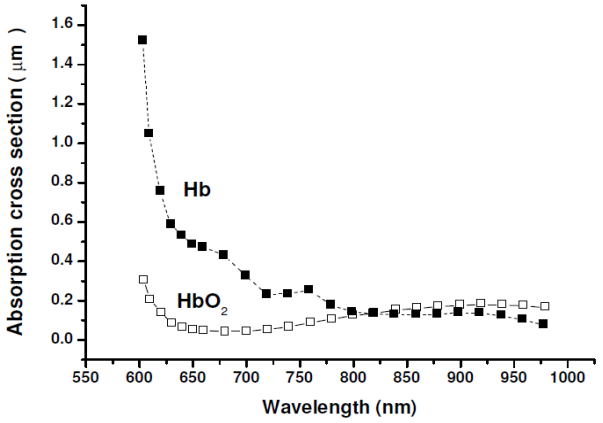


Figure 4. Absorption characteristics of Oxygenated Hemoglobin (HbO_2) and Deoxygenated Hemoglobin (Hb) within the light spectral domain extending from 550nm to 1000nm [4, 34-36]

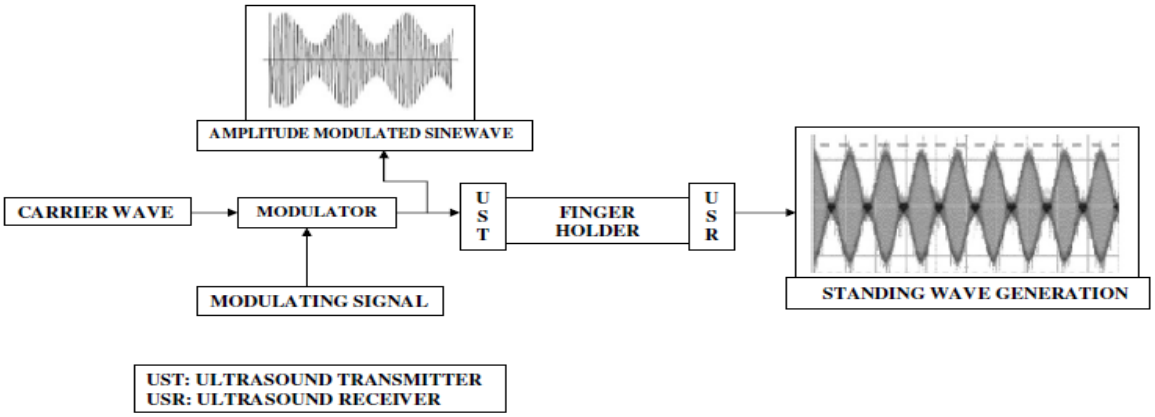


Figure 5. Shows the generation of amplitude modulated ultrasonic waves in MUS-IR Experimental unit

Ultra Sound Transmitter (UST):

Ultrasound Transmitter of 40 kHz central frequency had been selected and applied here. Actually the 40 kHz ultrasonic wavelength is medically safe for its use in human beings [24, 31, 32].

Moreover, amplitude modulated standing wave pulses as generated from the respective modulating unit serve as the signal input to UST unit. Consequently, the UST functional unit produces output signal in the form of ultrasonic amplitude modulated standing wave towards the finger holder unit. This phenomenon had been depicted here in the Figure No.5 as provided above.

Finger Holder Unit:

It upholds the human finger in precise geometrical position required for accurate IR light, ultrasonic wave transmission purposes. It helps in holding steady perpendicular orientation of IR light and ultrasonic unit with respect to finger positioning during signal acquisition periods. Moreover, it minimizes the unwanted errors such as motion related artifacts, wrong finger positioning, etc.

Ultra Sonic Receiver (USR):

Again, the Ultra Sonic Receiver of 40 kHz central frequency had been selected and applied here. Actually the 40 kHz ultrasonic wavelength is medically safe for its use in human beings [24, 31, 32]. USR unit checks the quality of ultrasonic waves generated from the UST unit. It is very important as it plays the key factor in blood glucose level determination purposes.

Infra Red (IR) detector:

It picks up the transmitted IR light signals and records the blood glucose specific vibrational patterns for its relevant concentration determinations.

Signal amplifier & processing unit:

This part performs the signal conditioning, amplifications and unwanted noise filtrations functions. Subsequently, the resultant signals were analyzed through the MATLAB toolbox to determine blood glucose concentration related embedded information. The peak to peak voltage amplitude spectrum variations in FFT (Fast Fourier Transform) domain with respect to BGL (Blood Glucose Level) concentration were observed here. Actually, those typical voltage amplitude pattern variations serve as a functional indicator for respective change in the BGL levels.

Result Display:

This part of MUS-IR unit displays the noninvasive BGL (Blood Glucose Level) in mg/dl.

Clinical status of the Volunteers:

A group of 10 (seven males, three females, aged 35±6.5 years, of height 173±5.5cm, weight 70±11.5 kg) adult volunteers were selected. From which 02 volunteers are normal and healthy adults. Other 08 adult volunteers are with history of Prediabetic symptoms like IGT and IFT. Written consent had been obtained from all the volunteers. Institutional Ethical Committee approved the pilot study.

Clarke and Parkes Error Grid Analysis:

Clarke Error Grid analysis had been utilized to evaluate medical importance of the differentiations between blood glucose level predicting technique under examination and the established invasive blood glucose reference method. Clarke et al in the year 1986 A.D presented this novel analytical approach [37-42] and represents a Cartesian plot based diagrammatic approaches to characterize values of the reference (invasive) technology versus the predicted (predicted) technology [37-42]. As for illustration, if a human volunteer's Blood Glucose Levels is predicted to be 121mg/dl for a particular moment where the reference BGL value is 113mg/dl, this fact will be produced by the particular point as (113,121) in the XY Cartesian domains [37-42]. In this fashion, the diagonal line such as Y=X symbolizes the ideal determinations, points under and over the diagonal line, designates over assessment and under assessment of the real BGL values. Interestingly, the Cartesian XY graph had been divided into several grid zones based on the degree of severity of miss judgments [37-42]. The Error Grid name also signifies these facts. Clarke et al divided the respective Cartesian graph into 05 different zones (A to E) [37-42] respectively, with the following interpretations as follows:

Zone A: signifies predicted blood glucose values which diverge as of the reference blood glucose values by 20% or less. It also include hypoglycemic ranges (<70mg/dl) of both the predicted and reference BGL values respectively [37-42]. These BGL values are medically accurate and suitable. For that, required medical supervision will be proper [37-42].

Zone B: signifies predicted BGL values which differ as of the reference BGL values by more than 20%. Within this zone we are nearer to medically erroneous BGL values but the medical supervision has an elevated likelihood of being accurate [37-42].

Zone C-E: the BGL values occupying those zones are extremely hazardous, as the determination or estimation is far away to be medically significant and the designated medical attention will differ from the accurate medical action required [37-42].

Parkes et al in the year 2000 A.D reentered the concept of respective 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 had been divided into 05 parts such as Zone A to Zone E respectively [43-45].

Zone A signifies medically correct determinations, with no consequence over medical supervision [43-45].

Zone B signifies changed medical action, minute or no consequences over medical treatment [43-45].

Zone C signifies changed medical action, probable to influence medical treatment [43-45].

Zone D signifies changed medical action, might comprise imperative medical jeopardy [43-45].

Zone E signifies changed medical action, might comprise unsafe effects [43-45].

Recently in the area of diabetes technology assessments, existence of the widely acceptable Consensus Error Grids (EGs) for checking errors between reference and predicted blood glucose level determinations is not available. For that reason, we had utilized both the available (Clarke and Parkes) Error Grids (EGs) for result analysis and evaluation purposes [37-45]

4. Experimental Results and Discussions

The experiments were conducted in two phases.

The Phase I includes determination of invasive (reference) and noninvasive (predicted) BGL values of both the Normal and Prediabetic volunteers after overnight fasting and after 02 hours of meal consumption.

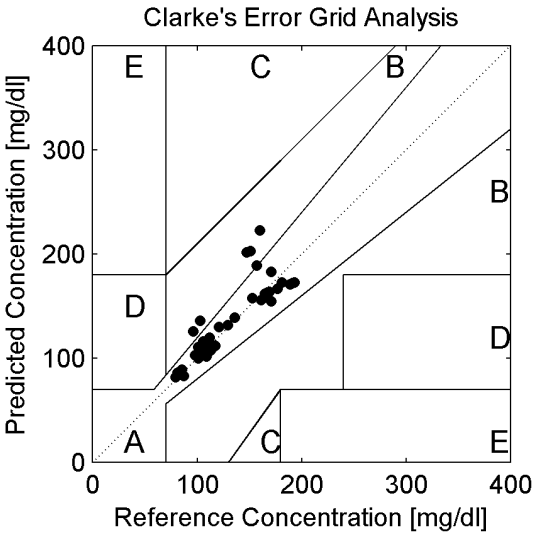
The Phase II includes determination of invasive (reference) and noninvasive (predicted) BGL values of both the Normal and Prediabetic volunteers next day after overnight fasting and 02 hours after 75gm/100ml of glucose solution consumption.

For invasive blood glucose level determinations the Accu-chek Active blood glucose monitoring system of Roche Diagnostics GmbH had been utilized here. Similarly, for noninvasive blood glucose level predictions we had utilized our indigenously designed and developed MUS-IR (Modulated Ultra Sound-Infra Red) unit.

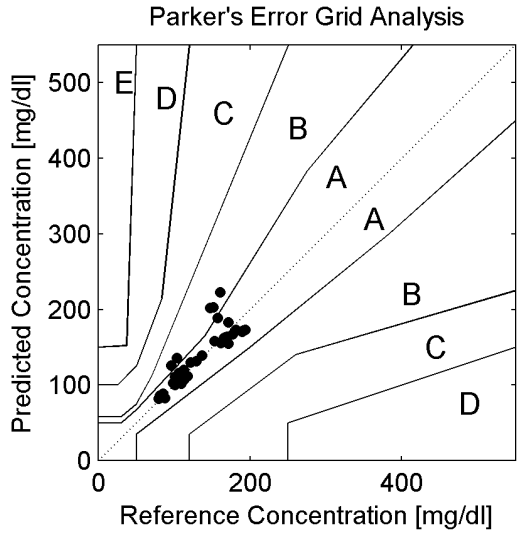
Table No.1 and 2 shows the comparison of invasive (reference) and noninvasive (predicted) Blood Glucose Levels (BGL) as obtained from the Normal and Prediabetic subjects during Phase I and II experimental pilot studies respectively.

Both the invasive (reference) and noninvasive (predicted) results were plotted over Clarke and Parkes Error Grids for critical analysis purposes.

Figure No. 6(a) and 6 (b) depicts the Clarke and Parkes Error Grid Analysis based graphical plotting of the Invasive (reference) and Noninvasive (predicted) BGL values of the Normal and Prediabetic volunteers as acquired during the experimental pilot study respectively.



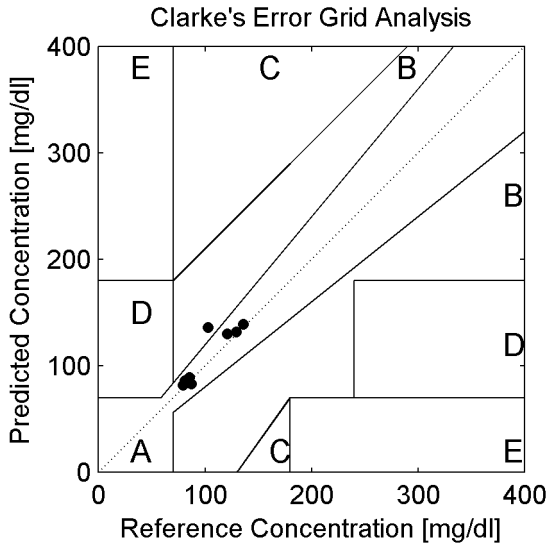
(a)



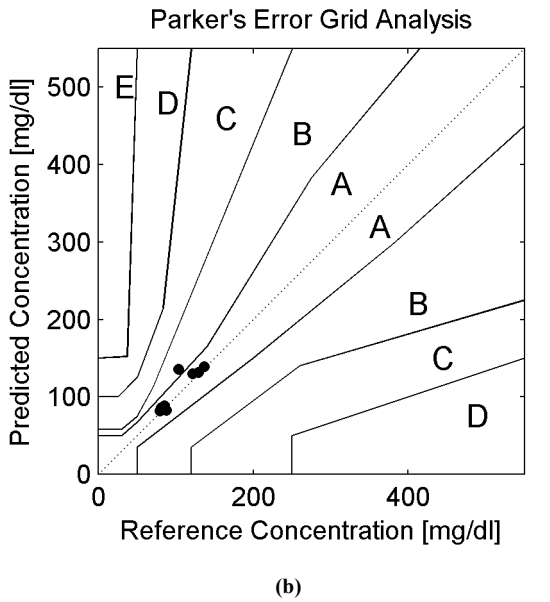
(b)

Figure 6. Represents Graphical depiction of Clarke and Parkes Error Grid Analysis of the invasive (reference) and noninvasive (predicted) BGL values as obtained from the Normal and Prediabetic subjects during the experimental pilot study respectively

Likewise, the Clarke Error Grid analysis based relevant BGL determining accurateness dependent proportional values as acquired from figure No.6 (a) are grouped as follows: A zone=85.00%, B zone=15.00%, C zone=00.00%, D zone=00.00% and E zone=00.00% respectively. Similarly, the Parkes Error Grid analysis based relevant BGL determining accurateness dependent proportional values as acquired from figure No.6 (b) are grouped as follows: A zone=85.00%, B zone=15.00%, C zone=00.00%, D zone=00.00% and E zone=00.00% respectively.



(a)

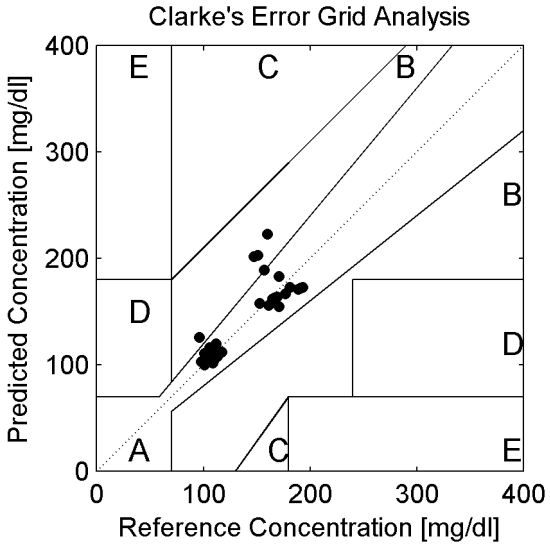


(b)

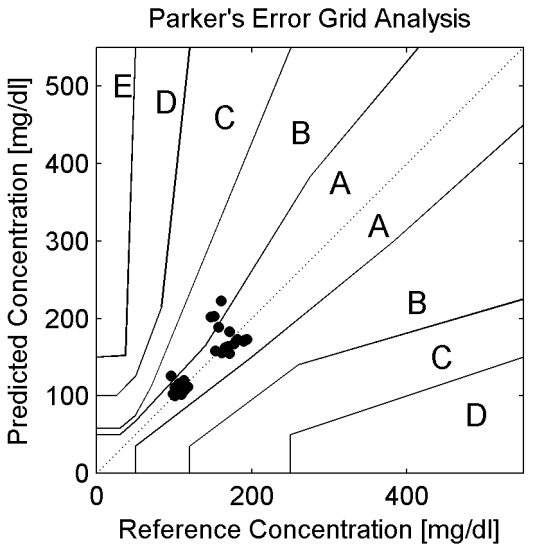
Figure 7. Represents Graphical depiction of Clarke and Parkes Error Grid Analysis of the invasive (reference) and noninvasive (predicted) BGL values as obtained from the Normal subjects during the experimental pilot study respectively

Clarke and Parkes Error Grid Analysis based graphical plotting for all the Normal volunteer's invasive (reference) and noninvasive (predicted) BGL values as obtained during this experimental pilot study had been depicted in Figure No.7 (a) and 7 (b) respectively.

The Clarke Error Grid analysis based respective BGL determining accuracy dependent percentage values from figure No.7 (a) are categorized as follows: A zone=87.50%, B zone=12.50%, C zone=00.00%, D zone=00.00% and E zone=00.00% respectively. Correspondingly, the Parkes Error Grid analysis based respective BGL determining accuracy dependent percentage values from figure No.7 (b) are categorized as follows: A zone=87.50%, B zone=12.50%, C zone=00.00%, D zone=00.00% and E zone=00.00% respectively.



(a)



(b)

Figure 8. Represents Graphical depiction of Clarke and Parkes Error Grid Analysis of the invasive (reference) and noninvasive (predicted) BGL values as obtained from the Prediabetic subjects during the experimental pilot study respectively

Graphical plots in Figure No.8 (a) and 8 (b) respectively depict the Clarke and Parkes Error Grid Analysis for the Prediabetic volunteer's invasive (reference) and noninvasive (predicted) BGL values.

Moreover, the Clarke Error Grid Analysis based accuracy dependent percentage values of the BGL invasive (reference) and noninvasive (predicted) readings are classified as follows: A zone=84.3750%, B zone=15.6250%, C zone=00.00%, D zone=00.00% and E zone=00.00% respectively. Similarly, the Parkes Error Grid Analysis based accuracy dependent percentage values of the BGL readings are classified as follows: A zone=84.3750%, B zone=15.6250%, C zone=00.00%, D zone=00.00% and E zone=00.00% respectively.

Results obtained from analysis depicts that Prediabetic subject's blood glucose levels were elevated than normal physiological ranges but not as high compared to diabetic subjects during both the Fasting stage and 02 hr after meal or glucose solution consumption respectively. Similarly, for Normal subjects the blood glucose levels during Fasting stage and 02 hr after meal or glucose solution consumption are always within normal physiological range extending between (80-140) mg/dl.

Moreover, all the invasive and noninvasive BGL values occupy the medically acceptable A and B zones respectively. This fact indicates dependable performance of our indigenously developed MUS-IR unit. All these results also correlate our previous blood glucose determination experimental values [24, 25, 31, 32]. The vital factor driving this 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 embedded signals from the transmitted infrared light.

The combined use of ultrasound and infrared light provides a new dimension for noninvasive detection of blood glucose levels. Few unwanted, erroneous signals had been obtained due to various types of factors like skin tissue related pigmentations, background light intensity, pulsatile flow of blood, machine related drifts, time dependent drifts, motion related artifacts, other physiological or pathological factors, etc. All these interfering sources modify the blood tissue complex induced bio signals and provide erroneous impact over blood glucose level determinations.

Table 1. Shows the comparison of invasive (reference) and noninvasive (predicted) Blood Glucose Levels (BGL) as obtained from the Normal subjects during Phase I and II experimental pilot studies respectively

NORMAL SUBJECTS	BLOOD GLUCOSE LEVEL (mg/dl)							
	Phase I				Phase II			
	BGL (mg/dl) After Overnight Fasting		BGL (mg/dl) 2hr After Meal Consumption		BGL (mg/dl) After Overnight Fasting		BGL (mg/dl) 2 hr after 75 gm/100ml Glucose Solution Consumption	
	Invasive method	Noninvasive method	Invasive method	Noninvasive method	Invasive method	Noninvasive method	Invasive method	Noninvasive method
SUBJECT1	81	86	103	136	79	82	121	130
SUBJECT2	85	89	136	139	87	83	129	132

Table 2. Shows the comparison of invasive (reference) and noninvasive (predicted) Blood Glucose Levels (BGL) as obtained from the Prediabetic subjects during Phase I and II experimental pilot studies respectively

PREDIABETIC SUBJECTS	BLOOD GLUCOSE LEVEL (mg/dl)							
	Phase I				Phase II			
	BGL (mg/dl) After Overnight Fasting		BGL (mg/dl) 2hr After Meal Consumption		BGL (mg/dl) After Overnight Fasting		BGL (mg/dl) 2 hr after 75 gm/100ml Glucose Solution Consumption	
	Invasive method	Noninvasive method	Invasive method	Noninvasive method	Invasive method	Noninvasive method	Invasive method	Noninvasive method
SUBJECT3	101	100	177	167	106	116	151	203
SUBJECT4	107	109	169	164	109	102	171	183
SUBJECT5	115	112	165	162	107	104	189	171
SUBJECT6	107	104	161	156	101	111	167	163
SUBJECT7	113	108	153	158	110	105	193	173
SUBJECT8	110	114	181	173	96	126	160	223
SUBJECT9	98	103	191	172	112	120	147	202
SUBJECT10	105	110	171	155	117	112	157	189

5. Conclusions

The hybridized potential aspect of utilizing amplitude modulated ultrasound and Infra Red technique for determining noninvasive blood glucose levels had been reported in this research paper. For cross validations of acquired noninvasive BGL values, the invasive glucometer of Roche diagnostics had been applied here. Furthermore, the 940nm LED and 40 kHz-generating central frequency based Ultrasound Transmitter forms the main instrumental base for this noninvasive technology. For validating the performance of noninvasive blood glucose detecting technology the Error Grid Analytical approaches had also been applied here. Error Grid Analysis shows that all the invasive (reference) and noninvasive (predicted) BGL values occupy within the medically significant A and B zones.

At present all new noninvasive BGL determining techniques requires invasive glucose sensors for calibration purposes. Hope our nascent noninvasive BGL determining technology will be successful in near future with all the aspects.

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The Error Grid Analysis of invasive and indigenously developed noninvasive technique based blood glucose readings obtained from the effect of various glucose concentration sample solutions over blood glucose levels on the human subjects

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Abstract

Noninvasive blood glucose monitoring technology with significant clinical accurate measurement capability should have a beneficial impact over medical care system for diabetes and its related risk managements. Moreover blood or other body fluid less technology should increase patient compliances as well as robust control over fluctuating blood glucose levels. Herein, we represent the noninvasive technology and evaluate its efficiency for detecting the respective glucose concentration induced blood glucose levels on the human subjects. The purpose of this experiment was to observe the effect of various glucose concentration sample solutions like 25gm/100ml, 50gm/100ml and 75gm/100ml over blood glucose levels on the fasting 10 human subjects during the time duration of 1hour and 2hour after respective glucose solution consumptions. Moreover, the invasive and indigenous noninvasive technology based blood glucose level readings correlations were compared and evaluated through Clarke and Parkes Error Grid analytical approaches.

The experimental findings indicate that blood glucose levels increase with increase in concentration of glucose solutions in human subjects. At the same time, the blood glucose levels were regulated and controlled by the individuals' body glucose homeostasis response mechanisms. Similarly the Clarke and Parkes Error Grid analysis of the obtained invasive and noninvasive blood glucose readings showed that the measurement readings occupy the medically significant A and B zones respectively. Experimental study indicates the impact of glucose concentration over blood glucose levels and potential use of indigenously developed noninvasive technology based on amplitude modulated ultrasound with infrared technology for noninvasive blood glucose level determinations.

Keywords: Error Grid Analysis, Invasive, Noninvasive, Blood Glucose, Amplitude Modulated Ultrasound, I.R.

Introduction

Diabetes mellitus represents the medical condition in which excess amount of blood glucose circulates within the human body. It is often characterized as the hyperglycemic stage resulted due to impaired insulin release or action[2,13,25,30]. Uncontrolled and abnormally elevated blood glucose concentrations for longer durations exert negative impact over the blood vessels, kidneys, eyes, nerves, heart etc. Severe pathological conditions like micro or macro Vascular diseases, Diabetic nephropathy, Diabetic retinopathy, Diabetic neuropathy, Diabetic foot ulcerations, Heart strokes etc.[2, 13,25,30] are the long term consequences of diabetes as the disease progresses[13,30]. On the other side, too much or excessive insulin usage for maintaining normal blood glucose levels may sometime lead to life threatening situation known as excessive hypoglycemic attacks.

According to IDF (International Diabetes Federation), the 38.2 crores of recent world populations were diabetic. This figures will touch 59.2 crores mark within or before 25 years from now.[15] Unfortunately 17.5 crores of today's world populations are unaware of diabetes prevalence in them and progressing towards diabetic related long term clinical consequences.[15] Furthermore, 80% of the diabetic diseased population belongs to the lower and middle income territories of the world[15]. All these factors provide severe economical burden over the individuals suffering.

Rigid monitoring over the blood glucose levels in diabetic patients is vital for significant clinical management[2,13,15,25,30]. The invasive glucometer provides self monitoring of blood glucose levels which employ tissue puncturing for collecting and analyzing blood samples[11-13]. This procedure is painful and involves skin-tissue infection related clinical risks[13,30]. To avoid such circumstances and for ease of self operations, several noninvasive blood glucose detecting technologies had been evolved in recent few decades.[2,25] Such technologies comprise of Infrared Absorption, Near Infrared scattering, Raman spectroscopy, Fluorescent spectroscopy, Thermal gradient spectroscopy, Polarimetry, Polarization heterodyning techniques, Photonic crystal Technology, Photo acoustic Technology, Photo-thermal Technology, Optical Coherence Tomography (OCT) techniques and Ultrasound-modulated optical technique[2,25,28]. The test sites for noninvasive blood glucose determinations comprise

mainly of the tip of fingers, finger web, forearms, ears, eyes etc.[5]. The experimental studies also include various skin layers and body fluids.[5]

The blood glucose molecule produces a very feeble signal which requires lots of designing complexity to acquire such weak signals.[20,28,29] In order to overcome such difficulties, the amplitude modulated ultrasound with infrared techniques had been introduced here. This research article presents an indigenously developed noninvasive blood glucose detecting technology and evaluates its efficiency for detecting the respective glucose concentration induced blood glucose levels on the human subjects.

Materials and Methods

The principle combined effect of amplitude modulated ultrasound and infrared technique towards noninvasive blood glucose level detections: In this instrumental architecture principally we had utilized 940nm LED (Light Emitting Diode) as an Infrared (IR) light emitter and its respective IR detector. For ultrasonic wave generations we had selected a pair of 40 kHz Piezo-sensor based ultrasonic transmitter and receiver.

When two oppositely propagating ultrasonic waves combine with each other, the ultrasonic standing wave formation occurs[3-5]. These travelling waves can be generated from two separate transducers or from a transducer source and its respective reflector[3-5,10,15,16,21,24,26,27,31]. The pressure amplitude of a standing wave has maximum and minimum values twofold above the distance of a unit wavelength. Within the propagating segment, discontinuities such as molecules achieve location specific acoustic potential energy due to its presence in the respective ultrasonic zone. Consequently, the progressions of the suspended molecules were observed towards the zone of least acoustic potential energy. Pressure nodes are usually nearer to these zones with respect to the molecules which are distanced from each other by path length of half a wavelength[3-5,10,15,16,21,24,26,27,31].

Similarly, when the molecular diameter is smaller than the wavelength of propagating ultrasound, the main force of radiation (F_r) acting on the molecule of volume (V_c), positioned by path of distance (z) from the pressure node is obtained from the gradient of the molecular acoustic potential energy[3-5,10,15,16,21,24,26,27,31] and is expressed as:

$$F_r = -\left[\frac{\pi p_o^2 V_c \beta_w}{(2\lambda)}\right].\phi(\beta,\rho).\sin(4\pi z/\lambda) \quad (1)$$

Here, peak amplitude of the acoustic pressure has been symbolized as (P_o). The ultrasound wavelength in the suspending segment had been denoted as (λ). Compressibility of the suspending segment had been characterized as (β_w)[3-5,10,15,16,21,24,26,27, 31]. The mathematical expression of this phenomenon had been signified as:

$$\phi(\beta,\rho) = \left[\frac{5\rho_c - 2\rho_w}{2\rho_c + \rho_w} - \left(\frac{\beta_c}{\beta_w}\right)\right] \quad (2)$$

Similarly (β_c) stands for compressibility of the molecules. Notations (ρ_c) and (ρ_w) represent the respective densities of the molecules and the suspending segments.

Multiple factors were required to be measured when infrared spectral ranges were analyzed. Each and every sample delivers their unique IR signatures at a definite wave numbers[5,24]. The well known Lambert-Beer law had been incorporated here to measure the specific absorption (A) properties at a definite light wave number (v)[5,24]. This observable fact had been expressed here as:

$$A(v) = -\log I(v)/I_0(v) \quad (3)$$

The background impact of light intensity had been denoted as (I_o) here. Similarly (I) represents the specific light intensity at the particular wave number (v) of actual sample measurements.

Noninvasive blood glucose detecting Instrumental system

The IR LED of 940nm had been selected for noninvasive blood glucose level predictions. Actually figure nos.1 and 2 depict that the interference effect of other optically active substances like skin pigmentations induced melanin contents, water molecules, oxygenated hemoglobin and deoxy-hemoglobin etc. was reasonably less in that optical window range extending from 700nm to 1100nm[5,17,28,29]. Furthermore, the absorption pattern of oxygenated hemoglobin and deoxy-hemoglobin as depicted from the fig. 3 is more or less similar in the wavelength band range between 900nm to1000nm respectively[1,19]. Consequently, the influence of oxygenation over the light absorption will be less in this zone[1,5,17,19,28,29]. All this fact had directed us for selecting 940nm as the principle operating wavelength for noninvasive blood glucose level determinations[5,17,28,29].

The ultrasonic transmitter built in aluminum casings with 40±1.0 kHz as centre frequency with driving voltage (RMS) of 30V had been utilized here. More over it is safe for its utilization over human beings. Additionally, easy commercial availability of both the items had encouraged us for their use in our experiments[5,6].

Instrumental system descriptions: Block diagram of the Modulated Ultra Sound-Infra Red (MUS-IR) unit for noninvasive blood glucose determinations had been represented in figure 4. Main functional parts of the instrumental system had been illustrated here as follows:

Square wave generator: This unit provides the square wave pulses in synchronous mode to the IR LED (Light Emitting Diode) light source.

IR LED: Infrared LED of 940nm (Thor Labs) had been utilized here as light source for the noninvasive blood glucose level determinations.

Modulator: This part had been connected with two other functional units like carrier wave and modulating signal unit respectively. It generates amplitude modulated signal as an input to the ultrasound transmitter unit.

UST (Ultra Sound Transmitter): The piezocrystal based ultrasound transmitter of 40 kHz as central frequency had been utilized here. Amplitude modulated input to the UST produces amplitude modulated ultrasonic waves. These unique waves were directed towards the finger holder section of the instrumental system.

Finger Holder: This part holds the human finger in correct fashion as required for the experimental purposes. The light and ultrasound sources were perpendicular in their respective geometrical orientations.

USR (Ultra Sound Receiver): This part provides cross checking of the unique standing wave pattern generated from the UST unit during calibration process of the instrument. The unique standing wave patterns of the amplitude modulated ultrasound play the important role for blood glucose level determinations in noninvasive manner. Consequently, the 40 kHz ultrasonic receiver had been incorporated here.

IR Detector: The detector incorporated here is very responsive in the infrared spectral region. It senses the minute changes due to the variation in the blood glucose levels.

Signal Amplifier: This part of the instrument amplifies the acquired bio-signal. At the same time it removes the unwanted noises from the acquired signal for obtaining good SNR (Signal to Noise ratio) values.

Signal Processing: This part performs the signal processing and conditioning procedures for extracting blood glucose related information from the acquired bio-signals.

Display: This part displays the predicted noninvasive blood glucose level values in gm/dl.

Invasive blood glucose determination: Invasive measurements were performed by the well known and established invasive glucometer known as Accu-chek Active blood glucose monitoring unit of Roche Diagnostics[11,12].

Human subject's medical profiles: Total of 10 human subjects was selected for this experiment. Eight of them were males and two are females aged between 22 to 29 years. Their height and weight vary in the range between 157cm to 165cm and 65kg to 79kg respectively. Objectives of the experimental protocols were briefed before the subjects; they all understood the procedures and their written consents were obtained. The Institutional Ethical Committee had approved the pilot study.

Experimental Protocol

Determine the effects of 25gm/100ml, 50gm/100ml, 75gm/100ml of glucose solution over fasting human individuals throughout the time period of 1hr and 2hr after its consumptions. Additionally, evaluate the working efficiency of the indigenously developed noninvasive technique based blood glucose level readings with respect to the standard invasive glucometer.

The experiments were conducted in the morning and the subjects were instructed to fast (water permitted) for 10 to 12 hours before the tests.

Step A: The noninvasive and invasive fasting blood glucose level of the human subjects was acquired at 0 min.

Step B: A glucose solution of 25gm/100ml (Approx.) of water was provided for drinking to the human subjects in a given time span of 5 minutes after step A.

Step C: This phase involves acquirement of noninvasive and invasive blood glucose level readings from all the 10 subjects after 1 hour and 2 hour of glucose solution consumptions respectively. Same steps were followed for 50gm/100ml and 75gm/100ml of glucose solution over 10 human subjects for next 2 days respectively.

For noninvasive determinations, all the obtained bio-signals were consecutively processed through the toolbox of MATLAB for obtaining the blood glucose level information. The peak to peak amplitude values in the FFT (Fast Fourier Domain) domain change with respect to the change in the blood glucose levels. This peak value serves as the functional indicator for the noninvasive blood glucose level determinations in this experimental procedure. Similarly, for invasive determinations Accu-chek active[11,12] based blood glucose level readings were obtained and recorded accordingly.

Error Grid Analysis: The Clarke Error Grid Analysis had been utilized here to critically access the medical importance of differences between the noninvasive blood glucose measurement technique under examination and invasive blood glucose monitoring glucometer as the reference measurements. Clarke et al.[7] in the year 1986 had presented this methodology where the Cartesian diagram had been utilized to compute the predicted (noninvasive) blood glucose values against the invasive (real) blood glucose values[6-9,18]. For instance, when the noninvasive (predicted) blood glucose value is 123mg/dl and the actual (invasive) blood glucose value obtained is 88 mg/dl, it will be expressed through the point as (88,123) in the Cartesian

XY coordinates. In this fashion the diagonal line where Y=X, expresses the ideal measurements[14]. The data points below and above the diagonal line represent the overestimation and underestimation of the real blood glucose values respectively.

Furthermore, the XY graph had been subdivided into several Grid zones which had been based on the degree of the miss calculations[14]. Clarke et al.[7] had divided 5 zones in the respective XY-Cartesian graph with the following interpretations:

Zone A: It characterizes the blood glucose values that diverge away from the actual reference values by means of 20% or less and also the blood glucose values in the hypoglycemic ranges (less than 70mg/dl). These type of output values are medically correct and medical attention will be proper[6-9,14].

Zone B: It characterizes the blood glucose values that diverge from the actual reference values by means of more than 20%. This domain represents the starting of errors but medical treatment had a high possibility of being accurate. The data points under the Zone B are also medically significant and tolerable[6-9,14].

Zone C-E: The blood glucose values in those zones had been characterized as potentially dangerous and unacceptable. The predicted values were distance away from the real values and the medical treatment will be erroneous. High probabilities of medically significant errors occur due to those erroneous values in those respective zones[6-9,14].

Parkes et al.[22] revisited the concept of the respective zones with a new dimension regarding error grids by utilizing the knowledge of a large group of expert medical physicians[14,22,23]. Parkes Error Grid analysis differs for Type 1 diabetic subjects and Type II diabetic subjects respectively. The procedures of surveying 100 medical physicians of diabetes expertise were conducted to consign any errors starting from zone A to E as provided by the Clarke Error Grid. The Parkes Error Grid had been divided into 5 different zones[14,22,23] such as:

Zone A represents medically correct blood glucose value determinations with no effect over the medical treatment.

Zone B represents misrepresented medical action, slight or less effect over the medical treatment [14, 22, 23].

Zone C represents misrepresented medical action, prone to provide moderate effect over the medical treatment.

Zone D represents misrepresented medical action, might provide significant medical risk.

Zone E represents misrepresented medical action, might provide dangerous penalty.

At present, in the area of endocrinology common consensus for determining errors in the blood glucose value calculations do not exist[14]. For that we had utilized both the Error Grid based Analytical approaches for evaluating our respective results.

Results and Discussion

The effect of 25gm/100ml, 50gm/100ml, 75gm/100ml of glucose solution over the human subjects is seen within the time duration of 1hr and 2hr after the glucose solution consumptions respectively. The pattern of those values as seen from the table no. 1, 2 and 3 indicates that with increase in glucose concentration the blood glucose level increases but simultaneously the glucose levels were normalized by the body's physiological mechanisms.

At the same time, the noninvasive and invasive blood glucose levels were also measured to evaluate the working efficiency of the indigenously developed blood glucose detecting technology. The obtained values were critically analyzed through Clarke Error Grid and Parkes Error Grid analysis respectively. The graphical plotting of Clarke and Parkes Error Grid Analysis has been represented in figure 5 and 6 respectively. The values obtained from the Clarke Error Grid analysis were characterized as A zone=87.7778%, B zone=12.2222%, C zone=00.00%, D zone=00.00% and E zone=00.00% respectively.

Similarly, the values obtained from the Parkes Error Grid analysis were characterized as A zone=87.7778%, B zone=12.2222%, C zone=00.00%, D zone=00.00% and E zone=00.00% respectively.

The Error Grid Analysis shows that all the blood glucose values occupy the medical significant and acceptable zones like A and B respectively. All this directs towards the reliable efficiency of the indigenously developed amplitude modulated ultrasound and infrared technique based noninvasive blood glucose determining technology.
Similarly, the result of these experimental studies resembles our previous pilot study values[4,5,25-27]. The important driving factor for this technology includes (a) appropriate utilization of the ultrasonic waves which is in amplitude modulating mode here; (b) Productive and standardized determination of blood glucose induced IR light signal patterns for noninvasive blood glucose level predictions.

The hybrid collaboration of amplitude modulating ultrasonic waves and the infrared methodology increases the blood glucose measuring perceptive with good and medically acceptable correctness[4,5,25-27]. A few erroneous signal based data had been acquired due to numerous typical factors like skin pigmentation issues, surrounding light, blood flow, machine drift, time drift, artifacts, other

biological factors etc.[2,28,29] All these aspects alter the blood optical characteristics and induce effect over the actual blood glucose level measurement procedures.

Conclusion

The increase in effect of blood glucose level with increase in glucose concentration solution consumptions by the subjects and their respective physiological mechanism to control and lowering the individual's blood glucose levels is observed. Moreover, the working capability of the indigenously developed noninvasive blood glucose determining technology had been evaluated through the Clarke and Parkes Error Grid Analysis. The result obtained occupies the A and B zones in both the Error Grid (Clarke and Parkes) analysis which represents the good working efficiency of the indigenously developed noninvasive blood glucose detecting technology.

Acknowledgement

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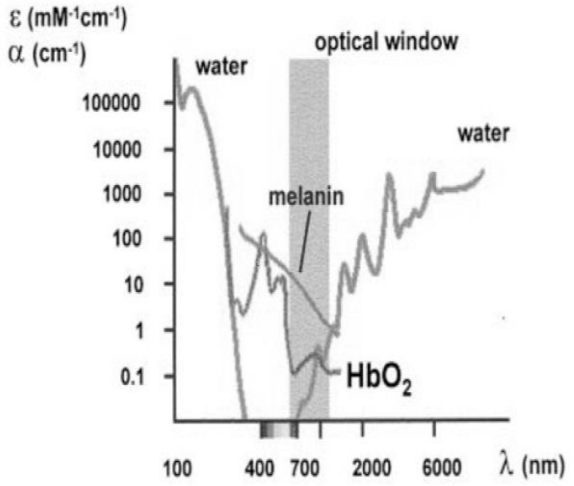

Figure 1: The range of major intracellular absorbers induced tissue optical window phenomenon in the wavelength spectrum starting from 100nm to 6000nm correspondingly[17].

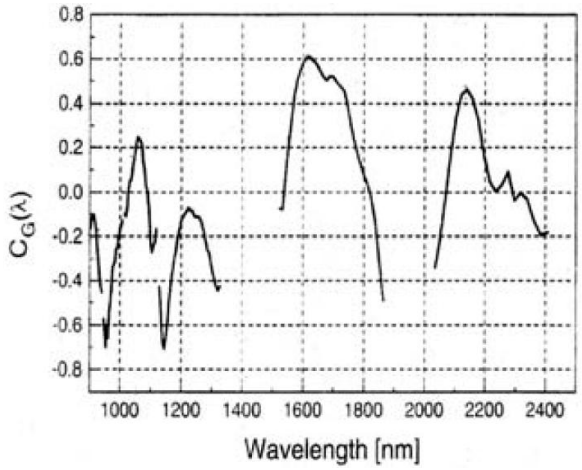

Figure 2: The absorption profile pattern of the glucose molecule in the wavelength spectrum starting from 1000nm to 2400nm correspondingly[29].

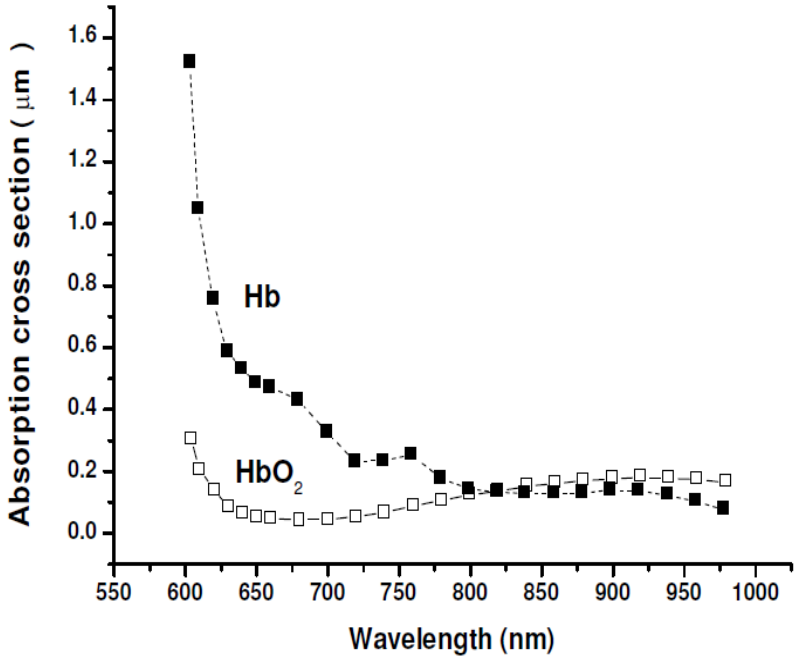

Figure 3: The absorption outline of the Hb (hemoglobin) and HbO_2 (oxygenated hemoglobin) in the wavelength spectrum starting from 550nm to 1000nm correspondingly[1,19].

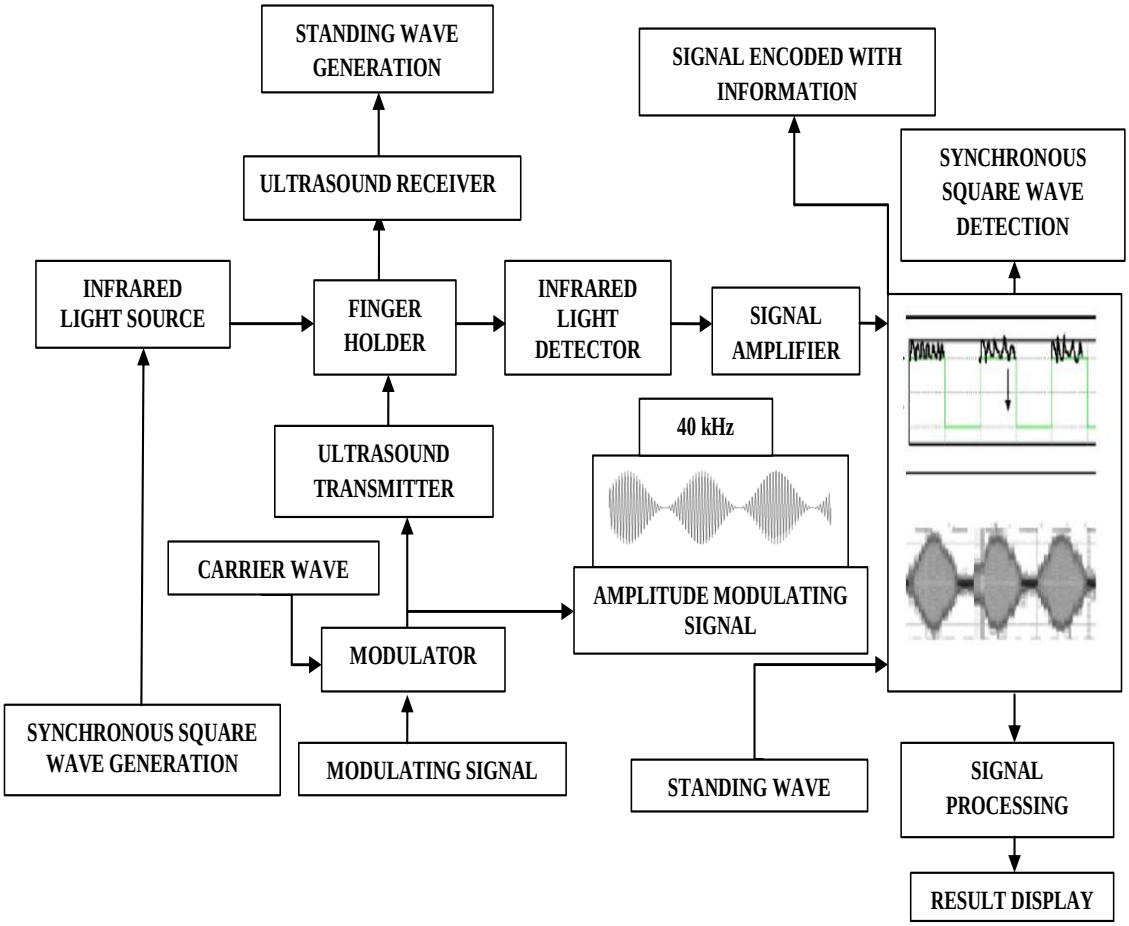

Figure 4: Outline of the noninvasive blood glucose determining instrumental system utilized during the experimental study.

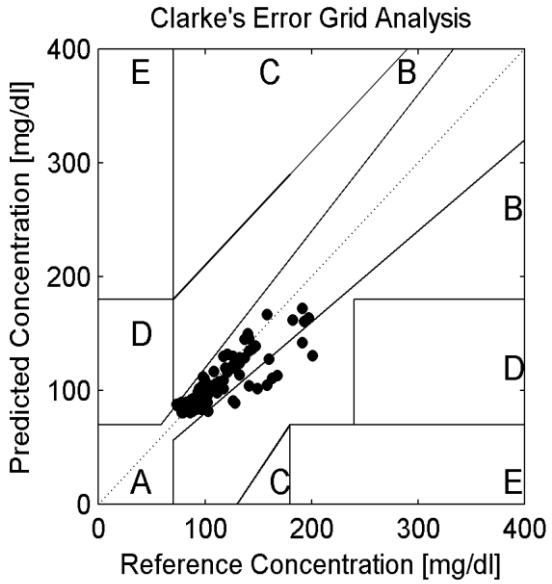

Figure 5: The Clarke's Error Grid based Analysis of the Blood Glucose Level values as acquired by the noninvasive (Predicted Concentration) and invasive (Reference Concentration) methods from all the subjects.

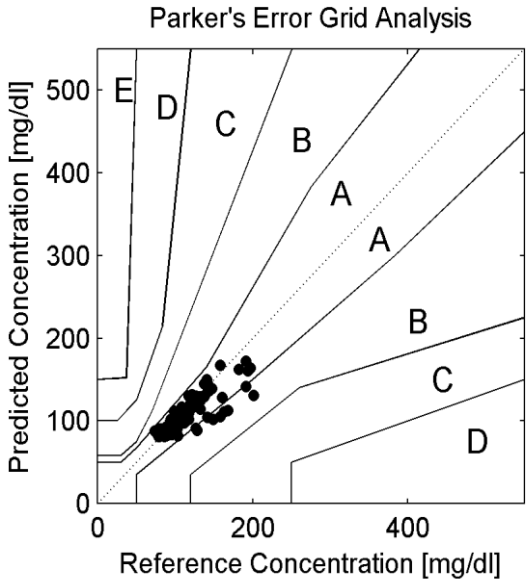

Figure 6: The Parkes Error Grid based Analysis of the Blood Glucose Level values as acquired by the noninvasive (Predicted Concentration) and invasive (Reference Concentration) methods from all the subjects.

Table 1
The respective invasive and noninvasive blood glucose levels (mg/dl) of all the subjects during fasting stage and after 1 hour, 2hour of 25gm/100ml of glucose solution consumption respectively

Subjects	Blood Glucose Level (mg/dl) during Fasting stage		Blood Glucose Level (mg/dl) after 1 hour of 25gm/100ml of glucose solution consumption		Blood Glucose Level (mg/dl) after 2 hour of 25gm/100ml of glucose solution consumption	
	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)
Subject1	102	96	117	109	88	93
Subject2	92	86	127	120	94	102
Subject3	78	85	126	122	97	104
Subject4	81	86	132	124	112	105
Subject5	96	87	141	135	113	108
Subject6	92	86	144	137	100	108
Subject7	92	84	147	139	98	112
Subject8	94	87	137	145	108	117
Subject9	78	81	141	146	117	130
Subject10	79	82	140	150	121	132

Table 2
The respective invasive and noninvasive blood glucose levels (mg/dl) of all the subjects during fasting stage and after 1 hour, 2hour of 50gm/100ml of glucose solution consumption respectively.

Subjects	Blood Glucose Level (mg/dl) during Fasting stage		Blood Glucose Level (mg/dl) after 1 hour of 50gm/100ml of glucose solution consumption		Blood Glucose Level (mg/dl) after 2 hour of 50gm/100ml of glucose solution consumption	
	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)
Subject1	77	86	110	106	98	100
Subject2	83	90	107	104	93	99
Subject3	94	91	111	98	103	82
Subject4	82	90	149	102	100	86
Subject5	77	85	117	102	102	90
Subject6	74	88	141	104	98	87
Subject7	77	89	158	105	88	86
Subject8	86	90	163	111	81	87
Subject9	79	81	168	113	78	86
Subject10	80	85	160	128	99	111

Table 3
The respective invasive and noninvasive blood glucose levels (mg/dl) of all the subjects during fasting stage and after 1 hour, 2hour of 75gm/100ml of glucose solution consumption respectively.

Subjects	Blood Glucose Level (mg/dl) during Fasting stage		Blood Glucose Level (mg/dl) after 1 hour of 75gm/100ml of glucose solution consumption		Blood Glucose Level (mg/dl) after 2 hour of 75gm/100ml of glucose solution consumption	
	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)
Subject1	87	92	114	104	87	84
Subject2	91	94	129	122	88	85
Subject3	88	82	137	129	96	85
Subject4	96	84	201	131	128	89
Subject5	99	87	191	142	126	91
Subject6	78	90	182	162	132	114
Subject7	74	87	197	164	121	116
Subject8	73	88	158	167	119	120
Subject9	86	81	191	172	132	129
Subject10	81	87	193	161	126	130

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Estimation of fasting Blood glucose levels by invasive and indigenously developed noninvasive technology and its correlation with the glycated hemoglobin (HbA1c) biomarker in healthy and diabetic subjects

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Abstract
This research article represents the cross sectional study about the fasting glycemic index of normal and hyperglycemic subjects by invasive and indigenously developed noninvasive technology. Moreover, correlation between the fasting glycemic index and glycated hemoglobin (HbA1c) biomarker profile of both the normal and diabetic subjects has been analyzed and discussed. The fasting glycemic index measurements have been performed by the indigenous developed noninvasive method which utilizes both the amplitude modulated ultrasound and infrared techniques. The standing wave pattern of the ultrasound causes molecular vibration in the focused zone.

The infrared light based technology has been utilized to detect those glucose sensitive vibrational patterns to predict the glycemic index of the normal and diabetic subjects. Invasive fasting glycemic index and profile of glycated hemoglobin (HbA1c) biomarker were determined by the established invasive methods. The result emphasizes that both the invasive, noninvasive fasting glycemic index values change as the actual fasting glycemic index changes. Similarly, the glycated hemoglobin (HbA1c) biomarker profile of the diabetic subjects varies largely as compared to the normal subjects.

Keywords: Fasting glycemic levels, invasive, noninvasive, glycated hemoglobin (HbA1c), diabetic subjects.

Introduction

In the year 2013, according to the IDF (International Diabetes Federation) the diabetic population in India and worldwide is estimated to be around 6.51 and 38.2 crores respectively[4,17,28]. This diabetic population prevalence in India and worldwide during the year 2035 may rise to 10.9 and 59.2 crores respectively[4,17]. Moreover globally at present in 2013, the direct diabetes related treatment cost for different age groups had been more or less near to 581 billion international dollars[17]. Various projections indicate that it will be near to at least 678 billion international dollars status by the year 2035[17]. About half portion of the global diabetic population which had been estimated around 17.5 crores peoples was undiagnosed for this metabolic disorder[17]. In the year 2013, global mortality rate indicates that nearly 5.1 and 1.1 million people died worldwide and in India respectively due to diabetic related complexity issues[17]. All this data reveal that every six seconds one person dies worldwide due to diabetic related disorders[17].

As of 2013 data, our country India in the South-East Asian zone had been the major regional contributor for diabetes related mortality rate[17]. One Indian study reveals that mean glycated hemoglobin (HbA1c) biomarker level had been around 9.2% between 20,554 Type II Diabetic volunteers[21]. This marks towards the poor and weak glycemic management in our country[21,25]. The consequences for this poor glycemic control in India count for 23.6% more macro and micro vascular neuropathy patients over the heart disease related patients[21,25].

The Glycated hemoglobin formation involves the amino-terminal valine moiety of hemoglobin β–chain combining with the glucose or hexose molecules. This bio-chemical process is slow, enzyme independent, irreversible substrate-concentration dependent and post translational in nature[5]. The glycated hemoglobin (HbA1c) biomarker efficacy for predictions, diagnosis, long term monitoring of Diabetes Mellitus profile had been an eminent methodology for rigorous investigations and discussion [22]. HbA1c level indicates the individuals plasma blood glucose homeostasis for past 60-90 days.[5, 11, 18, 21, 22, 25, 29]

Moreover, impacts of the subject's current food diet regimen, psychological status cause negligible effect on the HbA1c levels [5, 11, 18, 21, 22, 25, 29]. The count for HbA1c levels was the expedient and dependable diagnostic tools for accurate physiological glycemic levels projections [11, 21, 22, 25, 29]. Higher values of HbA1c levels had been critically associated with diabetic related patho-physiological complications like cardiopathy, nephropathy, retinopathy, neuropathy etc.[5, 11, 18, 21, 22, 25, 29] This metabolic disorder had taken the epidemic status worldwide triggering the research for the management of its long term complications and its related mortality, morbidity issues.

Material and Methods

Noninvasive technique based fasting blood glucose determination - Indigenously developed noninvasive MUS-IR unit operating principle for fasting blood

glucose level detections: In this technology we had utilized amplitude modulated ultrasonic waves and infrared light beam of desired wavelength. When ultrasonic sound beam amplitude modulating in nature propagates through the biological blood tissue complex matrix, it initiates vibrational properties into the medium[5-8,14,19]. The force of radiations acting over the small diameter based molecules as compared with the ultrasonic wavelength were obtained from acoustic potential energy gradients of those molecules[8,19,20,26,27,35] and represented as follows:

$$F_r = -\left[\frac{\pi p_o^2 V_c \beta_w}{(2\lambda)}\right].\phi(\beta,\rho).\sin(4\pi z/\lambda) \quad (1)$$

where F_r = character of the radiating force applied; V_c = molecular volumes of those molecules present in that blood tissue complex medium; z = distance; P_o = peak amplitude of the acoustic pressure applied and λ = ultrasonic sound wavelength.

When the influences of the compressibility factors (β_w) of the suspending medium were accounted[5-8,19,26,27,30,31,35], the mathematical expressions were represented as follows:

$$\phi(\beta,\rho) = \left[\frac{5\rho_c - 2\rho_w}{2\rho_c + \rho_w} - \left(\frac{\beta_c}{\beta_w}\right)\right] \quad (2)$$

where β_c = compressibility of the molecules; ρ_c = molecular densities of the suspending molecules in that blood tissue complex medium and ρ_w = molecular densities of the suspending blood tissue complex medium.

When infrared light had been utilized to detect the glucose specific vibrational patterns for glucose concentration measurement, the standard Lambert-Beer law [8, 26, 27, 30, 31, 35] principles were followed. The mathematical expression is as follows:

$$A(v) = -\log I(v)/I_0(v) \quad (3)$$

where A= Absorption profile; v= wave number of light wavelength; I_0= surrounding medium light intensity and I= light intensity aftermath propagation through the blood tissue complex medium.

Noninvasive MUS-IR unit instrumental System Description: In this part, the indigenously developed noninvasive blood glucose instrumental system had been elaborated. The facts behind selection of light wavelength and ultrasonic transducer operating frequency were discussed. Subsequently, the instrumental setup description and procedures of the test performed had been explained.

Light wavelength and ultrasonic transducer operating frequency selection: The light emitting diode of 940nm had been chosen here. The impact of major intracellular absorbers like melanin, water, oxyhemoglobin, deoxy hemoglobin etc. were moderately low in the optical window range between 700nm to 1100nm as depicted from the Figure 1 and 2 respectively [1,20,23,32,33].

Moreover, the absorption spectrum of the oxy hemoglobin and deoxy hemoglobin shows less dissimilarity in the range between 900nm to 1000nm as seen from the figure 3. The noise and interference from the other similar glucose like molecules were small in the range 700-1100nm of optical tissue window[1,20,23,32,33]. As in Figure 2 glucose absorption peak near to 940nm wavelength drives the factor for 940nm light spectrum selection as the suitable source for blood glucose level predictions[1,20,23,32,33]. Similarly, the safety aspect and wide availability of 40 kHz ultrasonic piezocrystal had also motivated us for its application in the experimental tests[8,28,30,31].

Block level depiction of the MUS-IR unit instrumental setup: Figure 4 depicts the block level description of the instrumental setup. The instrumental setup consists of amplitude modulating ultrasonic standing wave generator unit and the infra red light source-detector assembly. The square wave pulses supplied by the synchronous square wave generator focus typical square wave IR light pattern to the finger holder. Similarly amplitude modulating unit modulates the ultrasonic wave and directs those sound waves towards the finger holder. Both the light and ultrasonic units were perpendicular to each other. The infrared light focuses on the point of interaction of the amplitude modulating ultrasonic sound waves in the human finger based medium. The IR detector captures the resultant light signal.

After light signal acquisitions process, the signals undergo noise filtration and analysis through software toolbox of MATLAB. The peak to peak voltage amplitude spectrums in the FFT (Fast Fourier Transform) domain were observed here. The FFT Voltage amplitudes serve as a main function for blood glucose level determinations in noninvasive MUS-IR unit[8,28,30,31]. The blood glucose embedded information was decoded and calculated to predict the respective glucose concentrations.

Invasive technique based fasting blood glucose determination: The invasive fasting blood glucose levels were determined by the Accu-Chek Active blood glucose monitoring device manufactured and marketed by Roche diagnostics GmbH. The invasive glucometer working accuracy had been based on the glucose dehydrogenase enzyme based typical electrochemical reactions and its measurements[15,16].

HbA1c determination methodology utilized: For in vitro quantitative glycosylated hemoglobin level determination in human blood we had utilized Ion Exchange Resin method[2,3,24,34]. During column chromatography, the fast fraction glycosylated hemoglobins like HbA1a, HbA1b and HbA1c elute first in the columns due to lower isoelectric points. The bulk hemoglobin portions present there were

known as HbAo which is non-glycosylated in nature. The whole blood hemolysed sample preparations were introduced to the loosely binding cation-exchange resins beds[2,3,24,34]. Labile fractions were expelled out during preparation of whole blood hemolysate samples and binding processes. During the mixing process, HbAo binds actively with the ion exchange beds leaving behind the GHb portion as a supernatant fraction.[2,3,24,34]

Supernatant fractions rich in GHb portions were collected using filter separator after the mixing process. Absorbance of the Glycosylated Hemoglobin (GHb) fraction and the Total Hemoglobin (THb) fraction were measured at 415nm to determine the percentage of glycosylated hemoglobin[2,3,24,34]. Subsequently, the absorbance ratio of the Glycosylated hemoglobin and the Total hemoglobin fraction of the Control and the Test were measured to estimate the percentage of Glycosylated hemoglobin in the samples. The range between (04.00%-20.0%) the Glycosylated hemoglobin procedure shows linearity. After that the standard conversion tables for GHbA1 in % to HbA1c in % values were followed to predict the HbA1c values[2,3,24,34].

Medical status of the Volunteers: A total of 20 volunteers (subjects), out of which 10 volunteers are normal healthy nondiabetic adults (seven males, three females, aged 26.6±2.0 years, of height 176±4.2cm, weight 74.6±12 kg), 10 volunteers with hyperglycemic conditions (six males, four females, aged 34±5.5 years, of height 172±4.5cm, weight 69±13.5 kg) were taken. Institutional Review Board permitted the pilot study. Informed written consent was obtained from all the volunteers before collecting the fasting samples.

Experimental protocol: Each and every volunteer was informed before 05 days of the experiments to continue their normal physical exercises and food habits. The volunteers were directed to have 12 hours overnight fasting before the examinations. The bio-specimen in form of blood samples were collected from the right arm artery for fasting invasive blood glucose level detection and Glycated hemoglobin level determination methodology. Simultaneously, the readings for noninvasive fasting blood glucose level determinations were obtained from the right hand index finger by the indigenously developed MUS-IR (Modulated Ultra Sound-Infra Red) unit.

Results and Discussion

Diabetes mellitus refers to a serious medical situation in which body blood glucose had been elevated and it prevails in uncontrolled manner. Tight glycemic level and diet control along with proper medications were extremely necessary for safe management of this metabolic disorder[4,28]. Well managed blood glucose levels prevent patients from various life threatening diseases of heart, blood vessels, kidney, eyes, neurons etc.[4,28] Technology advancement in the patient diagnostic field had emerged the concept of noninvasive blood glucose detection methodology[4,28]. The fasting (pre-prandial) result of our experimental clinical studies indicates that noninvasive method based MUS-IR unit had been working properly.

The noninvasive, invasive readings correlations were very good and acceptable as seen from table 1 and 2 and figure 8 and 9 for normal (1-10) and diabetic (11-20) subjects accordingly. The HbA1c values of the hyperglycemic subjects as seen in table 2 were higher as compared to the values in the table 1 of normal healthy subjects. Similarly figure 10 reveals the same fact of poor blood glucose level maintenance in hyperglycemic subjects (11-20) as compared with the normal subjects (1-10) HbA1c values over past 2 to 3 months. The Clarke error grid based analytical approach had been utilized here to evaluate medical significance of dissimilarity between the noninvasive blood glucose determination methodology based predictions under assessment and the established invasive blood glucose determinations as the reference one. This technique employs the Cartesian method based diagrammatic approaches. Under this technique, values predicted by the technique under evaluation were plotted on Y-axis and the values obtained from the reference method were plotted on X-axis.

The diagonal line linking the two technical aspects characterizes the zone of ideal concurrences. The Clarke error grid plot consists of five types of zones like zone A, zone B, zone C, zone D and zone E respectively. Zone A symbolizes for the predicted noninvasive blood glucose values that diverge from the reference invasive blood glucose values by margin of 20%. The values inside this domain are medically acceptable and contribute towards significant medical treatment. Zone B signifies the beginning of errors and the values again are in increment by 20%. Zone B values are also medically acceptable. The values within the zone from C and E are likely to be unsafe, and it can call for potentially serious, erroneous medical diagnosis and treatment[9,10,12,13]. Here in this paper, figure 5 represents the Clarke error grid based percentage of total data for fasting blood glucose levels as obtained from the normal subjects (1-10). Values falling in the regions of A, B, C, D, E are 80.00%, 20.00%, 00%, 00% and 00% respectively.

Similarly, figure 6 corresponds to the percentage of total data for fasting blood glucose levels as obtained from the hyperglycemic or diabetic subjects (11-20). Values falling in the regions of A, B, C, D, E are 80.00%, 10%, 0%, 10% and 0% respectively. Again, the data points of normal (1-10) and hyperglycemic (11-20) subjects had been plotted altogether on the Clarke error grid platform as depicted from figure no. 7. The values obtained are as follows: A zone = 80%, B zone = 15%, C zone = 00%, D zone = 05% and E zone = 00% respectively. The result of this pilot study matches our earlier experimental values[7,8,28,30,31].

The vital factor for MUS-IR unit working includes (i) Suitable application of Amplitude Modulated Ultrasonic Standing waves (ii) Constructive and regular measurement of transmitted Infra red light signal based data for blood glucose level predictions. The collaboration of ultrasound and infrared techniques added up to the blood glucose measurement sensitiveness with high accuracy and precision [7, 8, 28, 30, 31]. These entire phenomenons direct us to use this hybrid technology for noninvasive blood glucose level detection purposes [7, 8, 28, 30, 31]. A small number of erroneous signals were obtained due to various factors like pigmentation of skin, motion artifacts, unwanted light, pulsatile blood flow and other physiological factors etc. All these factors modify the blood optical properties to deviate the actual noninvasive readings.

Conclusion

We had represented the working principle of the MUS-IR unit for noninvasive blood glucose monitoring, which is based on modulated ultrasound and infrared technology. To evaluate the MUS-IR unit performances, the clinical pilot study were performed on healthy and diabetic volunteers. The noninvasive blood glucose readings showed a strong correlation with the invasive blood glucose readings and HbA1c values confirms the individual glucose tolerance loads.

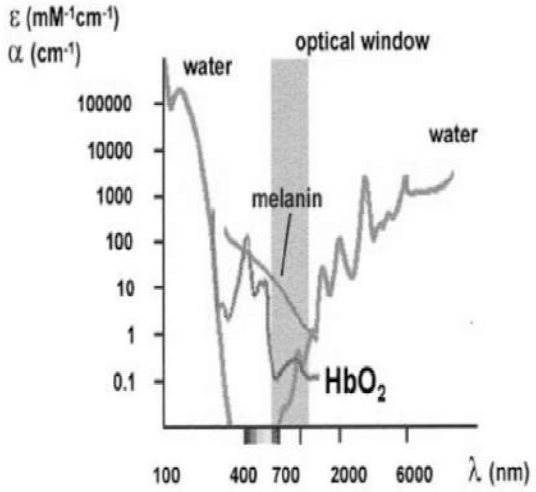

Figure 1: The tissue optical window phenomenon in the wavelength range from 100nm to 6000nm respectively[20].

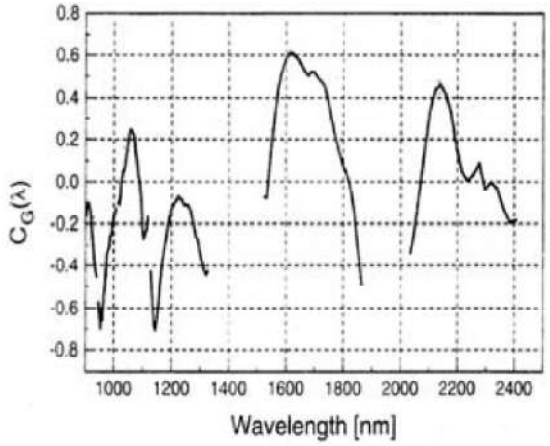

Figure 2: The glucose absorption pattern in the wavelength range from 900nm to 2400nm respectively[33].

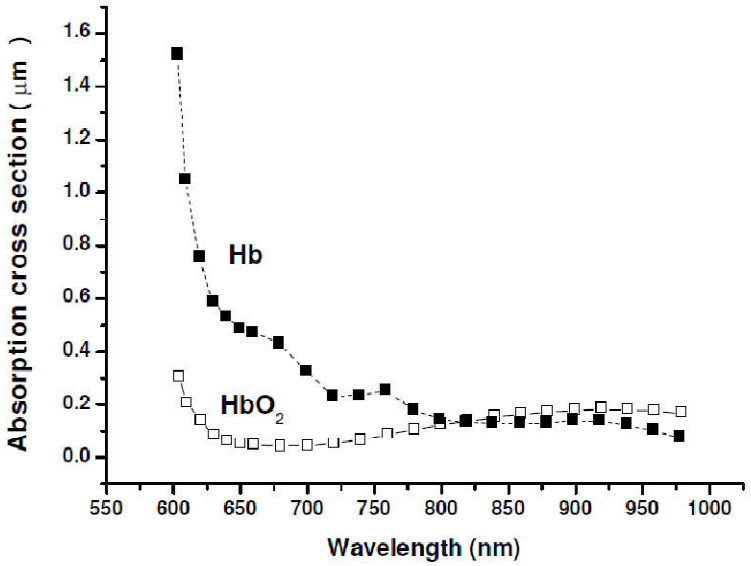

Figure 3: The absorption cross section pattern of the Hb (deoxy hemoglobin) and HbO_2 (oxygenated hemoglobin) in the wavelength ranges from 550nm to 1000nm respectively [1].

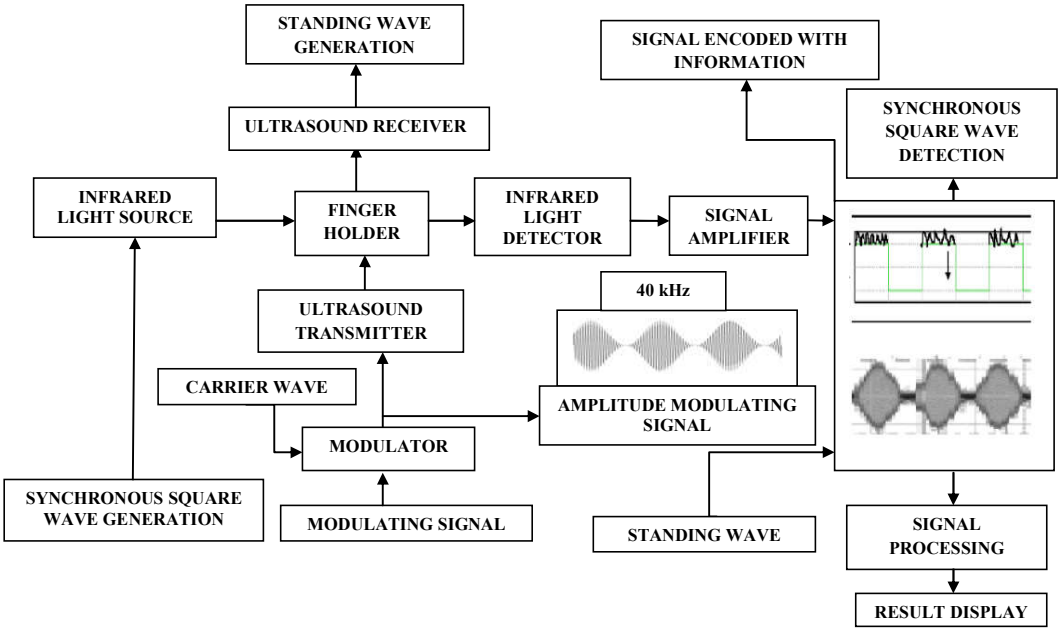

Figure 4: Sketch of the Amplitude Modulated Ultrasound and Infrared Technique based noninvasive blood glucose determining instrumental setup (MUS-IR Unit) utilized during the pilot study.

Table 1
Healthy volunteers invasive, noninvasive blood glucose values and GHb levels, HbA_{1c} values in percentage (%) respectively

S. N.	Healthy Volunteers	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)	GHb levels in %	HbA_{1c} Values in %
1.	Subject 1	**86.0**	99.0	**7.0**	**5.13**
2.	Subject 2	**76.0**	90.0	**6.7**	**4.88**
3.	Subject 3	**86.0**	83.0	**7.0**	**5.13**
4.	Subject 4	**71.0**	88.0	**6.5**	**4.71**
5.	Subject 5	**87.0**	84.0	**7.1**	**5.22**
6.	Subject 6	**95.0**	91.0	**7.4**	**5.47**
7.	Subject 7	**88.0**	89.0	**7.1**	**5.22**
8.	Subject 8	**90.0**	87.0	**7.2**	**5.30**
9.	Subject 9	**92.0**	88.0	**7.3**	**5.39**
10.	Subject 10	**105.0**	81.0	**7.7**	**5.72**

Table 2
Diabetic volunteers invasive, noninvasive blood glucose values and GHb levels, HbA_{1c} values in percentage (%) respectively

S. N.	Diabetic Volunteers	Invasive blood glucose values as obtained by invasive glucometer (mg/dl)	Noninvasive blood glucose values as obtained by MUS-IR Unit (mg/dl)	GHb levels in %	HbA_{1c} Values in %
1.	Subject 11	**141.0**	139.0	**9.0**	**6.81**
2.	Subject 12	**145.0**	136.0	**9.1**	**6.89**
3.	Subject 13	**181.0**	167.0	**10.4**	**7.98**
4.	Subject 14	**140.0**	144.0	**9.0**	**6.81**
5.	Subject 15	**148.0**	146.0	**9.2**	**6.98**
6.	Subject 16	**166.0**	154.0	**9.9**	**7.56**
7.	Subject 17	**159.0**	161.0	**9.6**	**7.31**
8.	Subject 18	**235.0**	143.0	**12.3**	**9.58**
9.	Subject 19	**165.0**	161.0	**9.8**	**7.48**
10.	Subject 20	**242.0**	142.0	**12.6**	**9.83**

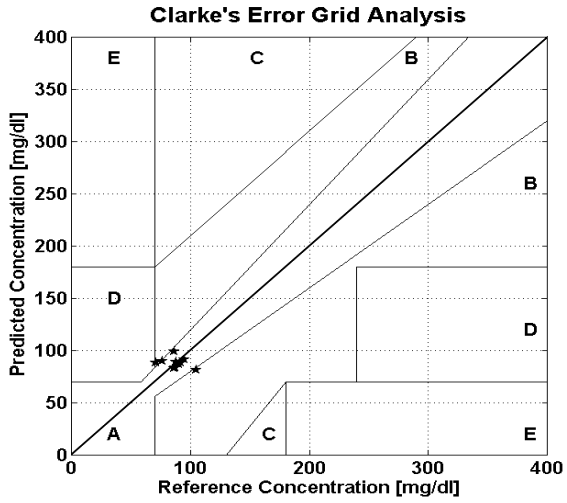

Fig. 5: Clarke's Error Grid Analysis of Invasive (Reference Concentration) and Noninvasive (Predicted Concentration) Blood Glucose levels as obtained from the Normal Healthy Subjects (1-10) respectively.

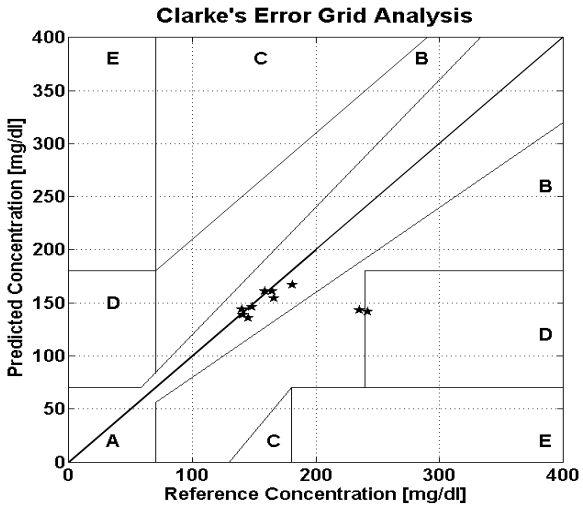

Fig. 6: Clarke's Error Grid Analysis of Invasive (Reference Concentration) and Noninvasive (Predicted Concentration) Blood Glucose levels as obtained from the Diabetic Subjects (11-20) respectively.

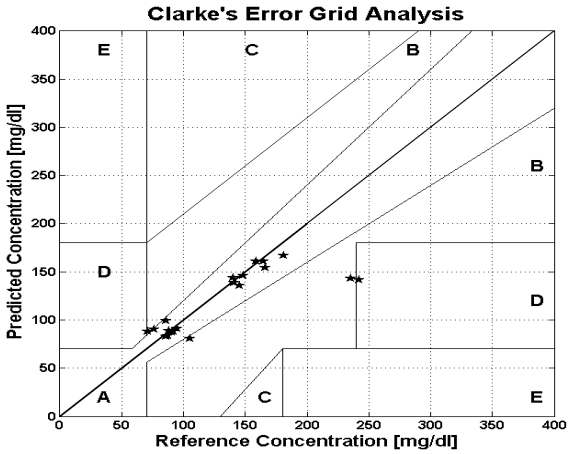

Fig. 7: Clarke's Error Grid Analysis of Invasive (Reference Concentration) and Noninvasive (Predicted Concentration) Blood Glucose levels as obtained from the Normal Healthy Subjects (1-10) and Diabetic Subjects (11-20) altogether respectively.

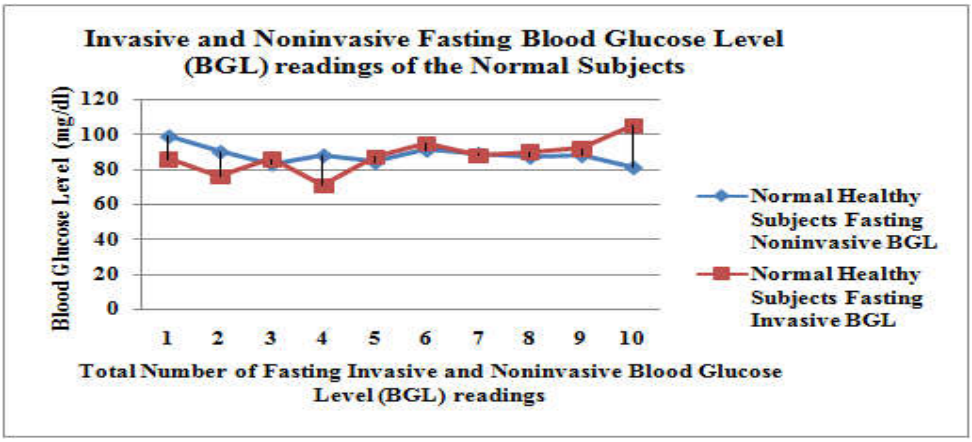

Fig. 8: Plotting of the Noninvasive and Invasive Fasting Blood Glucose Level (BGL) readings of the Normal Subjects (1-10) respectively.

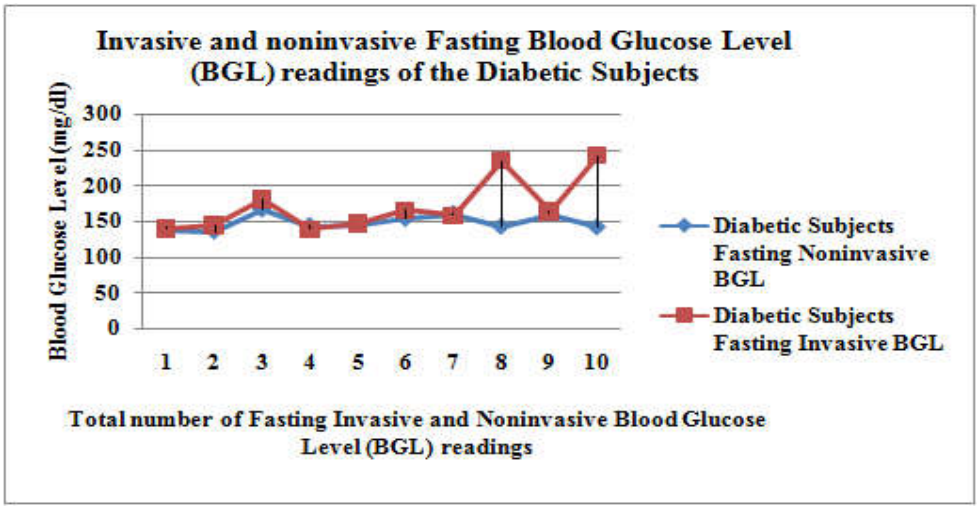

Fig. 9: Plotting of the Noninvasive and Invasive fasting Blood Glucose Level (BGL) readings of the Diabetic Subjects (11-20) respectively.

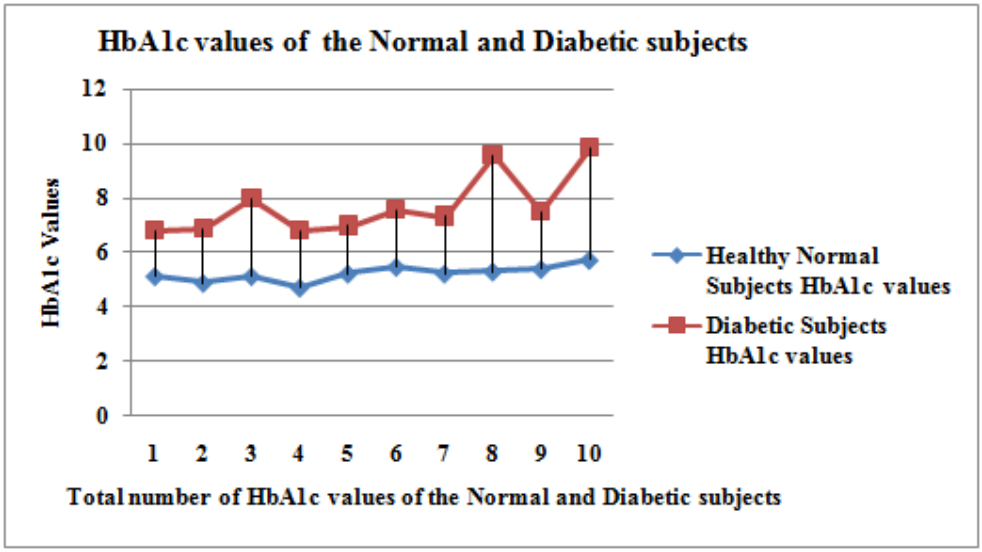

Fig. 10: Plotting of HbA1c values of the Normal (1-10) and Diabetic (11-20) Subjects respectively.

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(57) Abstract :
A truly new technique for non invasive glucose measurement is the need of hour. Precise in vivo blood glucose level monitoring skill would be precious for robust management of diabetes in all age group of patients. None of the existing principles offer enough accuracy to replace finger prick technology. Our method utilizes modulated ultrasound & optical technique (light of wavelength in the range of visible to infrared band). Amplitude modulated ultrasonic waves are used to excite the finger, as a result different constituent molecules vibrates at their specific response frequency depending upon their weight, shape & size, these specific vibrations are detected using light, the output response signal is in the form of modulated light signal, that carries information about the concentration of different constituent molecules. This modulated light response signal is collected using photo-sensor, and suitably processed using signal processing algorithm to extract the information of blood glucose level.

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