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SIMULTANEOUS DETERMINATION OF BLOOD SUBSTRATES BY FTIR SPECTROSCOPY COUPLED WITH LINEAR REGRESSION ANALYSIS

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ABSTRACT

Background: The search for a simple and accurate analytical method to determine the concentration of blood serum components is of major importance in clinical laboratories. Fourier Transform InfraRed (FTIR) spectrometry is a global, sensitive, and highly reproducible physicochemical analytical technique that identifies structural moieties of biomolecules on the basis of their absorption in the infra red region of the electromagnetic spectrum. **Objective:** Since a biomolecule is determined by its unique structure, each biomolecule exhibits a unique FTIR spectrum, representing the vibrations of its structural bonds. FTIR analytical applications have allowed determination of blood contents using a single spectral measurement. **Materials & Methods:** In this work, simultaneous determination of concentrations for the major components in human blood serum, namely glucose, protein, triglycerides, cholesterol, urea, and creatinine has been investigated using Fourier Transform InfraRed (FTIR) spectroscopy. The FTIR spectra of 35 blood samples have been recorded in the mid frequency region, 4000 – 450 cm^{-1}. The spectral measurements of 25 samples with known clinical parameters have been employed with Linear Regression Statistical Analysis using SPSS software to quantitatively correlate IR spectral features with the clinical analytical results. **Results:** The resulting quantification methods have been then validated with the remaining 10 specimens. **Conclusion:** The scatter plots obtained has validated that IR spectroscopy has the potential to become the potential tool in the clinical laboratories for instantaneous and simultaneous determination of human blood serum testing.

Keywords: Blood, FTIR spectroscopy, linear regression analysis, scatter plots, glucose; cholesterol

1. INTRODUCTION

The role of spectroscopy in medical diagnostics is very significant with the technical advancement in the instrumentation techniques and efficient data evaluation software. Most physicochemical analytical methods, such as Raman spectroscopy [1, 2], mass spectrometry [3], and nuclear magnetic resonance spectroscopy [4, 5] have been evaluated as tools to determine glucose concentration in whole blood, plasma, or serum. Fourier Transform InfraRed (FTIR) spectroscopy is an effective and efficient instrumental diagnostic technique in the analysis of biotic fluids. It possesses many

advantages over the regular clinical methods, as minimum sample requirement, instantaneous results, avoid of costly disposables, non invasive and reagent free method. As part of a recent surge in health-related research, FTIR spectroscopy is being explored as a possible means to gauge various chemical and cellular properties [6-8]. In broad terms, these explorations fall into two categories, with one being diagnostic (e.g. cancer detection) and the second quantitative, i.e. estimation of clinical parameters. Heise et al [9] have demonstrated that the Fourier Transform infrared spectrometry coupled with an ATR sampling device can be used to determine the amount of glucose in blood or plasma. Cyril Petibois et al [10] determined Glucose in serum samples. Gunasekaran et al [11, 12] studied lipid disorder in women blood samples and renal failure blood samples. Anthony Shaw et al [13] determined the concentration of serum LDL cholesterol and HDL cholesterol with the help of statistical techniques. Continuous monitoring of blood samples during chemotherapy in cancer treatment by FTIR Spectroscopy is found to be highly informative and useful.

The main aim of the present study is to bring out the potential of IR spectroscopy to become the clinical method of choice for quick and simultaneous determination of blood serum. In the present work, the main purpose has to assess the feasibility of infrared (IR) spectroscopy for the simultaneous quantification of six major metabolites of human blood serum, namely glucose, LDL cholesterol, urea, triglycerides, total protein and creatinine.

2. MATERIALS AND METHODS

2.1. Sample preparation for FTIR study:

Blood samples of 35 volunteers of the age group of 30 – 50 years who underwent Master Health Check up at Apollo Hospital, Chennai have been employed in the present study. For all the samples, the clinical parameters namely glucose, protein, triglycerides, cholesterol, urea, and creatinine are known. After centrifugation of blood at 10, 000g for 10 min at 4°C in a refrigerated centrifuge, the serum aliquots were transported to the laboratory in a portable freezer until analysis. Due permission was obtained from the institutional ethics committee before the start of the work. Informed consent was obtained from the participants in the study explaining the objectives of the study.

2.2. Spectral Analysis:

The FTIR spectra for all the samples have been recorded in the mid frequency region 4000 – 400 cm^{-1} using PERKIN – ELMER Spectrum One FTIR spectrophotometer at Sophisticated Analytical Instrumentation Facility, Indian Institute of Technology, Madras. 20µl volume of each serum sample has been spread evenly over the surface of a circular Thallium Bromide (TlBr) window which measures 9mm diameter and 2mm thickness. All the specimens were air dried for 30 minutes prior to the measurement so that strong absorption band of water in the mid IR region is hindered. The sampling window was scanned as the background and 32 scans were co added with a spectral resolution of 1 cm^{-1}.

The collected signal was transferred to the PC. The data were processed by windows based data program – Spectrum one software. The spectra were base line corrected and they were normalized to acquire identical area under the curves and the maximum absorbance values of the corresponding characteristic bands were noted.

2.3. Construction of a Statistical model:

The FTIR spectrum of a sample exhibits characteristic absorption bands depending on the various functional groups present in it. In the present work, the specific vibrational bands that are characteristic nature of the clinical parameters, namely, glucose, LDL cholesterol, urea, triglycerides, total protein and creatinine are noted. With the spectral absorptions of the different vibrational bands as one variable and the known clinical parameters as another variable a mathematical model has been proposed. In the present work, linear regression analysis has been carried out using SPSS software with the test group of 25 samples. The clinical parameters have been then calculated for the experimental group of the remaining 10 samples and it is found that the values obtained by the present work agrees well with the reported clinical values that validate the significance of the present work.

3. RESULTS AND DISCUSSION

Figure 1 presents a representative FTIR spectrum of a human blood serum in the mid frequency region of 4000 – 400 cm-1. Previous studies have shown that this spectral region features the characteristic absorption bands of main blood serum metabolites such as glucose, cholesterol, urea, triglycerides, protein and creatinine [14-16]. It shows coupled absorption bands at 930 -1080 cm^{-1} range interval associated with the elongations of vibrations of C – O bonds which is characteristic of the presence of glucose. The lipid absorption appearing in the 1700 – 1740 cm^{-1} range interval is mainly due to the ester C = O groups of LDL cholesterol. The N – H stretching vibrations occurs at 3110 – 3550 cm^{-1} interval, indicating the presence of urea in the serum.

The two prominent amide absorptions are seen in the 1650 - 1540 cm^{-1} interval arising from C = O stretching termed as amide I band and from N – H bending termed as amide II band. These two vibrations indicate the presence of peptide groups in proteins. The symmetric C – H bending at 1400 – 1600cm^{-1} interval due to the active presence of $–CH_3$ group indicate the presence of creatinine in serum. The absorptions originating from 2750 - 2950cm^{-1} interval is due to the ester C – O – C asymmetric vibrations and asymmetric C – H (methyl) stretching vibrations of phospholipids such as triglycerides.

The absorbance values of the vibrational bands FTIR spectra and the clinical report of the particular clinical parameter of the serum samples, say for example glucose is used in the construction of the regression formula using SPSS software which is derived as

$$y = a + B_1X_1 + B_2X_2 + \ldots.$$

where,

'a' is a constant generated by the software which is unique for each parameter,

'B' represents the individual contributions of each independent variable to the prediction of the dependent variable i.e the values of the significant vibrational frequencies and

'X' represents the spectral absorbance of the vibrational frequency B.

In the same way, the regression equation is obtained for the calculation other parameters namely, LDL cholesterol, urea, triglycerides, total protein and creatinine. The value of 'a' for the six parameters as generated by the software is given in Table 1.

The success of this method depends on the ability of the estimation of the clinical

parameters of any given experimental sample accurately and correctly using a single FTIR spectrum of the blood sample. To validate this, an experimental group comprising of 10 blood serum samples was formed. Substituting the spectral absorption 'X' and the significant vibrational frequencies 'B' and the corresponding constant 'a' generated by the regression equation, the different components of the blood namely, glucose, protein, triglycerides, cholesterol, urea, and creatinine h for each individual blood serum sample have been calculated. The result thus obtained was compared with the clinical records of the samples and it is found that the results matched with clinical records with greater accuracy. This has been pictorially represented in the form of scatter plots drawn between the calculated value and the clinical value for the control group samples which is shown from Figure.2 to Figure.7.

The scatter plots obtained for the six parameters showed that there is a high degree of accuracy between the calculated value and clinical value for the control group blood serum samples. The statistical result obtained for the six parameters are given from Table 2.

4. CONCLUSION

Thus it is found that the role of FTIR spectroscopy in medical diagnostics is highly relevant and significant. Generally, linear regression statistical analysis in the mid IR spectral region produces a more reliable prediction as mid IR spectrum consists of relatively well resolved band shapes due to the narrow band width. The standard error percentage value calculated quantitatively shows that mid IR spectroscopy is more reliable than by using the conventional methods. Therefore the present study has successfully demonstrated the potential of mid IR spectroscopy in determining the concentrations of several major metabolites in the clinically relevant range of concentration.

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Figure.1 Representative FTIR spectrum of a human blood sample

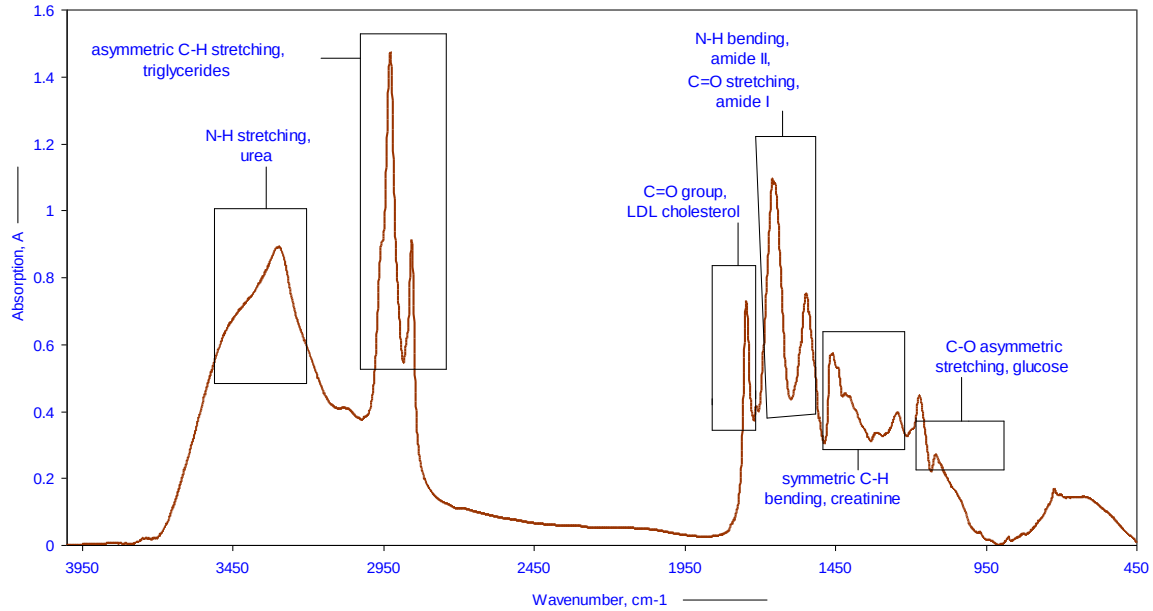


Table 1: Value of 'a' for the six parameters generated using SPSS software

PARAMETER	'a' value
Serum Creatinine	24.451
Total Protein	72.996
Serum Triglycerides	-4156.940
Blood Urea	-2172.182
LDL – Cholesterol	-2050.652
Blood Glucose	1725.399

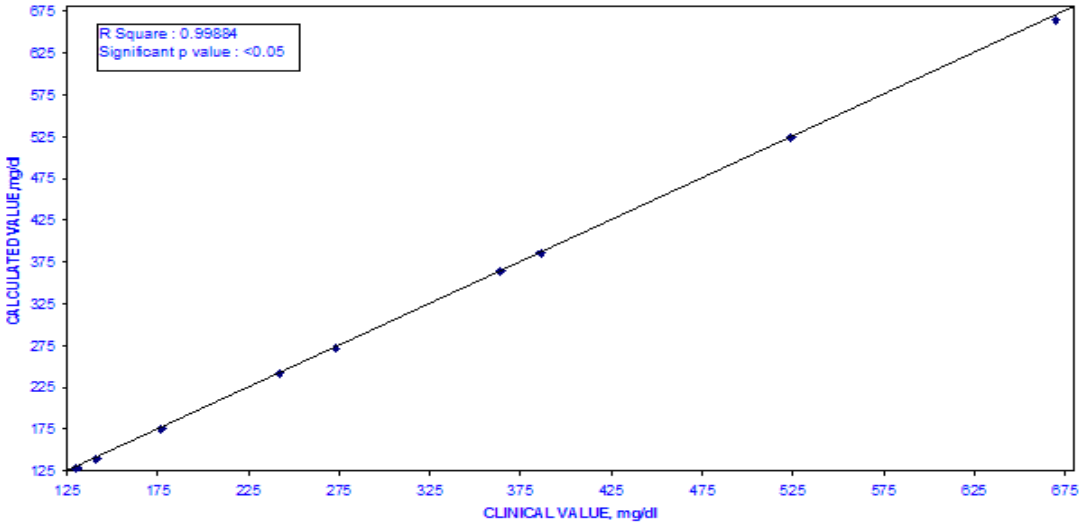


Figure.2 Statistical scatter plot for Blood Glucose

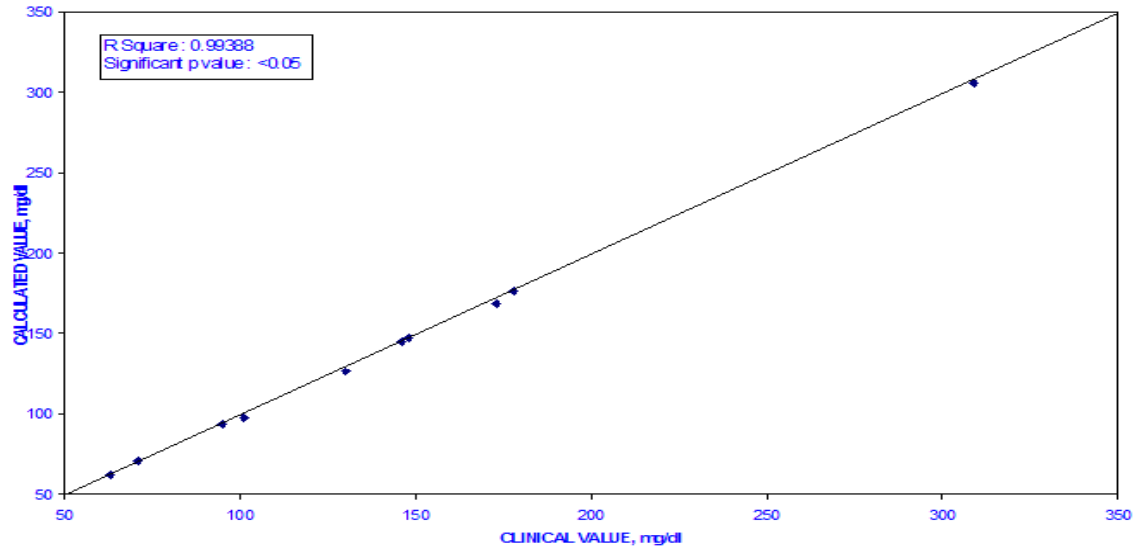
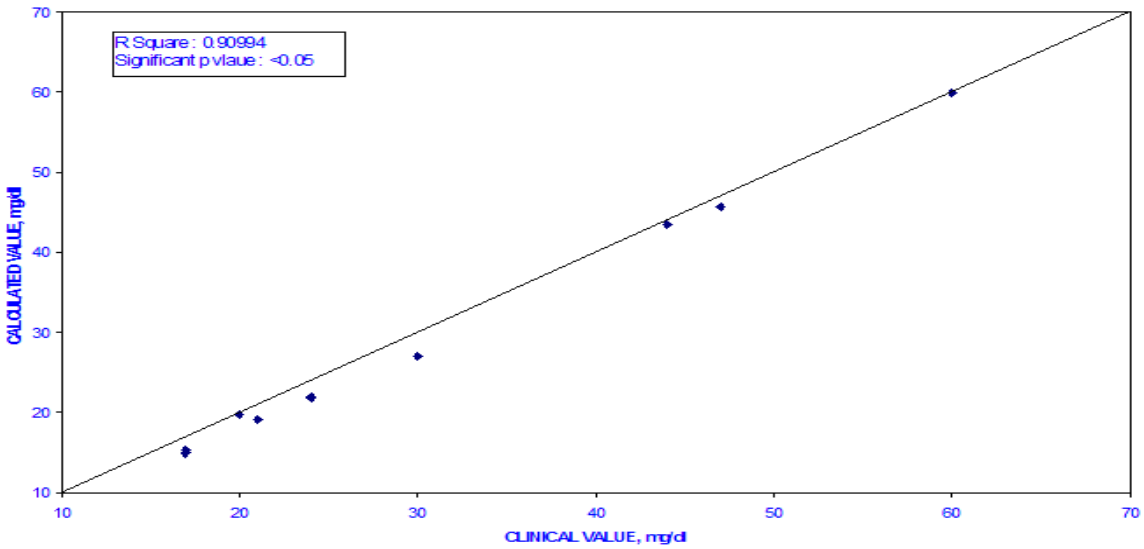


Figure.3 Statistical scatter plot for LDL Cholesterol

Figure.4 Statistical scatterplot for Blood Urea



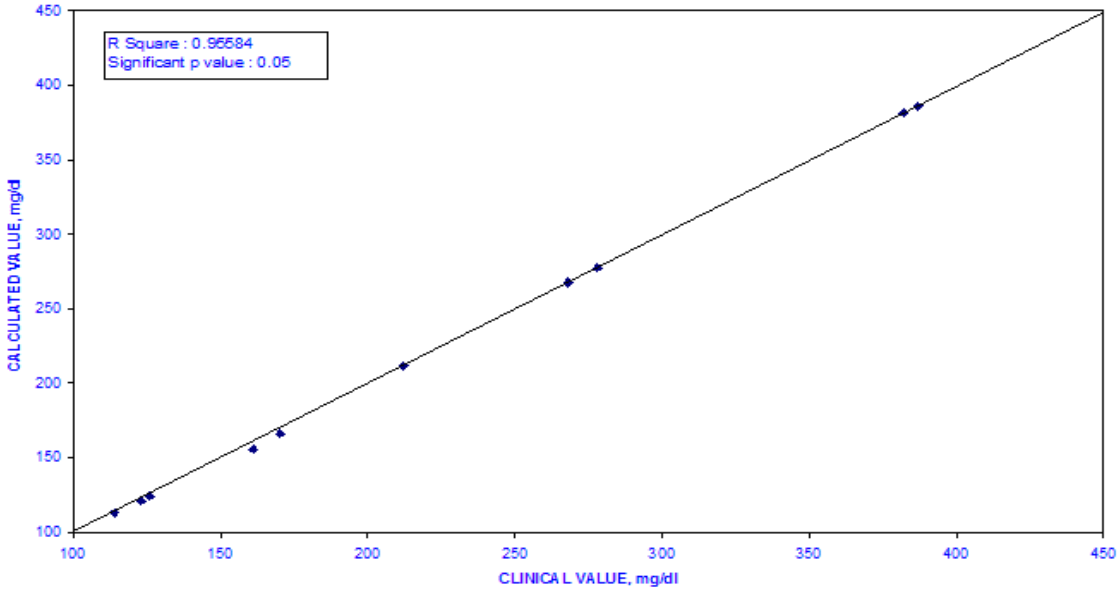


Figure.5 Statistical scatterplot for Serum Triglycerides

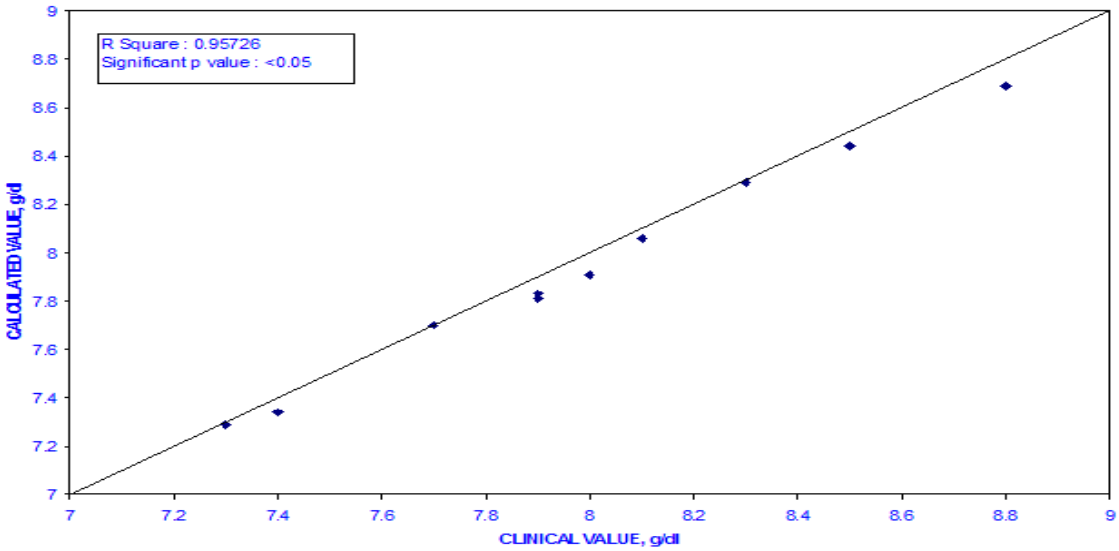


Figure.6 Statistical scatterplot for Serum Total protein

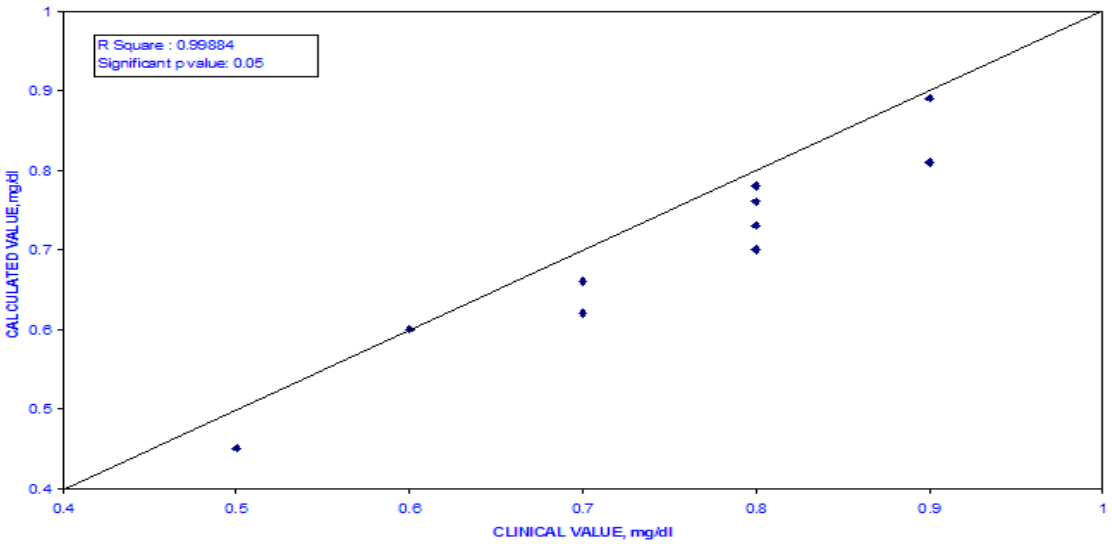


Figure.7 Statistical scatterplot for Serum Creatinine

Table 2: Statistical report for the six serum parameters

Model Summary							
	Method	R	R Square	Adjusted R Square	Std.Error of the Estimate	F	Sig.F
Blood Glucose	Enter	0.9886	0.9774	0.9060	0.3263	13.688	0.0019
LDL Cholesterol	Enter	0.9323	0.9475	0.9301	0.3308	12.802	0.0055
Blood Urea	Enter	0.9760	0.9675	0.9213	0.7308	1.216	0.0419
Serum Triglycerides	Enter	0.9545	0.9274	0.9039	12.9192	0.254	0.0073
Total Protein	Enter	0.9475	0.8977	0.9121	0.2808	1.848	0.0292
Creatinine	Enter	0.9886	0.9774	0.9060	0.3263	13.688	0.0019