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FTIR and UV Visible Spectrophotometric Approach to Discriminate Leukemic Sera

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Blood being the chief circulatory medium in human body, participates in every functional activity by virtue of its circulation through every organ. Almost in all diseases the blood undergoes major changes in chemical and biochemical properties. The study of blood by spectroscopic techniques can be used not only for understanding the biological nature of the disease, but also for the diagnosis of the disease. In the present work, Fourier Transform Infra Red (FTIR) and UV-Visible spectroscopic techniques are employed to study the spectral differences between a healthy serum and that affected with leukemia. The intensity ratio parameters (IRP) among the peaks are calculated in both the methods and it is found to be an indicator to differentiate a leukemic serum from the healthy one. Also to substantiate the findings, univariate statistical analysis has been made.

Key Words: Blood, Leukemia, FTIR spectrum, UV-Visible spectrum, IRP, Univariate statistical analysis.

INTRODUCTION

Asepsis is of prime importance in medical diagnostic analysis and hence call for the use of inexpensive disposables, which do not hike the cost of diagnosis. The advantage in the ever improving sensitivity of contemporary spectrometers with sophisticated computational techniques proved that FTIR and UV-Visible spectroscopy could be exploited to explore the various biochemical alterations on the molecular and structural differences of the biofluids of the human body. It has been demonstrated that using FTIR and UV techniques, glucose, total protein, albumin, cholesterol, triglycerides and urea can be assayed with blood serum[1-3]. The promise of the FTIR analytical method has been made in the characterization of different forms of cancer and in the discrimination of cancerous cells[4,5]. FTIR spectroscopy is already a renowned standard technique that provides useful information in

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biodiagnostics, although it is not yet accepted fully in the clinical laboratory[6,7]. The objective of the present work is to employ the FTIR and UV-Visible spectroscopic techniques to detect the changes in blood sera of leukemic subjects. The potential of the spectroscopic technique as a diagnostic tool to differentiate the healthy sera from the leukemic has also been discussed[8].

EXPERIMENTAL

Blood samples have been collected from healthy volunteers and leukemia patients. Patients were adults of age between 20 and 45 years, registered at Government Royapettah Hospital, Chennai, India. The collected blood samples were centrifuged to get the serum. For FTIR spectral recording, a volume of 1 mL of serum has been diluted with 4 mg/L aqueous solution of potassium thiocyanate (KSCN) solution and 20 µL of each diluted sample was spread evenly over the surface of a circular KBr window. All the specimens were air dried for 0.5 h prior to recording the IR spectra to eliminate the absorption band of water in the mid IR region. The spectra were recorded in the region 4000-400 cm^{-1} using Bruker IFS 66V FTIR spectrophotometer at SAIF, IIT Madras. The collected signals were transferred to PC and data were processed by Windows based data program. All the spectra were baseline corrected and normalized to equal intensities and therefore compensated for the imprecision in the film preparation.

Since better spectra with more absorption peaks could be observed for the whole blood than the serum in UV-Visible spectrometry, the UV-Visible spectral analysis was done for the whole blood of both normal and leukemic patients. For UV-Visible spectral studies, each blood sample was diluted with normal saline at a concentration of 5 µL/mL. The spectra were scanned in the region between 200-700 nm using ELICO SL 159 UV-Visible spectrophotometer at Spectrophysics Research Laboratory, Pachaiyappa's College, Chennai, India. The sources that produce ultra violet rays in the spectrometer were deuterium and tungsten halogen lamps with the monochromator as Czerny-Turner type with 1200 lines/mm holographic grating. The detector has a wide range of photodiode with greater efficiency and stray light loss is about < 0.1% at 220 nm with NaI 10 g/L. The photometric range of the device is ± 2.5 Abs with an accuracy of ± 0.005 Abs at 1.0 Abs. Even after 2 h warm-up, the baseline flatness is maintained to be ± 0.003 Abs/h. The data processing is done by PC. Quartz cuvettes of 10 mm path length were used. The device compensates for solvent absorption and losses of radiation by scattering and reflection. The decision of the solvent is made in such a way that the solvent should not absorb in the same region as the solute[9]. The spectra were recorded under identical conditions.

RESULTS AND DISCUSSION

Infrared vibrational band frequency assignment of human serum: The representative normalized FTIR absorption overlay spectra of normal and leukemic serum samples in the frequency region 4000-400 cm^{-1} is presented in Fig. 1. The infrared spectrum of serum provides useful information of biomolecules like structure, functional groups, types of bonds and its interactions. A satisfactory vibrational band assignment of absorption bands of the spectra is done with the help of the group frequency of the various constituents of the serum samples[1,10,11]. Table-1 presents the vibrational band assignment of human serum.

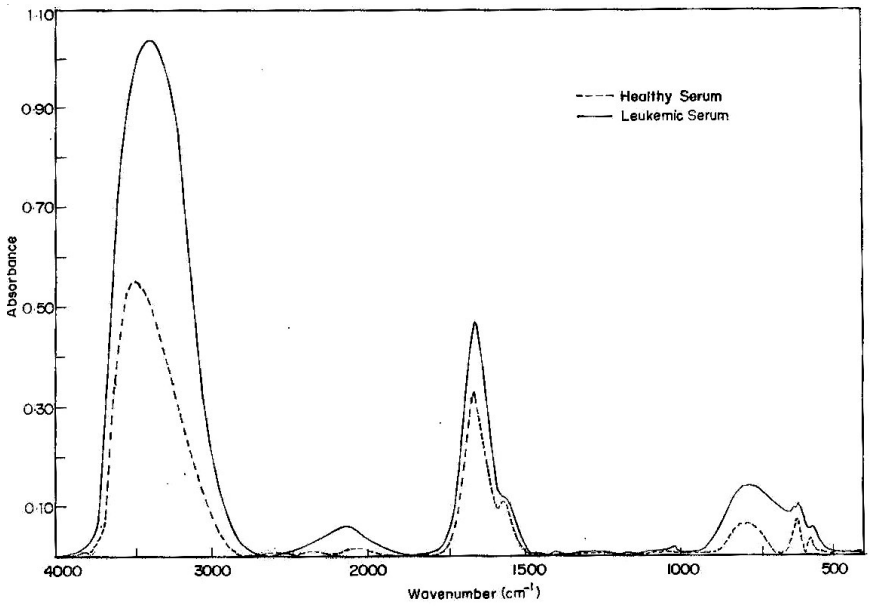


Fig. 1. FTIR Overlay spectrum of healthy and leukcmia serum

The vibrational band at 3400 cm^{-1} is due to N-H stretching vibration of the secondary amides of protein. The asymmetric and symmetric stretching vibrations of the methyl group of the proteins and lipids are present in the region 3050-2800 cm^{-1}. It emerges from CH stretching vibrations of fatty acyl chains of all cellular lipids[11]. The other two vibrational bands in the C-H stretching region are found to be present near 2922 and 2851 cm^{-1}, which are due to the asymmetric and symmetric stretching vibrations of the methylene group, respectively[12]. The essential amide bands dominate in the region 1700-1500 cm^{-1}. The strong absorption band at 1655 cm^{-1} is assigned to C=O stretching of amide I of the proteins[13]. The presence of

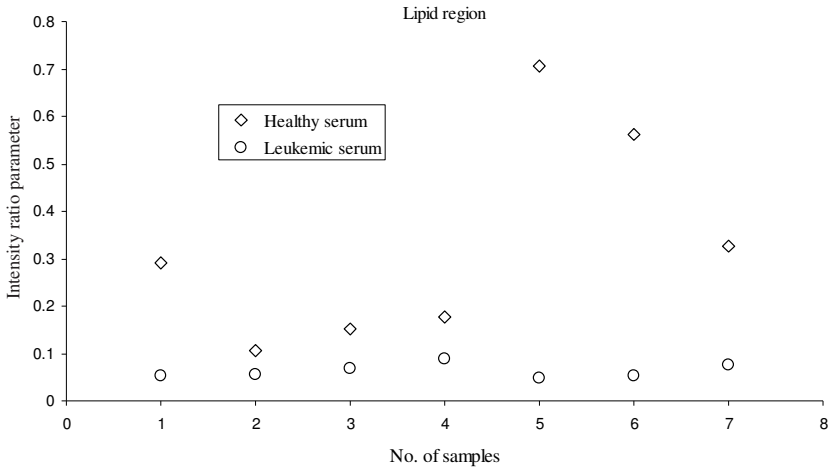
TABLE-1
INFRARED BAND ASSIGNMENT OF HUMAN SERUM

Frequency (cm^{-1})	Assignments
702	N-H out-of-plane deformation of protein
1035	C-O stretch of β anomer, (major glucose band)
1079	C-O stretch of α anomer
1107	Endocyclic C-O-C vibration
1153	Ring vibrational modes of C-O-C and C-O-H bonds
1169	C-O stretch of COH tyrosine protein
1315	CH_2 vibrations of α anomer
1365	CH_2 vibrations of β anomer
1400	CH_3 symmetric bending vibration of protein
1435	C-H bending
1456	CH_3 asymmetric bending vibration of protein
1655	C=O stretching/C-N stretching/ N-H bending of proteins (Amide I band)
1548	N-H bending strongly coupled with C-N stretching (Amide II band)
2851	CH_2 symmetric stretching
2871	CH_2 asymmetric stretching
2922	CH_3 symmetric stretching of proteins and lipids
2956	CH_3 asymmetric stretching of proteins and lipids
3400	N-H asymmetric stretching of secondary amides of proteins

band at 1548 cm^{-1} is due to the N-H bending vibrations of amide II that are strongly coupled to the C-N stretching vibrations of the protein amide group. The peaks at 1456 cm^{-1}, 1400 and 1315 cm^{-1} arise mainly from the asymmetric and symmetric deformations of methyl groups of proteins[14]. The peak at 1400 cm^{-1} may also be considered due to COO^- stretch of ionized amino acid chains, suggesting an increased contribution from carboxylate[15]. The lipid phosphate band due to the asymmetric P-O stretching of PO_2 occurs at 1240 cm^{-1}. The absorption bands at 1325, 1365, 1435 cm^{-1} arise due to the CH bending of CH_2 groups in a and β anomers[16]. For glucose the optimal frequency range of 1250-925 cm^{-1} is used, since the mid IR spectrum of glucose includes several strong absorption bands in this region. The absorption peaks present at 1169, 1153, 1107, 1079 and 1035 cm^{-1} are considered to be due to the different C-O stretching vibration of C-O-H and C-O-C bonds. The medium strength vibrational band present at 702 cm^{-1} is assigned to N-H out of plane bending with the contribution of C-N torsional vibrations.

FTIR spectral analysis: FTIR spectra of both normal and leukemia sera show the corresponding bands in their specific regions qualitatively. But, quantitatively there is a considerable spectral difference between normal and leukemia sera. The absorbance is directly proportional to the concentration according to Beer Lambert law. Hence the different serum samples are analyzed quantitatively by calculating the intensities ratio among the absorption peaks. In this study the complete spectral region is divided into three, which corresponds to glucose region (1500-925 cm^{-1}) and protein region (1700-1500 cm^{-1}) and lipids or fat region (3400-2800 cm^{-1}). Based on the differences in the spectral signatures of the normal and leukemia sera, three intensity ratio parameters have been introduced. They are $R_1 = I_{3400}/I_{2951}$ in the lipid region, $R_2 = I_{1653}/I_{1548}$ in the protein region and $R_3 = I_{1028}/I_{1033}$ in the glucose region[17].

It is a well known fact that C-H stretching vibrations of methyl and methylene groups of tripalmitin dominate this region. An absorption band due to N-H stretching is established at 3300 cm^{-1} in normal subject, while for the leukemia subject there is a less considerable shift of about 50 cm^{-1}. Intensity ratio parameter $R_1 = I_{3400}/I_{2951}$ is introduced in the lipid region, the intensity ratio due to the NH stretching vibration of secondary amides of protein to the asymmetric stretching vibrations of the methyl group of lipids. The change in IRP for normal and leukemic sera clearly shows that the conformation structure of methylene chains of triglycerides got changed in leukemia serum. Fig. 2 presents the scatter plots of IRP R_1 in the lipid, R_2 in protein and R_3 in glucose regions, respectively.



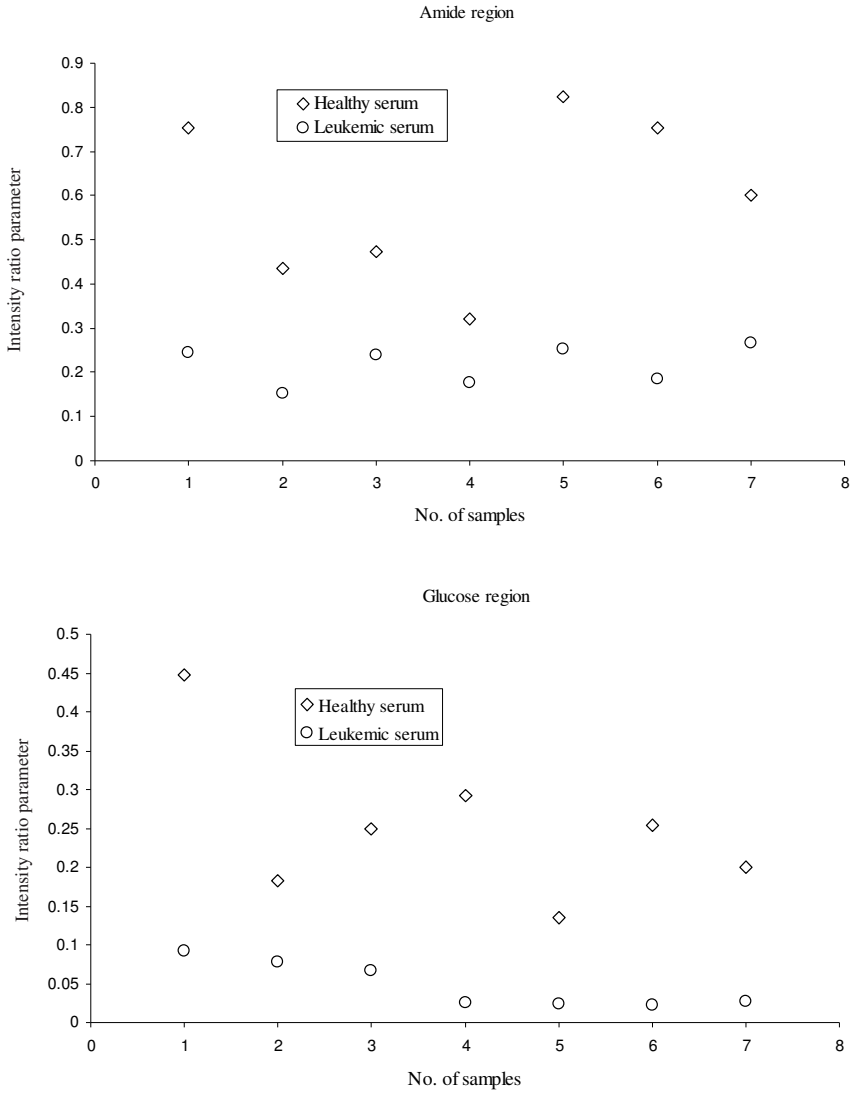


Fig. 2. Scatter plots of the IRP using FTIR spectra of healthy and leukemic sera

Generally, spectral investigations are made using different univariate methods involving ratios of peak intensities, shift in band position and, area under the curve[15,18,19]. According to Beer-Lambert's law, the absorbance of a constituent is directly proportional to its concentration. Assuming Beer-Lambert law is obeyed for the spectroscopic analysis, the present work deals with one of the univariate statistical analysis, *i.e.*, analysis of intensity Ratio Parameter (IRP). Table-2 presents the results of univariate statistical analysis of the leukemic sera in all the three regions.

TABLE-2
FTIR SPECTRAL RESULTS OF INTENSITY RATIO PARAMETERS IN THE THREE REGIONS OF HEALTHY AND LEUKEMIC SERUM SAMPLES

Region	Intensity ratio parameter	Average ± SD
	I_{3400}/I_{2951}	
Lipid	Normal	0.3320 ± 0.2243
	Leukemic	0.0632 ± 0.0417
	I_{1653}/ I_{1548}	
Amide	Normal	0.5945 ± 0.1904
	Leukemic	0.2168 ± 0.0441
	I_{1028}/ I_{1033}	
Glucose	Normal	0.2515 ± 0.1010
	Leukemic	0.0479 ± 0.0303

UV-Visible spectral analysis: The UV-Vis spectral study of blood in healthy and diseased subjects has already been reported[3,20,21]. As blood under-goes physical and biochemical changes in many diseases, the patho physio-logical changes in the healthy and diseased blood are analyzed by the characteristic absorptions in the UV-Visible spectral region. The over-lay UV-Visible spectrum of the healthy and leukemic blood is presented in Fig. 3. Six peaks are observed at 210, 278, 344, 417, 543 and 578 nm due

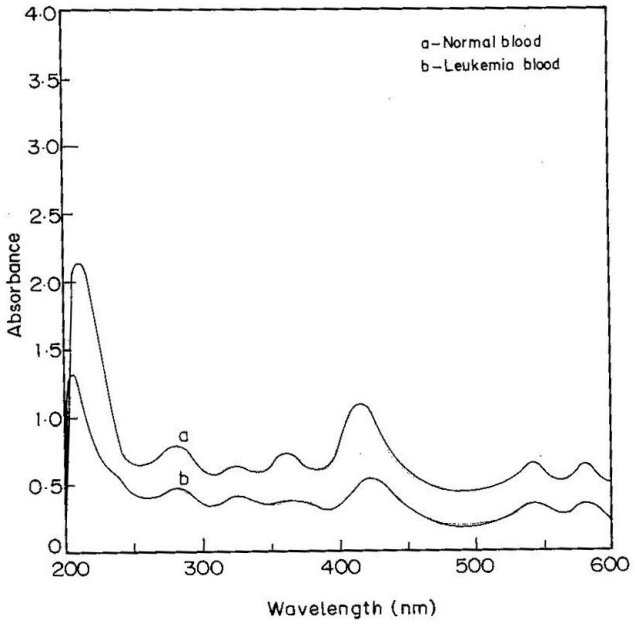


Fig. 3. UV-Visible overlay spectrum of healthy and leukemia blood

to constituents of blood[20,22]. The amide chains of the proteins present in the blood absorbs strongly *ca.* at 210 nm. Also the strong absorption peak at around 280 nm is due to amino acids *i.e.*, tyrosine and tryptophan[3]. The carbohydrate metabolism in blood can be assigned to the absorption maxima at 344 nm due to NADH and NADPH. These are reduced forms of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) which function in the action of many dehydrogenases[22]. While getting reduced these enzymes undergo structural change which causes the absorption at around 344 nm. Also the absorptions at 417, 543 and 578 nm are due to d-f transition of CO-Oxy hemoglobin[22]. There is a great change in the optical density of healthy and diseased blood.

Table-3 gives the statistical results of the analysis of UV-Visible spectral data and the scatter plots in protein and oxy hemoglobin region in Fig. 4 clearly shows the discrimination of the leukemic blood from that of the healthy one.

TABLE-3
UV-VISIBLE SPECTRAL ANALYSIS OF ABSORBANCE RATIO PARAMETERS OF HEALTHY AND LEUKEMIC SERA

Region	Intensity ratio parameters	Average ± SD
Protein	A_{280}/A_{210}	
	Normal	0.4973 ± 0.00893
	Leukemic	0.2446 ± 0.02929
Oxy hemoglobin	A_{543}/A_{417}	
	Normal	0.6143 ± 0.01187
	Leukemic	0.7972 ± 0.02360
	A_{543}/A_{417}	
	Normal	0.6183 ± 0.01358
	Leukemic	0.8053 ± 0.02406

Conclusion

For the early detection and diagnosis of diabetes, FTIR spectroscopy is already a well established biodiagnostics tool. In this study, it has been demonstrated that the study of IR and UV-Vis spectra of serum samples may be used to differentiate between the healthy and leukemic subjects. Some remarkable differences are elucidated in terms of FTIR spectral profiles, absorption bands, wave numbers and the intensity ratio parameters and satisfactory analysis has been made. It can be observed that in the case of leukemia samples, the absorbance for the various fundamental modes of vibrations of the three vital regions is lesser than that for the normal samples. Further this is also confirmed by a clear discrimination between healthy

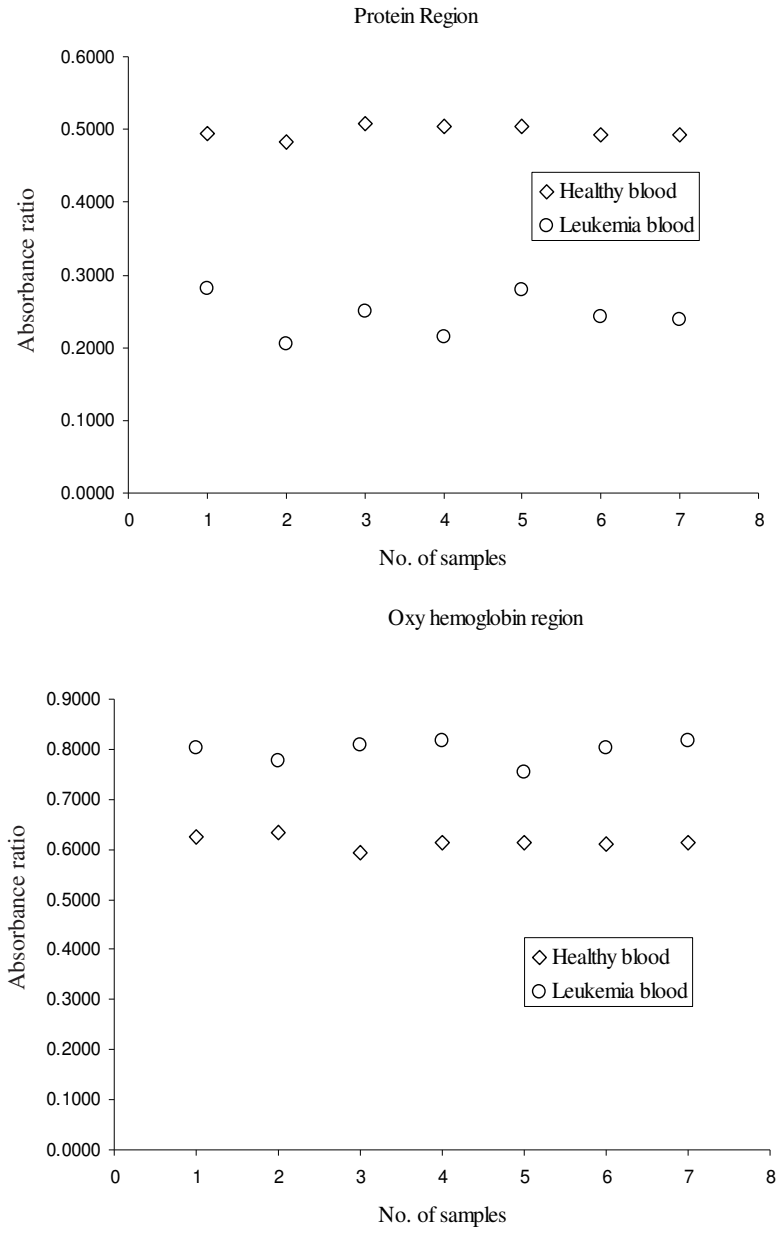


Fig.4. Scatter plot of the absorbance ratio of healthy and leukemic sera

and leukemia serum samples with scatter plots drawn in the three important spectral regions namely lipid, amide and glucose spectral regions. The results of UV-Visible spectral analysis of healthy and leukemic blood samples are well in accordance with the FTIR spectral interpretations.

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