

FTIR and UV-Visible Spectral Study on Normal and Diseased Blood Samples

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The application of spectroscopy for the study of biomedical compounds has increased tremendously in recent years. Blood is the chief circulatory medium in our body and participates in every functional activity by virtue of its circulation through every organ. The study of blood is one of the fascinating branches of clinical medicine. With the onset of a disease, the relative content of the biomolecules of blood changes, thereby producing pathophysiological changes in the functions. Spectroscopic techniques can be effectively employed as a diagnostic tool in clinical chemistry and it can be an alternate method in clinical analysis. In the present work FTIR and UV-Vis spectroscopic techniques are employed to study the spectral differences between a normal healthy serum and that affected with some diseases like diabetes, cholesterol, thyroid and urea. The internal standards among the absorption peaks are calculated in both the methods and it is found to be an indicator to differentiate a normal from a diseased serum sample.

Key Words: Blood, Diabetes mellitus, Cholesterol, Urea, Thyroid, FTIR spectrum, UV-Visible spectrum, Internal standard.

INTRODUCTION

Blood is a fluid which circulates in a closed system of blood vessels. Blood contains about 55% of plasma and 45% of cellular fraction consisting of formed elements. The cellular fraction is composed of three types of cells, viz., erythrocytes or red blood corpuscles (45,00,000–60,00,000), leukocytes or white blood corpuscles (5000–10,000) and thrombocytes or blood platelets (2,00,000– 4,00,000). The fluid portion plasma contains a large number of organic and inorganic substances such as proteins, vitamins, minerals, lipids, etc. Although the main function of blood is to transport various materials to all the cells of the body, blood also provides the temperature regulating and defence mechanism^{1,2}. There is a wide fluctuation in the number of various formed elements present in the blood of a normal healthy person and a person affected with a disease. With the onset of a disease the relative content of the biomolecules changes, thereby producing pathophysiological change in their functions. Spectroscopic methods of blood analysis have an alternate technique to the clinical methods since they require less sample and provide more information. In this work a normal healthy blood sample and that affected with abnormal values of glucose (diabetes mellitus), cholesterol, urea and thyroid hormones are analysed by employing FTIR and UV-Vis spectroscopic techniques.

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EXPERIMENTAL

Blood samples have been collected from normal healthy persons of same age group. The samples affected with abnormal values of glucose, cholesterol, urea and thyroid hormones have been obtained from patients from a medical care centre at Chennai, India. All the samples have been analyzed in the serum form. A volume of 1 mL of serum has been diluted with 4 mg/L aqueous solution of potassium thiocyanate solution (KSCN) and this mixture has been spread evenly on the surface of a circular KBr window. All the specimens are air dried for 30 min prior to measuring the infrared spectra to eliminate the absorption band of water. The FTIR spectra of the serum samples have been recorded over the region 4000–400 cm^{-1} using Perkin-Elmer FTIR spectrophotometer at Medopharm, Chennai, India. All the spectra have been baseline corrected and are normalised in the absorbance mode to acquire identical data.

For UV-Vis spectroscopic measurements, the serum sample is diluted with normal saline at a concentration of 2 $\mu\text{L}/\text{mL}$ and the spectra are recorded using Elico SL-159 UV-Vis spectrophotometer, at Spectrophysics Research Laboratory, Pachaiyappa's College, Chennai, in the region 200–400 nm.

RESULTS AND DISCUSSION

The spectra of normal serum and that of diabetes, cholesterol, urea and thyroid serum samples are all distinct from one another, but are dominated mainly by the absorption of the protein constituents which provides the selectivity in infrared based serum analysis. Figs. 1–4 present the FTIR spectra of diabetes, cholesterol, urea and thyroid serum samples along with the normal serum respectively. A satisfactory vibrational band assignment of the absorption bands of the spectra is done with the idea of the group frequency of the various constituents of the serum samples^{3,4}. Table-1 presents the vibrational band assignment of human serum. The vibrational band at 3300 cm^{-1} is due to the N–H stretching vibration of the secondary amides of protein. The asymmetric and symmetric stretching vibrations

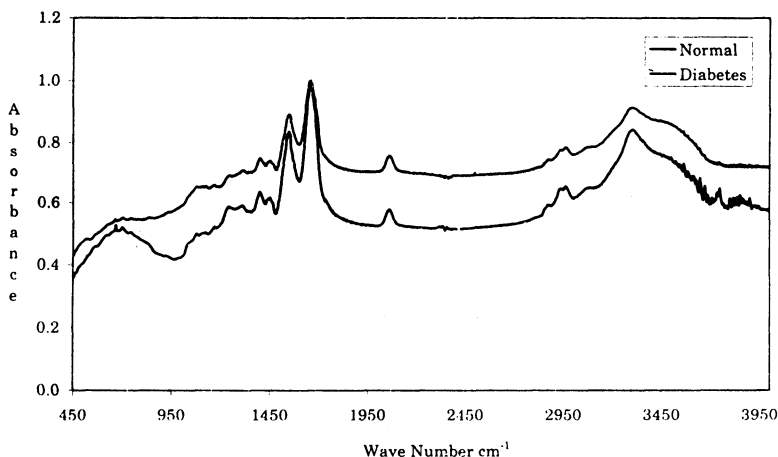


Fig. 1. FTIR spectra of diabetes and normal serum

of the methyl group of the proteins and lipids are found to be present at 2956 and 2896 cm^{-1} respectively. 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.

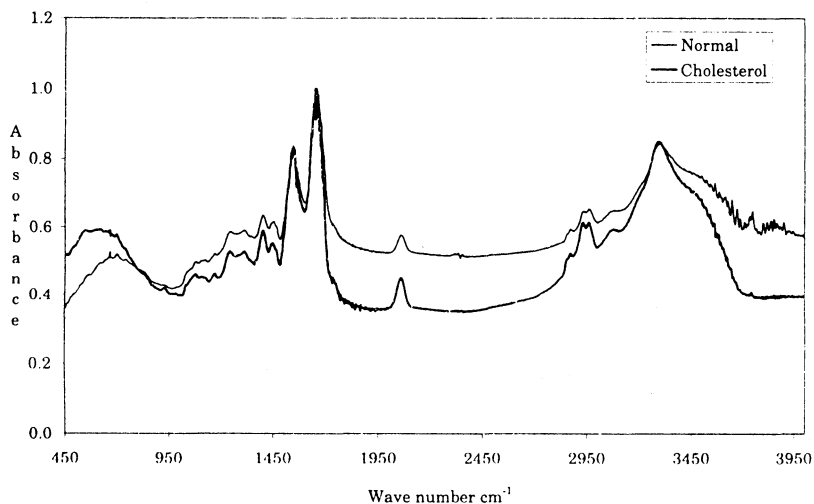


TABLE-1

INFRARED VIBRATIONAL BAND FREQUENCY ASSIGNMENT OF HUMAN SERUM

Vibrational band (cm^{-1})	Assignment
3304	N—H stretching of secondary amides of protein: amide A
2955	CH_3 asymmetric stretching of proteins and lipids
2936	CH_2/CH stretching
2869	CH_3 symmetric stretching of proteins and lipids
2851	CH_2/CH stretching
1656	C=O stretching, (80%) weakly coupled with C—N stretching (10%) and NH deformation (10%)-amide I
1545	NH deformation (60%) strongly coupled with C—N stretching (40%): amide II
1456	CH_3 asymmetric deformation
1403	CH_3 asymmetric deformation COO^- stretching of amino acids
1315	CH_3 symmetric deformation
1240	Asymmetric PO_2 stretching of lipid phosphates
1169	C—O stretching
1128	C—O stretching
1081	C—O stretching
955	PO_2 symmetric stretching of lipid phosphates
701	NH asymmetric deformation coupled with CH_2 rocking: amide V
625	O=C—N deformation (40%) coupled with other ring deformations (60%): amide IV

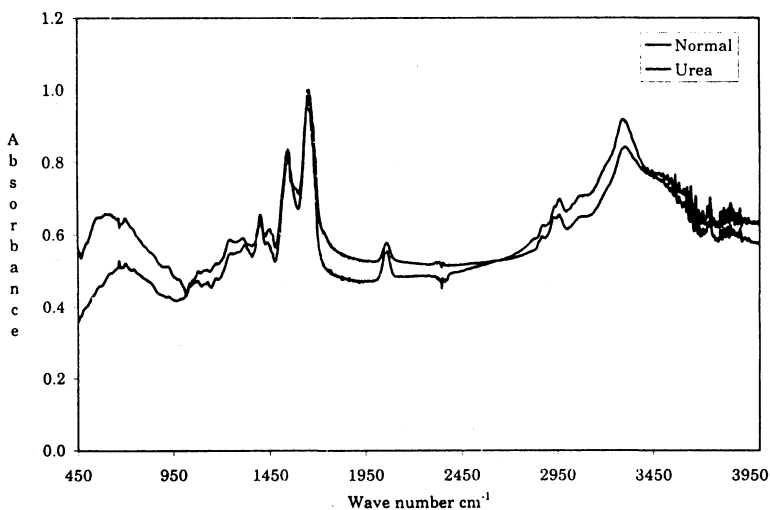


Fig. 3. FTIR spectra of urea and normal serum

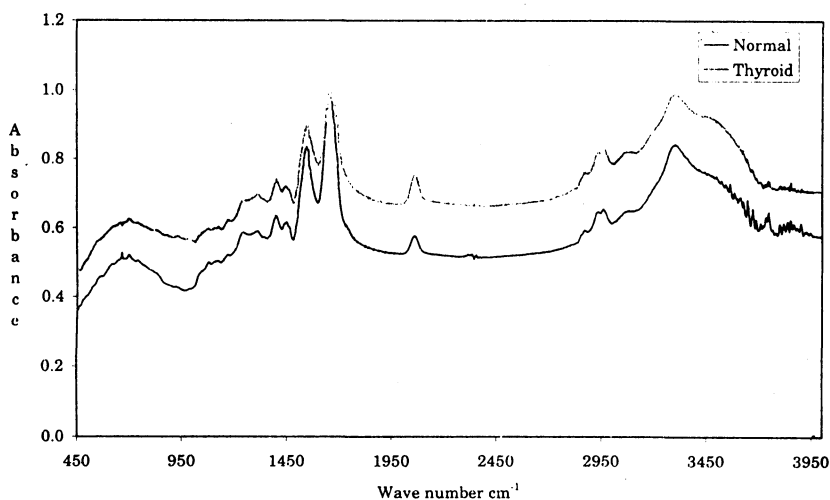


Fig. 4. FTIR spectra of thyroid and normal serum

The strong absorption band present at 1655 cm^{-1} is attributed to $\text{C}=\text{O}$ stretching of amide-I of the proteins. In the same way the presence of the band at 1548 cm^{-1} is due to the amide-II or $\text{N}-\text{H}$ bending vibrations that are strongly coupled to the $\text{C}-\text{N}$ stretching vibrations of the protein amide groups. The peaks at 1456 , 1400 and 1315 cm^{-1} are considered to be due to the asymmetric and symmetric deformations of the methyl group of proteins. 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. The lipid phosphate band due to the asymmetric $\text{P}-\text{O}$ stretching vibration is found to occur at 1240 cm^{-1} . The spectral region $1250-925\text{ cm}^{-1}$ is predominantly occupied by the $\text{C}-\text{O}$ stretching vibrations of glucose. The absorption peaks present at 1169 , 1153 , 1107 ,

1079 and 1035 cm^{-1} are considered to be due to the different C—O stretching vibrations of C—O—H and C—O—C bonds. The weak absorption band at 955 cm^{-1} is considered to be due to P—O symmetric stretching of the phosphate bond of proteins. The medium strong vibrational band present at 702 cm^{-1} is assigned as N—H out-of-plane bending with the contribution of C—N torsional vibrations.

The infrared spectrum provides various useful information of a biomolecule like structure, functional groups, types of bonds and its interactions. Hence the FTIR spectra of all the serum samples both normal and various diseases show the corresponding absorption bands in their specific regions qualitatively. But quantitatively there is a considerable spectral difference between the normal and diseased serums. The absorbance is directly proportional to the concentration. Hence the different serum samples are analyzed quantitatively by calculating the intensity ratio among the absorption peaks. In order to quantify the spectral difference four intensity ratio parameters have been introduced. They are $R_1 = I_{2869}/I_{2955}$ due to the asymmetric and symmetric stretching vibrations of the methyl group of the lipids, $R_2 = I_{1545}/I_{1656}$ which is due to the ratio of intensities of amide-II and amide-I bands of the amino acids and hence proteins, $R_3 = I_{1315}/I_{1403}$ due to the ratio of the intensities of symmetric and asymmetric deformation vibrations of the methyl groups of protein and lipids and $R_4 = I_{1169}/I_{1245}$ due to C—O stretching of glucose and P—O stretching of lipid phosphate. Though the infrared spectra of all the serum samples are similar, considerable differences are found to be present in the internal standards among the absorption peaks in various diseases. For example, the values of R_1 , R_2 , R_3 and R_4 are found to be around 0.90, 0.83, 0.93 and 0.88 respectively for a normal healthy serum. But in the case of diabetes, the values are found to be high as 0.94, 0.89, 0.95 and 0.96 respectively. In the other diseases it is observed that the internal standards are found to be less than that of normal serum samples. Table-2 summarizes the internal standard calculations of the normal and different diseased samples.

Absorption spectroscopy is ideally suited for quantitative measurements as the absorbance of a solute depends linearly on its concentration. The spectral properties of a molecule depend on the molecular environment and the mobility of the chromophores. The UV-Vis spectrum of blood contains information on the absorption and scattering properties of particle suspensions. UV-Vis spectroscopy has been successfully employed in the characterization and conformation of proteins and nucleic acids by many workers. The UV-Vis spectroscopic study of blood in healthy and diseased subjects has been already reported^{5,6}. As blood undergoes physical and biochemical changes in many diseases, the pathophysiological changes are analyzed by the characteristic absorptions in the UV-Vis spectral region. The UV-Vis spectra have been recorded for the normal blood samples as well as the diseased samples of diabetes, cholesterol, urea and thyroid as has been done already in FTIR spectral measurements.

TABLE-2
INTERNAL STANDARDS AMONG SOME INFRARED ABSORPTION PEAKS FOR
NORMAL AND DISEASED SERUM SAMPLES

Category of the sample	$R_1 = \frac{I_{2869}}{I_{2955}}$	$R_2 = \frac{I_{1545}}{I_{1656}}$	$R_3 = \frac{I_{1315}}{I_{1403}}$	$R_4 = \frac{I_{1169}}{I_{1245}}$
Normal 1	0.9306	0.8057	0.9048	0.8496
Normal 2	0.9126	0.8161	0.9204	0.8696
Normal 3	0.9074	0.8361	0.9304	0.8887
Normal 4	0.9236	0.8316	0.9082	0.8869
Normal 5	0.9069	0.8258	0.9258	0.8514
Diabetes 1	0.9555	0.9018	0.9554	0.9327
Diabetes 2	0.9484	0.8919	0.9466	0.9528
Diabetes 3	0.9380	0.8769	0.9596	0.9667
Diabetes 4	0.9455	0.8875	0.9286	0.9458
Diabetes 5	0.9564	0.8545	0.9318	0.9616
Cholesterol 1	0.8968	0.8304	0.8945	0.9046
Cholesterol 2	0.8520	0.8574	0.8954	0.8755
Cholesterol 3	0.8944	0.8496	0.9076	0.9185
Cholesterol 4	0.8646	0.8368	0.9124	0.9012
Cholesterol 5	0.8542	0.8575	0.9038	0.8955
Urea 1	0.8274	0.8203	0.8768	0.8568
Urea 2	0.8520	0.8075	0.8855	0.8642
Urea 3	0.8962	0.8111	0.8730	0.8719
Urea 4	0.8141	0.8245	0.8943	0.8514
Urea 5	0.9012	0.8101	0.8915	0.8624
Thyroid 1	0.9645	0.9304	0.9666	0.9752
Thyroid 2	0.9727	0.9231	0.9644	0.9401
Thyroid 3	0.9342	0.9554	0.9888	0.9849
Thyroid 4	0.9606	0.9428	0.9596	0.9525
Thyroid 5	0.9458	0.9508	0.9634	0.9326

Amino acids are the basic building blocks of proteins and are classified into indispensable and dispensable. Tyrosine is a dispensable amino acid as it can be synthesized in the body. Tryptophan is an indispensable amino acid which has to be supplied in the diet. Proteins absorb strongly at about 280 nm due to the amino acids tyrosine and tryptophan. The amide backbone of the proteins present in the blood absorbs strongly approximately at 210 nm. The UV-Vis spectra of all the serum samples exhibit the presence of only two strong absorption peaks at 210

and 280 nm as the samples are analyzed in serum form. But there is a marked difference in the absorption levels of normal and diseased samples as there are two absorption peaks in the UV-Vis spectra; the Q-factor or the ratio of absorbance among the peaks is also calculated. The internal standard among the absorption peaks is found to be around 0.11 in the case of normal serum. But it is found to be different in different diseased samples. In diabetes the Q-value is around, 0.08, in cholesterol it is 0.10 whereas in thyroid it is around 0.088 and in urea it is as low as 0.053. Table-3 summarizes the internal standard representation among the absorption peaks for the normal and diseased serum samples.

TABLE-3
INTERNAL STANDARDS REPRESENTATION AMONG THE WAVELENGTH
MAXIMA FOR NORMAL AND DISEASED SERUM SAMPLES

Sample	Absorbance of the wavelength maxima at		$\frac{A_{280}}{A_{210}}$
	210 nm	280 nm	
Normal 1	2.305	0.236	0.1024
Normal 2	2.360	0.271	0.1148
Normal 3	2.317	0.248	0.1070
Normal 4	2.327	0.251	0.1079
Normal 5	2.206	0.225	0.1020
Diabetes 1	2.095	0.140	0.0668
Diabetes 2	1.991	0.151	0.0758
Diabetes 3	2.038	0.150	0.0736
Diabetes 4	2.056	0.146	0.0710
Diabetes 5	2.045	0.147	0.0719
Cholesterol 1	2.181	0.244	0.1119
Cholesterol 2	2.146	0.216	0.1006
Cholesterol 3	2.046	0.206	0.1007
Cholesterol 4	2.086	0.197	0.0944
Cholesterol 5	1.992	0.192	0.0964
Urea 1	1.802	0.095	0.0527
Urea 2	1.825	0.106	0.0581
Urea 3	1.827	0.112	0.0613
Urea 4	1.938	0.124	0.0640
Urea 5	1.875	0.125	0.0666
Thyroid 1	1.825	0.161	0.0882
Thyroid 2	1.816	0.158	0.0870
Thyroid 3	1.912	0.168	0.0879
Thyroid 4	1.852	0.156	0.0842
Thyroid 5	1.835	0.142	0.0774

Conclusion

Spectroscopy has been employed as a diagnostic tool in the study of blood. FTIR and UV-Vis spectroscopic methods have been employed to study the normal and diseased blood samples. The internal standards among the absorption peaks are calculated and it clearly indicates how a diseased blood sample is different from a normal one.

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