Plant Archives Vol. 17 No. 2, 2017 pp. 871-876 *ISSN 0972-5210*

SPECTROSCOPIC INVESTIGATION ON NORMAL AND DISEASED BLOOD

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Abstract

FTIR and UV- visible spectroscopic techniques have gained significance in the understanding of the nature of the disease. Blood is made of fluid matrix called plasma and several types of cells. In diseased conditions pathological changes takes place, which leads to changes in plasma and cellular constituents. During abnormal conditions the blood samples undergo major changes in chemical and biochemical properties. Detection and measurement of these changes is of importance in clinical diagnosis and follow-up treatment. The FTIR and UV-visible spectroscopic techniques can be used as a tool to differentiate normal blood sample from that of diseased. The FTIR spectra of serum samples from normal and a subject having high urea and creatinine were recorded in the region 400-4000 cm-1. The internal standards were calculated to characterize the diseased from that of the healthy subject. The spectral differences were quantified by introducing four intensity ratio parameters. These are found to decrease when compared to that of the normal subject. An attempt has also been made to study the sample obtained from a hypercholesterolemia case using uv-visible spectral studies. There is a marked difference the characteristic absorbance in the region 200-400 nm corresponding to the constituents of the diseased sample. The internal standard calculated among the peaks show that the change observed on comparison with that of a normal subject is attributed to the disease. The change seen in the absorbance due to tryptophan and tyrosine around 280 nm could be allotted to disruption in the metabolism of tryptophan leading to the production of nicotinic acid. Nicotinic acid is known for its lipid reducing properties. An increased intake of nicotinic acid suppresses the production of VLDL, is suggested through the analysis. Hence nicotinic acid and or statin group of drugs seems to be used to treat high cholesterol related conditions.

Key words : Spectroscopic Investigation, diseased blood, serum samples, high cholesterol.

Introduction

Spectroscopy is a powerful tool in the hands of a spectroscopist. When one considers a molecule, it is found that it is associated with different types of motion. The molecule as a whole rotate, the bonds undergo vibrations and even the electron move. Each of these kinds of motion is quantised *i.e.*, the molecule can exist only in distinct states which corresponds to discrete energy contents. Absorption occurs only when the radiation supplying exactly the right 'packet' of energy impinges the compounds under study (1-2). Thus spectral line result, when a molecule absorbs a quantum of radiation hv and goes into an excited state or when a molecule in an excited state emits energy hv and goes into a lower level.

An isolated molecule in space has various forms of energy of its different kinds of motion and intra molecular interaction. the total energy of a molecule can be expressed as the sum of the constituent energies *i.e.*,

$$E_{tot} = E_{trans} + E_{rot} + E_{vib} + E_{elec} + \ldots$$

In short, the absorbed quanta of energy bring about different kinds of excitation in a molecule, and each requires its own distinctive energy ΔE.The time required for an electronic transition is about 10^{-15} s and the energies involved are at least 10 to 50 times greater than that for a typical vibrational transition (3). The electronic transitions are about 100 times faster than the vibrational transitions. In the present investigation IR and UV- visible spectroscopic methods are used as analytical and diagnostic tools.

Experimental

Fundamentals of Molecular Spectroscopy by C.N. Banwell. In the present work, investigation on normal

and diseased blood samples of subjects of the age group 60-65 has been done, using UV and IR analysis. The spectra thus obtained, for hypercholesterolemia and high urea and creatinine cases are distinct, from one another. Each of these cases has been compared with the sample procured from a normal subject. The molecular vibration characteristic of each distinct molecular species produce an absorption pattern to the overall spectrum obtained and the spectra were recorded. The IR spectra for hyperlipidimic sample, and FTIR spectra for urea and creatinine sample were obtained in the absorbance mode and normalized, to compensate any imprecision in the preparation of the film, and were air dried before the spectrum was recorded (6).

The hypercholesterolemia sample was also analyzed using UV-VIS spectrophotometer, in the region 200-400nm, and the internal standards were calculated. The human blood has significant changes in the blood parameters when affected with a certain pathological condition. The different analysis can be studied using infrared spectroscopy and clinical diagnosis could be affected. The two conditions that are investigated in the present work are discussed in detail.

Result and Discussions

In this paper figure (1) is the FTIR spectrm of the normal sample (N), figure (2) is the the infrared spectrum of the subjects having urea and creatinine *i.e.*, Diseased sample(D) and figure (3) is the FTIR spectrum of hypercholesterolemia sample. The spectrums obtained were given a satisfactory vibrational assignment given in the table (1). The samples were also analyzed on a basis of quantization. This is done as each constituent provides a unique absorption pattern to the spectrum obtained, by molecular vibrations characteristics of distinct molecular species. The spectra of both the samples are distinct from each other in this sense which provides the selectivity in the IR analysis of serum analytes. The quantitative information is carried by the relative intensities of the various constituent spectra contributing to the unique absorption profile of each serum specimen. The appearance of the spectra is similar, but they are dominated by the absorption due to protein constituents. The assignment of the vibrational band has been allotted by analyzing the established spectra of blood. The table (1) presents the vibrational band assignment of human serum.

In the present investigation, the band obtained at 3434 cm^{-1} is assigned to N–H stretching of the secondary amides of protein. The C–H stretching vibrations of methylene and methyl groups in lipids dominate over the region 3200 – 2800 cm^{-1}. The asymmetric and symmetric stretching vibrations of the methyl group of lipids occur at 2960 cm^{-1} and 2880 cm^{-1} respectively. In the present study on the blood serum of the diseased, the asymmetric and symmetric stretching vibrations of CH_3 occur at 2952 cm^{-1} and 2892 cm^{-1} respectively (4). It is observed that a considerable variation is seen in the absorbance levels. The asymmetric and symmetric vibrations of CH_2 are observed at 2922 cm^{-1} and 2856 cm^{-1} respectively for that of the normal subject. In the case of the diseased serum sample the asymmetric and symmetric vibrations of CH_2 are observed at 2928 $cm^{-1}$and 2857 cm^{-1} respectively.

The region between 1500-1700 cm^{-1} is usually dominated by amide I and amide II bands of proteins. In the present case the absorptions at 1650 cm^{-1} and 1651 cm^{-1} is due to C=0 stretching weakly coupled with C–N stretching and N–H deformation due to amide I for the normal and diseased serum respectively. The absorptions at 1549 cm^{-1} and 1556 cm^{-1} are assigned to N–H bending vibrations strongly coupled with C–N stretching vibrations of protein amide groups for the normal and diseased serum respectively. The region between 1400–900 cm^{-1} is dominated by C–O stretching vibrations of proteins and carbohydrates. The band at 1154–1175 cm^{-1} is due

Table 1: Infrared Vibrational Frequency Assignment of Human Serum

Frequency (CM^{-1})	ASSIGNMENT
3434 (S)	N-H Stretching of Secondary amides of protein
2960 (MS)	Asymmetric Stretching Vibrations of CH_3 of lipids
2922 (MS)	CH_2/ CH asymmetric stretching
2880 (M)	Symmetric stretching vibrations of CH_3 of lipids
2856 (M)	CH_2/ CH symmetric stretching
1650 (VS)	Amid I band which arises from C=O stretching, weakly coupled with C-N stretching and N-H deformation
1549 (S)	Amid II band which arises from N-H deformation strongly coupled with C-N stretching
1448 (M)	CH_3 asymmetric deformation of Proteins
1399 (MS)	CH_3 asymmetric deformation / COO – stretch of ionized amino acid chains
1315 (W)	CH_3 symmetric deformation of proteins
1241 (W)	Asymmetric PO_2 stretching of lipid phosphates
1167 (M)	C – O stretching of C – O – H in tyrosine of proteins
1127 (W)	C – O stretching of C – O – H and C- O – C bond
1081 (W)	C - O stretching of C – O – C bond

Table 2 : Internal Standards among Some Infrared Absorption Peaks For Normal And Diseased Serum Samples.

Category	$R_1 = I_{2880} / I_{2960}$	$R_2 = I_{1549} / I_{1650}$	$R_3 = I_{1315} / I_{1399}$	$R_4 = I_{1167} / I_{1241}$
Normal Sample	0.979	0.964	0.983	0.981
High Urea and Creatinine Sample	0.404	0.414	0.589	0.309

Table 3: Internal Standards among Some Infrared Absorption Peaks For Normal And Diseased Serum Samples.

Category	$R_1 = I_{1549} / I_{1650}$	$R_2 = I_{1315} / I_{1399}$	$R_3 = I_{1167} / I_{1241}$
Normal Sample	0.964	0.983	0.981
Hypercholesterolemia sample	0.3048	0.5520	0.3172

Table 4 : Internal Standard among the Wavelength Maxima For Normal And Diseased Sample.

Category	Absorbance of the Wavelength maxima around		$Q = A_{280} / A_{210}$
Normal Sample	1.844	0.450	0.2440
Hypercholesterolemia	2.204	0.220	0.0998

to the C-O stretching of C–O–H group of Tyrosine of protein. Therefore in the present work the band at 1167 cm^{-1} and 1170 cm^{-1} obtained in the spectra of normal and diseased serum respectively allotted to C–O stretching of C–O– H in the tyrosine of protein. The peak at 1448 cm^{-1} and 1315 cm^{-1} is assigned to methyl deformation from proteins (9-10, 12). The band at 1399 cm^{-1} and 1401 cm^{-1} in the spectra of normal and diseased serum could be due to asymmetric methyl deformation. The peak around 1400 cm^{-1} may also be considered to be due to COO- stretch of ionized amino acid chains. The lipid phosphate bands due to asymmetric P–O stretching vibration and symmetric P–O stretching vibration are found to occur at 1241 cm^{-1} and 1081 cm^{-1} respectively (7). The C–O stretching vibrations of glucose occupy the region 1250–925 cm^{-1}. The absorption peak at 1127 cm^{-1}, 1167 cm^{-1} are assigned to C-O stretchings of C–O -H and C-O–C bonds in the normal serum. In the case of diseased serum, the bands are observed at 1124 and 1170 cm^{-1} respectively

I. Internal Standard Calculations

The IR spectrum analysis gives information about the functional groups, types of bonds and their interactions and coupling in biological specimens. The FTIR spectrum of both the normal and that of the diseased subjects are similar but quantitatively produce different absorption peaks. The absorbance is directly proportional to the quantity of the analyte present. The different serum samples are analyzed quantitatively by calculating the intensity ratio among the absorption peaks. In the present work an attempt is made to differentiate the normal and the diseased samples and the intensity ratio of absorbance peaks are calculated in the table (2).

To obtain the differences due to quantization, four intensity ratio parameters are introduced and studied. They are $R_1 = I_{2869} / I_{2955}$ due to asymmetric and symmetric stretching vibrations of the methyl group of lipids. $R_2 = I_{1545} / I_{1656}$ due to the ratio of intensities of Amide – II and Amid – I bands of the amino acids and therefore 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, $R_4 = I_{1169} / I_{1245}$ due to

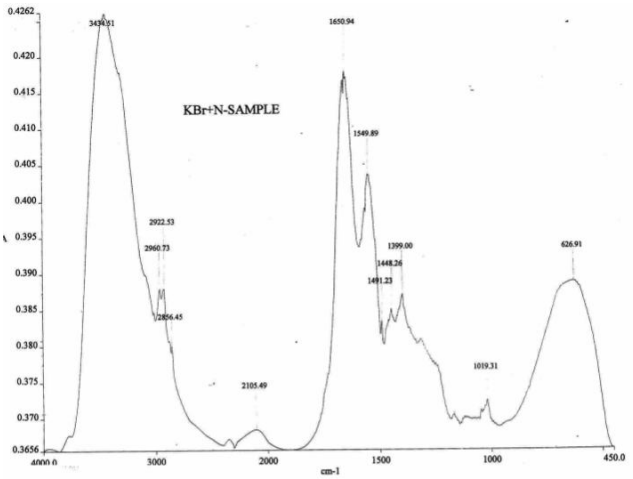

Fig. 1: FTIR Spectrum of Normal Sample (N)

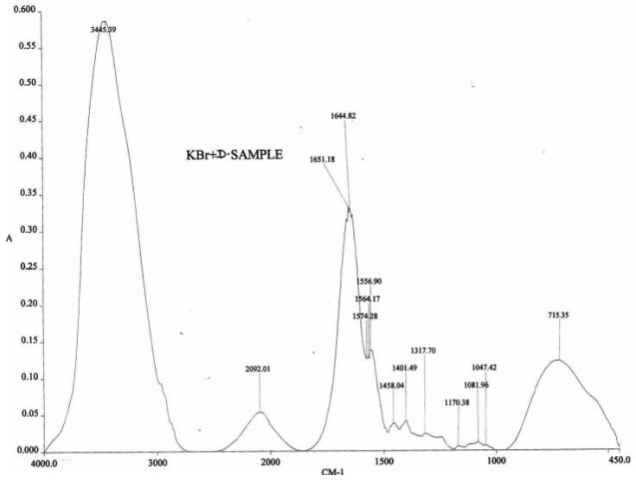

Fig. 2 : FTIR Spectrum of High Urea and Creatinine Sample (D)

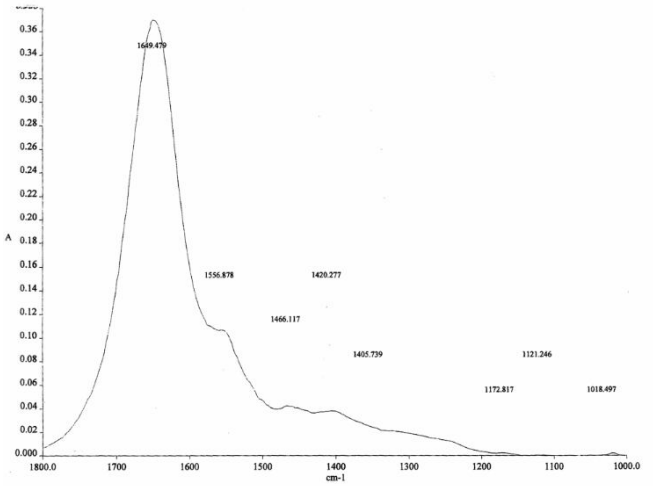

Fig. 3: FTIR Spectrum of Hypercholesterolemia Sample

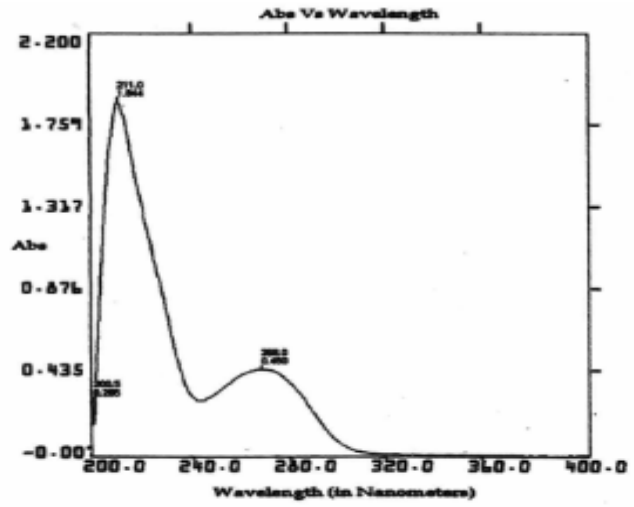

Fig. 4: UV-VIS Spectrum of Normal Sample

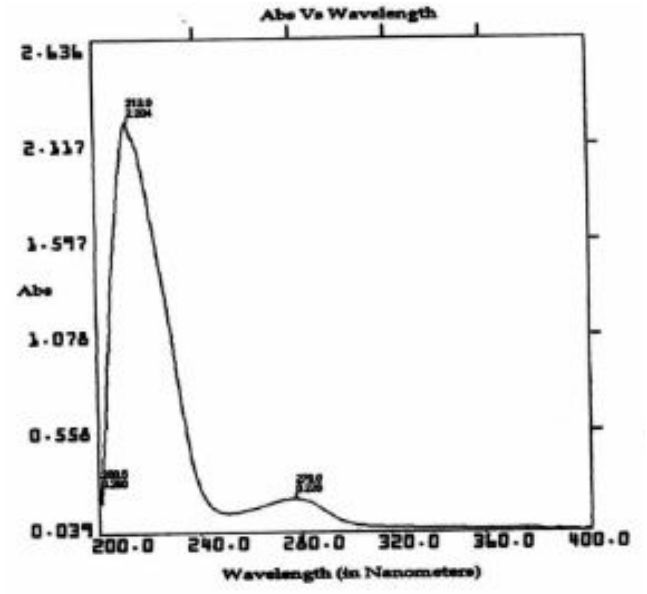

Fig. 5 : UV-VIS Spectrum of Diseased Sample

C–O stretching of glucose and P–O stretching of lipid phosphate. Significant differences are found to be present in the internal standards calculated among the absorption peaks for different diseased samples in the table.

The values of R_1, R_2, R_3 and R_4 of normal and diseased sample (high urea and creatinine) is tabulated (2). The values of that of the diseased subject are found to decrease as compare to that of the normal sample.In the present IR analysis of the hypercholestrimic sample, the initial standard values are found to decrease as compared to that of the normal sample and all the values are given in the table (3).

II. Ultraviolet – Visible Spectral Analysis

The UV–Visible spectrum of a specimen contains information on the absorption and scattering properties of the particle suspensions. The interpretation of the ectra can be done by using the information obtained in rms of chemical composition of the specimen, stribution of the particles etc. The spectral properties us observed depend on the molecular environment and so dependant on the mobility of the chromophores. UV–IS spectrometry suits well for quantitative measurements the absorbance of a solute depends linearly on its ncentration (5, 11). Blood undergoes biochemical anges in many diseases according to the different thological conditions. These changes are analyzed by e characteristic absorption in the UV–VIS spectral gion, obtained due to the different constituents present. the present investigation, UV–spectral analysis has en done on a hypercholesterolemia sample and then alyzed. Figure (4) and figure (5) are the UV-VIS spectral analysis of normal and diseased samples.

UV–VIS spectral measurements have been done on biological specimens and have been successfully used in the characterization and conformation of proteins and nucleic acids. The constituents of the blood give their characteristic absorbance peaks distributed throughout the spectrum obtained. Amino acids are classified into indispensible and dispensable. They are the building blocks of proteins. Tyrosine is a dispensable amino acid since it can be synthesized inside the body itself. Tryptophan comes under indispensible amino acids, which has to be supplemented through intake. It is an essential amino acid which has the metabolic distinction of producing a vitamin, nicotinic acid, as a product of its metabolism, since nicotinic acid is not however produced in sufficient amounts it has to be supplemented through diet, through adequate quantities of tryptophan can completely replace the vitamin. Proteins absorb strongly at about 280 nm due to the amino acids tyrosine and tryptophan. Tryptophan

undergoes catabolism and is synthesized into niacin (vitamin) and leads to the production of the coenzymes NAD^+ and $NADP^+$.

Tyrosine is used in the synthesis of melanin pigments. Thyroxin, an active hormone of the thyroid gland is synthesized from tyrosine of the protein thyroglobulin. The amide backbone of the proteins present in the blood absorbs strongly around 210 nm (8). NAD^+ (Nicotin Amide Adenine Dinucleotide) and $NADP^+$ (Nicotimide Adenine Dinucleotide Phosphate) are two important pyridine nucleotide coenzymes present in the blood. NAD^+ works in conjunction with the enzymes of the respiratory chain but NADP+transfers hydrogen in processes involving biosynthesis of lipids, steroid, hormones etc. The reduced forms of NAD+ and NADP+ are NADH and NADPH respectively. These coenzymes absorb at 349 nm. Hemoglobin is a blood pigment which is a combination of protein globin which have the prosthetic group. This hemoglobin combines with oxygen to form oxyhemoglobin. The amount of hemoglobin is directly proportional to the partial pressure of oxygen. Hemoglobin combines with oxygen in the lungs where the partial pressure of oxygen is more and then it dissociate into the tissues where the partial pressure of oxygen is less. The oxyhemoglobin exhibits three absorbance peaks at 417, 542, 577 nm. The serum samples analyzed exhibit two absorbance peaks at 210 nm and 280 nm.

Though the spectrums for the two samples are similar there are significant differences, due to biochemical changes that have occurred in the sample due to the pathological condition of the subject. The internal standards among the peaks are obtained and are compared between the normal and the diseased sample in the table (4). The ratio of absorbance among the peaks is found to around 0.244 in the case of the sample obtained from the normal subject. The value for the hypercholesterolemia case is found to be lower, placed at 0.099. The internal standard representation for that of the normal and diseased subject is presented in the table.

The changes seen in the absorbance around 280 nm due to the tryptophan and tyrosine peak could be allotted to disruption in the metabolism of tryptophan leading to the production of nicotinic acid. The decreased absorbance is due to the decreased production of nicotinic acid. Nicotinic acid is known for its lipid reducing properties. An increased intake of nicotinic acid suppress the production of VLDL, is suggested through the analysis. Hence nicotinic acid and or statin group of drugs may be used to treat high cholesterol related conditions.

Cholesterol is used for the biosynthesis of steroid hormones, vitamin D. Its recent notoriety is because of its involvement in the hardening of arteries. It is deposited in the walls of the arteries and destroys their normal elasticity. In the present investigation on the hypercholesterolemic sample using IR analysis, it is found that the absorbance ratio is found to change indicating the degenerating pathological condition of the subject. The synthesis of the enzyme HMG–CoA reductase in the liver is suppressed which leads to the inactivation of the existing enzyme molecules, sine the enzyme catalyses the formation of mevalonate which is the committed step in cholesterol synthesis, inactivation of this enzyme means suppression of cholesterol synthesis, inactivation of this enzyme means suppression of cholesterol biosynthesis. The same condition is also derived sing UV–VIS spectrometric studies done on the specimen, under study.

The ratio of the absorbances is found to decrease on comparison with the sample obtained from a normal subject. We know that the band at 280 nm is due to the absorption by tryptophan and tyrosine amino acids. The change of absorbance at the peak at 280 nm could be assigned due to the metabolism of tryptophan. Tryptophan in the presence of tryptophan pyrrolase produces formyl kynurenine finally to 3–hydroxyl anthranalic acid and then to nicotinic acid (13 -16). Dietary cholesterol and also cholesterol synthesized in the intestinal mucosa are esterified at the 3–hydroxyl group in the intestinal mucosa.

Esterified cholesterol and other lipids are incorporated into VLDL and chylomicrons. These are absorbed into the blood streams. Liver also synthesizes cholesterol and secretes it into the blood stream along with triglycerides as VLDL. Nicotinic acid reduces the flux of free fatty acids by inhibiting adipose tissue lipolysis, thereby inhibiting VLDL production of liver. Thereby a decreased presence of nicotinic acid helps VLDL production and this is seen by UV–VIS spectrometric analysis, in the present investigation. An increased intake of nicotinic acid suppresses the production of VLDL through this pathway, is suggested, through the analysis.

Conclusion

The analysis of biological fluids (blood samples) has a long tradition I providing information to suggest or corroborate diagnosis. But here complementary technique is tried by interpretation of IR spectra. The spectra are viewed as finger prints that correlate directly with the presence or absence of disease. Because the spectra are complex, pattern characteristic of specific disease are rarely discernible from visual examinations of the spectra. In the present study on a hypercholesterolemia case and a subject having high urea and creatinine the

corresponding IR spectra were collected and subjected to two interlinked procedures, *viz.* feature extraction and classification, and then analyzed. The changes seen in the pattern through the intensity parameters with reference to the control subject is attributed to the diseased condition of the subject.

The UV–Visible spectral studies was done on the hypercholesterolemia case and the decreased absorbance corresponding to the tryptophan peak, an amino acid, seen as related to the disruption in this metabolism, leading to a depressed production of nicotinic acid. An increased intake of nicotinic acid suppress the production of VLDL, is suggested through the analysis. Thus statin group of drugs and or nicotinic acid may be used to treat cholesterol related symptoms.

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