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FTIR AND UV-VISIBLE SPECTRAL STUDY ON NORMAL BLOOD SAMPLES

Kanagathara N[1], M Thirunavukkarasu[1], Esther Jeyanthi C[2], P.Shenbagarajan[2]*

[1] Department of Physics, Vel Tech Multi Tech Dr.Rangarajan Dr.Sakunthala Engg.College, Avadi, Chennai-62
[2] Department Of Physics, Vel Tech Dr.Rr & Dr.Sr Technical University, Avadi, Chennai-62
*Corresponding Author Email: kanagathaara@gmail.com

Research Article

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ABSTRACT

Spectroscopic techniques can be effectively employed as a diagnostic tool in clinical chemistry and it can be an alternate method in clinical analysis. 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, FTIR and UV-Vis spectroscopic technique is employed to study the spectral differences in the serum of normal blood samples.

KEYWORDS: Blood; Serum; FTIR spectrum; UV-visible spectrum

Introduction

In recent past, Mid Infrared, UV-Visible spectroscopic methods efficiently been used in the field of Medical science for qualitative analysis of biological samples such as blood plasma and sera or tissues. Implementation of these techniques reduces time, resources and cuts cost. Spectroscopy is emerging as a potential diagnostic tool in the medical and pharmacological fields to provide information about the different chemical and morphological structures of healthy and pathological tissues. Blood being the chief circulatory medium of our body, reflects the physiological and pathological changes that take place in the tissues, which leads to the changes in the various plasma and cellular constituents. Spectroscopy has received quite a lot of attention not only for understanding the biological nature of the disease, but also for the diagnosis of the disease in recent years. Almost in all diseases the blood undergoes major changes in chemical and biochemical properties. The application of spectroscopy for the study of biomedical compounds has increased tremendously in recent years since this gives the metabolic photography of the subject.

Materials and Methods

Instrumentation, Reagents &Chemicals:

Blood samples of healthy subjects were collected from healthy volunteers of different age groups and each blood sample was allowed to coagulate naturally without adding any anticoagulant agents for about half an hour. The serum was separated from every sample and centrifuged at a speed of 1200 rpm

in REMI electric centrifuge. And the serum was subjected to both FTIR and UV-Vis spectroscopic technique.

EXPERIMENTAL PROCEDURE:

FTIR METHOD:

FTIR spectra of sera samples were recorded in the frequency range 4000 – 450 cm-1 on a Perkin Elmer One Spectrometer at Indian Institute of Technology, Chennai equipped with an air cooled DTGS detector. IR transparent Thallium Bromide material without the serum was scanned as the background for each spectrum and 16 scans were co-added at a spectral resolution of 1 cm-1. FTIR spectra were obtained by spreading a small volume of serum on a Thallium Bromide plate (IR transparent material) and allowed to dry for few minutes to remove the water bands. To minimize problems from avoidable baseline shifts, the spectra were baseline corrected and normalized.

UV-VIS METHOD:

UV-Vis spectra were recorded in the region 350-200 nm using Elico SL-159 Double beam UV-Vis spectrophotometer. In this instrument, the UV light region is scanned normally over the range 400-200 nm and the visible portion is from 600-400 nm. Over a short period of time, the spectrometer automatically scans all the components of wavelengths.

FTIR INTREPRETATION:

The sample constituents namely lipid, protein and carbohydrates form the characteristic bands on the infrared spectrum in the frequency ranges 3100 – 2800 $cm^{-1,}$1800-1400 cm^{-1} and 1400-900 cm^{-1} respectively. Vibration band assignment has been carried out on the infrared spectrum of sera on comparing the position relative intensity and shape of the bands with the bands of related molecules. And considerable differences in infrared absorbance of these bio molecules have been observed in the present investigation of different normal sera. Fabian et al[15] have predicted the spectral region 3300 – 700cm^{-1} for serum in blood samples.

There are two very strong prominent amide absorptions one at 1652 cm^{-1} due to C=O symmetric stretching[13] and corresponds to Amide I band and another at 1542 cm^{-1} due to strong N-H in plane bending[13] and termed as an Amide II band. The strong characteristic band at 3295cm^{-1} [12] due to N-H symmetric stretching confirmed the existence of amino acid group. The medium band at 2873cm^{-1}due to C-H asymmetric and symmetric stretching of CH_3 group established the presence of lipids and the medium bands at 2854 cm^{-1} due to C-H symmetric stretching of CH_2 group established the presence of lipids / fatty acids[7]. The medium bands at 1400 cm^{-1} , 1456 cm^{-1} signified the presence of amino acid due to C=O symmetric stretching of COO^{--} and asymmetric C-H scissoring of $–CH_3$ group. The medium weak bands at 1313 cm^{-1}, 698 cm^{-1} represent the presence of amide III & amide IV due to C-H / N-H deformation[15] of C-H out plane bending. The characteristic medium weak bands at 1172 cm^{-1}, 1165 cm^{-1}, and weak bands at 1077 cm^{-1} gives rise to the existence of glucose due to C-O symmetric stretching, C-C symmetric stretching, C-O symmetric stretching respectively. The weak band at 1030 cm^{-1} due to C-N symmetric stretching represents aminoacids and methyne gives rises to weak band at 667 cm^{-1} due to NH_2 wagging corresponding to amino acid group.

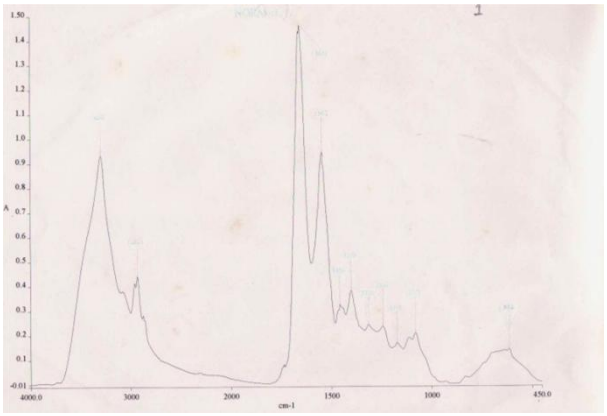
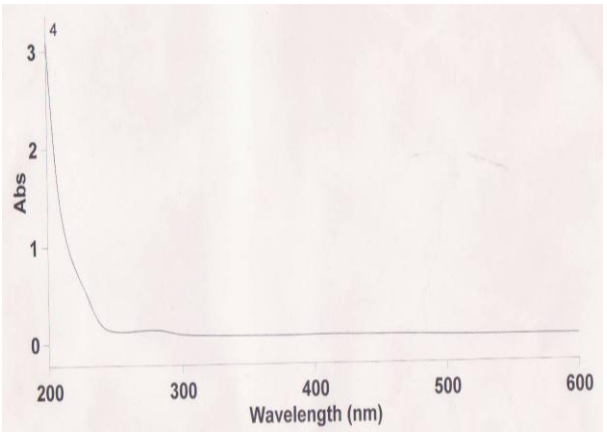
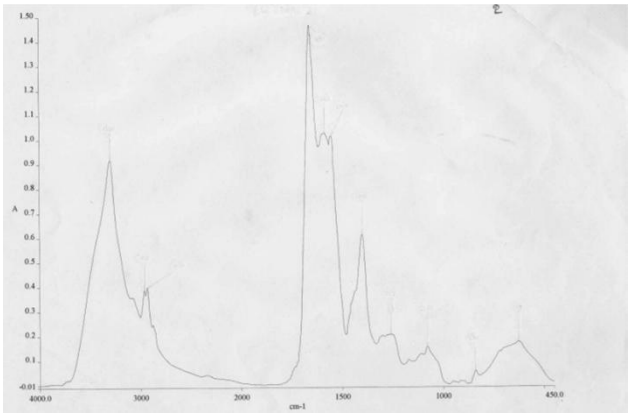
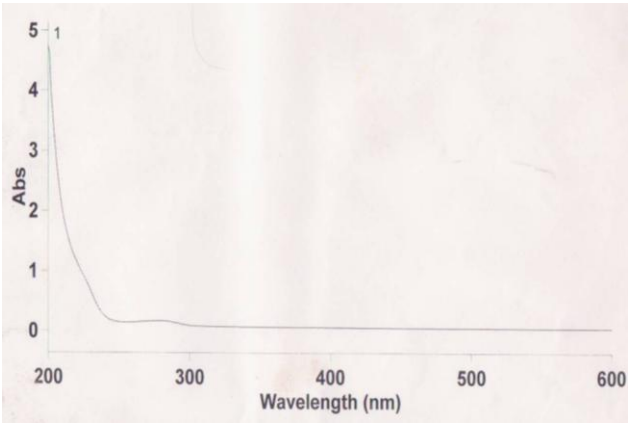
UV-VIS INTREPRETATION:

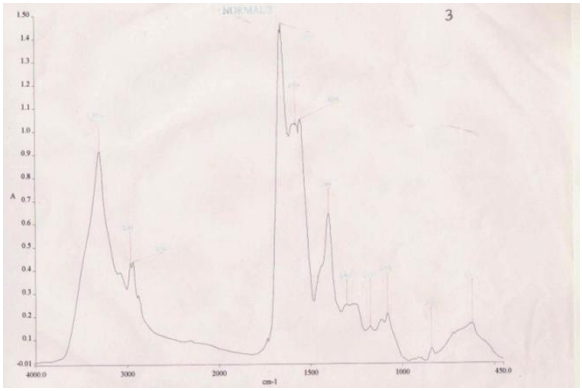
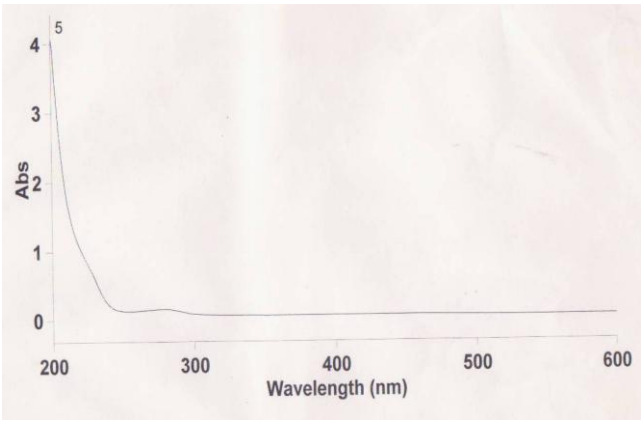
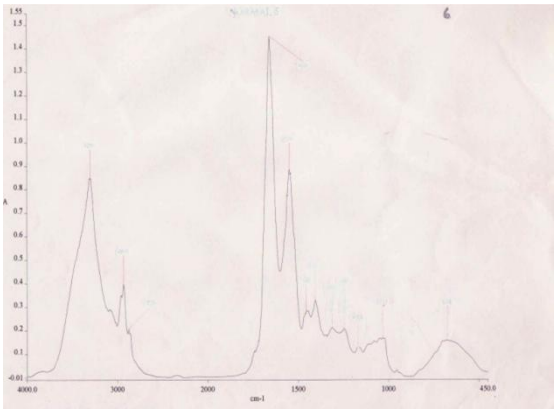
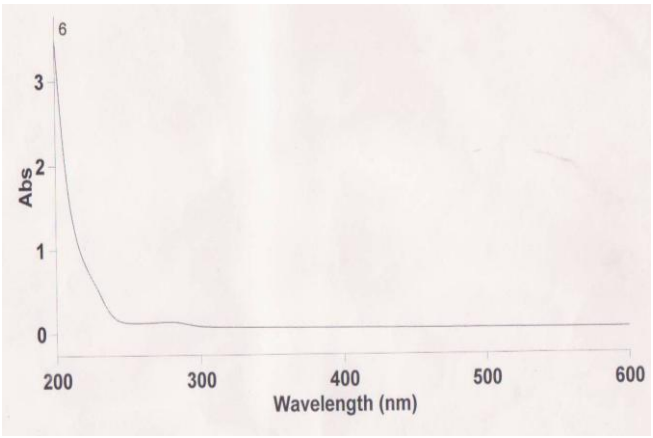
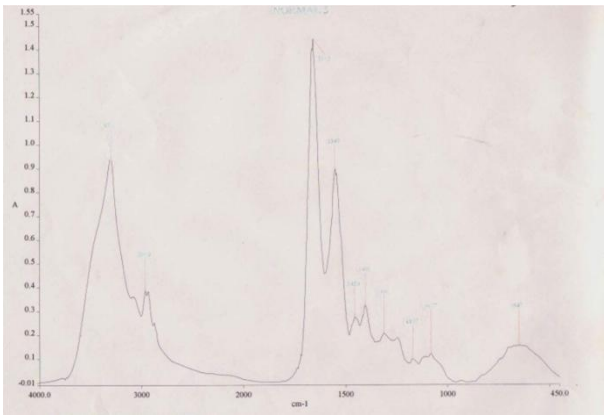
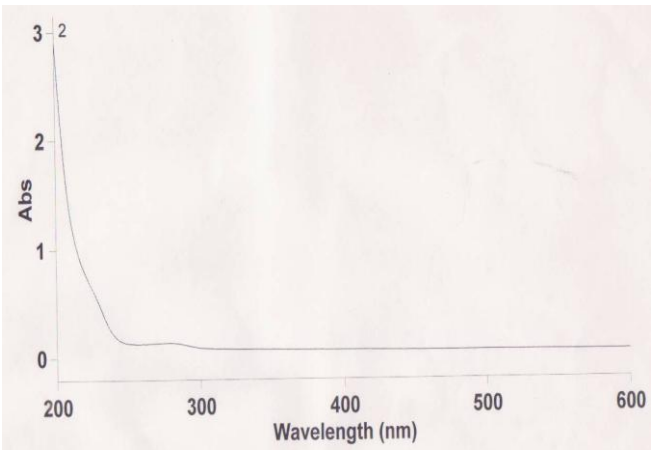
UV-Vis spectral analysis for characterization of blood sera and plasma of patients of various diseases have been carried out by many researchers recently[4-5, 7] UV-Vis absorbance's of sera samples were measured at

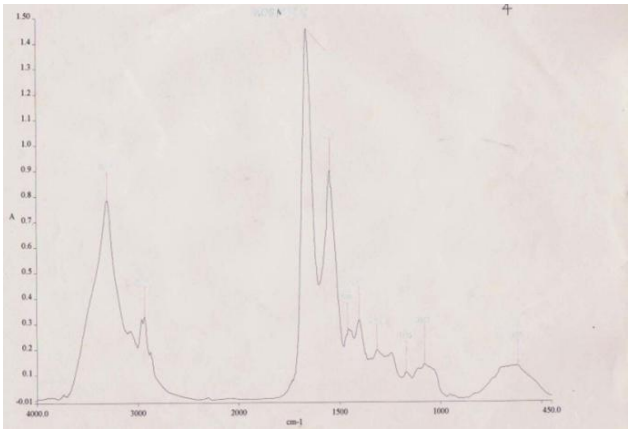
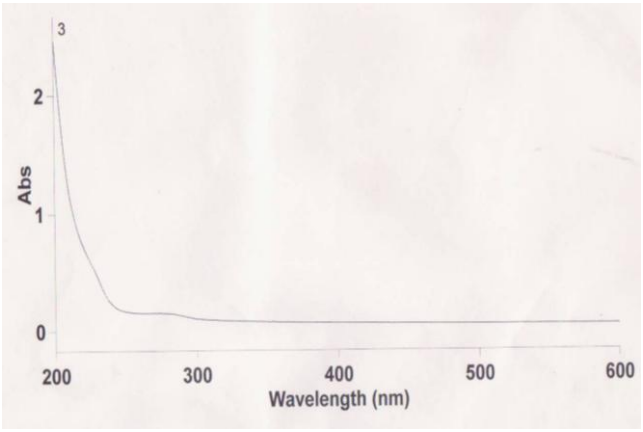
characteristic wavelength (λ_{max}) 210 and 279 nm corresponding to total protein and tryptophan respectively[5]. From this study, it has been found that the absorbance's of each sera are slightly different from that of other. It has been concluded that the decrease in the UV-Vis absorbance could be due to the structural changes of amino acids chain of protein molecule. Gunasekaran et al[1,5] using UV-Vis spectroscopic method have measured the optical density of blood at various stipulated periods of time and observed that optical density of blood constituents decreases with time except CO-hemoglobin which occurs at wavelength maximum 418 nm. The optical absorption is a classic method of studying a protein and it consists in measuring the absorption function of wavelength. The protein absorbance band in the UV domain (about 280 nm) determines the characteristic spectrum of the blood sera.

TABLE 1: VIBRATIONAL BAND ASSIGNMENT

Frequency cm^{-1}	Description	Component group
667	NH2 wagging	Amino acid
698	C-H out plane bending	Amide IV
1030	C-N symmetric stretching	Amino acid
1077	C-O symmetric stretching of glucose region	Cyclo propane
1172	C-O symmetric stretching	Carbonyl (carbohydrates)
1242	Asymmetric P=O stretching vibration of PO2	Lipid phosphates
1313	C-H / N-H deformation vibrational modes methyl groups	Amide III
1400	C=0 symmetric stretching of COO–	Amino acid
1456	Asymmetric C-H scissoring of –CH3	Amino acid
1542	N-H in plane bending vibration strongly coupled to C-N stretching vibration of protein	Amino acid (amide II)
1652	C=O symmetric stretching	Amide I
2854	C-H symmetric stretching of CH2 group	Lipids
2873	C-H symmetric stretching of CH3 group	Lipids
2960	C-H asymmetric stretching of CH3 group	Fatty acid/ lipids
3295	N-H symmetric stretching	Amino acid
3301	N-H asymmetric stretching	Amino acid (amide A)

FTIR spectrum :	UV-Vis Spectrum
Sample 1 	Sample 1 
Sample 2: 	Sample 2 

Sample 3 	Sample 3 
Sample 4 	Sample 4 
Sample 5 	Sample 5 

Sample 6	Sample 6
	

Results and Discussions

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. Gunasekaran et al. [4] have successfully employed FTIR and UV-Vis spectroscopic techniques to study the spectral differences between healthy serum and serum of diseases like diabetes, cholesterol, thyroid and urea and by internal standard calculation method they have differentiated healthy serum against disease serum. . It has been reported that the absorption spectra of the diseased blood show marked changes from that of normal blood which is the evidence for the manifestation of disease

Conclusion

Analysis of the blood sera spectrum for different samples under FTIR and UV-Vis spectroscopic technique showed that there are some differences between each and every spectrum. There will be a linear relationship between the protein content and the maximum absorption spectrum in the UV region. Due to the fact that the protein absorbance band at 280 nm determines the characteristic spectrum of the blood plasma and the absorption maximum strongly depends on the blood plasma protein concentration. The present study suggests that the spectrophotometric analysis of the blood Serum is a useful tool for determination of diseases in human body. Since FTIR spectrum gives the molecular finger print out of the sample, compare to the clinic test, it play a vital role in bio medical field for diagnostic purpose.

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*Address for the Correspondence:
Kanagathara Narayanan
Department of Physics
(Biological Sciences category)
Vel Tech Multi Tech Dr.Rangarajan
Dr.Sakunthala Engg.College,
Avadi, Chennai-62
E.mail: kanagathaara@gmail.com