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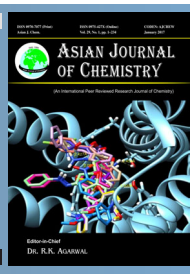

FTIR Spectral and Statistical Studies on Alcoholic and Non-Alcoholic Blood Samples

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This work focuses on Fourier transform infrared (FTIR) spectroscopic technique which is employed to study the spectral differences between the non-alcoholic and alcoholic blood samples. The studies conducted on the blood samples of alcoholic patients and the results were compared with the non-alcoholic patients using FTIR spectroscopic technique combined with statistical analysis. It is observed that the general shape of the spectra is the same but there is considerable change in the absorption of the characteristic peaks. As a measure to characterize the non-alcoholic and alcoholic blood, the intensity ratio calculation have been carried out among some of the specific absorption peaks for both non-alcoholic and alcoholic samples. It is found that the values are different in both the samples. Thus the role of FTIR spectroscopy in the clinical analysis of blood samples has been established both qualitatively and quantitatively. Statistical analysis is performed to find whether the absorption ratios differ in the healthy and diseased groups employing analysis of variance (ANOVA) and independent t-test.

Keywords: FTIR spectrum, Blood, ANOVA.

INTRODUCTION

The importance of Fourier transform infrared (FTIR) spectroscopy in the clinical analysis has increased tremendously in the recent past, due to the development of sophisticated instruments and efficient data evaluation software. FTIR spectroscopy is a well known diagnostic tool which is used for the analysis of biological fluids. It has many advantages over the regular clinical methods, as very small amounts of sample are required. It has high sensitivity, gives instantaneous, accurate and precise results and requires minimum manpower. FTIR spectroscopy has been used by scientists as a powerful tool to study inorganic and organic compounds [1]. It has been applied in biology for studying the structure and conformation of molecules like proteins, nucleic acids and lipids [2-4]. The mid infrared-IR region is useful in the identification of disease patterns of human sera [5], Components of serum, such as glucose, total protein, cholesterol, urea *etc*. [6]. The main aim of this study was to determine the spectral variation of the alcoholic and non-alcoholic blood samples. Characteristic absorption peaks in the FTIR spectrum of the blood sample is due to the specific functional groups which are present in the sample. The variations in the spectrum were analyzed quantitatively by the intensity ratio calculation. It was observed that the values were significantly different in the non-alcoholic and the alcoholic samples. The results are further validated with statistical analysis by applying the independent t-test, which indicated that the spectral variations were statistically significant. Thus, the role of FTIR spectroscopy in the clinical analysis of blood could be established.

EXPERIMENTAL

Blood samples (2 mL) from 25 patients addicted to alcohol as well as non-alcoholic subjects were collected. The blood samples are centrifuged and the serum is separated and is used for spectral recordings.

The FTIR spectrum of the blood samples were measured at Sophisticated Analytical Instrumentation Facility IIT, Chennai-600 036, India using Spectrum-One Perkin-Elmer FTIR spectrophotometer. The spectra are recorded in the mid infrared region of 4000-400 cm^{-1} in the absorption mode. The FTIR spectra are obtained by spreading a small volume of serum on a thallium bromide cell (IR transparent material) and the water content is removed by drying it for few minutes [7]. The dried serum forms a thin uniform film on the cell [8]. A complete vibrational band assignment of the absorption bands of the spectra was done with the idea of the group frequency of the various constituents of the blood samples [9].

RESULTS AND DISCUSSION

Many researchers have been working on the spectra of human blood and blood serum in order to characterize the blood and attempted to understand the pathophysiological conditions of the body. Based on these studies, various important groups of vibrations were identified in the FTIR spectrum of blood sera.

Table-1 presents the satisfactory vibrational band assignment of blood samples.

TABLE-1
INFRARED VIBRATIONAL BAND ASSIGNMENT OF BLOOD SAMPLE

Wavenumber (cm^{-1})	Vibrational band assignment
3295	N-H stretching of secondary amides of protein: amide A
2960	CH_3 asymmetric stretching of proteins and lipids
2934	CH_2/CH stretching
2874	CH_3 symmetric stretching of proteins and lipids
2851	CH_2/CH Stretching
1660	C=O stretching coupled with C-N stretching and NH deformation-amide I
1554	NH deformation strongly coupled with C-N stretching amide II
1457	CH_3 asymmetric deformation
1398	CH_3 asymmetric deformation COO^- stretching of amino acids
1315	CH_3 symmetric deformation
1240	PO_2 asymmetric stretching of lipid phosphates
1169	C-O stretching
1128	C-O stretching
1081	C-O stretching
955	PO_2 symmetric stretching of lipid phosphates
699	NH asymmetric deformation coupled with CH_2 rocking amide V
625	O=C-N deformation coupled with other ring deformation amide IV

The vibration band found at 3296 cm^{-1} is because of the N-H stretching vibration of the secondary amides of protein. The asymmetric and symmetric stretching vibrations of the methyl group of proteins and lipids are found at 2960 and 2874 cm^{-1} respectively. The C-H asymmetric and symmetric vibrations of the methylene group are present near 2934 and 2851 cm^{-1}. The strong absorption found at 1660 cm^{-1} is assigned to C=O stretching of amide-I of the proteins [10]. Similarly the presence of the band at 1554 cm^{-1} corresponds to the amide-II or NH bonding vibration that are strongly coupled to the CN stretching vibrations of the protein amide groups. The absorption peaks at 1457 and 1315 cm^{-1} are due to the asymmetric and symmetric deformations of the methyl group of proteins. The absorption peak at 1398 cm^{-1} may also be considered due to COO^- stretching of ionized amino acid chains, suggesting an increased contribution from carboxalate. The lipid phosphate band at 1240 cm^{-1} corresponds to the asymmetric PO_2 stretching vibration. The spectral region 1169-1081 cm^{-1} is occupied by the C-O stretching vibrations of glucose. The absorption peaks present at 1169, 1153, 1107, 1079 and 1035 cm^{-1} are due to the different C-O stretching vibrations. The absorption band observed at 955 cm^{-1} is due to PO_2 symmetric stretching of the phosphate bond of proteins is found to be weak. The strong vibration band present at 625 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. Fig. 1 represents the FTIR spectrum of a non-alcoholic blood sample.

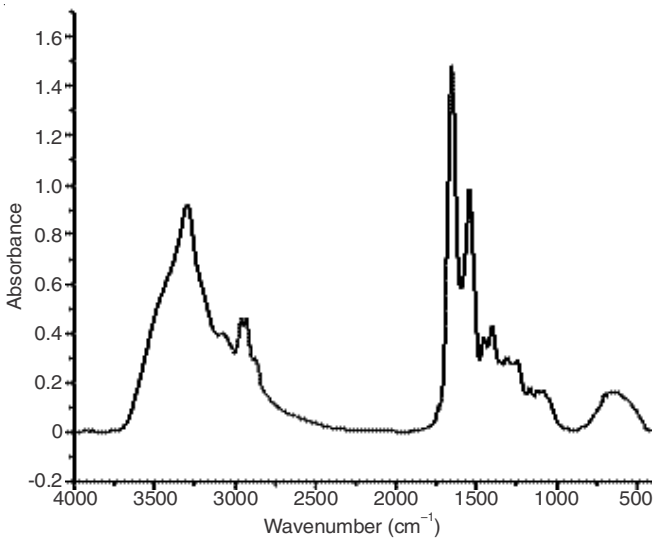

Fig. 1. FTIR spectrum of non-alcoholic blood sample

It is observed that the FTIR spectrum shows vibrational bands characteristics of the various group frequencies. Fig. 2 represents the FTIR spectrum of alcoholic and non-alcoholic blood samples superimposed on each other. The spectrum of alcoholic and non-alcoholic blood samples are found to be the same with respect to the positions of the peaks but are different in terms of the absorption levels of the peaks. The amount of absorption in the alcoholic blood samples is decreased than that of non-alcoholic ones. The difference in the absorption of the non-alcoholic and alcoholic samples characterize the samples qualitatively.

In order to quantify the results further, intensity ratio calculation among some of the specific absorption peaks of

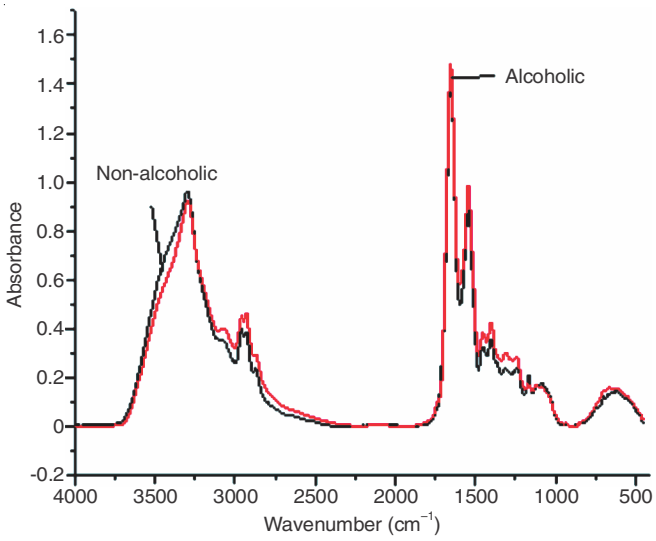

Fig. 2. FTIR spectra of alcoholic and non-alcoholic blood sample

the sample is employed [11]. Seven intensity ratio parameters *i.e.* $A_{3295}/_{2960}$, $A_{2960}/_{1655}$, $A_{1655}/_{1546}$, $A_{1546}/_{1452}$, $A_{1452}/_{1403}$, $A_{1317}/_{1081}$, $A_{1081}/_{700}$ are calculated, respectively among the prominent absorption peaks. It is observed that these values are decreased in alcoholic samples than that of the normal ones. Tables 2 and 3 show the intensity ratio calculations of alcoholic and non-alcoholic blood samples. Thus it is observed that both individual spectral absorption of the peaks and also intensity ratios are decreased in alcoholic samples compared to that of non-alcoholic ones.

TABLE-2
INTENSITY RATIO CALCULATIONS OF THE SPECIFIC MODES OF VIBRATION OF ALCOHOLIC BLOOD SAMPLE

Sample	$A_{3295}/_{2960}$	$A_{2960}/_{1655}$	$A_{1655}/_{1546}$	$A_{1546}/_{1452}$	$A_{1452}/_{1403}$	$A_{1317}/_{1081}$	$A_{1081}/_{700}$
1	2.2805	0.2721	1.5986	2.9207	0.8369	1.8096	0.9122
2	2.2332	0.2521	1.5036	2.8654	0.9021	1.8096	0.9436
3	2.2207	0.2666	1.5937	2.9163	0.9048	1.7641	0.9186
4	2.2966	0.2515	1.5802	2.6685	0.9104	1.8157	0.9258
5	2.2755	0.2283	1.5731	2.9222	0.9151	1.7432	0.9568
6	2.1913	0.2941	1.5828	3.1843	0.8904	1.7099	0.9138
7	2.2593	0.2384	1.5611	2.9811	0.9036	1.8554	0.9191
8	2.3192	0.2321	1.5651	2.6699	0.9113	1.8473	0.9987
9	2.3422	0.2269	1.5423	2.8434	0.9007	1.7557	0.9272
10	2.3394	0.2942	1.5572	2.4753	0.9291	1.7984	0.9618
11	2.2193	0.2943	1.5302	2.8014	0.8987	1.8582	0.9168
12	2.2979	0.2955	1.5671	2.9715	0.9257	1.8225	0.9287
13	2.2376	0.2955	1.5281	2.2378	0.9202	1.8363	0.8754
14	2.2581	0.2955	1.5671	2.9715	0.9246	1.8559	0.9569
15	2.3692	0.2261	1.5457	2.4687	0.9313	1.8348	0.9175
16	2.2816	0.3359	1.5635	2.5448	0.9053	1.8522	0.9634
17	2.2709	0.2927	1.5904	2.8051	0.9981	1.8505	0.9149
18	2.3692	0.3961	1.5571	2.5687	0.9313	1.8642	0.9256
19	2.3083	0.3011	1.5045	2.8746	0.8466	1.8463	0.8925
20	2.2873	0.2621	1.5663	2.6511	0.8881	1.8553	0.9756
21	2.2805	0.2721	1.5686	2.9207	0.8369	1.7965	0.9365
22	2.2087	0.2936	1.5598	2.8847	0.8338	1.8654	0.8453
23	2.3805	0.2857	1.5641	3.0727	0.8621	1.8025	0.9865
24	2.2581	0.2955	1.5671	2.9715	0.9246	1.8756	0.9222
25	2.3692	0.3961	1.5571	2.6687	0.9313	1.8765	0.9365
Average	2.2861	0.2837	1.5597	2.7944	0.9025	1.8240	0.9308

TABLE-3
INTENSITY RATIO CALCULATIONS OF THE SPECIFIC MODES OF VIBRATION OF NON-ALCOHOLIC BLOOD SAMPLE

Sample	$A_{3295}/_{2960}$	$A_{2960}/_{1655}$	$A_{1655}/_{1546}$	$A_{1546}/_{1452}$	$A_{1452}/_{1403}$	$A_{1317}/_{1081}$	$A_{1081}/_{700}$
1	2.4255	0.2545	1.5354	2.5674	0.9854	1.5675	1.268
2	2.4657	0.2345	1.5865	2.5765	0.9432	1.5682	1.257
3	2.4986	0.2786	1.5342	2.5634	0.9254	1.5345	1.246
4	2.4678	0.2674	1.5673	2.6435	0.9123	1.5734	1.277
5	2.4346	0.2578	1.5611	2.6645	0.9456	1.6723	1.235
6	2.4765	0.2576	1.5765	2.7546	0.9345	1.5724	1.277
7	2.4678	0.2745	1.5345	2.5435	0.9365	1.5945	1.3
8	2.4634	0.2634	1.5234	2.6345	0.9654	1.5345	1.268
9	2.4784	0.2756	1.5348	2.6545	0.9123	1.5786	1.253
10	2.4845	0.2354	1.5623	2.7567	0.9642	1.5782	1.255
11	2.4245	0.2535	1.5353	2.5674	0.9754	1.5865	1.278
12	2.4647	0.2346	1.5855	2.5765	0.9433	1.5668	1.247
13	2.4976	0.2788	1.5442	2.5634	0.9244	1.5355	1.248
14	2.4668	0.2679	1.5683	2.6435	0.9133	1.5744	1.276
15	2.4346	0.2598	1.5616	2.6645	0.9458	1.6783	1.235
16	2.4766	0.2546	1.5769	2.7546	0.9375	1.5754	1.277
17	2.4668	0.2748	1.5345	2.5435	0.9355	1.5945	1.299
18	2.4613	0.2634	1.5224	2.6345	0.9654	1.5385	1.268
19	2.4787	0.2766	1.5348	2.6545	0.9128	1.5756	1.254
20	2.4745	0.2544	1.5633	2.7567	0.9647	1.5752	1.256
21	2.4251	0.2588	1.5616	2.6645	0.9355	1.5774	1.277
22	2.4654	0.2579	1.5765	2.7546	0.9385	1.5955	1.235
23	2.4988	0.2598	1.5445	2.5435	0.9664	1.5365	1.277
24	2.4675	0.2646	1.5236	2.6345	0.9653	1.5756	1.3
25	2.4346	0.2348	1.5345	2.6545	0.9652	1.5752	1.268
Average	2.4640	0.2597	1.5514	2.6387	0.9445	1.5774	1.2652

Statistical analysis: Statistics is the study of the collection, analysis, interpretation, presentation of data. ANOVA and independent t-test are the two statistical methods employed in this study. In statistics, analysis of variance (ANOVA) is a collection of statistical models used in order to analyze the differences among group means and their associated procedures and the independent samples (or two-sample). t-test is used to compare the means of two independent samples [12]. The statistical method used for the validation of the FTIR spectroscopic analysis is the independent sample t-test by using the SPSS software package. It is an inferential statistical test that determines whether there is a statistically significant difference between the means in two unrelated variables. The independent sample t-test is first employed between the non-alcoholic and alcoholic samples. Table-4 describes the summary of the statistics (minimum, median, maximum, average, above average, below average) describing the amount of variation present in the sample.

In Table-4, from the above and below average category, it is observed that in ratios NONAL-6 and ALCO-R6 of the non-alcoholic and alcoholic samples, the observations are 56 % and 44 % respectively. The maximum of observations in the above average group is 68 % (17) in NON-ALR1 and is 64 % (16) in ALCO-R3 and ALCO-R4, similarly the maximum of observations in the below average group is 56 % (14) in ALCO-R1and NONAL-R5.The minimum values range from 0.2345 (NON-ALR2)-2.5435 (NON-ALR4) and 0.2261 (ALCO-R2)-2.2378 (ALCO-R4) in non-alcoholic and alcoholic groups. One the other end the maximum values range from 0.27889 (NONAL-R2)-2.7576 (NONAL-R4) and 0.3961 (ALCO-R2)-3.1843 (ALCO-R4) for non-alcoholic and alcoholic groups. Table-5 represents the independent t-test. It also represents whether significant difference exist between means of healthy and diseased groups observed at 7 different levels of ratios. It is concluded that between non-alcoholic and alcoholic samples, expect in R3, there exist a mean level difference in all other groups. Thus from the statistical results, it was quantitatively observed that the intensity of the absorbance which was obtained from the FTIR spectrum of the alcoholic and non-alcoholic blood samples are significantly different.

Conclusion

Fourier transform infrared (FTIR) spectroscopy was explored as a means to distinguish alcoholic blood samples from that of the non-alcoholic samples. Characteristic band alterations were identified in both the samples. Similarly absorbance ratios among specific bands were calculated. Some clear differences are observed in terms of spectral profiles, absorption bands, wave numbers and the intensity ratio parameters and satisfactory analysis has been made. The results show that FTIR can be used as a tool in the qualitative and quantitative investigation of biological fluids to distinguish sample sets from non-alcoholic and alcoholic groups. The internal standard method is used in characterizing the samples quantitatively. The spectral results were validated by applying statistical calculations, namely the independent t-test. The independent t-test is able to provide a better significant ratio level for the alcoholic and non-alcoholic samples for a given spectral peak in the entire frequency region of 4000-400 cm^{-1}. Thus, in the present work, it has been proved that the independent t-test can be very well combined with FTIR spectroscopy for the analysis of alcoholic and non-alcoholic blood samples. Although the results obtained in this study could be considered only as preliminary results, it forms a promising basis for a future study including a large number of samples. The advantage of this technique is only a small amount of serum is required and the results can be obtained in a very short time. As it is also cheaper when compared to clinical tests it is worthwhile to continue developing this technique as an efficient and reliable tool for the diagnosis and identification of alcoholic

TABLE-4
STATISTICS OF ALCOHOLIC AND NON-ALCOHOLIC SAMPLES

Parameters	Non-alcoholic R1	Alcoholic R1	Non-alcoholic R2	Alcoholic R2	Non-alcoholic R3	Alcoholic R3	Non-alcoholic R4
Minimum	2.4245	2.1913	0.2345	0.2261	1.5224	1.5036	2.5435
Median	2.4668	2.2805	0.2598	0.2927	1.5445	1.5641	2.6435
Maximum	2.4988	2.3805	0.2788	0.3961	1.5865	1.5986	2.7567
Average	2.464	2.2862	0.2597	0.2838	1.5513	1.5598	2.6388
Above Average	17	11	13	14	13	16	13
Below Average	8	14	12	11	12	9	12
Parameters	Alcoholic R4	Non-alcoholic R5	Alcoholic R5	Non-alcoholic R6	Alcoholic R6	Non-alcoholic R7	Alcoholic R7
Minimum	2.2378	0.9123	0.8338	1.5345	1.7099	1.2345	0.8453
Median	2.8654	0.9432	0.9053	1.5752	1.8363	1.2675	0.9258
Maximum	3.1843	0.9854	0.9981	1.6783	1.8765	1.2998	0.9987
Average	2.7944	0.9446	0.9025	1.5774	1.8241	1.265	0.9309
Above Average	16	11	15	14	14	14	10
Below Average	9	14	10	11	11	11	15

TABLE-5
INDEPENDENT t-TEST (p-value, N = 25)

	R1	R2	R3	R4	R5	R6	R7
Non-alcoholic	15.30	-2.60	-1.34	-3.38	4.92	-22.06	43.31
Alcoholic	0.00	0.01	0.19	0.00	0.00	0.00	0.00

and non-alcoholic samples. Thus, FTIR technique can be used as one of the methods for analyzing the alcoholic and non-alcoholic blood samples.

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