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FTIR Spectral Analysis of Plasma Homocysteine Levels Among Smokers

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The present work aims to report application of Fourier-transform infrared spectroscopy for the analysis of blood plasma of smoker's inorder to detect spectral parameters which might serve as biomarker for identifying and detecting homocysteine levels. The analysis led to the identification of specific modes of vibration pertaining to homocysteine in blood plasma. The absorbance values at these specific modes of vibration were significantly increased for those smokers having elevated homocysteine levels when compared to healthy non-smokers with optimal levels of homocysteine. The internal ratio parameter was calculated using the absorbance values of the wavelength corresponding to specific modes of vibration. It provided an excellent classification between non-smokers and smokers with elevated homocysteine levels which correlated completely with the clinical data. These parameters could be used as a basis for deriving a spectral method for determining and measuring plasma homocysteine spectroscopically.

Key Words: FTIR spectroscopy, Plasma, Homocysteine, Smokers.

INTRODUCTION

Homocysteine is a thiol-(sulfhydryl-) containing non essential amino acid which converts itself to several beneficial compounds required for energy including ATP, cysteine and S-adenosylmethionine (SAM-e). If homocysteine is not completely broken down it begins to cause oxidative damage to the walls of the arteries, oxidation of blood fats and abnormal blood clotting by making the platelets stick together[1]. Homocysteine also enhances the binding of lipoprotein-a to fibrinogen initiating a series of biochemical reactions eventually leading to blockages[2]. Blocking may be followed by heart attacks, strokes and other circulation calamities. The enzyme co-factors especially vitamin B_6, vitamin B_{12} and folic acid play a vital tool in the metabolism of homocysteine[3].

Smoking is associated with an increase in plasma homocysteine levels. Both are associated with an increase in the risk of cardio vascular diseases[4]. Smoking destroys the B complex vitamins that are the cofactors for homocysteine metabolism. Smokers generally have lower levels of folic acid and vitamin B_{12}[5]. Thus cigarette smoking which is one of the leading and avoidable cause of death and morbidity has been associated with increased homocysteine levels[6].

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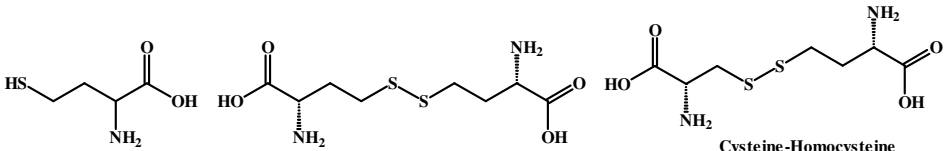
FTIR spectroscopy has been used by chemists as a powerful tool to characterize inorganic and organic compounds[7]. It has been applied in biology for studying the structure and conformation of molecules like proteins, nucleic acids and lipids[8-10]. The mid-IR region has been shown to be useful in the identification of disease patterns using the FTIR spectrum of human sera[11]. Precise quantification of serum components, such as glucose and total protein, cholesterol and urea has been achieved using mid IR spectroscopy[12,13].

Homocysteine is found in several forms after being released into plasma. More than 70 % is conjugated to proteins. About 5-15 % is present as homocystine. Another 5-15 % forms mixed disulphides with other lower molecular weight thiols. Only less than 1 % of homocysteine exist in its free reduced form[14,15]. The structure of homocysteine, homocystine, cysteine-homocysteine is shown in figures below.



Homocysteine Homocystine Cysteine-Homocysteine

Though different biomolecules in the blood plasma has been analyzed by many, not much work has been done in spectroscopically analyzing the plasma homocysteine. In present study, the FTIR spectra of plasma samples obtained from healthy non-smoking individuals and smokers who have elevated levels of plasma homocysteine have been examined.

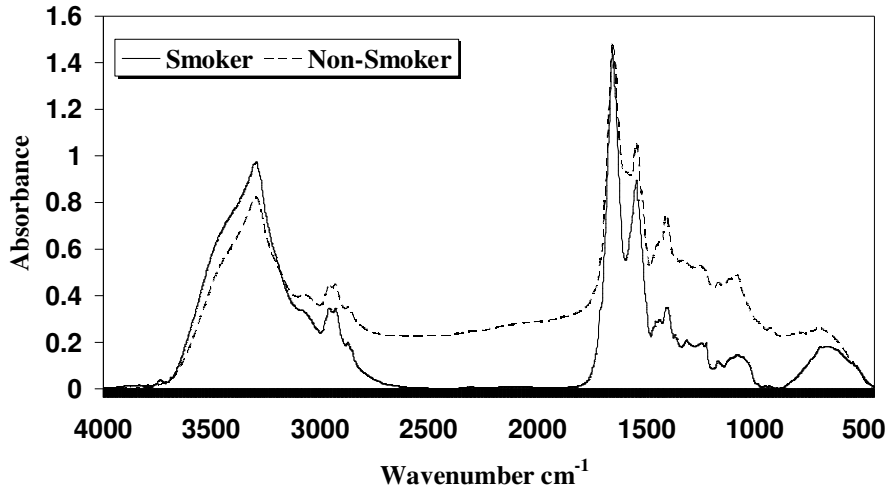
EXPERIMENTAL

Clinical analysis: Subjects included in the study were 10 young men between the age group of 20-30 years. They were medically healthy, but were smoking atleast 20-30 cigarettes a day. Also 10 non-smoking healthy individuals in the same age group were chosen. 2 mL of blood of each individual were collected in EDTA vacutainers. The blood was centrifuged immediately and the plasma was separated. It was subjected to a clinical test (Immunoassay-chemiluminescence) and the homocysteine levels were measured[16] clinically in the reference range of 10 to 12 μmol/L. Almost all the smokers had homocysteine levels greater than 12 μmol/L.

FT-IR spectra acquisition: The capillary blood samples (*ca.* 2mL) of the smokers and non-smokers were collected. The blood was immediately centrifuged for 3 min to separate plasma from erythrocytes. The samples were then stored at -20 ºC before analyses. After the samples returned to room temperature (about 15 min at 25-30 ºC) a volume of 1 mL of serum was diluted with an equal volume of 4 mg/L aqueous potassium thiocyanate solution. 20 μL of each diluted sample was spread evenly over the surface of a thallium chromide pellet. All the specimens were air dried for 0.5 h prior to measuring the spectra[17]. The strong absorption band of water in the mid IR-region poses hindrance and hence to eliminate this, the serum samples

were air dried. The dried serum forms a thin uniform film on the pellet[18]. Infrared transparent thallium chromide without the sample was scanned as background for each spectrum and 16 scans were co-added at a spectra resolution of ± 1 cm^{-1}.

The spectra were baseline corrected and they were normalized to acquire identical area under the curves. The spectra were recorded in the wave number range of 4000-400 cm^{-1}.



Overlaid spectra of smokers and non-smokers

RESULTS AND DISCUSSION

The wide multiple band between 3300 and 2300 cm^{-1} corresponds to the anti-symmetric and symmetric stretching frequencies of N-H[19]. An absorbance peak was noticed at 3295 cm^{-1} due to N-H stretching vibrations. The spectra were dominated by absorbance bands at 1542 and 1656 cm^{-1} *i.e.* the amino acid and amide I bands, respectively[20]. The peak at 1542 cm^{-1} was due the bending vibration of NH_2. The amide I band showing a peak at 1656 cm^{-1} was due to stretching vibrations of C=O. The absorbance at 2930 and 1456 cm^{-1} were due to the asymmetric bending and asymmetric stretching vibrations of the CH_2 molecule. The bands at 2996-2819 cm^{-1} were assigned to symmetric and asymmetric stretching vibrations of CH_2.

The absorbance peak at 1480-1360 cm^{-1} was attributed to stretching vibrations characteristic of amino acids (COO^-)[21]. The C-S vibrations resulted in a band at 710-570 cm^{-1} with a maximum absorption at 698 cm^{-1}. No significant peaks could be detected for the weak vibrations corresponding to the disulphides[21,22] (S-S) at 540-500 cm^{-1}. The absorption bands corresponding to the weak stretching vibrations of thiols (S-H) were also insignificant due to its dimeric nature[19].

Calculation of internal ratio parameter: Among the various mathematical methods applied for classification in biology and medicine internal standard para-

meter calculation is one of the simplest procedures. In present study this technique was used to classify the smokers with elevated homocysteine level from that of normal smokers with the help of the FTIR spectra of corresponding groups. The internal standards for the specific modes of vibration for the healthy samples and smokers with elevated homocysteine levels are given in Tables 1 and 2, respectively.

TABLE-1
INTERNAL RATIO PARAMETER OF THE SPECIFIC MODES OF VIBRATION OF PLASMA HOMOCYSTEINE AMONG SMOKERS

Samples	A_{3295}/A_{2930}	A_{2930}/A_{2848}	A_{1656}/A_{1542}	A_{1542}/A_{1456}	A_{1456}/A_{1402}
1	2.584715	1.924859	1.515385	2.917227	0.818325
2	2.798182	2.334146	1.610783	3.075534	0.827982
3	2.704053	2.225575	1.510242	2.490326	0.898843
4	2.317635	1.987837	1.551827	2.672954	0.886110
5	2.346627	1.945423	1.528144	2.684107	0.856380
6	2.356404	2.328875	1.562669	2.592363	0.879021
7	2.537290	1.998542	1.565656	3.026421	0.836416
8	2.176262	1.965560	1.435424	2.591851	0.874085
9	2.956644	2.681174	1.550807	2.815115	0.843785
10	2.530962	2.191533	1.611147	1.608436	0.966002

TABLE-2
INTERNAL RATIO PARAMETER OF THE SPECIFIC MODES OF VIBRATION OF PLASMA HOMOCYSTEINE AMONG NON-SMOKERS

Samples	A_{3295}/A_{2930}	A_{2930}/A_{2848}	A_{1656}/A_{1542}	A_{1542}/A_{1456}	A_{1456}/A_{1402}
1	1.823965	1.435782	1.402103	1.743024	0.809425
2	1.832395	1.410380	1.400159	1.722979	0.794742
3	1.897690	1.466973	1.416947	1.646457	0.804314
4	1.953679	1.495148	1.478510	1.812973	0.784920
5	1.855217	1.440649	1.454564	1.868884	0.806120
6	1.983106	1.450566	1.422938	2.136884	0.803340
7	1.937435	1.593123	1.518946	2.190086	0.743534
8	2.082022	1.445264	1.521421	2.153607	0.702598
9	1.421141	1.253339	1.458657	1.406286	0.886331
10	2.242788	1.434151	1.559232	2.089642	0.702286

Conclusion

In the present study we examined the potential of FTIR spectroscopy for identifying elevated levels of plasma homocysteine among smokers by comparing with healthy non smokers with optimal levels of plasma homocysteine. We were able to identify specific modes of vibrations pertaining to plasma homocysteine and distinguish between the healthy and smoking groups by calculating the internal standards for the specific modes of vibration. 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. Furthermore, for this technique only a small amount of plasma is required and the results can be obtained in 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 plasma homocysteine levels.

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