FOURIER TRANSFORM INFRA RED SPECTROSCOPIC STUDIES ON EPILEPSY, MIGRAINE AND PARALYSIS

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(Received: January 7, 2010 – Accepted in Revised Form: November 11, 2010)

Abstract In the present paper, we have studied immunglobulin (IgG) of epileptic children, migraineous and paralytic patients. We have compared our results with normal healthy controls. We found that the bands ranges from 1151.94 to 1168.28 cm^{-1} due to phospholipids (P-O-C) group appear in some of the migraineous and paralytic patients only. These bands are absent in normal and epileptic patients. The vibrational energy of these phospholipid bands is almost constant and is approximately equal to 13.87kJ $mole^{-1}$. The force constant is found in the range of 455.04 to 468.04 cm^{-1}. Amide A band is intact and found in all the normal and diseased samples. Hydrocarbon, carbide and peroxide were also present in all the diseased and healthy controls. The absence of few bands in these disorders is the distinct features of these samples. Amide IV band is also found in paraplegic patient at 767.76 and 771.97 cm^{-1}. The vibrational energy is found 9.18 kJ $mole^{-1}$ and 9.23 kJ $mole^{-1}$ and force constant is found 32.30 Nm^{-1} and 32.65 Nm^{-1}.

Keywords Phospolipid, ImmunoglobulinG, Vibrational Energy, Epilepsy, Migraine, Hemiplegia

چکیده در این مقاله ایمونوگلوبین کودکان مبتلا به مرض صرع، میگرنی و بیماران فلج اطفال مورد مطالعه قرار گرفت. این نتایج با نمونه های سالم و طبیعی مقایسه شدند. تنایج نشان داد که باندهای در محدوده ۱۱۵۱/۹٤ تا ۱۱٦۸/۲۸ (بر سانتیمتر) که مربوط به گروه فسفولیپید ها هستند در بعضی از میگرنی ها و بیماران فلج اطفال مشاهده شد. این باند ها در نمونه های نرمال و بیماران مبتلا به صرع وجود ندارند. انرژی ارتعاشی این باندهای فسفولیپید تقریبا ثابت بوده و در حدود ۸۷/ ۱۳ کیلوژول بر مول است. ثابت نیرو در محدوده ٤۵۵/۰٤ تا ٤٦۸/۰٤ (بر سانتیمتر) است. باند آمید A صدمه نیده بوده و در همه نمونه های طبیعی و بیمار مشاهده گردید. همچنین هیدروکربن، کربید و پروکسید در همه نمونه های سالم و بیمار وجود داشتند. عدم حضور تعدادی از باندها در این نمونه های معلول مشخصه بارز این نمونه هاست. باند آمید IV همچنین در بیماران مبتلا به صرع در طول موج ۷٦۷/۷٦ و ۷۷۱/۹۷ (بر سانتیمتر) وجود دارد. انرژی ارتعاشی ۹/۱۸ و ۹/۲۳ کیلوژول بر مول بوده و ثابت نیرو ۳۲/۳۰ و ۳۲/٦۵ می باشد.

1. INTRODUCTION

Some diseases generate specific changes in the metabolic pattern of blood or other body fluids. These changes may produce characteristic spectroscopic markers which can be used for identification and classification in seconds to minutes.

The ability to diagnose the early onset of disease, quickly, non-invasively and unequivocally has several benefits. Some of the clinical findings currently in use are not reliable. There are so many diseases covered under metabolic disturbances. It is very important to measure metabolism directly.

Vibrational spectroscopic techniques including Fourier Transform Infrared (FTIR) spectroscopy is potential tool for non-invasive optical tissue diagnosis and protein conformations. The applications of spectroscopic techniques in biological studies have increased a great deal in recent years.

Wide field of medical and biological studies has been covered by spectroscopic techniques in the past few years. Studies related to spectroscopic techniques, both the reliable experimental procedure and characterization of spectral peak positions and their assignment along with accurate peak detection and definition are of great importance. It appears that there is a remarkable parity in their spectral interpretation of comparable ones in their collected spectra of different types of the human body disorders blood samples.

We have used this sophisticated technique in the present study for understanding the changes occurring at the molecule of immunoglobulinG [IgG]. IgG is a protein and the literature regarding the study of conformational analysis of protein structure is plenty. The significance of the present work is to provide a brief data on studying the pathological changes occurring in the IgG samples of epilepsy, paraplegia and migraine.

Our main motive of the present research work is to use FTIR spectroscopy in understanding the structure of IgG in complex body disorders.

1.1. Basic Theory of FTIR Spectroscopy

Chemical bonds absorb internal energy at specific frequencies (or wavelengths). The basic structure of compounds can be determined by the spectral locations determined by their infrared absorptions.

It is not possible to excite the vibrational levels of the molecule by the visible light, even though it has high energy. We can however excite these levels in the infrared region (4000 to 400 cm^{-1}). The atoms remain in unison against attractive and repulsive forces existing in a molecule with the help of different types of bonds at same distances. One needs the energy to affect or break bonds in stretching or for altering the angle between them. Interaction with electromagnetic radiations leads to transitions from lower energy state to higher energy state.

The major components of energy of a molecule are:

1. The vibration of the constituents
2. The rotations of the molecules
3. The motion of the electrons in the molecule

Energy transition must satisfy the Bohr condition

$$F = -K(r - r_{eq.}) = -Kx \quad \text{(Hook's law)} \qquad (1)$$

where K stands for the force component and r for the distance between the nuclei.

The energy of the vibration is:

$$E = \frac{1}{2}kx^2 \qquad (2)$$

The energy absorbed in transitions from state E_1 to state E_2 is:

$$\Delta = E_2 - E_1 = h\nu \qquad (3)$$

If the system behaves like a harmonic oscillatory of mass μ_R, its frequency in Hertz will be:

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu_R}}$$

Or

$$\frac{\nu}{c} = \bar{\upsilon} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu_R}} \qquad (4)$$

$\mu_R = \dfrac{M_1 M_2}{M_1 + M_2}$ is reduced mass.

$$\Rightarrow \frac{1}{\mu_n} = \frac{1}{m_1} + \frac{1}{m_2} + ... + \frac{1}{m_n} = \frac{1}{\Sigma m_n}$$

The vibrational energy is given by:

$$E_v = \left(\upsilon + \frac{1}{2}\right)h\upsilon = \left(\upsilon + \frac{1}{2}\right)\frac{hc}{\lambda} \text{ Joule} \qquad (5)$$

ν is the vibrational quantum number. The lowest energy will be at $\nu = 0$. The constraints of selection rules allow only the heteronuclear diatomic molecules to give vibrational spectra.

The force constant [1] is given by:

$$k = 4\pi^2 c^2 \mu_R \nu^2 \qquad (6)$$

By measuring at a specific frequency over time, changes in the character or quantity of a particular bond can be measured. The functional groups within the sample will absorb the infrared radiation and vibrate in one of a number of ways, either stretching, bending, deformation or combination vibrations [2,3].

The intensities of the bands in the spectrum are proportional to the concentration of their respective functional groups

Lambert-Beer's law shows

$$I = I_0\, e^{\varepsilon bc}$$

$$\frac{I}{I_0} = A = \varepsilon bc$$

where, I_0 is the incident radiation, I is the transmitted radiation, A is the absorption of the band, b is the+ path length, ε is the molar proportionality constant called molar absorptivity. It is the characteristic of each functional group, and c is the concentration of the functional group.

1.2. Existing Work in the Support of Fourier Transform Infrared Spectroscopy Mishra and kumar [1] have studied nociceptive networks affilictes with bacterial, mycobacterial and neurological disorders using this powerful tool to study the alterations in hemoproteins and immuoglobulinG and found that the structure of immunoglobulinG was completely disturbed in the diseases which they have taken as samples for the study.

Some of the researchers [4,5] have reported in the literature carbohydrates, amino acids, fatty acids, lipids, proteins and polysaccharides can be analyzed simultaneously with the help of FTIR spectroscopy.

FTIR can be used to optically probe the molecular changes associated with diseased samples [6-8].

The studies including cervix [9-17], lungs [18-20], breast [21-25], skin [26-30], gastro-intestinal tissue [31-34], brain [35-37], oral tissue [38], lymphoid tissue [39], lymphocytes (childhood leukemia) [40], non Hodgkin's lymphoma [41], prostate [42,43], colon [44-47], fibroblasts [48], bacteria [49,50], tumor cells [51], DNA [52], anti-cancer drug [53], tissue processing [54], cancer detection [55], tissue preservation [56], cytotoxicity and heating [57], plant tissue [58], gall stones [59], glucose measurement [60] and bones [61] have been carried out earlier using FTIR.

FTIR spectra of synovial fluids could be used as a diagnostic aid for arthiritic disorders [62-64]. Hyalurenic acid can be measured by the method of spectroscopy. This acid leaks from the joint's synovial fluid in osteoarthritis [62]. Diem et al. [65] have reported that significant number of studies have been carried out on tissues, cell and biofluids in an emergent area of research termed as infrared pathology. Goodacre et al. [66] have referred the use of this sophisticated technique as metabolic finger printing.

Petibois et al. [67-72] have used this technique and studied the metabolic profiling of athletes. They have used serum, blood and plasma for FTIR spectroscopy. The toxicity of drug can be measured with the help of FTIR.

Narasimhan et al. [73] have studied the diagnosis of renal stones with underlying metabolic abnormalities using FTIR spectroscopy in children. They found that this tool of spectroscopy can give a clue to the nature of stones.

Le et al. [74] have studied FTIR technique for the diagnosis of gastric inflammation and malignancy in endoscopic biopsies based on FTIR. Melmiciuc et al. [75] have studied FTIR for the analysis of vegetable tanned ancient leather. They found that the IR spectra of leather extract content in addition to a series of bands which are common for those found for the oak extract.

Hameed et al. [76] have studied FTIR in the determination of antioxidant efficacy in sunflower oil and reported that atmospheric oxygen can react spontaneously with lipids and other organic compounds causing structural degradation, which is ultimately responsible for the loss of quality in several chemical or natural products with industrial importance. This phenomenon could be retarded by the addition of synthetic or natural antioxidants. Bruchard et al. [77] have studied formation of insulin amyloid fibrils followed by FTIR simultaneously with CD and electron microscopy. They observed changes in the shape and frequency of the amide I band as a function of time. The amide I band was very sharp and located at 1,651 cm^{-1}. The shape and position of the band are consistent with the presence of largely helical or disordered structure studied by Krimm and Bandekar [78], Arrondo et al. [79], Vecchio et al. [80]. FTIR results were in good agreement and indicative of the partial unfolding of the protein. They have also suggested that the β-structure in the insulin fibrils could be predominantly parallel rather than antiparallel on the molecular level. FTIR findings show that insulin prior to heat treatment has substantially native like α-helical characteristics.

Movasoghi et al. [81] have reviewed the literature and supplied the relevant information

regarding the peak position intensities and frequencies of the bands and provided a data base, which is useful in the field of research and industries globally.

Jackson and Mantsch [82] have studied the use and misuse of FTIR spectroscopy in the determination of protein structure. This technique can be used as a tool for the structural characterization of proteins.

Rumana et al. [83] have studied FTIR spectroscopy to distinguish wood of trees from different growth habitats for wood certification.

Kong and Yu [84] have studied the structure of proteins using FTIR and established a correlation between infrared spectra and secondary structure of proteins.

Neurological disturbances such as epilepsy, migraine and paraplegia are some of the highly challenging problems of science. The present work is a humble attempt to explore this problem with the help of FTIR spectroscopy.

We aim to investigate the nature, strength and situation of chemical bonds in the IgG molecule in these disorders. We are interested in comprehending hitherto non-understood changes, which are responsible for deteriorating the situation of epileptic, paralytic and migraineous disorders. For this purpose, we have used FTIR spectroscopy, which has been a powerful tool for studying the side chain conformations. FTIR has a strong potential for study of the hydrogen bonds of proteins and polypeptides.

Till now, most of the information concerning the conformation of immunoglobulin has come from X-ray, circular dichroism (CD) and infrared spectroscopy. It has been shown by several workers that the immunoglobulin contains β-structure and irregular conformation, but does not have α-helical conformation. Abaturov et al. [85] have reported the observed frequencies of the four amide bands of IgG, its peptide chains and proteolytic fragments. The purpose of this paper is to show that FTIR spectroscopy can be used to understand and characterize the conformational changes occurring at the molecular and electronic level.

2. MATERIALS AND METHODS

Blood samples of the epileptic children, migraineous and paralytic patients along with normal healthy controls were collected from the Department of Neurology, Safdarjang Hospital, New Delhi–110 016 after the approval of ethical committee of the hospital.

A 10 ml of freshly drawn blood was collected in siliconised screw capped test tubes. Separation of IgG was done by protein A Sepharose method.

Samples were prepared for infrared spectroscopic measurements by taking about 1 mg of the human IgG which was prepared in the Biotechnology laboratory of our college and grinded with 100-150 mg of KBr, finally dried to remove moisture and pressed at elevated temperature under high pressure into a small disc. A clear pellet was obtained. The infrared spectra of prepared samples were recorded in the range from 400 to 4000 cm^{-1} with single beam Fourier transform infrared spectrophotometer, Perkin Elemer Model-1710 at Deptt. of Chemistry , University of Roorkee Uttrakhand,India. This instrument has the following units and features. IR source had temperature stabilized ceramic source operating at 1400K. The abscissa accuracy and ordinate precision are 0.01 cm^{-1} and 0.1% T, respectively. This instrument has a resolution of 1 to 64 cm^{-1} [1cm^{-1} with a memory option fitted]. The ambient temperature and relative humidity are 15 to 35 ^{o}C and75% max, respectively. There are three units which are given here:

(a) Centre processing unit
(b) Cathode ray tube
(c) Fast recovery deuterated triglyceride sulphate detector

3. RESULTS AND DISCUSSION

Results obtained from FTIR studies of normal and pathological samples are summarized in Table 1. Investigations demarcate specific regions for a particular type of disorder. The vibrational energy and force constants of the exhibited bonds along with the probable assignments are also given.

TABLE 1. Comparative chart of FTIR spectra of IgG in normal and pathological samples; E - Epilepsy, M – Migraine, P – Paralysis

S. No.	Wave number (n) (cm^{-1})/ $\bar{u}$	Type of sample	Vibrational Energy (E) (kJ $mole^{-1}$)	Force Constant (K) (Nm^{-1})	Group/Probable assignment
1	3458.82	N	41.37	655.55	N-H Amide B.
2	3472.23	N	41.53	660.64	- do-
3	3440.64	N	41.15	648.67	- do-
4	3450.00	E	41.26	652.22	- do-
5	3443.02	E	41.18	649.56	- do-
6	3457.30	E	41.35	654.96	- do-
7	3480.74	E	40.67	633.41	- do-
8	3461.11	E	41.39	656.41	- do-
9	3450.65	E	41.27	652.46	- do-
10	3417.49	E	40.87	639.98	- do-
11	3429.46	E	41.02	644.47	- do-
12	3390.93	E	40.55	630.07	- do-
13	3448.13	E	41.24	651.49	- do-
14	3380.04	E	40.42	626.02	- do-
15	3444.05	E	41.19	649.95	- do-
16	3455.72	E	41.37	654.36	- do-
17	3435.88	M	41.09	646.88	- do-
18	3417.73	M	40.88	640.07	- do-
19	3374.80	M	40.36	624.09	- do-
20	3415.63	M	40.85	639.28	- do-
21	3444.05	M	41.19	649.96	- do-
22	3435.88	M	41.09	646.88	- do-
23	3440.36	M	41.15	648.57	- do-
24	3439.96	M	41.14	648.42	- do-
25	3415.45	M	40.85	639.21	- do-
26	3454.13	P	41.31	653.39	- do-
27	3335.04	P	39.89	609.83	- do-
28	3423.50	P	40.94	642.23	- do-
29	3456.06	P	41.33	654.88	- do-
30	3460.56	P	41.39	656.21	- do-
31	3419.44	P	40.90	640.70	- do-
32	3430.08	P	41.02	644.70	- do-
33	3441.35	P	41.16	648.94	- do-
34	3448.15	P	41.24	651.51	- do-
35	3417.78	P	40.88	640.09	- do-
36	3440.14	P	41.14	648.49	- do-
37	3431.04	P	41.03	645.06	- do-
38	3452.74	P	41.29	653.25	- do-
39	3444.05	P	41.19	649.96	- do-
40	2806.67	N	33.57	427.01	C-H, Hydrocarbon
41	2810.76	N	33.62	428.25	- do-
42	2814.84	N	33.66	429.49	- do-
43	2815.89	P	33.68	429.82	- do-
44	2716.78	P	32.49	400.09	- do-
45	2815.84	P	33.68	429.80	- do-
46	2729.17	P	32.64	403.75	- do-
47	2810.76	P	33.62	428.25	- do-

S. No.	Wave number (n) (cm^{-1})/ ū	Type of sample	Vibrational Energy (E) (kJ $mole^{-1}$)	Force Constant (K) (Nm^{-1})	Group/Probable assignment
48	2815.77	P	33.68	429.78	- do-
49	2729.04	P	32.60	403.71	- do-
50	2913.05	P	34.86	459.90	- do-
51	1650.40	N	19.74	149.25	N-H Amide I & Amide II
52	1632.95	N	19.53	146.11	- do-
53	1585.03	N	18.95	137.66	- do-
54	1632.57	N	19.52	146.04	- do-
55	1588.10	N	18.99	138.20	- do-
56	1654.49	N	19.78	149.99	- do-
57	1631.64	N	19.51	145.87	- do-
58	1589.27	N	19.00	138.40	- do-
59	1649.11	E	19.72	149.02	- do-
60	1649.46	E	19.72	149.02	- do-
61	1645.89	E	19.68	148.43	- do-
62	1650.45	E	19.74	149.26	- do-
63	1650.51	E	19.74	149.27	- do-
64	1649.85	E	19.73	149.15	- do-
65	1651.99	E	19.75	149.54	- do-
66	1650.46	E	19.74	149.26	- do-
67	1650.06	E	19.73	149.19	- do-
68	1559.59	E	18.65	133.28	- do-
69	1649.91	E	19.73	149.16	- do-
70	1558.67	E	18.64	133.12	- do-
71	1649.95	E	19.73	149.17	- do-
72	1558.86	E	18.64	133.15	- do-
73	1649.63	M	19.73	149.11	- do-
74	1546.77	M	18.50	131.09	- do-
75	1638.30	M	19.59	147.07	- do-
76	1625.89	M	19.43	144.85	- do-
77	1641.26	M	19.63	147.60	- do-
78	1638.10	M	19.59	147.03	- do-
79	1634.06	M	19.54	146.31	- do-
80	1645.52	M	19.68	148.37	- do-
81	1638.79	P	19.60	147.16	- do-
82	1638.14	P	19.59	147.04	- do-
83	1649.30	P	19.72	149.05	- do-
84	1649.41	P	19.72	149.07	- do-
85	1649.27	P	19.72	149.05	- do-
86	1649.58	P	19.73	149.10	- do-
87	1649.62	P	19.73	149.11	- do-
88	1649.72	P	19.73	149.13	- do-
89	1654.49	P	19.78	149.99	- do-
90	1638.14	P	19.59	147.04	- do-
91	1589.49	P	19.01	138.44	- do-
92	1649.83	P	19.73	149.15	- do-
93	1634.05	P	19.54	146.30	- do-
94	1590.27	P	19.02	138.57	- do-
95	1654.49	P	19.78	149.99	- do-
96	1632.18	P	19.52	145.97	- do-
97	1588.73	P	19.50	138.30	- do-

S. No.	Wave number (n) (cm^{-1})/ $\bar{u}$	Type of sample	Vibrational Energy (E) (kJ $mole^{-1}$)	Force Constant (K) (Nm^{-1})	Group/Probable assignment
98	1642.23	P	19.64	147.78	- do-
99	1590.94	P	19.02	138.69	- do-
100	1649.21	P	19.72	149.04	- do-
101	1647.73	P	19.70	148.77	- do-
102	1649.57	P	19.73	149.10	- do-
103	1383.54	N	16.54	676.71	C-C, Carbide compounds, such as amino acid derivatives
104	1349.99	N	16.14	644.29	- do-
105	1383.56	N	16.54	676.73	- do-
106	1350.08	N	16.14	644.37	- do-
107	1380.74	N	16.51	673.96	- do-
108	1348.06	P	16.10	642.43	- do-
109	1381.81	P	16.52	675.02	- do-
110	1383.18	P	16.54	676.36	- do-
111	1350.34	P	16.15	644.62	- do-
112	1383.51	P	16.54	676.65	- do-
113	1350.37	P	16.15	644.65	- do-
114	1383.54	P	16.54	677.69	- do-
115	1350.13	P	16.14	644.24	- do-
116	1383.55	P	16.54	678.68	- do-
117	1350.42	P	16.15	644.76	- do-
118	1372.53	P	16.41	645.98	- do-
119	1168.28	M	13.97	468.04	P-O-C, Phospholipids
120	1158.92	M	13.86	460.57	- do-
121	1160.11	M	13.87	461.52	- do-
122	1151.94	M	13.77	455.04	- do-
123	1156.02	P	13.82	458.27	- do-
124	1156.03	P	13.82	458.28	- do-
125	1151.94	P	13.87	455.04	- do-
126	1108.66	N	13.26	579.37	O-O, Peroxide
127	1098.84	N	13.14	569.15	- do-
128	1041.62	N	12.45	511.41	- do-
129	1073.30	E	12.83	542.69	- do-
130	1086.57	E	12.99	556.50	- do-
131	1078.40	E	12.89	548.16	- do-
132	1074.31	E	12.85	544.01	- do-
133	1095.57	E	13.10	565.00	- do-
134	1093.46	E	13.07	563.59	- do-
135	1082.48	E	12.94	552.33	- do-
136	1072.77	E	12.83	542.45	- do-
137	1053.88	M	12.60	523.53	- do-
138	1066.29	M	12.75	535.93	- do-
139	1066.14	M	12.75	535.78	- do-
140	1070.22	M	12.80	539.89	- do-
141	1055.95	M	12.63	525.58	- do-
142	1123.34	M	13.43	594.81	- do-
143	1069.37	P	12.79	539.03	- do-
144	1053.88	P	12.60	523.53	- do-
145	1071.11	P	12.81	541.39	- do-

S. No.	Wave number (n) (cm^{-1})/ $\bar{u}$	Type of sample	Vibrational Energy (E) (kJ $mole^{-1}$)	Force Constant (K) (Nm^{-1})	Group/Probable assignment
146	1102.91	P	13.19	573.37	- do-
147	1078.40	P	12.89	548.17	- do-
148	1102.04	P	13.18	572.46	- do-
149	1068.64	P	12.78	538.28	- do-
150	1081.41	P	12.93	551.23	- do-
151	1072.29	P	12.82	541.98	- do-
152	1119.18	P	13.38	590.41	- do-
153	1076.86	P	12.88	546.61	- do-
154	1098.83	P	13.14	569.14	- do-
155	1074.71	P	12.85	544.43	- do-
156	1069.80	P	12.79	539.46	- do-
157	767.76	P	9.18	32.30	N-H, Amide IV to VI
158	771.97	P	9.23	32.65	- do-

Nociceptive networks have very strong affinity towards the biochemical aspects of the human body and reflect the entire functioning or malfunctioning of the physiological system. Due to this main fact we have used vibrational spectroscopy in the present research article.

Abaturov et al. [85] have reported the observed frequencies of the four amide bands of IgG, its peptide chains and proteolytic fragments. The frequencies and the shape of the amide bands of sulfonated IgG and $F(ab')_2$ fragment were identical to those of the complete molecule. Position of the band maximum e.g. namely amide I, amide II, amide A and amide B in IgG, light chain of IgG and F_{ab} fragments of IgG have been found to be 1644, 1550, 3295 and 3055 cm^{-1}, respectively.

The complex shape and the asymmetry of the Amide I band are indications of high heterogenicity of the secondary structure of IgG. The maxima of this band (1644 cm^{-1}) arises due to irregular conformation and the shoulder (1637 cm^{-1}) observed with the better resolution is probably due to β-structure [86].

The inflexion at 1665 cm^{-1} shows the presence of an irregular and β-conformations as the major elements of the secondary structure of IgG. The band frequencies of the papain fragments of IgG are almost identical to the intact IgG molecule. However, we notice a little difference in the shape of the amide I band.

This band possesses greater half-width in the infrared spectrum of the F_c fragment; compared to the full IgG and the F_{ab} fragments. The shape of the amide II band in the infrared spectra of the F_{ab} and F_c fragments is very near to the full IgG. There is no strong difference in the position and shape of the amide bands of IgG as compared with both of the papain fragments. This exhibits their secondary structures which are very much similar.

High content of irregular conformations [87, 88] and complex pattern lead to the three dimensional structure of IgG. We have two portions in IgG with different compactness. The more compact one has stronger hydrogen bonding and less motility (e.g., regions having regular structure); the less compact type possesses weaker hydrogen bonding and greater motility (e.g., portions having irregular structures).

Two types of structures have been found to exist within IgG using small-angle X-ray scattering. These structures possess different electron densities leading to non-uniform compactness. High stability of the secondary structure of IgG over wide pH range originates from the small content of ionized groups and insignificant interaction between them [89].

We are providing some of the information regarding amide band. These amide bands are called vibrational bands and are complex in nature. Amide bands depend on the details of the force field, nature of side chains and hydrogen bonding. Some of the researchers [78] provide a tabular form of some characteristic infrared band of protein, which are given in Table 2.

TABLE 2. Main Features of Infrared Band Present in Protein Structure

S. No.	Frequency (cm^{-1})	Assignment	Description
1	3300	AmideA	N-H Stretching
2	3100	AmideB	N-H Stretching
3	1600-1690	AmideI	C=0 Stretching
4	1480-1575	AmideII	CN Stretching
5	1229-1301	AmideIII	N-H Bending
6	625-767	AmideIV	N-H Bending
7	640-800	AmideV	OCN Bending
8	537-606	AmideVI	Out of plane Bending
9	200	AmideVII	C=O Bending Skeletal torsion

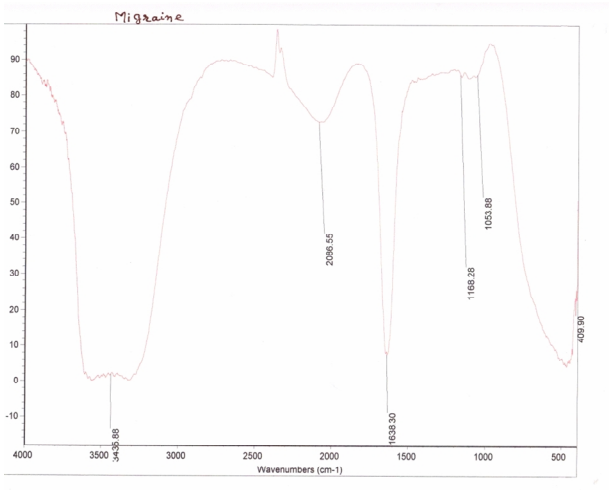

Figure 1. FTIR Spectrum of a Person Suffering from Migraine

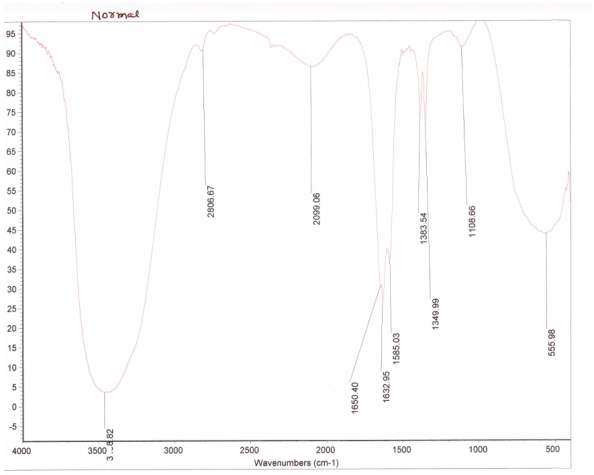

Figure 2. FTIR Spectrum of a Person Who is Healthy

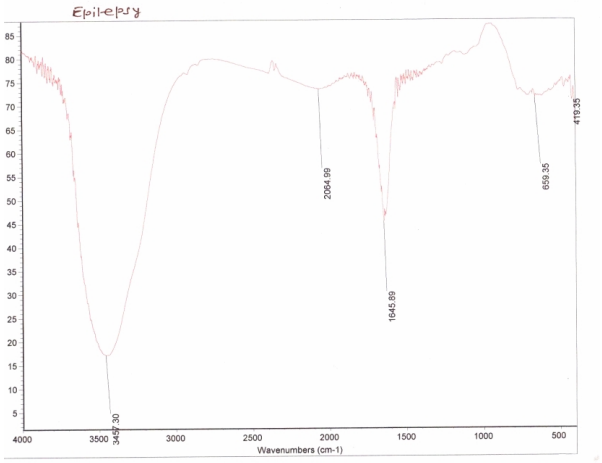

Figure 3. FTIR Spectrum of a Person Suffering from Epilepsy

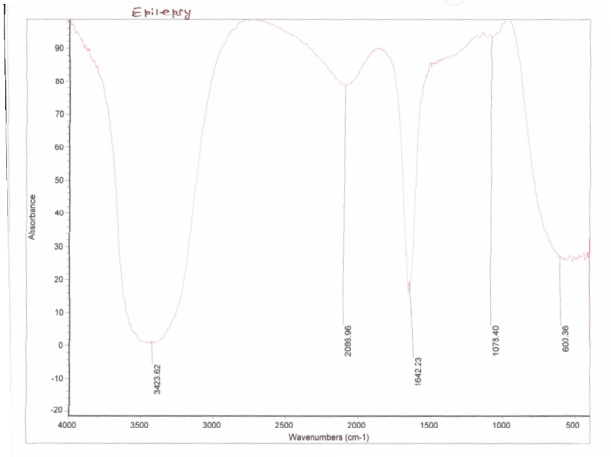

Figure 4. FTIR Spectrum of a Person Suffering from Paraplegia

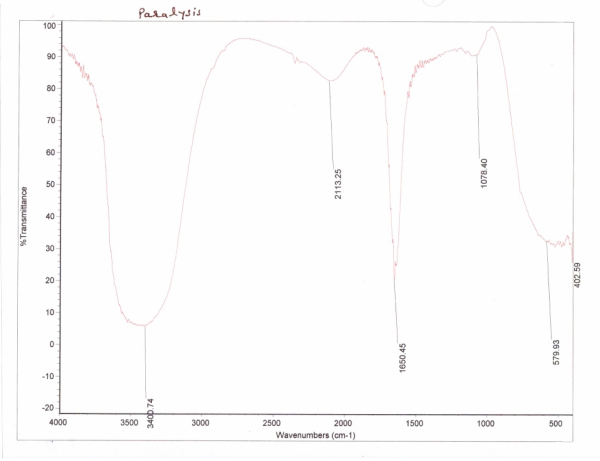

Figure 5. FTIR Spectrum of a Healthy Control

Findings of IgG samples of neurogically diseased patients are reported one by one.

We would like to present some of the typical FTIR spectra here to support our present findings in Figures 1 to 5.

The amide A band (about 3500 cm^{-1}) in proteins is found in all the neurologically diseased samples and healthy controls at range from 3415.45 to 3460.56 cm^{-1}. It can be safely said that amide A band does not deviate from the original position in all the spectra. We have also found that amide II band appears in four patients suffering from epilepsy. The vibrational energy is almost constant and is equal to18.65 kJ $mole^{-1}$. Force constant is 133.15 Nm^{-1}. Amide I band is found intact clearly in all the cases of epilepsy, migraine and paralysis. The vibrational displacement of the amide I and II are highly localized in the CONH group. The amide I band found in the range from 1625 to 1649.11 cm^{-1} are close to the absorption frequencies of the deuterated polypeptides having the β-conformation and have also been observed in proteins which contain a part of β-structure.

The absence of hydrocarbon band (C-H) in all the epileptic and migraine patients is a clear cut differentiation from the normal healthy controls. This band falls in the range from 2810.76 to 2913.05 cm^{-1}. The vibrational energy is constant and approximately equal to 33.68 kJ $mole^{-1}$. The force constant is found in the range from 403.71 to 429.82 Nm^{-1}.

The absence of carbide compound (C-C) in all cases of migraine and epilepsy is a distinct feature of this study. While, this band is found to appear in all the paraplegic and hemiplegic patients. This band is found in the range from 1350.13 to 1387.54 cm^{-1}.

The band due to P-O-C (phospholipids) is found in some of the cases of migraine along with hemiplegia and paraplegia. These frequencies are found to be absent in all the cases of epilepsy and normals. The disappearance of this band in normals is a distinct feature of these samples.

The band due to O-O (peroxide) compound is found intact in the range from 1041.62 to 1119.18 cm^{-1} in all cases of epilepsy, migraine and paralysis and healthy controls. The vibrational energy is 12.45 kJ $mole^{-1}$ and force constant is 511.41 Nm^{-1}.

A band called amide IV due to N-H is found only in two cases of paralytic disorder at 767.76 and 771.97 cm^{-1}. This amide IV band is absent in all cases of paralysis, epilepsy and migraine and controls. These two patients were under medical treatment in the preliminary stage.

4. CONCLUSION

FTIR spectroscopy is a well established experimental method for studying the structural composition and dynamics of proteins. A correlation between the spectra and protein structure has been well documented. These spectra also give information on the protein stability and dynamics. These spectra are complex in nature. Side chain absorption is to be taken into account in the analysis of protein spectra.

It should be noted here that some amino acid residues, especially arginine, asparagines, glutamine, asparatic and glutamic acids, lysine, tyrosine, histidine and phenylaline have very fast absorption in the amide band region.

Many diseases generate specific changes in the metabolic pattern of blood or other body fluids. These changes may produce particular spectroscopic indications which can be used for identification and classification of diseases in seconds to minutes.

Amide IV band is also found in the present work only in two cases of paralysis. Absence of this band in other cases is a remarkable change. These patients were followed by the proper medication with standard drugs.

The bands due to phospholipids [P-O-C] is found only in the migraineous and paralytic patients and absent in epilepsy and healthy controls. This technique is able to detect these bands and give clear cut indicating something is hidden inside the IgG molecule.

In the present work, we have succeeded in identifying the basic atomic level transformations occurring in different neurological disturbances. It is possible, on the basis of our investigations, to use this technique for differential diagnosis of the various pathological disorders and to some extent the stage of disease. It has already been pointed out that the infrared spectra exhibit the presence of specific bands peculiar to a particular disease such as epilepsy, migraine, headache and paraplegia as compared to the normal samples.

The FTIR experimental findings show that in these neurological perturbations, stability of the secondary structure is completely disturbed. It follows that a large extent of conformations of the two parts of IgG is independent of the intact IgG molecule. This fact is in line with the Noelken-Nelson-Buchley-Tamford model [90], which is supported by the electron microscopic studies.

It is concluded that this method is reliable and efficient to detect changes at the molecular level. Specific changes could be seen in the structure of protein molecule with the help of detailed theory of infrared spectroscopy measurements.

5. ACKNOWLEDGEMENT

The authors are thankful **to Dr. P.K. Saxena** Principal, D.A.V. (P.G.) College, Muzaffarnagar for providing the facility of doing work. We are also thankful to the Medical Superintendent, Safdarjang Hospital, New Delhi, for arranging the blood samples of the diseased and healthy controls. We would like thanks to Mr. M.G.Mittal Director, Self finances Department, D.A.V. (P.G.) College, Muzaffarnagar for granting the permission to do the experimental work. Authors are grateful to Dr. Manju Chauhan, Head, Department of Biosciences, D.A.V. (P.G.) College, Muzaffarnagar, for providing the facility of purification of IgG.

We are thankful to the anonymous reviewers for their valuable suggestions.

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