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# Analysis of serum immunoglobulins using Fourier transform infrared spectral measurements

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**Abstract**
Fourier Transform Infra Red (FTIR) Spectroscopy is a non-invasive, reagent free diagnostic tool in the analysis of biological fluids. The results can be best employed in the qualitative and quantitative investigation of biological fluids. Multiple myeloma is a disorder in which malignant plasma cells accumulate in bone marrow and produce excess immunoglobulin that leads to many complications. The present work is attempted in the study of normal and three different myeloma affected blood samples IgA, IgG and IgM using FTIR Spectroscopy. Internal Standard method is adopted in characterizing the samples quantitatively.

**Keywords:** Immunoglobulins; Multiple Myeloma; Non-invasive; Reagent free diagnostic tool; Fourier Transform Infrared Spectroscopy.

## Introduction

The use of Infrared Spectroscopy for biomedical applications has increased tremendously in the recent years. Fourier Transform Infra Red (FTIR) Spectroscopy is a non-invasive, reagent free diagnostic tool in the analysis of biological fluids. The results can be best employed in the qualitative and quantitative investigation of biological fluids like blood, serum, saliva, urine etc. The promise of IR Based analysis is that it can rapidly and simultaneously quantify several components without any specific reagents (Shaw et al., 1998; Heise et al., 1998; Heise, 1994; Yan-Ping Zhou et al., 2007; Deleris and Petibois, 2003).

The quantitative analysis of body fluids is a major field in clinical chemistry. Blood is the chief transporting medium in a human system. Its composition is the preferred indicator with regard to patho-physiological condition of the system. IR based analysis is based on the rich infrared absorption pattern of the constituents of blood, that characterize the analytes themselves. These absorption patterns provide the basis to distinguish among the constituents and to separately quantify them. They possess the advantage that very small sample volume requirement, good precision over entire physiological range, avoid of costly disposables, wealth of information from a single spectral measurements (Boussaidi et al., 2009; Franck et al., 1996; Ward et al., 1992; Shaw and Mantsch, 2000; Petibois et al., 2001; Petibois, 2000; Petibois, 2002; Buddinova et al., 1997).

Multi-component assay of human plasma has been evaluated for the determination of blood substrates (Rohleder et al., 2005). Shaw et al. (2005) has used this technique for the quantification urea, creatinine and total protein from dried blood sample. Petibois et al. (1999) determined glucose in serum samples. Gunasekaran et al. (2007) studied lipid disorder in women blood samples and renal failure blood samples. Continuous monitoring of blood samples during chemotherapy in cancer treatment by FTIR Spectroscopy is found to be highly informative and useful (Khanmohammadi et al., 2007). FTIR Spectroscopy coupled with statistical calculation has been employed by the researchers in the estimation of plasma proteins (Deleris and Petibois, 2003; Petibois et al., 2001). In line with the spectral method of analysis of blood, the present work is proposed to evaluate a new approach in the

analysis of serum immunoglobulin in multiple myeloma mid- IR spectroscopy.

Blood is the chief circulatory medium of human system. The study of blood is the important aspect in clinical chemistry. Blood contains 55% plasma and 45% cellular fractions. The fluid portion plasma contains 7% proteins as albumin, globulin and fibrinogen. Globulin is derived from lymphocytes and they are responsible for the production of antibodies and other immune substances, which are useful in the defense of the body against the invasion of the disease-producing microorganism. IgA, IgD, IgG, IgE and IgM are the five major isotypes of immunoglobulins, which are named as to the type of the respective polypeptide chains. Multiple myeloma is a disorder in which malignant plasma cells accumulate in bone marrow and produce excess immunoglobulin that leads to many complications. Clinically immuno-fixation electrophoresis is done for the estimation of immunoglobulin levels in blood, which involves lot of analytical reagents, costly disposables and manpower (Lee et al., 2002; Chatterjea and Shinde, 2006). The present work is attempted in the study of normal and myeloma affected blood samples using FTIR Spectroscopy both qualitatively and quantitatively. The type of spectral signatures qualitatively differentiates the different types of immunoglobulins whereas the intensity ratio among the absorption bands quantitatively characterizes them.

**Materials and Methods**

Blood samples were collected from normal healthy subjects from the OPD of Apollo Hospitals, Chennai who were admitted to hospital for routine check up. The samples were collected from age group 40 - 50 years. Blood samples were also collected from age-matched persons with increased immunoglobulin levels admitted to hospital with severe infection diagnosed as myeloma. In the present work, three different immunoglobulin high samples viz. IgA, IgG and IgM have been chosen for the analysis. All the sampling procedures were performed between 08.00 to 09.00 am after overnight fasting. The samples were kept as such for two hours. After that, the samples were centrifuged and the serum was separated. In each category, 10 samples were used for spectral analysis. The samples were stored at refrigerator at a uniform temperature of 20$^{o}$C until analysis.

The FTIR Spectral measurements of all the samples were carried out at Sophisticated Analytical Instrumentation Facility IIT, Madras, Chennai-36, 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. After the samples returned to room temperature 20 µL of the aliquots were diluted with 80 µL of water. The diluted samples are homogenized, with an agitator. 50 µL of each solution was spread evenly on the thallium bromide crystals window. The samples were air dried for water evaporation to eliminate the stray absorption bands due to water and holder is mounted in the sample window of the spectrometer. The spectrometer is equipped with a globar source, KBr beam splitter and DTGS cooled detector. The sampling window is scanned as the background and 32 scans are co added with a spectral resolution of 1 cm$^{-1}$. All the spectra were baseline corrected and normalized to acquire identical area under the curve.

**Results and Discussion**

The infrared spectrum is the essence of reflection of the infrared color pattern characteristics of the sample (Liu et al., 2002). The basis of quantification is that each constituent contribute a unique absorption pattern to the overall spectrum governed by the unique set of molecular vibration characteristics of each distinct molecular specimen. The quantitative information is carried out by the relative intensities of the various contributing spectra to the unique absorption profile of each specimen (Low-Ying et al., 2002).

A representative FTIR absorption spectrum of serum sample is shown in Fig. 1. The characteristic vibrational peaks are mainly dominated by the of the protein constituents of the sample. (Deleris and Petibois, 2003; Petibois et al., 2001). A vibration band assignment is done with the idea of the group frequencies of the various analytes present in the sample. The spectral region 3600 – 3000 cm$^{-1}$ comprises of C-H, O-H and N-H stretching vibrations of the proteins. The prominent absorption peak 3300 cm$^{-1}$ is due to the N-H stretching mode (amide A) of proteins. The asymmetric and symmetric stretching C-H vibrations of methyl and methylene group are found to be present around 2930 – 2875 cm$^{-1}$. The strong absorption band at 1650 cm$^{-1}$ correspond to C=O stretching vibrations (amide I) whereas the vibration band at 1542 is attributed as amide II arising of N-H bending vibrations strongly coupled with C-N stretching of proteins (Gunasekaran and Sankari, 2004).

The absorption peaks in the region 1400 – 1200 cm$^{-1}$ arise due to the C–H

deformation of methyl and methylene group of the proteins. The asymmetric and symmetric P-O stretching vibrations are found to be around 1245 $cm^{-1}$ and 956 $cm^{-1}$ respectively. The spectral region 1250-925 $cm^{-1}$ is predominantly occupied by C–O–C asymmetric and symmetric vibrations of phospholipids of proteins (Randhawa, 2003).

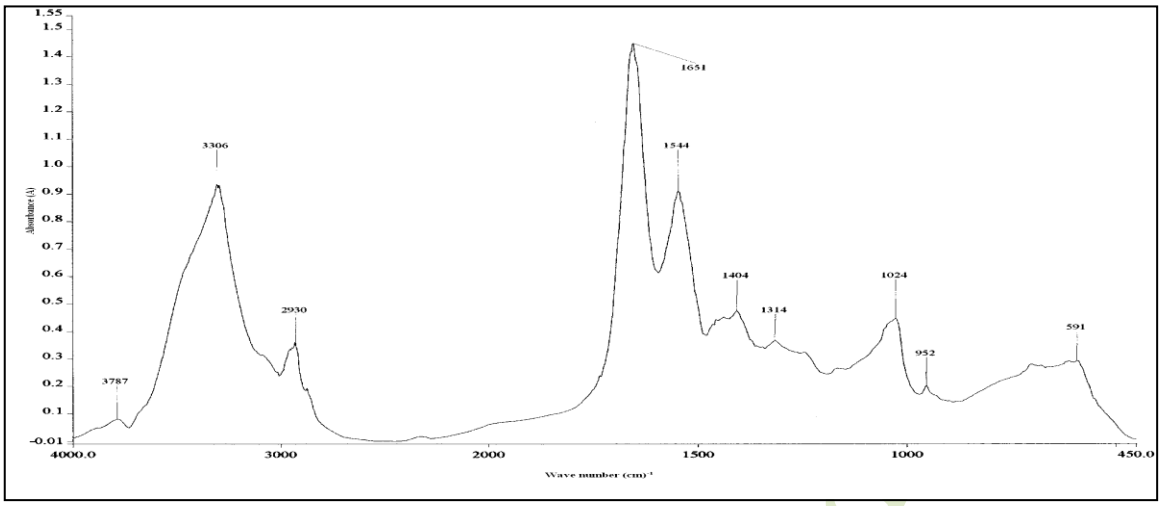


Figure 1: FTIR spectrum of blood sample.

As the IR spectrum exhibits vibrations bands characteristics of the various group frequencies, the spectrum of a normal blood sample and that of a myeloma affected blood samples are the same with respect to the positions of the peaks but different in terms of the absorption levels of the peaks. Fig. 2 presents average FTIR spectrum of normal and myeloma IgG blood samples superimposed on each other. It is observed that the spectral features are the same as expected, but the amount of absorption is decreased in IgG samples than that of normal ones. In order to quantify the results further five intensity ration parameters $R_1$ ($I_{2874}/I_{2930}$), $R_2$ ($I_{1542}/I_{1654}$), $R_3$ ($I_{1315}/I_{1404}$), $R_4$ ($I_{1165}/I_{1244}$) and $R_5$ ($I_{953}/I_{1025}$) are calculated among the prominent absorption peaks due to proteins. It is observed that these values are also decreased in myeloma samples than that of the normal one.

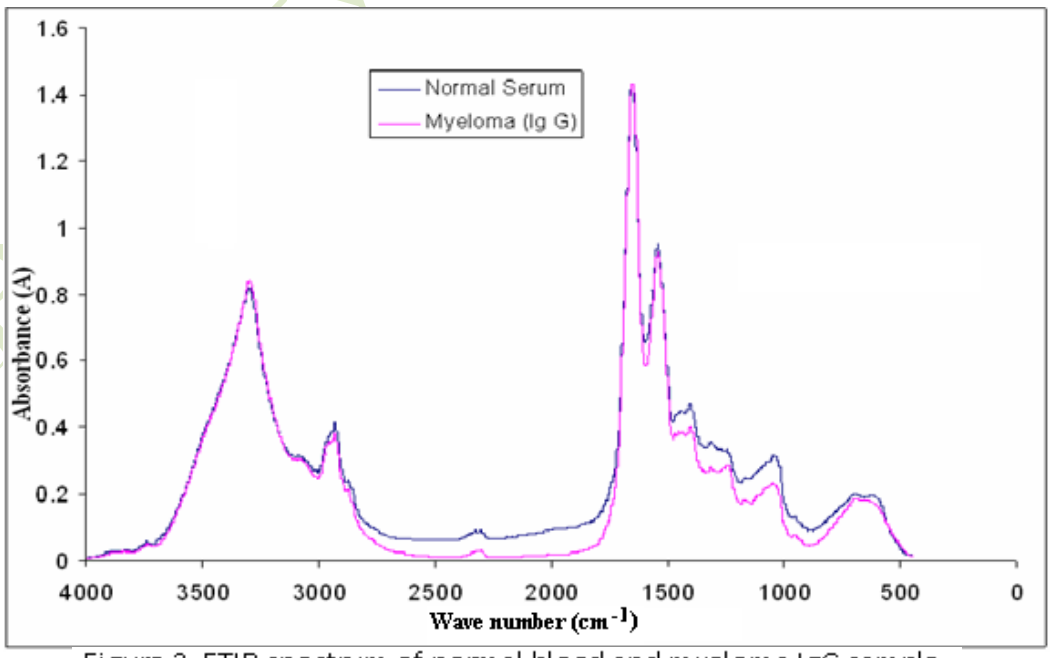


Figure 2. FTIR spectrum of normal blood and myeloma IgG sample

The experiment is repeated in other types of myeloma samples, IgA and IgM respectively. Figure 3 presents FTIR spectra of normal and IgA blood samples. The figure shows the absorption levels of the various peaks are increased in IgA sample than that of normal sample. Table 1 verifies the same absorption by intensity ratio calculation.

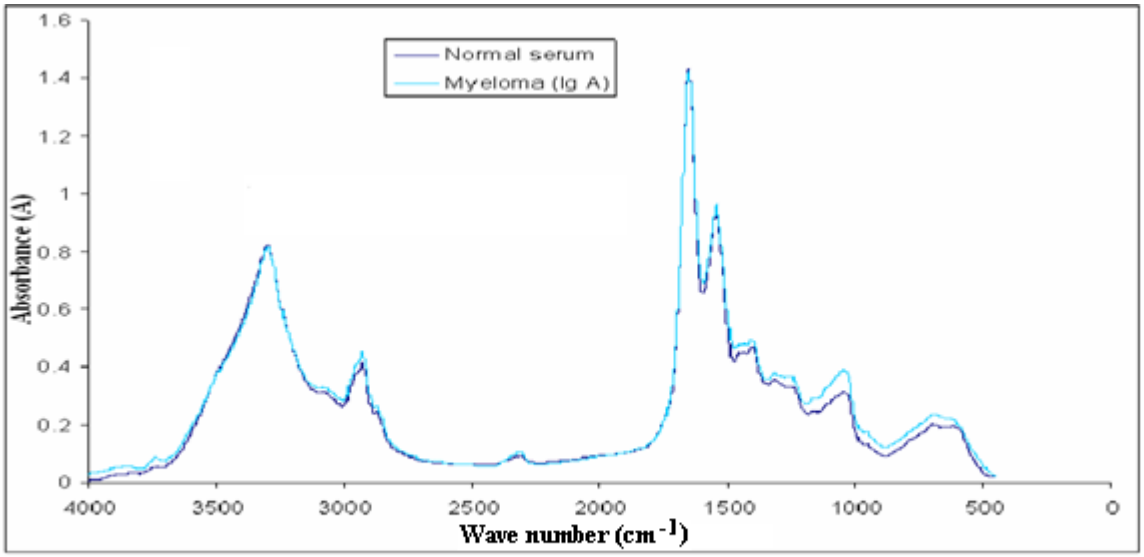


Figure 3. FTIR spectrum of normal blood and myeloma IgA sample

Fig 4 represents the FTIR spectral analysis of normal and IgM blood samples. It is observed that spectral absorption of the peaks are decreased in IgM samples and hence the intensity ratio calculation also. Table 1 presents intensity ratio calculation for all the three types of myeloma samples in the same spectral regions as a comparison. Fig 5 presents variations as a bar diagram.

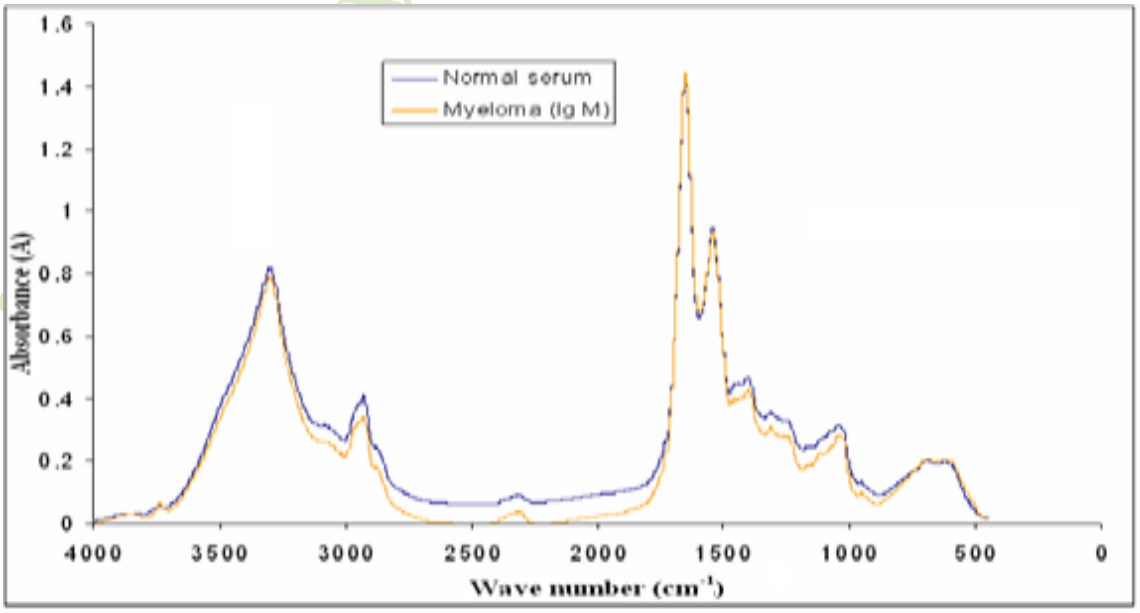


Figure 4. FTIR spectrum of normal blood and myeloma IgM sample

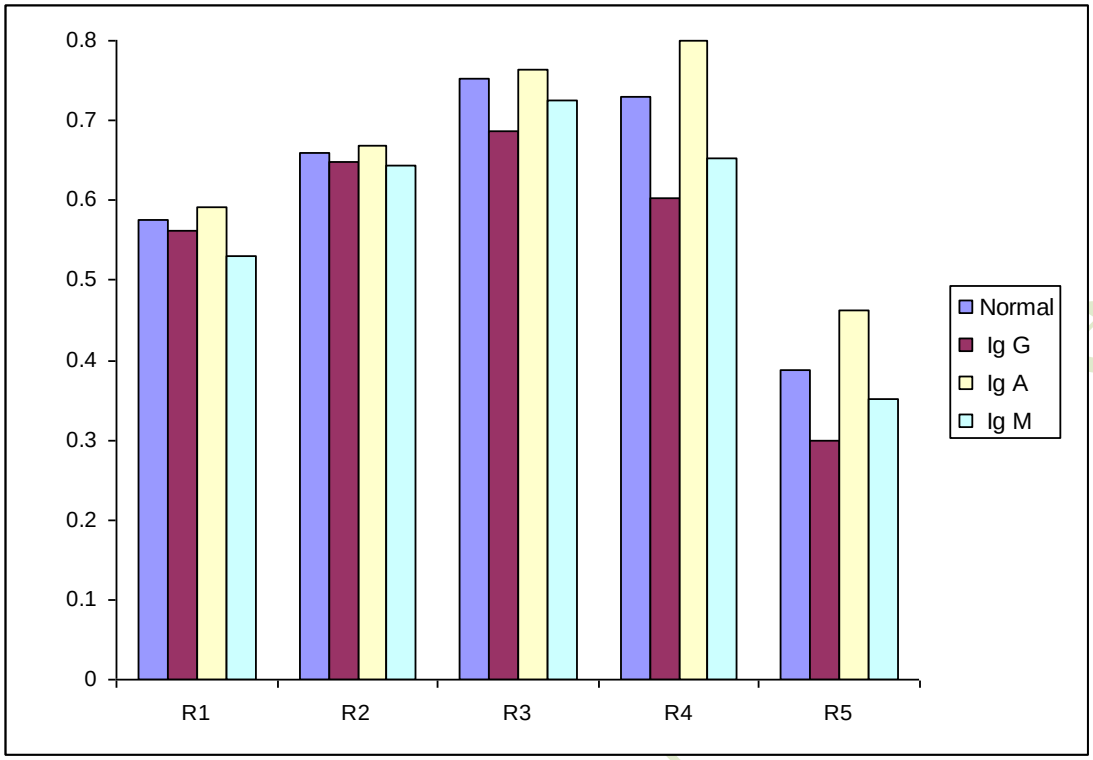


**Figure 5:** It shows the comparison of Intensity Ratio Parameters between Normal and Myeloma Samples.

**Table 1:** It shows the comparison of Normal and three different myeloma samples using Intensity Ratio Parameters.

| Ratios | Normal Serum | Myeloma Ig G | Myeloma Ig A | Myeloma Ig M |
|---|---|---|---|---|
| $R_1 = I_{2874}/I_{2930}$ | 0.5746 | 0.5617 | 0.5906 | 0.5306 |
| $R_2 = I_{1542}/I_{1654}$ | 0.6591 | 0.6483 | 0.6689 | 0.6441 |
| $R_3 = I_{1315}/I_{1404}$ | 0.7515 | 0.6869 | 0.7647 | 0.7244 |
| $R_4 = I_{1165}/I_{1244}$ | 0.7303 | 0.6023 | 0.7993 | 0.6529 |
| $R_5 = I_{953}/I_{1025}$ | 0.3885 | 0.2983 | 0.4618 | 0.3508 |

**Conclusion**

The role of FTIR spectroscopy in the clinical analysis of normal and immunoglobulin high blood samples is clearly demonstrated both qualitatively and quantitatively. It is demonstrated that among the three immunoglobulins, IgG and IgM present decreased level of absorption of the vibrational peaks whereas IgA exhibits increased absorption with reference to the normal samples, which is verified using Internal Standard calculations also.

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