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A FTIR Spectroscopic Study on Quantitation of Albumin in Human Blood Serum

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ABSTRACT: The measurement of concentration of Albumin has been achieved using FTIR Spectroscopy. The FT-IR spectra of human blood serum samples are recorded using liquid cell in Mid IR region 4000-400 cm⁻¹. The normal blood serum is treated with Albumin at different concentrations viz 100, 200, 300, 400, and 500 and gm/dL and the FTIR spectra are recorded, which confirms the specific peak for Albumin. A graph between concentration of Albumin and intensity of absorption shows a linear relation. There is an increase in the intensity of absorption.

KEY WORDS: FT - IR Spectroscopy, Albumin, Serum.

I. INTRODUCTION

IR spectroscopy has been used by Biophysicist and Chemist as a powerful tool to characterize compounds. It has been applied in biology for studying the structure and conformation of molecules like proteins, nucleic acids and lipids. The advances made in instrumentation have paved the way for its utilization in medicine. Besides the application of FT-IR for tissue diagnostics, the investigation of body fluids has been gaining importance. The mid -IR region is very useful in the identification of disease patterns using the FT-IR spectrum of human blood serum. Precise quantification of several components such as Albumin, total protein, and urea can be achieved using FT-IR spectroscopy

II. RELATED WORK

Zhdanov et al [9] made a comparative study to determine the role of the skeletal base configuration of carbohydrate molecules, using IR and Raman spectroscopy. Theoretical calculations of the vibrational spectra of series of carbohydrates differed in the configuration of CO (CH) bonds in various positions of the pyranosering. The normal vibrations of carbohydrate molecules had, with few exceptions, close or coinciding frequencies; however, they differed greatly in the shape and contribution to the potential energy distribution(PED) of individual groups and bonds. Despite the cooperative character of the vibrations, each compound was characterized by a specific set of frequencies with a prevailing contribution to the PED of particular CO and CC bonds of the molecule. They concluded that vibrations have a peculiar localization and that steric factors play an important role in the vibrational spectra of carbohydrates.

Minesh Patel et al [10] Studied poly acrylic acid and mucus using infrared, ¹H and ¹³C nuclear magnetic resonance, and X-ray photoelectron spectrometers and differential scanning calorimetry, which supported the hypothesis that hydrogen bonds, formed between the carboxylic acid functionality of the muco- adhesive material poly(acrylic acid) and the glycoprotein component of mucus, play a significant role in the process of muco-adhesion. They found fewer

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H-bonded interactions between the components than within the bulk of the pure muco-adhesive agent and pH of the medium influenced the structures of both the poly acrylic acid and the mucus, which, in turn, determine the nature and the extent of muco-adhesive interactions.

Davis and Mauer [11] highlighted the principles of FT-IR spectroscopic analysis of bacteria, the advantages and disadvantages of FT- IR applied to bacterial analysis, various sampling techniques, spectral manipulation, statistical analysis of spectra, and applications in pathogen detection.

Jana kopikova et al [12] used FT-IR spectroscopy for the estimation of isolated high molecule fractions and also for the identification of food hydrocolloids in confectionery jellies and food supplements. The simple comparison of spectra of standards and samples proved that this technique is useful for the monitoring of food hydrocolloids in particular food products.

III. MATERIALS & METHODS

The blood samples were collected from healthy donors without adding anticoagulant and serum was separated by removing blood clot. The serum was treated with research grade (SD fine chem) glucose an hydras at concentrations 100,200,300,400 and 500 gm/dL. Infrared spectrum was recorded in Fourier Transform Infrared spectrophotometer (ShimadzuFTIR - 8400S) in the range of 4000 cm⁻¹ to 400 cm⁻¹. The resolution was kept at 4 cm⁻¹ and scanning time was fixed at 38 Sec. A total number of 10 scans were carried out on each sample.

IV. RESULTS & DISCUSSION

Fig1shows FTIR spectra of human blood serum treated with Albumin of concentration 100, 200, 300, 400, 500 mg/dl. Spectra show a series of bands, in the range of 4000 cm⁻¹ and 400 cm⁻¹, related to functional groups of proteins, lipids and carbohydrates and other inorganic materials present in the blood serum. Fig1(a) to Fig1(e) are FTIR spectra of blood serum treated with Albumin of concentration 100, 200, 300, 400 and 500 gm/dl respectively. Characteristic band for Albumin can be seen approximately at 1440 cm⁻¹.

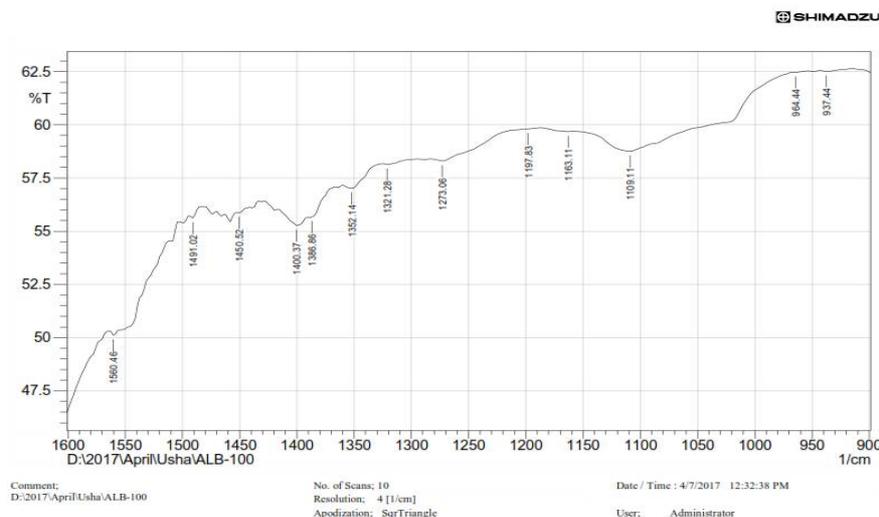


Fig.1.(a). FTIR spectrum of human blood serum treated with Albumin of concentration 100 mg/dl

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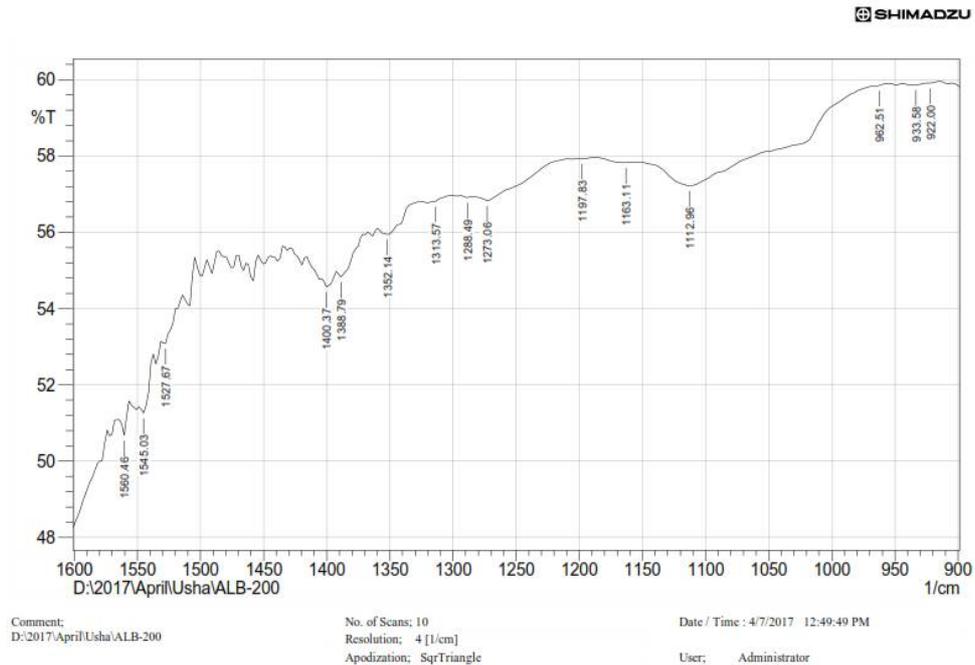


Fig. 1(b). FTIR spectrum of human blood serum treated with Albumin of concentration 200 mg/dl

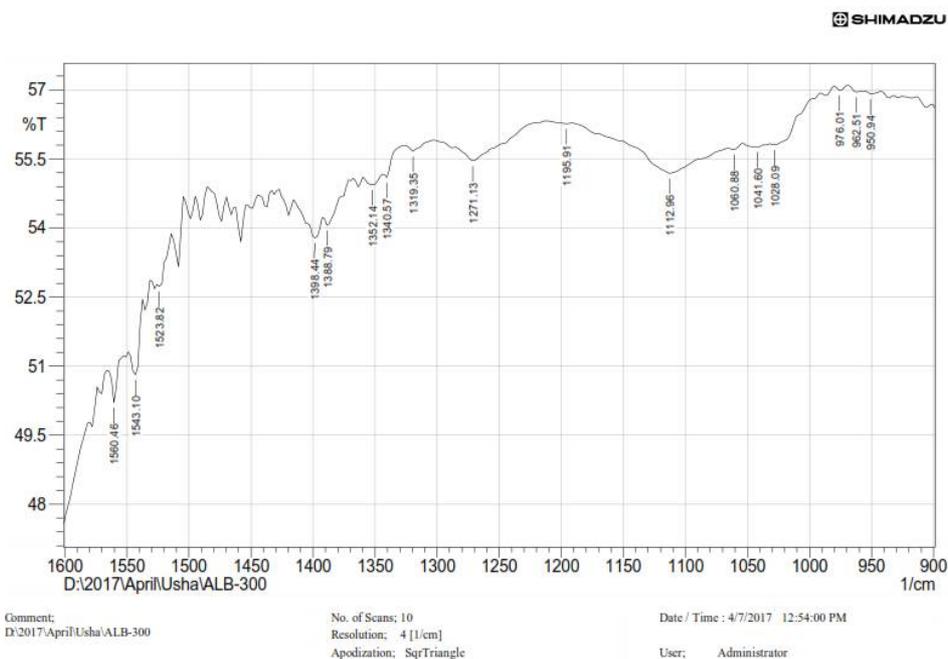


Fig. 1(c). FTIR spectrum of human blood serum treated with Albumin of concentration 300 mg/dl

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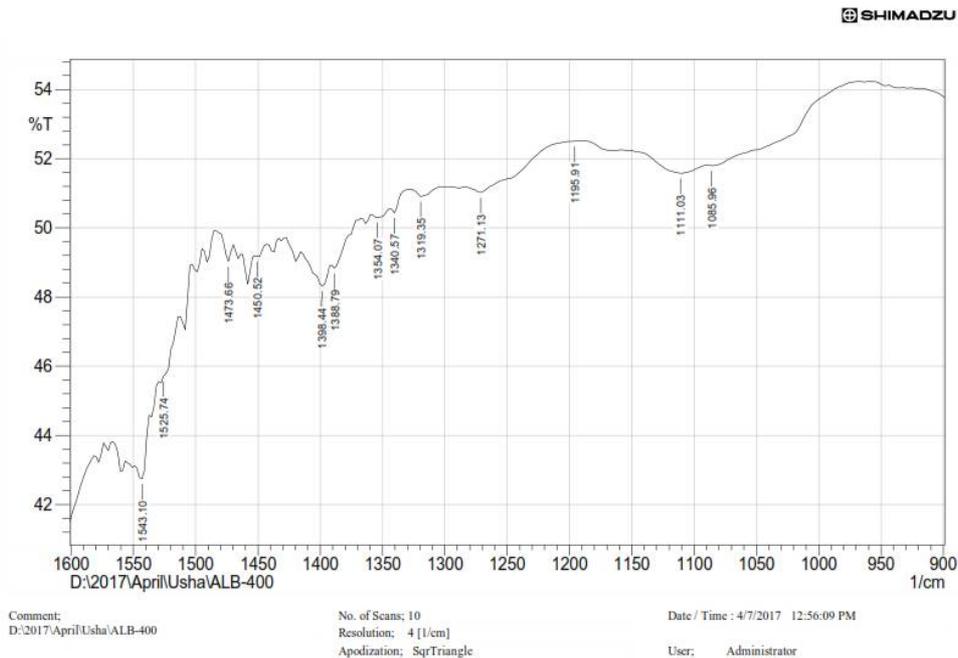


Fig. 1(d). FTIR spectrum of human blood serum treated with Albumin of concentration 400 mg/dl

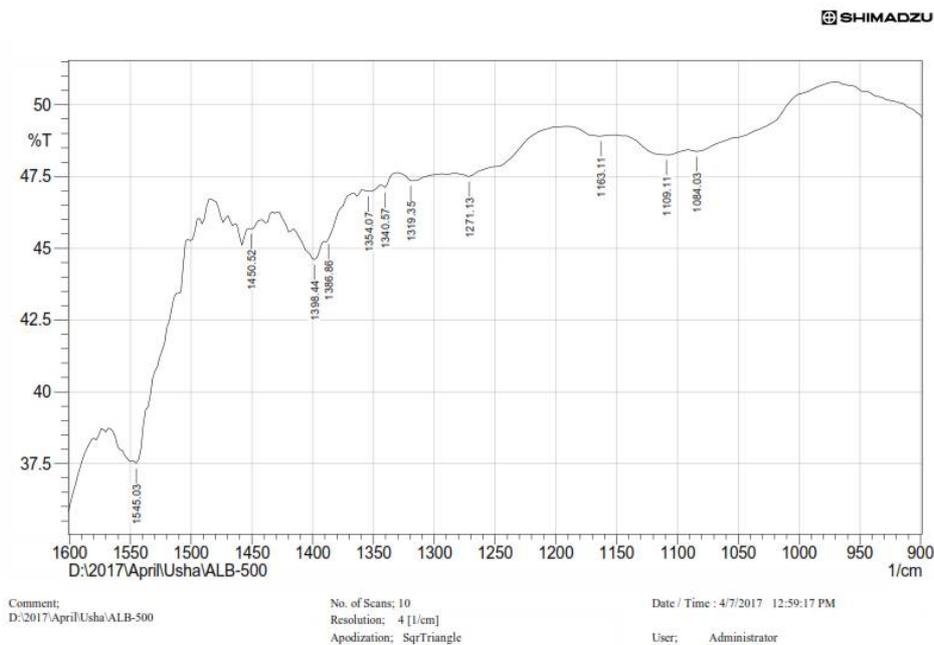


Fig. 1(e). FTIR spectrum of human blood serum treated with Albumin of concentration 500 mg/dl

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Table 1 gives FTIR data obtained from Fig1. at 1440 cm^{-1} . The table reveals IR absorbance, calculated from transmittance (%), in blood serum treated with Albumin of concentration ranging from 100 to 500 mg/dl with an interval of 100 mg/dl. A plot is drawn between Absorbance on Y – axis and glucose concentration (mg/dl) on X – axis (Fig.1). It is a straight line with +ve slope and also +ve Y – intercept, the linear regression equation of which is $Y = 0.0002 X + 0.218$, where A is absorbance and X is glucose concentration, and $R^2 = 0.88$.

Table 1 – Absorbance of IR bands of serum treated with Albumin of different concentration (gm/dl) Absorbance

Concentration of Albumin (mg/dl)	Absorbance
100	0.25
200	0.27
300	0.26
400	0.31
500	0.34

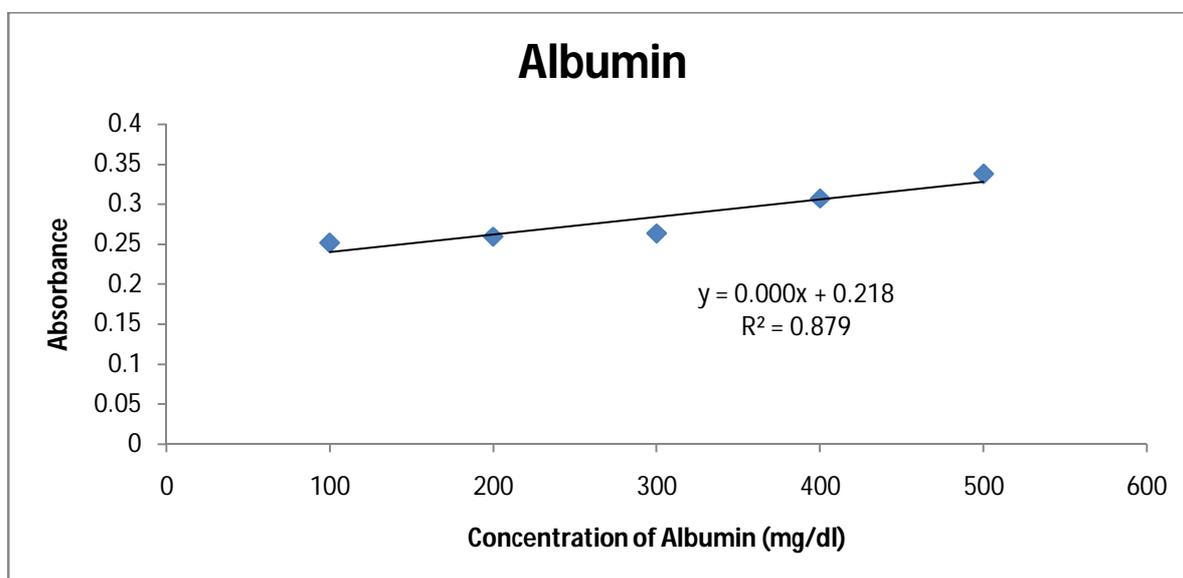


Fig 2.A plot between Absorbance and concentration of Albumin added to blood serum

The study concludes that FT-IR spectrum is a finger print of molecular constituent of the sample. The absorbance positions of band sat wave numbers reveal the qualitative characteristics of chemical bonds and structure of the molecule, while the intensities of absorption provide quantitative information of the sample. The present study suggests that the most specific band for Albumin is around at 1440 cm^{-1} in human blood serum

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