

# A FTIR Spectroscopic Study on Quantitation of Glucose in Human Blood Serum

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**ABSTRACT:** The measurement of concentration of glucose has been achieved using FTIR Spectroscopy. The FT-IR spectra of human blood serum samples are recorded using liquid cell in Mid IR region  $4000-400\text{ cm}^{-1}$ . The normal blood serum is treated with glucose at different concentrations viz 2, 4, 6, 8 and 10 gm/dL and the FT-IR spectra are recorded, which confirms the specific peak for glucose. A graph between concentration of glucose and intensity of absorption shows a linear relation. There is an increase in the intensity of absorption.

**KEY WORDS:** FT - IR Spectroscopy, Glucose, 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 glucose, total protein, and urea can be achieved using FT-IR spectroscopy.

## II. RELATED WORK

Lucie Stovickova et al [1] investigated the blood plasma of diabetic patients and healthy controls by Raman optical activity and electronic circular dichroism and measurements were combined with Raman and infrared spectroscopy. The obtained data was evaluated using linear discriminant analysis focusing on the spectral ranges that correspond to the structure and conformation of proteins and other plasmatic biomolecules. They reported that chiroptical spectroscopy gave more detailed information about the 3D structure of biomolecules; and therefore, might be a promising complement to conventional diagnostic methods.

David Scott et al [2] employed infrared spectroscopy as a novel diagnostic tool in the prediction of diabetic status by analyzing the molecular and sub-molecular spectral signatures of saliva collected from subjects with diabetes and healthy controls. Spectral analysis revealed differences in several major metabolic components such as lipid, proteins, glucose, thiocyanate and carboxylate that clearly demarcated healthy and diseased saliva. They established that infrared spectroscopy can be used to generate complex biochemical profiles in saliva and identify several potential diabetes-associated spectral features.

Medhat Ibrahim et al [3] employed molecular modelling using semiempirical methods and density functional theory to calculate the structure and vibrational spectra of D-glucose and D-fructose in their open chain, and  $\alpha$  and  $\beta$  monohydrate forms. The calculated data showed that both molecules were not linear; ground state and the number for

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the point-group C is equal to 1. The results indicated that there were similarities in bond lengths and vibrational modes of both molecules. They concluded that DFT could be used to study both the structural and vibrational spectra of glucose and fructose.

Wanjie Zhang et al [4] investigated the effects of two - dimensional correlation spectroscopy (2DCOS) on chance correlations in the spectral data, generated from the correlations between glucose concentration and some undesirable experimental factors, such as instrument drift, sample temperature variations, and interfering compositions in the sample matrix. They demonstrated the utility of the proposed analysis with a series of aqueous solutions using near - infrared spectra over the overtone band of glucose.

Srisuthep et al [5] obtained infrared and Raman spectra of malto oligosaccharides. Malto oligosaccharides and higher could not be readily distinguished by their infrared spectra. The Raman spectra of crystalline glucose and maltose showed distinct vibrational bands, but maltotriose and all higher oligosaccharides exhibited such high fluorescence that the vibrational bands were obscured.

Syed Ismail Ahmad et al [6] made an attempt to estimate concentration of glucose in human urine. They reported specific band at 1034  $\text{cm}^{-1}$  for glucose and also a relation between glucose concentration in urine and Transmission (%) of IR band.

Shen et al [7] used Fourier transform infrared (FTIR) transmission spectroscopy for the determination of glucose concentrations in whole blood samples from twenty eight patients. A four-vector partial least squares calibration model, using the spectral range 950 -1200  $\text{cm}^{-1}$ , yielded a standard error of prediction of 0.59 mM for an independent test set. For blood samples from a single patient, they found that the glucose concentration was proportional to the difference between the values of the second derivative spectrum at 1082  $\text{cm}^{-1}$  and 1093  $\text{cm}^{-1}$ , suggesting that these two specific wavelengths can be used for determining glucose concentrations in blood.

Cyril Petibois et al [8] developed a new method to determine glucose concentration in dried sera and studied 32 serum samples after fourfold dilution and desiccation before FT-IR analyses on a spectrometer operated at a resolution of 2.0  $\text{cm}^{-1}$ . They integrated all spectral windows at the surface of the spectrum in the C - O region and measured glucose in the sera by a glucose oxidase method for comparison. Further, they concluded that FT-IR spectroscopy is an accurate method to determine glucose concentration and could be widely used to simultaneously identify and quantify several metabolites in biological fluids or tissues.

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 a series of carbohydrates differed in the configuration of CO (CH) bonds in various positions of the pyranose ring. 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,  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance, and X-ray photoelectron spectroscopies 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 H-bonded interactions between the components than within the bulk of the pure muco-adhesive agent and

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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.

Vijaya Ushasree and Adeel Ahmad [13] reported IR spectroscopic data on blood of patients suffering from *Diabetes mellitus*. IR analysis was made on whole blood, plasma and serum. The characteristic spectral bands pertaining to glucose in the medium of blood were identified.

### 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 anhydrase at concentrations 2, 4, 6, 8 and 10 gm/dL. Infrared spectrum was recorded in Fourier Transform Infrared spectrophotometer (Shimadzu FTIR - 8400S) in the range of 4000  $\text{cm}^{-1}$  to 400  $\text{cm}^{-1}$ . The resolution was kept at 4  $\text{cm}^{-1}$  and scanning time was fixed at 38 Sec. A total number of 10 scans were carried out on each sample.

### IV. RESULTS & DISCUSSION

Fig. 1. shows FTIR spectra of human blood serum treated with glucose of concentration 2, 4, 6, 8, 10 gm/dl. Spectra show a series of bands, in the range of 4000  $\text{cm}^{-1}$  and 400  $\text{cm}^{-1}$ , related to functional groups of proteins, lipids and carbohydrates and other inorganic materials present in the blood serum. Fig. 1 (a) to Fig.1(e) are FTIR spectra of blood serum treated with glucose of concentration 2, 4, 6, 8 and 10 gm/dl respectively. Characteristic band for glucose can be seen at 1033.88  $\text{cm}^{-1}$ , approximately 1034  $\text{cm}^{-1}$ .

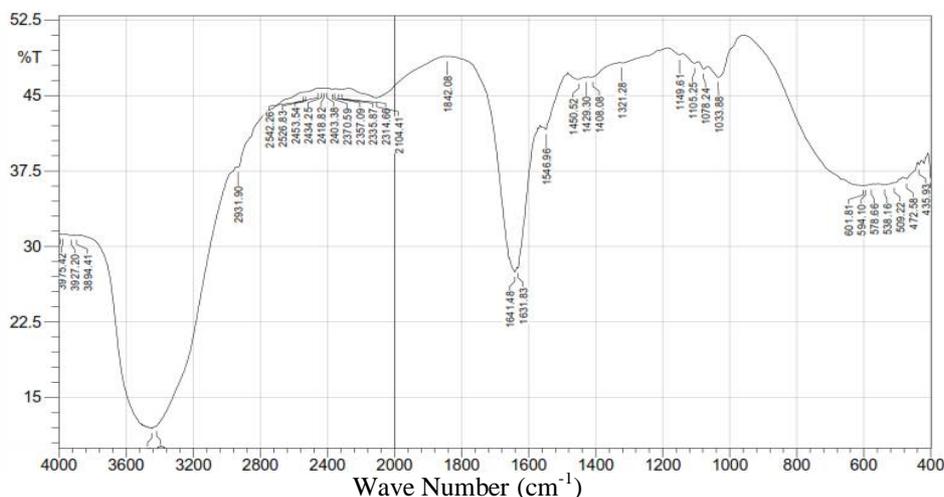


Fig. 1. (a). FTIR spectrum of human blood serum treated with glucose of concentration 2 gm/dl

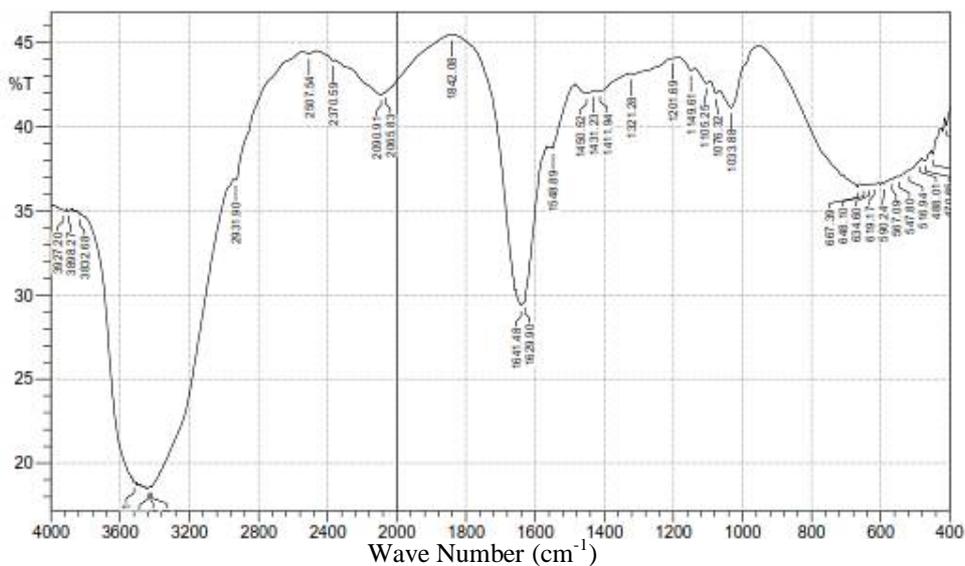


Fig. 1. (b). FTIR spectrum of human blood serum treated with glucose of concentration 4 gm/dl

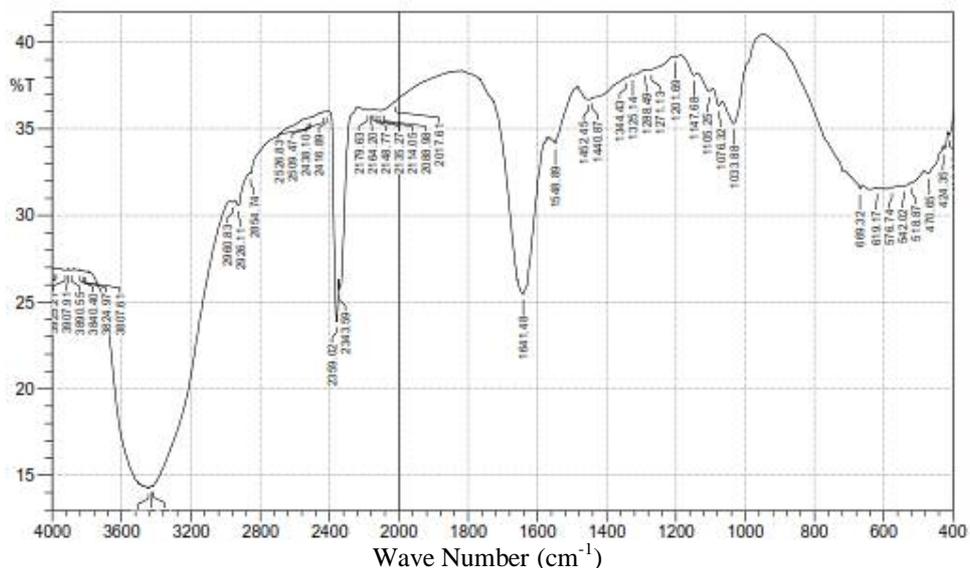


Fig. 1. (c). FTIR spectrum of human blood serum treated with glucose of concentration 6 gm/dl

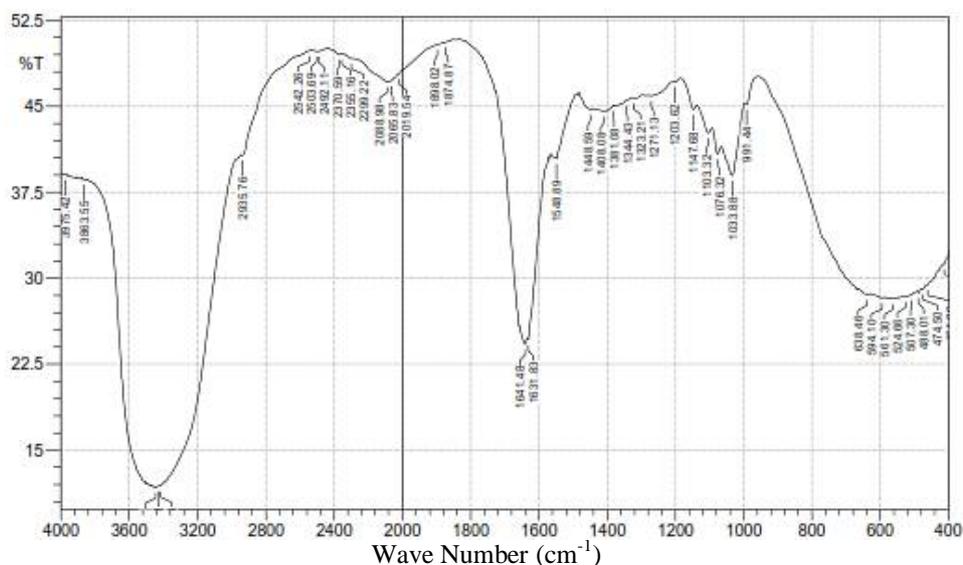


Fig. 1. (d). FTIR spectrum of human blood serum treated with glucose of concentration 8 gm/dl

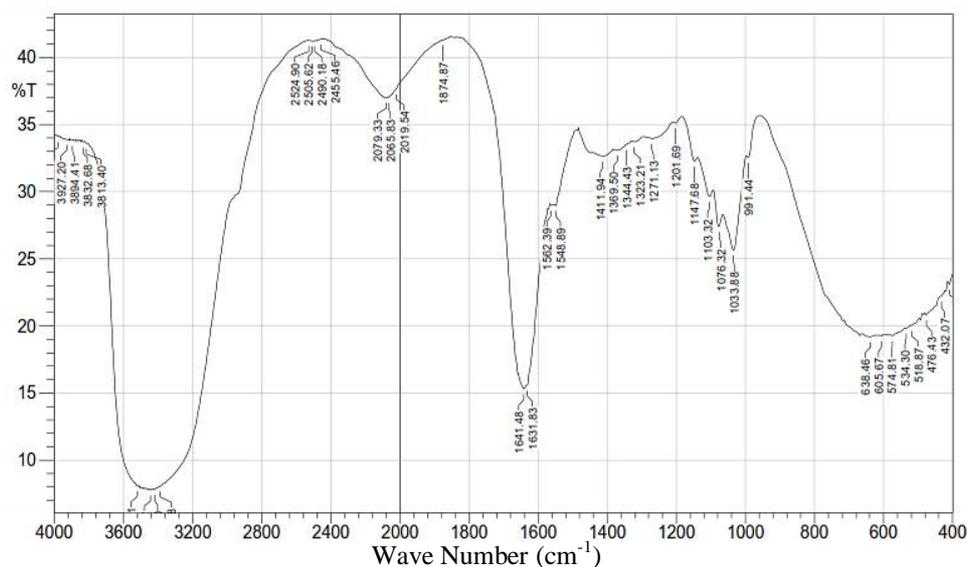


Fig. 1. (e). FTIR spectrum of human blood serum treated with glucose of concentration 10 gm/dl

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Table 1 gives FTIR data obtained from Fig. 1 at  $1034\text{ cm}^{-1}$ . The table reveals IR absorbance, calculated from transmittance (%), in blood serum treated with glucose of concentration ranging from 2 to 10 gm/dl with an interval of 2 gm/dl. A plot is drawn between Absorbance on Y – axis and glucose concentration (gm/dl) on X – axis (Fig.2). It is a straight line with +ve slope and also +ve Y – intercept, the linear regression equation of which is  $A = 0.0254 X + 0.2862$ , where A is absorbance and X is glucose concentration, and  $R^2 = 0.98$ .

Table 1 – Absorbance of IR bands of serum treated with glucose of different concentration

Glucose concentration (gm/dl)	Absorbance
2	0.330
4	0.386
6	0.452
8	0.496
10	0.529

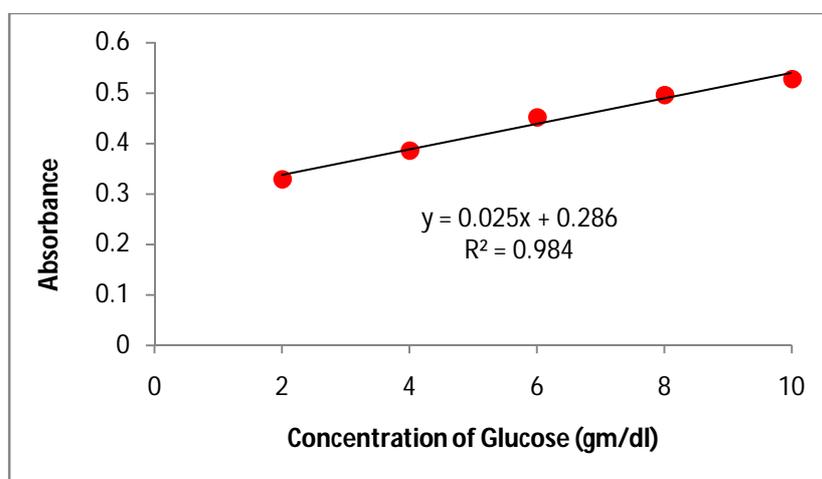


Fig. 2. A plot between IR absorbance and concentration of glucose in human blood serum

The study concludes that FT-IR spectrum is a finger print of molecular constituent of the sample. The absorbance positions of bands at 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 glucose is around at  $1034\text{ cm}^{-1}$  in human blood serum.

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