

RESEARCH ARTICLE

A STUDY ON INFRARED SPECTROSCOPY OF HUMAN BLOOD OF PATIENTS SUFFERING FROM DIABETES MELLITUS

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Accepted 22nd May, 2017; Published Online 30th June, 2017

ABSTRACT

The paper reports IR spectroscopic data on blood of patients suffering from *Diabetes mellitus*. IR analysis has been made on whole blood, plasma and serum. The characteristic spectral bands pertaining to glucose in the medium of blood are identified.

Key words: FTIR spectroscopy, Diabetes mellitus, Human blood, Plasma, Blood Serum.

INTRODUCTION

FTIR spectroscopy is being used by chemists as a powerful tool to characterize organic and inorganic compounds. It has been applied in biology for studying the structures and conformation of molecules like proteins, nucleic acids and lipids. The advances made in instrumentation such as development of IR microscopy, definitely, paved the way for the use of FTIR spectroscopy in the discipline of medicine. The role of FTIR spectroscopy in diagnostic aspects involving body fluids, besides tissue diagnostics, is gaining importance. The mid IR region has been proved to be useful in the identification of disease patterns by the use of IR spectra of human blood serum. Precise measurement of glucose, total protein, lipids, cholesterol, urea and pigments like bilirubin could be possible with FTIR spectroscopy. Cyril Petibois *et al.*, 1999 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 cm⁻¹. They integrated all spectral windows at the surface of the spectrum in the CO 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. Syed Ismail Ahmad *et al.*, 2010 made an attempt to estimate concentration of glucose in human urine. They reported specific band at 1034 cm⁻¹ for glucose and also a relation between glucose concentration in urine and Transmission (%) of IR band. Shikha Rathore (2014) analyzed,

spectroscopic ally in IR region, normal and laser exposed human blood in order to examine the influence of low power red coherent light at molecular and cellular levels. Wanjie Zhang *et al.*, (2013) 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 interferent compositions in the sample matrix. They evaluated the validity of the spectral data set, instead of assessing the calibration models, and then to provide a complementary procedure for better verifying or rejecting the data set. Further, they demonstrated the utility of the proposed analysis with a series of aqueous solutions using near-infrared spectra over the overtone band of glucose and results concluded that spectral variations from chance correlations induced by those experimental factors could be determined by the 2DCOS method. The aim of the present study is to evaluate the utility of FTIR spectroscopy for the estimation of glucose in the blood of patients suffering from Diabetes mellitus.

MATERIALS AND METHODS

The blood samples were collected from patients suffering from Diabetes mellitus. The FT-IR spectrum of blood was recorded. First, spectral grade pure KBr powder was dried in an oven up to 60°C for 24 hours. Then 1gm powder was taken in an agate motor and was ground until it becomes fine powder. The ground powder was mixed with blood sample and transferred into the bore of a cylinder so that it was distributed across the polished face of lower plate. The polished face of the second plate towards the powder was inserted in to the bore by a plunger. The die assembly was connected to a vacuum pump and was kept under vacuum for approximately 2 min so as to

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remove air from the sample disk. The die was dismantled and the KBr disk was removed without touching its faces. Here, FTIR spectrometer of make *Bruker Optics* and model *Tensor 27* was used. The resolution was kept at 4 cm^{-1} and scanning time was fixed at 38 Sec. A total number of 32 scans were carried out on each sample. The scanning range fixed from $4000 - 400\text{ cm}^{-1}$ for each sample. And also the ranges $2000-1400\text{ cm}^{-1}$, $1400 - 600\text{ cm}^{-1}$ and $1200 - 1000\text{ cm}^{-1}$ were carried out.

RESULTS AND DISCUSSION

Fig.1. shows a typical FTIR spectrum of blood of patient suffering from Diabetes mellitus. The spectrum shows a series of IR bands in the range of 4000 cm^{-1} to 400 cm^{-1} . The IR spectral data is presented in Table 1. The data shows wave numbers and corresponding transmittance (%) along with characteristic vibrations of functional groups.

Table 1. FTIR data on Blood of diabetic patient

Wave Number (cm^{-1})	Transmittance (%)	Characteristic vibrations of functional groups
3304	18	N-H in $\nu_{\text{N-H}}$
3064	42	Amide-B, N-H stretching, Methoglobin
2960	43	C-H str (asymmetry) of $-\text{CH}_3$ in fatty acids, Phospholipids, Cholesterol esters
2930	60	-C-H symmetric stretching of $-\text{CH}_2$ Platelets
2873	56	-C-H symmetric stretching of CH_3
2361	95	CO_2 (atmospheric absorption) ν_{as} (str)
2094	90	$\text{C} \equiv \text{C}$ (Symmetry reduces intensity)
1656	13	Amide-I, α - helical structure
1544	20	Amide -II peak region protein (NH,C-N)
1454	47	CH_2 bend. CH_2CH_3 bending modes
1403	38	Amino acid, $\nu_{(\text{N}=\text{O})}$, CH_2, CH_3 bending modes
1395	41	Fibrinogen
1308	53	-P=O asymmetric stretching of PO_2^-
1315	52	-P=O asymmetric stretching of PO_2^-
1248	54	Normal erythrocyte membrane or $\nu(\text{C}-\text{O}^*)$ of $-\text{COOH}$
1170	66	C-O, C-H str C-O-H, C-O-C deformation of carbohydrates
1118	65	Triglycerides
1106	66	Oxy haemoglobin
1080	66	Urea
988	80	C-O, C-C, stretching C-O-H, C-O-C deformation of carbohydrates
954	79	Finger print region
932	79	C-O-H of glycogen
833	68	CO_3^{2-}
739	53	$-\text{CH}_2$ rocking mode of fatty acid
701	48	S-OR esters
663	82	CO_2 (atmosphere) $\nu_{\text{s}(\text{CO}_2)}$ bending
621	47	ν_{S_2} in free state

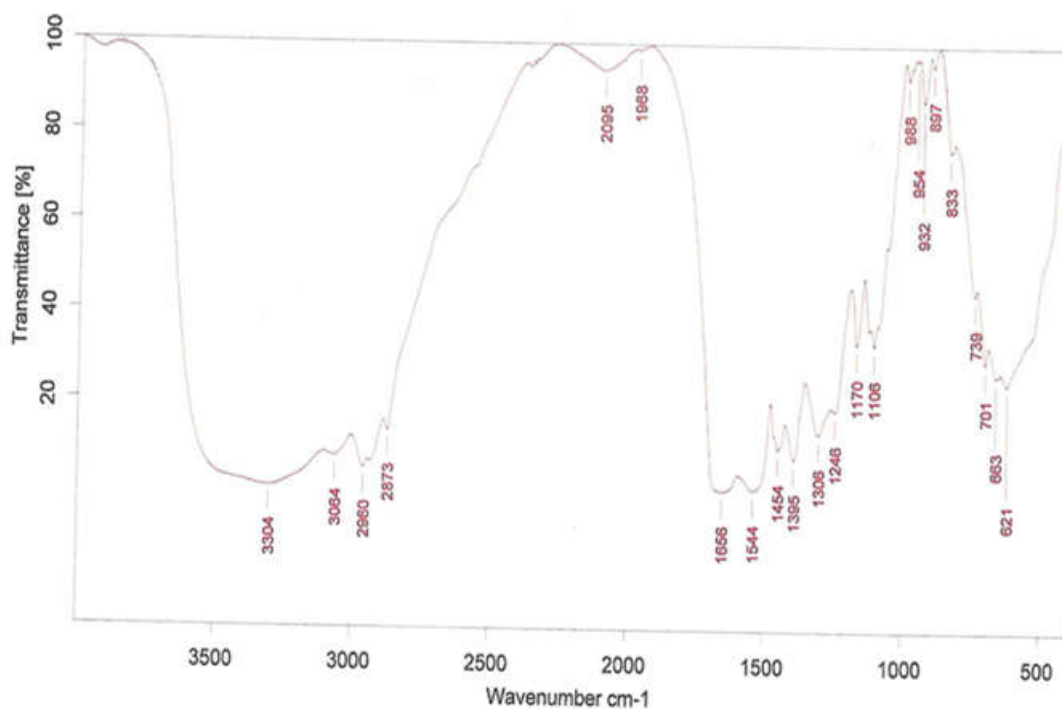


Fig. 1. A typical FTIR Spectrum of blood of diabetic patient

The IR spectrum serves as a 'finger print' of biological macromolecules present in the sample. It is to note that the intensities (% transmittance or % absorbance) of IR spectral bands provide quantitative information, while their absorption positions (in wave number) reveal qualitative information on the nature of chemical bonds, their structure and their molecular environment. In blood, major absorption bands arise from the functional groups such as NH, C=O, C-H and P=O concerned with proteins, lipids and nucleic acids. The region between 4000 – 3100 cm^{-1} is dominated by broad spectral characteristics bands arise from O-H stretching modes about at 3400 cm^{-1} and from N-H stretching modes due to Amide – A at 3300 cm^{-1} . The region between 3100 cm^{-1} and 2800 cm^{-1} reveals C-H stretching vibration of $-\text{CH}_3$ and CH_2 functional groups and hence dominated by fatty acids of cell membrane and also by some amino acid side chain vibrations. The bands in the region between 1470 cm^{-1} and 1350 cm^{-1} are concerned with various deformation modes of the functional groups, hence, are considered to present complimentary information. Further, the region between 1800 cm^{-1} and 1500 cm^{-1} is dominated by conformation sensitive Amide – I and Amide – II bands, which are very sensitive bands as far as biological complex systems are considered. As is known, IR spectroscopy is an averaging technique and as such Amide – I and Amide – II bands cannot provide structure information of a particular protein, but can suggest the dominant conformation α or β or both. The IR spectrum of a molecule shows its characteristic absorptions. In the case of glucose, the following absorption bands may be found: $\nu(\text{O-H})$ between 3570 and 3120 cm^{-1} , $\nu(\text{C-H})$ between 3085 and 3020 cm^{-1} , $\nu(\text{C-O})$ between 1230 and 1000 cm^{-1} , and $\nu(\text{C-O-C})$ between 1275 and 800 cm^{-1} . The two latter bands are known to be the most specific of glucose molecule in complex spectra (Bauer and Floyd, 1987; Ward *et al.*, 1997). The mid IR spectral bands of glucose and other carbohydrates have been assigned to C-C, C-H, O-H stretching and bending vibrations.

The finger print region of the IR spectrum of glucose is 800 cm^{-1} to 1200 cm^{-1} (Chris *et al.*, 2007). In the present study, the bands around 834 cm^{-1} , 1080 cm^{-1} , 1247 cm^{-1} are concerned with C-H bending vibrations. The band at 988 cm^{-1} is related to C-O, C-C stretching; and C-O-H, C-O-C deformation. The characteristic band for glucose in blood and serum is at 988 cm^{-1} .

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