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A STUDY ON INFRARED SPECTROSCOPY OF HUMAN BLOOD

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Abstract: The paper reports IR spectroscopic data on human blood and its constituents. IR analysis has been made on whole blood, plasma and serum. The characteristic spectral bands pertaining to fibrinogen, hemoglobin, erythrocyte membrane lipids and other plasma proteins are identified. The paper explores the possibility of disease analysis by IR spectroscopy.
Keywords: FTIR spectroscopy; Human blood; Plasma; Blood Serum.

1. Introduction

In recent past, mid infrared and UV - Visible spectroscopic methods were efficiently used in the fields of biological sciences [1, 2]. Implementation of these techniques reduces time, resources and cuts cost. IR spectroscopy is emerging as a potential diagnostic tool in the medical and pharmacological fields to provide information about the different chemical structures of healthy and pathological tissues [3]. Blood being the chief circulatory medium of our body, reflects the physiological and pathological changes that take place in the tissues, which lead to the changes in the various plasma, serum and cellular constituents. In view of this, an attempt is made to analyse human blood spectroscopically in the IR range.

2. Materials and Methods

2.1. Sample collection

Collection and handling of a sample is an integral part of obtaining valid results. Here a disposable plastic syringe was used to collect venous blood. Blood samples were collected from healthy volunteers. Blood collection tubes with anticoagulant (EDTA- Ethylene Diamine Tetra Acetate) were inverted gently as soon after collection as possible to prevent clotting. The blood samples were brought to the laboratory in siliconized bottles, keeping them in ice cooled thermos. The samples were kept in refrigerator at 4^0C until used. Investigations were done within two to three hours after collection.

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2.2. Preparation of sample

First, spectral grade pure KBr powder was dried in an oven upto 60^0C for 24 hours. Then 1 gm 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 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.

3. Results and Discussion

Fig. 1. presents FTIR spectrum of Human blood, which reveals a series of bands with different intensities and the spectral data is shown in Table 1. For the systematic analysis, IR spectrum is divided into three regions. Region I is from 4000 to 3000 cm^{-1}, concerned with water and hydroxyl group. This region is of considerable interest, because it reveals the nature of hydrogen bonding. Region II is 3000 to 1500 cm^{-1}, wherein bands for functional groups are observed. In this region, major IR absorption pertaining to fibrinogen occurs. Region III is 1500 – 200 cm^{-1}, which has significant importance in the context of biological minerals and their combinations.

The spectra of human blood indicate the presence of bands characteristics of water molecule and also of some functional groups concerned with proteins and lipids. The IR band at wave numbers 3294 cm^{-1} and 3065 cm^{-1} are related to Amide A and Amide B respectively. The dominating band at 1396 cm^{-1} may be originated due to the important protein of blood Fibrinogen. This band is related to the stretching C=O symmetric stretching vibrations of COO^-. A band around 2960 cm^{-1} is due to the -C-H asymmetric stretching of $–CH_3$ in Fatty acids, Phospholipids and Cholesterol esters. The band at 1106 cm^{-1} is related to HbO_2, exhibits $\nu_{(O2)}$ bond. The two most intensive bands are centered at 1652 cm^{-1} and 1547 cm^{-1} in the FTIR spectrum of human blood. They correspond to the Amide I and Amide II. Both bands are representative of secondary structures of proteins. Amide I peak arises from C=O hydrogen bonded stretching vibrations, and Amide II is attributed to C-N stretching; NH and CH_2 bending modes. Amide I and Amide II absorption bands are associated also with specific secondary sub- structures, such as α-helix, β-sheet, β-turn and random coil. The bands at

1307 $cm^{-1}$and 1248 cm^{-1} are related to Amide III bond components of proteins (C-N). The band at 1170 cm^{-1} corresponds to C-O-C asymmetric stretching vibrations of phospholipids. The bands at 1106 cm^{-1}, 1170 $cm^{-1}$and 1248 cm^{-1} are associated with triglycerides of human blood. The band at 2936 cm^{-1} is related to platelets due to -C-H symmetric stretching of $–CH_2$

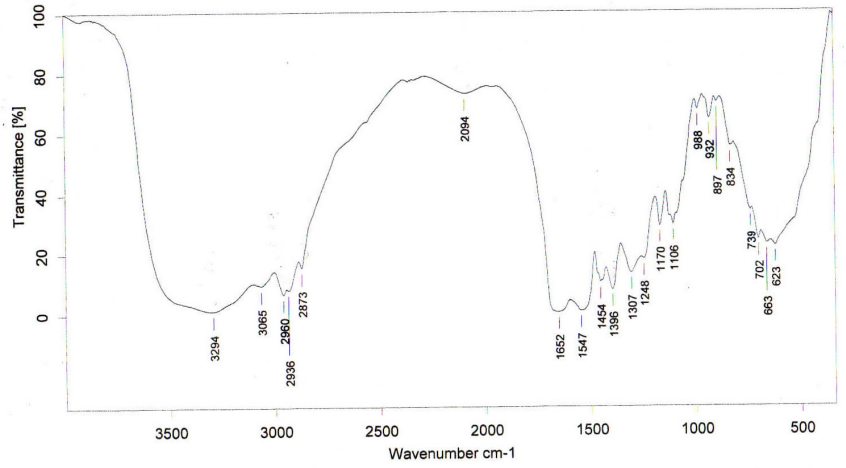

Fig. 1. FTIR spectra of human blood

Table 1- FTIR spectral data of human blood

Wave Number (cm^{-1})	*Characteristic vibrations of functional groups*
3294	Amide A, N-H stretching of proteins
3065	Amide B, N-H stretching
2960	-C-H asymmetric stretching of $–CH_3$ in **Fatty acids**, **Phospholipids**, **Cholesterol esters**
2936	-C-H symmetric stretching of $–CH_2$, **Platelets**
2873	-C-H symmetric stretching of $–CH_3$
2094	
1652	Amide I. – helical structures (C=O)
1547; 1454	Amide II Peak region – Protein (NH, C-N) CH_2 bend
1396	C=O symmetric stretching vibrations of COO^-, **Fibrinogen**
1307, 1248	Amide III band components of proteins (C-N)
1170	C-O-C asymmetric stretching vibrations of phospholipids
1106	HbO_2 exhibits $\nu_{(O2)}$ band, **Oxy hemoglobin**
1106, 1170, 1248	Triglycerides

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