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FTIR SPECTROSCOPIC ANALYSIS ON HUMAN BLOOD GROUPS

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Abstract: IR spectroscopic data on human blood of groups A, B, AB and O is presented. IR analysis is made on 90% packed erythrocytes. The characteristic spectral bands pertaining to antigens are discussed. The paper explores the possibility of identification of blood antigens spectroscopically.
Keywords: FTIR spectroscopy; human blood; blood groups.

1. Introduction

FTIR is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like molecular *finger print*. This method is used in the different fields of sciences [1, 2, 3]. The FTIR technique reduces time, resources and cuts cost. IR spectroscopy is as a potential diagnostic tool in the medical fields like a pharmacological and pathological. Andreas Barth [4] reviewed and discussed the application of infrared spectroscopy to the study of proteins. He focused on the mid-infrared spectral region in the study of protein reactions by reaction-induced infrared difference spectroscopy. Heinz Fabian and Werner Mantele [5] described IR instrumental techniques for steady state absorbance and reaction – induced difference spectra and reported sampling procedures available to obtain IR spectra of proteins, peptides, amino acids and more complex enzymes.

The present study is an attempt to explore the possibility of characterization of blood groups IR spectroscopically.

2. Materials and Methods

A disposable plastic syringe was used to draw venous blood. Blood samples were collected from healthy volunteers of blood group A, B, AB and O. 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 silliconized bottles, keeping them in ice cooled thermos. The samples were kept

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in refrigerator at 4^0C until used. Investigations were done within two or three hours after collection.

The blood samples were centrifuged at 1500 rpm in order to have 90% packed erythrocytes. Thus packed cells were separated and used for FTIR spectral analysis.

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. Thin KBr disk was prepared by transferring the powder into the bore of a cylinder, so that it was distributed across the polished face of lower plane. The polished face of the second plane towards the powder was inserted into the bore by a plunger. The 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. The smear of blood sample was taken on KBr disk for IR spectral analysis. Infrared spectrum was recorded in Fourier Transform Infrared spectrophotometer (Shimadzu FTIR - 8400S) in the range of 4000 cm^{-1} to 400 cm^{-1}.

3. Results and Discussion

Fig.1. shows FTIR spectra of 90% packed erythrocytes of blood groups A, B, AB and 0. Spectra show series of bands pertaining to proteins, carbohydrates, lipids and inorganic compounds in the spectral range of 4000 cm^{-1} to 400 cm^{-1}.

Table 1 presents FTIR data on functional groups pertaining to antigens of human blood of groups A, B, AB and O obtained from spectra shown in Fig. 1. The presented data is concerned with the components of antigens of human blood.

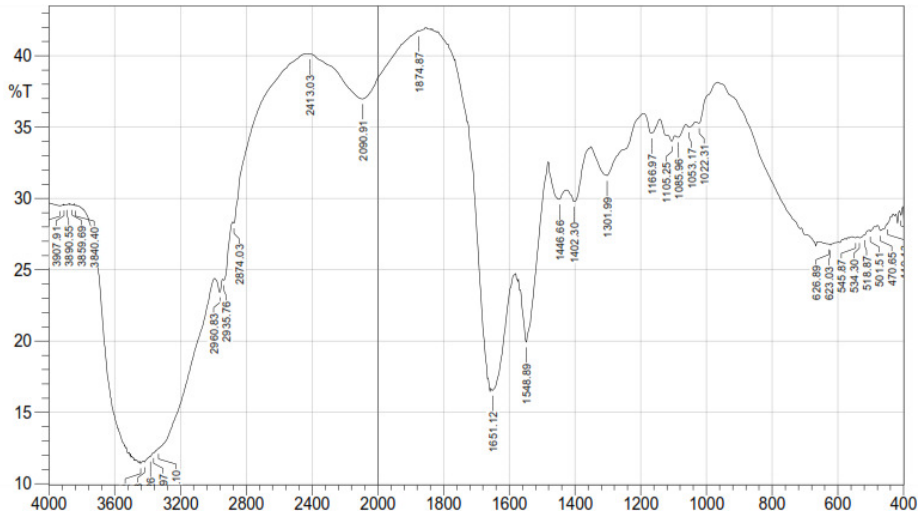

Fig. 1(a). FTIR spectrum of human blood of group A

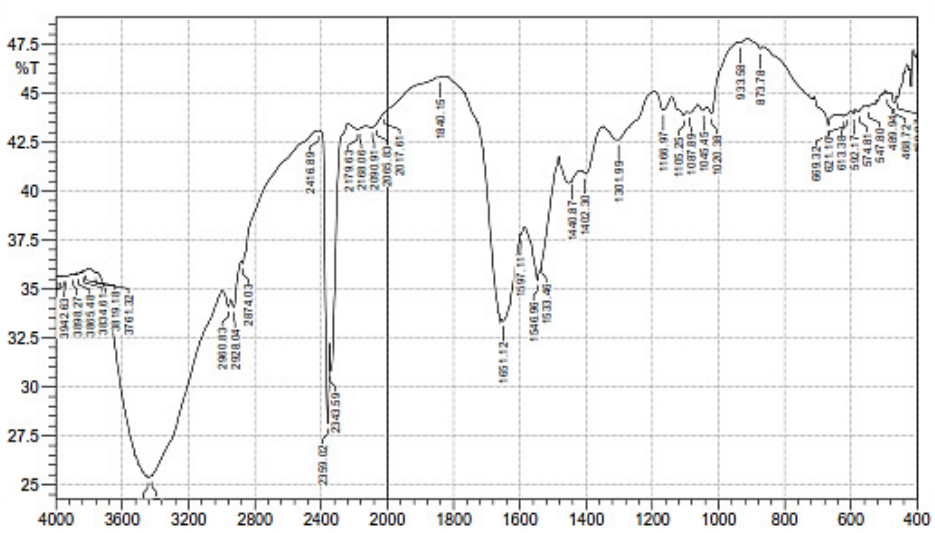

Fig. 1(b). FTIR spectrum of human blood of group B

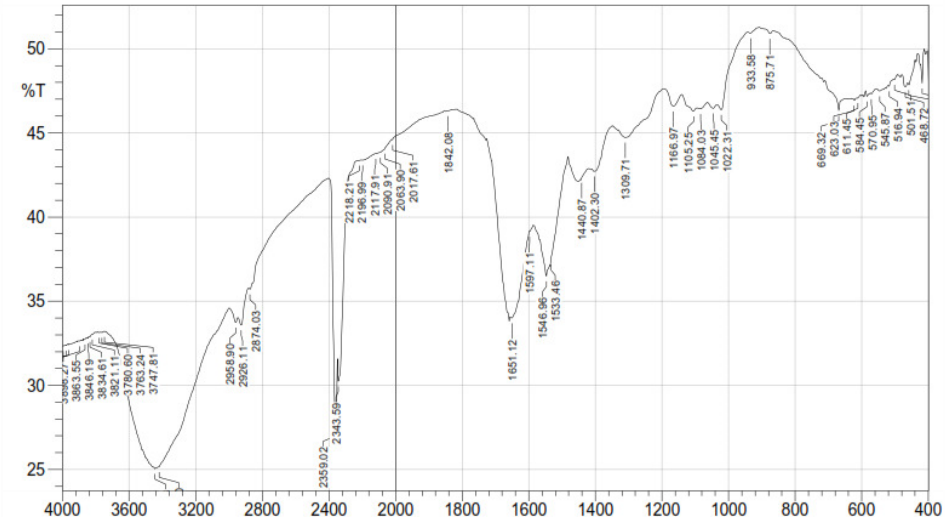

Fig. 1(c). FTIR spectrum of human blood of group AB

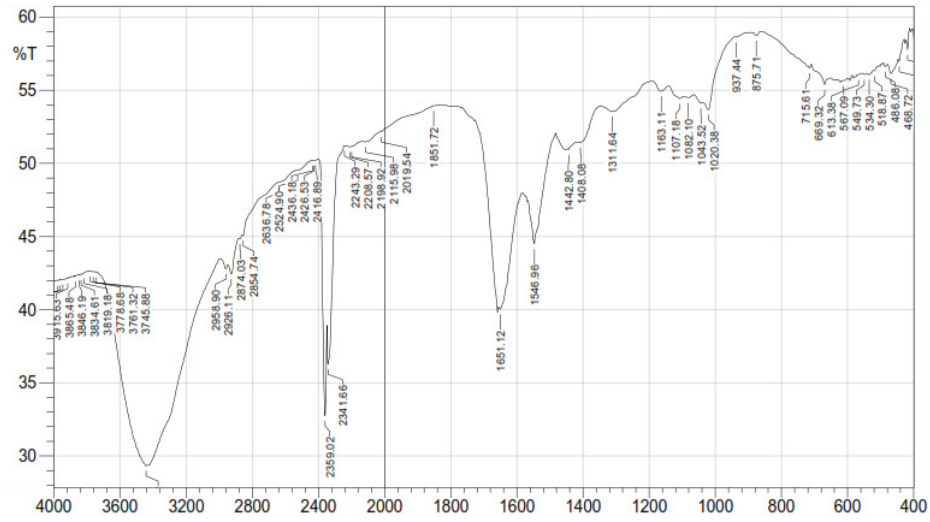

Fig. 1(d). FTIR spectrum of human blood of group O

Table 1 - Characteristic FTIR spectral data on antigens of human blood

a				Functional groups
A	B	AB	O	
1166	1166	1166	1163	**Fucose** linked to **galactose** viaglycosidic linkage
1022	1020	1022	1020	**Fucose** residues linked to **GlcNAc** via glycosidiclinkage
1022	-	1022	-	**GalNAc** glycosidically bonded to O antigen
-	1166	1166	-	Additional **Galactose** glycosidically bonded to O antigen

It is known that the most basic oligosaccharide attached is called the O antigen (also referred to as the H antigen). This O antigen is the base oligosaccharide found in all three blood types O, A, and B. The O antigen is of the form (—Lipid—Glucose—Galactose—*N*-acetyl glucosamine—Galactose—Fucose). Blood type O only has the O antigen attached to the red blood cells.

Blood type A is formed through the addition of the A antigen, which has *N*-acetylgalactosamine (GalNAc), α-1, 2 glycosidically bonded to the O antigen. Similarly for blood type B, the B antigen has an additional galactose forming a glycoside bond to the O antigen. In both the A and B blood types, the new antigen forms an α-1,3 linkage to the outermost galactose component of the O antigen through the help of glycosyl transferases. GalNAc transferase adds the extra N-acetylgalactosamine for the A antigen while Gal transferase adds the extra galactose for the B antigen.

The ABO blood group system is very much reflected in FTIR spectra of human blood. The bands at 1166 cm^{-1} and 1020 cm^{-1} are related to fucose linked glycosidically with galactose and GlcNAc respectively, concerned with O antigen. GalNAc linked to O antigen through glycoside linkage exhibits a band at 1022 cm^{-1} pertaining to A antigen. A band at 1166 cm^{-1}reveals additional Galactoseglycosidically bonded to O antigen for B antigen. The results of the present study is in agreement with those of Lewis antigens in secretion of mucins [6, 7].

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