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MAGNETIC PROPERTIES OF METHEMOGLOBIN

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Abstract: The paper presents a brief introduction to hemoglobin and its various forms. The paper provides the standard method of preparing methemoglobin solution. It also reports the data on magnetic susceptibility of methemoglobin, when subjected to magnetic field produced by pole pieces of electromagnet. The magnetic susceptibility is used to find the allied parameters namely mass susceptibility, molar susceptibility, Curie constant and magnetic moment. The study suggests that the samples of methemoglobin are substantially affected by the magnetic field and exhibits diamagnetic behavior.
Keywords: Methemoglobin, Cyanmethemoglobin, Mass susceptibility, Molar susceptibility, Curie constant, Quincke's method, Magnetic moment.

1. Introduction

Hemoglobin is the main component of the red blood cell. Its function is to transport oxygen from places of high oxygen pressure to places of low oxygen pressure, that is from lungs to tissues/organs. Hemoglobin is also responsible for the transport of CO_2 from places of low oxygen pressure to high oxygen pressure [1, 2]. Hemoglobin consists of one molecule of globin and 4 molecules of heme. Globin consists of two pairs of polypetide chain, and each heme contains one molecule of iron in ferrous state [2]. During this transportation, hemoglobin reacts with oxygen in lungs to form oxyhemoglobin, whereas in tissues with the release of oxygen they are resulted into reduced hemoglobin [3].

There are various forms of hemoglobin, as they readily reacts with acids, bases, oxidizing and reducing agents. The various forms include oxy-hemoglobin, carboxy-hemoglobin, methemoglobin, sulfhemoglobin and cyanmethemoglobin. These variation of hemoglobin is due to structural difference in globin protein. Cyanmethemoglobin is a compound results when methemoglobin combines with cyanide radical. Cyanmethemoglobin is recommended by the international committee for standardization in hematology [5].

As hemoglobin concentration is directly proportional to the oxygen combining capacity of blood. Hence, measurement of the hemoglobin concentration in the blood is important in

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diagnosing for diseases associated with anemia and for its treatment [3,6&7]. The hemoglobin is being studied extensively by physiologist, biochemists and hematologists, but the characteristics of hemoglobin have not drawn much attention of physicists. However, some reports are available on magnetophoresis [8, 9] and dielectrophoresis of erythrocytes and dielectric properties of human blood and erythrocytes [2]. Earlier, investigations were made by the author on magnetic properties of healthy human blood, and blood of healthy chicken [6, 7]. Therefore, the main aim of this investigation is to determine the magnetic properties of methemoglobin.

2. Materials and Methods

2.1 Sample collection and preparation

There are four basic methods of preparing and measuring of the hemoglobin concentrations. They are gasometric method, gravimetric method, chemical method and colorimetric method [4, 5]. In the present investigation, a type of colorimetric method, cynamethemoglobin methods is employed. The principle of this method involves mixing of blood initially with a solution of potassium ferricyanide and then later with a solution of potassium cyanide. Potassium ferricyanide oxidizes the iron present in the blood samples to methemoglobin, and then potassium cyanide combines with methemoglobin to form cyanmethemoglobin [4]. There are three reasons for selecting cynamethemoglobin method (also known as Drabkin's method) are as cyanmethemoglobin reagent is the most stable hemoglobin pigment, as the method is a standard in hematology, and as cynamethemoglobin reagent measures all forms of hemoglobin except sulfhemoglobin [5].

Blood samples of hemoglobin were prepared using cyanmethemoglobin reagent which was commercially available at Span diagnostic limited. 10 samples were prepared with 5 ml of cyanmethemoglobin reagent in each tube with 20 μl of blood sample. Test tubes were kept aside corked until a convenient time for estimation. The mixture was then kept in colorimeter/spectrophotometer to measure the O. D of the test against drabkin's solution at 540 nm. The blood hemoglobin in g% may be directly calculated as integral multiple of 0.251 with the reading on the O. D [3].

2.2 Experimental

There are three main classes of experiment by which the magnetic susceptibility of material may be measured. Quincke's method was selected for the investigation of magnetic susceptibility of samples prepared with cyanmethemoglobin reagent. The experimental setup of Quincke's method in detail is mentioned elsewhere [3, 6 & 7].

A Quincke's tube is a U - shaped glass tube with one limb very narrow and the other one wide. The length of the limb is about 20-30 cm and diameter of the limb is about 1 mm. After the preparation of the samples at temperature 28-30 ºC, each sample was systematically taken in Quincke's tube, and placed between the pole pieces of an electromagnet. The readings of the meniscus were observed by a microscope of the order of 10^{-3} cm, without field and with varying magnetic field, H. The magnetic susceptibility and allied parameters namely mass susceptibility, molar susceptibility, Curie constant and magnetic moment of distilled water were calculated with the formulae mentioned elsewhere [3, 6].

3. Results

Table 1 shows the calculated values of susceptibilities and allied parameters namely mass susceptibility, molar susceptibility, Curie constant and magnetic moment of distilled water, and ten methemoglobin samples prepared using cyanmethemoglobin reagent. Distilled water was employed in the investigation to calibrate the instrument. The average molecular weight of distilled water and prepared samples employed in the calculation is 18 and 60,000 respectively. The values of susceptibility and Curie constant are calculated at the temperature of 28-30 ºC.

Table 1: Data showing calculated susceptibilities of distilled water and 10 methemoglobin samples (MHb)

Sample	Mass susceptibility, χ_{mass} (x 10^{-7} cm^3g^{-1})	Molar susceptibility, χ_{molar} (cm^3 mol^{-1})	Curie constant	Magnetic moment, μ_m
Distilled water	9.457	1.697 x 10^{-5}	5.112 x 10^{-3}	0.202
Methemoglobin Samples:				
MHb1	9.457	1.697	5.112	0.202
MHb2	4.546	0.027	8.211	8.103
MHb3	5.066	0.030	9.149	8.554
MHb4	4.344	0.026	7.845	7.921
MHb5	6.288	0.038	11.355	9.530
MHb6	4.762	0.029	8.600	8.293
MHb7	7.113	0.043	12.846	10.136
MHb8	6.803	0.041	12.286	9.913

MHb9	6.146	0.037	11.100	9.422
MHb10	6.237	0.037	11.264	9.491
Mean:	5.618	0.034	10.147	9.008
S. D.:	±0.972	±0.006	±1.755	±0.780

4. Discussion

Hemoglobin is the major components of red blood cells, and is responsible for their red color. Its constitute about 34% concentration in erythrocytes. It is the most important respiratory protein by virtue of its ability to transport oxygen from the lungs to body tissues, and to facilitate the return transport of carbon dioxide. Hemoglobin is a tetramer; composed of four heme groups and 2 pairs of polypeptide chains called globins. Iron is bonded to four pyrrole nitrogens of protoprophyrin, and to an imidazole nitrogen of histidine residue from the globin side of the porphyrin. The sixth coordination position is available for binding with other small molecules such as O_2, CO or CO_2. As a result of this binding, the hemoglobin distorts in the process.

Methemoglobin refers to ferrihemoglobin (Fe^{3+}) which contains iron in the +3 oxidative state, whereas ferrohemoglobin (Fe^{2+}) is referred to reduced hemoglobin containing iron in +2 reduced and oxidized state.

Measurement of magnetic susceptibility of blood were performed initially by Faraday in 1845 [12]. During early 20th century, based on the experiment on quantum chemistry, it was pointed that the magnetic susceptibility measurements of hemoglobin and its derivatives played an important role in the study of the molecular structure of the heme group [10]. The magnetic susceptibility of hemoglobin explains the molecular and electronic structure of chemical bonds in the heme group, and the magnetic measurements made on human erythrocytes exhibited diamagnetic behavior [11].

In the same context, Table 1 clearly shows that data on methemoglobin samples exhibit diamagnetic behavior. This behavior is because of heme group consisting of Fe^{2+} or Fe^{3+} ion which is held in heterocyclic ring known as prophyrin, which is the main cause of electric and magnetic properties of blood cells, its constituent and derivatives. It is found that there is a non-linear relationship between the shifts in the sample with the variation of applied magnetic field. Thus, the non-linear relationships of the samples are attributed to the magnetization with the field. The Table 1 also gives the susceptibility values of distilled water, and it is found that water also exhibits diamagnetic behavior.

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