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Magnetic behavior of human erythrocytes at different hemoglobin states

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Abstract The effect of a static magnetic field on human erythrocytes at different hemoglobin states (normal, oxidized and reduced hemoglobin) was investigated. Three different blood samples, normal, iron deficiency anemic and beta thalassemia minor, were studied. Measurements of the magnetization curves of the erythrocytes for all blood samples in all states showed diamagnetic behavior; however, oxidation was found to enhance this behavior. These measurements have also shown that the normal and iron deficiency samples in the reduced states exhibit a less diamagnetic response in comparison with the normal state. This result indicates that the reduction process gave rise to a paramagnetic component of the magnetization. Analysis of the measured paramagnetic behavior, using a Brillouin function, gave an effective magnetic moment of 8 μ_B per reduced hemoglobin molecule for both normal and anemic samples. This result shows that both anemic and normal blood have similar magnetic behavior and the only difference is the number of hemoglobin molecules per erythrocyte. For the beta thalassemia minor blood sample, magnetic measurements showed that both the normal and reduced states have almost the same diamagnetic behavior. However, this diamagnetic response is less than that for the normal state of the iron deficiency anemic sample. This result may indicate a low oxygen intake for the blood in the normal state for the beta thalassemia minor blood. All magnetic measurements were made using a vibrating sample magnetometer using field steps of 0.001 T from 1 T to –1 T.

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Introduction

Previous work on human hemoglobin has shown that oxygenated hemoglobin contains iron in the ferric state (Fe^{3+}), which has diamagnetic behavior. However, deoxygenated or reduced hemoglobin contains iron in the ferrous state (Fe^{2+}), giving the hemoglobin molecules a magnetic moment of about 4.9 μ_B. The presence of this moment causes the red cells to be captured in a high magnetic field gradient (Roath et al. 1990).

Other studies (Chen et al. 1998) on the behavior of light absorption by different human blood states (deoxygenated and sickle hemoglobin) upon exposure to high external magnetic field have shown different absorption peaks. These results indicate clearly a pronounced difference in the magnetic behavior between deoxygenated and sickle hemoglobin.

Other reports on the behavior of intact human erythrocytes with a magnetic field showed cell alignment as they were exposed to a high magnetic field. Higashi et al. (1993) have shown that erythrocytes were influenced by the application of a uniform static magnetic field of strength 1 T, and they were oriented with their disk plane parallel to the magnetic field direction. Furthermore, the degree of orientation was not influenced by the state of hemoglobin (oxy: diamagnetic; deoxy and reduced: paramagnetic). Also, Kuchel et al. (2000) have reported clear evidence of red cell alignment in a magnetic field gradient for erythrocytes with normal shape and volume.

In this work the magnetization behavior of female human blood is examined in order to gain a better understanding of the magnetic response of erythrocytes to an applied external magnetic field. Three blood samples were examined: sample (1) was normal blood, sample (2) was iron deficiency anemia blood and sample (3) was beta thalassemia minor blood. The three samples were

investigated at different states of hemoglobin (normal, oxy and reduced hemoglobin).

Materials and methods

In this study, human female whole blood was drawn using EDTA tubes, where three blood samples were studied. Each blood sample was divided into three parts representing normal, oxidized and reduced states. The normal part was diluted 1:1 with normal saline. In order to oxidize the blood, a solution of 5% potassium ferricyanide was added in a ratio of 1:1 to the sample and then it was incubated for 5 min in a dark place at room temperature. The third part was treated with 2% sodium metabisulfite; this reducing solution was added to blood in a 1:1 dilution and incubated for 30 min at room temperature. The three parts were then centrifuged at 3000 rpm for 5 min. The supernatants were discarded and the sediments were frozen and lyophilized. The samples were then pressed to form disks of 1 cm diameter using a Riken presser (600 kg/cm^2).

To confirm oxidation and reduction of the blood, the absorbency of the blood at 540 nm was measured. Hemoglobin (Hgb) concentration, mean cell volume (MCV), mean cell hemoglobin and red blood count were measured for each of the three parts of blood at the Al-Khaldi Central Medical Laboratory (Hematology Department), Amman, Jordan. The values obtained for the samples examined are shown in Table 1.

Measurements of magnetization curves up to 1 T were made using a Micro-Mag 3900 VSM system that has a sensitivity of 5 e.m.u. on a 1 s averaging time scale.

Results and discussion

Samples characterization

From the data in Table 1 it is clear that there is a pronounced difference in the red cell values between sample (1), sample (2) and sample (3). The values for sample (1) are within the normal range for a female, while for samples (2) and (3) the Hgb and MCV are decreased; this is an indication of blood anemia. The patient that produced sample (3) has a family record of thalassemia, while for the patient that produced sample (2), no such record could be found. To differentiate between the two samples, the discriminant factor (DF) was calculated for both samples using the England-Frazer formula:

$$\mathrm{DF} = \mathrm{MCV} - (\mathrm{Hgb} \times 5) - \mathrm{RBC} - 3.4 \quad (1)$$

where RBC is the red blood cell count. The DF is a mathematical manipulation of the indices and generates a number which can be used to help differentiate between thalassemia minor and iron deficiency. If the DF is found to be positive, then the case is diagnosed as iron deficiency; if it is negative, then it is diagnosed as thalassemia minor. DF was found to be positive for sample (2) and negative for sample (3). To confirm that sample (3) is thalassemia minor, Hgb electrophoresis was performed and the results were given as Hgb A = 94.6% of the total, Hgb A2 = 4.1% of the total and Hgb F = 1.3% of the total.

From the above results it is clear that Hgb A is decreased while Hgb is increased, as expected for a beta thalassemia minor case. In summary, the three samples are: sample (1), normal; sample (2), iron deficiency anemia; and sample (3), beta thalassemia minor.

From the data in Table 1 the number of hemoglobin molecules N within an erythrocyte was calculated using a value of 68,000 for the molecular weight of the hemoglobin molecule (Hillman and Ault 1995). The values obtained for N are 267×10^6 molecules/erythrocyte, 164×10^6 molecules/erythrocyte and 171×10^6 molecules/erythrocyte for samples (1), (2) and (3), respectively.

Magnetic measurements

Figures 1 and 2 show the measured magnetization curves for sample (1) and sample (2), respectively. These data show that for both samples examined the blood in the normal state has diamagnetic behavior while in the reduced state they exhibit a lesser diamagnetic response. These data infer that the reduction process introduces a paramagnetic component into the magnetic behavior of blood cells. For sample (2), it can be also seen that the blood in the oxidized state has a larger diamagnetic susceptibility than the normal one, which is expected as a result of the oxidation process.

The magnetic behavior of the red blood cells in the reduced state is due to the diamagnetic contribution of the core electrons of the atoms that make up the hemoglobin molecules, the cell membrane and due to the paramagnetic contribution of electrons of the iron atoms. The magnetization curve of blood cells will be the sum of all these contributions.

In order to account for the paramagnetic component that is introduced by the reduction process, the data for samples (1) and (2) can be corrected by subtracting the diamagnetic component using the diamagnetic susceptibility obtained for the normal case. Hence the paramagnetic component of magnetization is given by:

$$M_\mathrm{P} = M_\mathrm{R} - \chi_\mathrm{d} H \quad (2)$$

where M_P is the paramagnetic component of magnetization, M_R is the magnetization for the reduced blood cells and χ_d is the diamagnetic susceptibility for the

Table 1 The values obtained for the blood samples examined

	Hemoglobin (Hgb) (±0.01) (g/dL)	Mean cell volume (MCV) (fL)	Mean cell hemoglobin (MCH) (pg)	Red cell count (±0.01) (10^6/μL)
Sample (1)	14.70	87.9	30.2	4.86
Sample (2)	9.20	62.9	18.5	4.98
Sample (3)	10.62	60.1	19.3	5.51

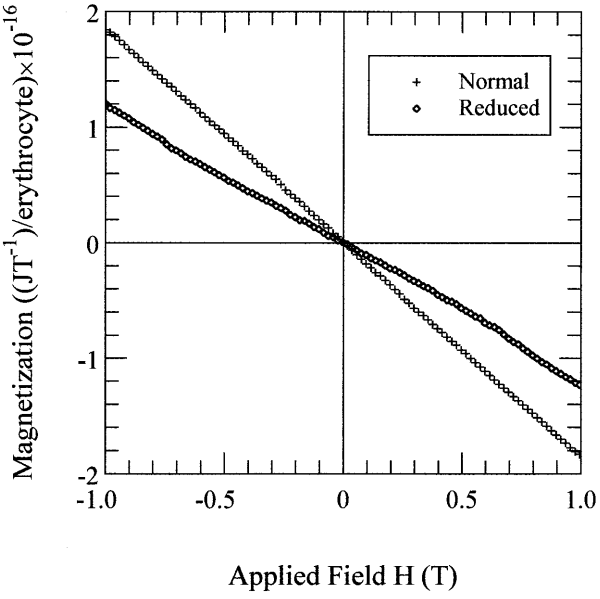

Fig. 1 Magnetization curves for sample (1)

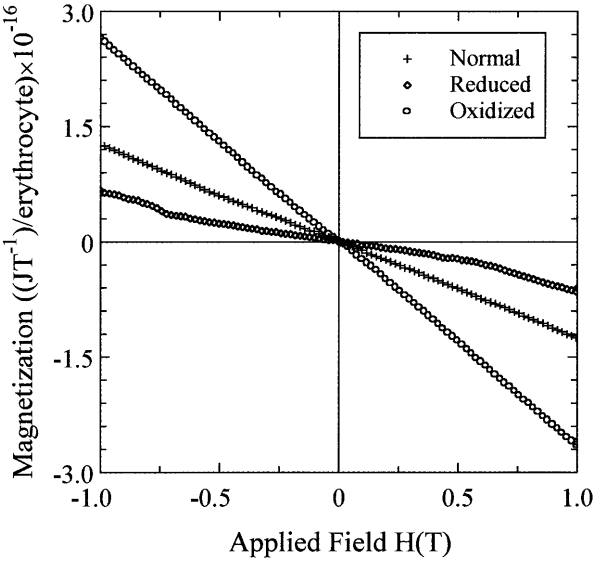

Fig. 2 Magnetization curves for sample (2)

normal blood cells. The obtained paramagnetic curves of reduced blood cells for samples (1) and (2) are shown in Fig. 3.

The paramagnetic behavior of reduced blood cells can be used to estimate the magnetic moment of each hemoglobin molecule in the blood cell by describing the response of these magnetic moments using the well-known Brillouin function $B(J,a)$ (Cullity 1972), which is usually given by:

$$\frac{M}{M_0} = B(J,a) = \frac{2J+1}{2J}\coth\left(\frac{2J+1}{2J}\right)a - \frac{1}{2J}\coth\frac{a}{2J} \tag{3}$$

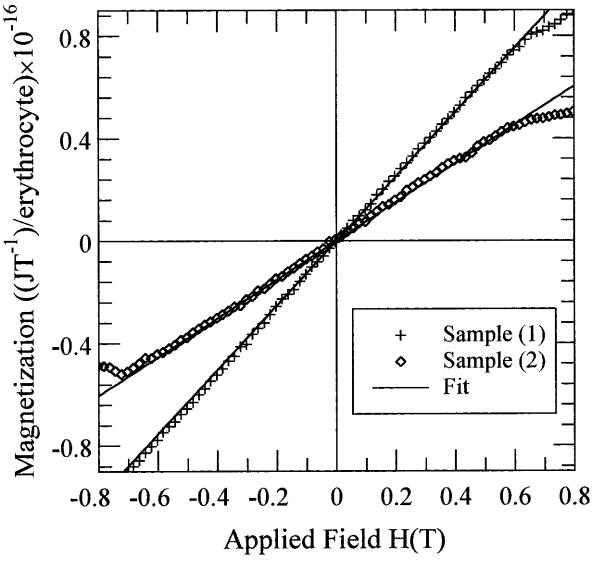

Fig. 3 The paramagnetic magnetization curves for sample (1) and sample (2). The *symbols* represent the experimental data. The *solid lines* represent the calculated magnetization curves using Eq. 3

where M_0 is the saturation magnetization of the red blood cell, which is given by:

$$M_0 = NgJ\mu_B \tag{4}$$

and:

$$a = \frac{gJ\mu_B H}{kT} = \frac{\mu_H H}{kT} \tag{5}$$

where μ_H is the maximum value of the magnetic moment of the hemoglobin molecule, μ_B is the Bohr magneton, J is the quantum number and g is the g factor.

At low magnetic field the Brillouin function reduces to:

$$B(J,a) = \frac{a(J+1)}{3J} \tag{6}$$

and the initial susceptibility is then given by:

$$\chi_i = \frac{N\mu_{\text{eff}}^2}{3kT} \tag{7}$$

where:

$$\mu_{\text{eff}} = g\sqrt{J(J+1)}\mu_B \tag{8}$$

Now using Eq. 6 for the initial susceptibility and the low field data in Fig. 3, a value of $\mu_{\text{eff}} = 8\ \mu_B$ was estimated for both samples (1) and (2).

Since the only magnetic ions in the hemoglobin molecule are due to iron, and the magnetic moments of these ions are entirely due to spin, orbital components being largely quenched, then $g = 2$. Using the obtained value of μ_{eff}, Eq. 7 gives a value of $J = 7/2$. Accordingly, the lines in Fig. 3 represent the calculated magnetization curves using Eq. 3 fitted to the parameters obtained for μ_{eff}, $g = 2$ and $J = 7/2$. The data show that the

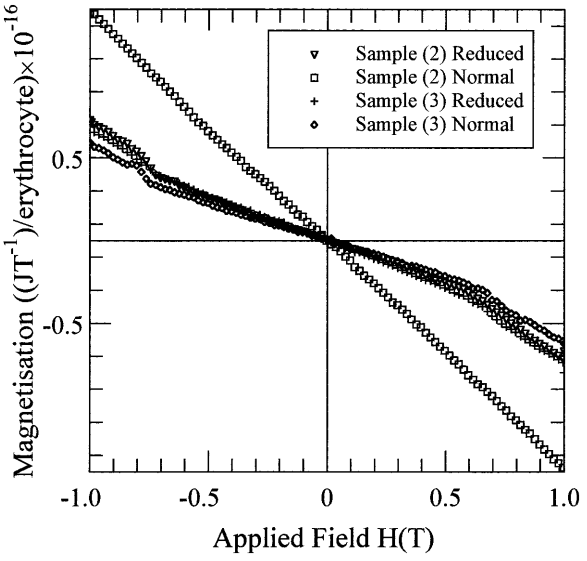

Fig. 4 Magnetization curves for sample (3) compared to those of sample (2)

experimental curves tend to saturate earlier than the predicted theoretical curves; however, the disagreement between the experimental data and the calculated curves is quiet small. The obtained value for μ_{eff} was not in good agreement with the estimated value given by Roath et al. (1990), where a value of $\mu_{\text{eff}} = 4.9$ was calculated on the basis that $g = 2$ and $J = 2$.

Figure 4 shows the magnetization curves for sample (3) compared to those for sample (2). The data show that both normal and reduced states for sample (3) have almost the same magnetization curves, which indicates a low oxygen intake of blood in the normal state. These results suggest that magnetization curves in this case cannot be used to distinguish between different blood states. These results were confirmed by measuring the absorbency of the blood at 540 nm, where the absorption was not altered as the sample was reduced. The same test was made for samples (1) and (2). The light absorption was found to increase as the samples were reduced.

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