Journal of Applied Sciences

ISSN 1812-5654

Journal of Applied Sciences 7 (9): 1279-1285, 2007
ISSN 1812-5654

Protective Effect of Ascorbic Acid on Molecular Behavior Changes of Hemoglobin Induced by Magnetic Field

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Abstract: With the use of electricity and industrialization of societies, humans are commonly exposed to static magnetic field induced by electric currents. The putative mechanisms by which Static Magnetic Field (SMF) may affect biological systems is that of increasing free radical life span in organisms. To test this hypothesis, we investigate the effect of ascorbic acid (Vitamin C) treatment on the changes in the molecular behavior of hemoglobin as a result of exposure of the animals to magnetic field in the occupation levels. By measuring the relative permittivity, dielectric loss, relaxation time, conductivity, radius and diffusion coefficient of aqueous solutions of hemoglobin. These measurements were calculated in the frequency range of (100 Hz-100 kHz) to give more information about molecular behavior. Twenty four male albino rats were equally divided into four groups 1, 2, 3 and 4. Animals of group 1, were used as control, animals of group 2, were exposed to (0.2T) magnetic field and that of group 3, 4, were treated with Ascorbic Acid by two doses group 3 (20 mg kg^{-1} body weight), group 4 (50 mg kg^{-1} body weight) orally half hour before exposure to magnetic field. The sub chronic exposure expanded (1 h day^{-1}) for 30 consecutive days. The results indicated that exposure of animals to magnetic field resulted in changes in the molecular behavior of hemoglobin molecule while treatment with ascorbic acid afforded comparatively more significant amelioration in these molecular changes, via decreasing the radical pair interaction of magnetic field with biological molecules.

Key words: Hemoglobin, ascorbic acid, dielectrics, magnetic field

INTRODUCTION

In the recent years, there has been a great deal of publicity concerning the possible health effects of magnetic fields (Tenford, 2003; Rongen, 2005). However there is still very little understanding of the interaction mechanisms between magnetic fields and living matter. The bioeffects of static magnetic fields are increasing because of the growing uses of such fields in research, medicine and industry (Dasdag *et al*., 2002; De Wilde *et al*., 2005). Researches carried out by Pacini *et al*. (1999) described the effects of the static magnetic field generated by 0.2 T magnetic resonance tomography on a normal human neuronal cell culture. Cells showed dramatic changes of morphology, developing branched dendrites and modifications in physiological functions of cells. Some reports studied the effect of magnetic field on electrical properties of hemoglobin which indicated changes in the molecular structure of Hb (Hafedh *et al*., 2002; Ali *et al*., 2003). Moreover, numerous reports indicate that SMF is involved in cancer induction as co-carcinogenic factor able to enhance the effects of other mutagenic substances (Chater *et al*., 2004; Amara *et al*., 2007).

It has been assumed that frequency as well as static Magnetic Fields (MF) can increase free radical concentrations in biological systems (Lee *et al*., 2004). An experimental observation showed that a low frequency (60 Hz), magnetic field increases free radical species in living cells (Eraslan *et al*., 2007).

Chemical effect of the static magnetic field consists of modification of the kinetics and recombination of radical pairs reaction. Free radicals are supposed to be involved in harmful reactions in biological systems (Buczynski *et al*., 2005). From this observation, the radical pair mechanism has been proposed as a working hypothesis for possible adverse effects of magnetic field on biological systems. According to the most accepted theory (Brocklehurst, 2002) the magnetic field splits the radical pairs into two energy levels, this increases the amount of radicals pairs that escape the recombination reaction, i.e., the concentration of free radicals.

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Experimental research has shown that weak magnetic fields may reduce the second-order decay rate constant of the reaction (Everson *et al.*, 2000; Brochlehurst, 2002).

Some important naturally occurring vitamins, particularly vitamin C (ascorbic acid), which is water soluble, low-molecular mass antioxidant in biological fluids interact directly with the oxidizing radicals and protect cells from Reactive Oxygen Species (ROS) (Itoh *et al.*, 2007). Vitamin C scavenges the aqueous ROS by very rapid electron transfer that inhibit lipid peroxidation (Serbecic and Beutelspacher, 2005). Hemoglobin may be expected to exhibit dispersions in the radio-frequency region (Polevaya *et al.*, 1999; Sosa *et al.*, 2005) due principally to the relaxation of the permanent dipoles. This allows deductions to be made about molecular shape and size as have been described for other proteins (Hayashi, 2003). Alternative dispersion mechanisms are not apparent at radio frequencies (100 kHz-100 MHZ) although proton fluctuation (Ali *et al.*, 2003) may contribute to the effective dipole moment. Any differences in conformation of hemoglobin with various bound legends would be of considerable physiological interest, apart from adding to the knowledge of the dielectric behavior of protein molecules. An examination of these dispersions is described here.

The present research aimed to investigate the effect of (0.2 T) magnetic field on the electrical properties of hemoglobin of albino rats and to predict the role of ascorbic acid in protection from magnetic field hazards, at an exposure intensity equivalent to the range occurring in the industrial and occupational environment.

MATERIALS AND METHODS

Experimental animals: Twenty four-mature male albino rats weighing at the beginning of the experiment 150-200 g were randomly divided into four equal groups of six rats each. Group 1 served as control. Group 2, static magnetic field exposed rats. Group 3, 4 ascorbic acid treated and (SMF) exposed rats with doses (20 and 50 mg kg^{-1} body weight) respectively. Animals were housed in cages in animal house of National Research Center (NRC) at 25°C, under a 12/12 light/dark cycle, with free access to basal diets and water. All groups were kept under similar environmental condition of temperature, acoustic noise, ventilation and received the same diet during the course of the experiments.

Exposure system: Homogenous magnetic field was generated by GMW magnet system model 3473-70 (made in New Zealand). Source of magnetic field were two parallel coils with pole diameter 150 mm and the gap between the two poles 0-96 mm, coil maximum resistance 0.87 ohm maximum power in air 70 A/59V. Water-cooled coils provide excellent field stability and uniformity when high power is required to achieve the maximum field capability for the electromagnet.

Static magnetic field exposure: The intensity of SMF was measured and standardized over the total floor area of the plastic cage at 200 mT (0.2T) by using a digital Tesla-meter (phywe, 13610.93, Göttingen Germany) with an axial hall probe which determines both static and electromagnetic field in the frequency range of DC-5 kHz and in the magnetic field intensity rang 10 μT-2.5 T. The cage is 15 cm long, 9 cm wide and 25 cm height. The cage was placed in the gab between the two poles. Animals were exposed to the SMF for 1 h day^{-1} during 30 consecutive days. The cage contained two rats for each exposure. The control rats were exposed to a sham (not energized) magnetic field.

Ascorbic acid treatment: Ascorbic acid (vitamin C, liquid supplement) was used. It was purchased from El-Nasr chemical company. The dose of ascorbic acid (AA) was 20 and 50 mg kg^{-1} body weight per day administered orally with the help of plastic tube directly into the oesophagopharyngeal region approximately half hour before applying the static magnetic field. These doses were used because the previous studies showed that 20 or 40 mg AA/kg BW were most efficacious against pesticide and heavy metal toxicities but the lower dose (10 mg kg^{-1} BW per day) of AA (equivalent to the human therapeutic dose according to body weight) was least efficacious (Khan and Sinha, 1996; Yousef *et al.*, 2004).

Blood sampling protocol: Control and treated rats were sacrificed 24 h after the last exposure. Blood was collected in heparinized chilled tubes and immediately centrifuged. Hemoglobin (Hb) solution was prepared according to the method of Trivelli *et al.* (1971).

Dielectric measurements: Dielectric measurements were carried out by using LCR meter type AG-4311B Ando Electric LTD Japan in the frequency range between 100 Hz and 100 kHz with an uncertainty about 2%. The capacitance C, the loss tangent tan δ and a.c resistance were measured directly from the bridge, from which the permittivity ε' and dielectric loss ε'' and also σ_{ac} were obtained.

The cell used is cylindrical capacitor type NFL1. The temperature of the cell was controlled to within ±0.1°C by circulation of water from an ultra thermostat through a jacket surrounding the cell the instrument was calibrated using standard materials (water, acetone and benzene).

Static conductivity measurement: Static conductivity was measured using a conductivity meter type digimeter L21/L21C aqualytic auto temperature. Measurements were performed at constant frequency (1500 Hz in the range of 0 to 200 μ Siemen cm^{-1}). The conductivity meter was calibrated before measurements using a standard solution.

Statistical analysis: The statistical analysis of the result were used according to Harnet (1984) by calculating; arithmetic means and standard deviations using student t-test. Measurements have been done for animals of all groups, the average reading of five runs were used to calculate the means and standard deviation for each group, which means that n = 6. The results were significantly different from control at level $p<0.05$.

RESULTS AND DISCUSSION

The relative permittivity ε' and dielectric loss ε'' were measured in the frequency range 100 Hz to 100 KHz for the aqueous solution of hemoglobin in four different group of samples (control, exposed to magnetic field, treated with ascorbic acid in two different doses 20 and 50 mg kg^{-1}). According to the equations (Gabriel *et al*., 1996) The dielectric constant (ε') for the investigated samples were calculated at each frequency from the measured values of the capacitance (C) through the equation:

$$\varepsilon' = \text{constant} \times C \quad (1)$$

the value of this constant depends on the cell constant and the permittivity of free space and also on the calibration of the cell with different known solution. The dielectric loss ε'' calculated by the equation:

$$\varepsilon'' = \tan\delta \times \varepsilon' \quad (2)$$

and ac conductivity (σ) for the samples were calculated at each frequency from the measured value of the resistivity R through the equation:

$$\sigma = C/R \quad (3)$$

where C is the volume of the sample

Figure 1a and b shows the variation of the relative permittivity ε', dielectric loss ε'' plotted on one scale and electrical conductivity σ on another scale for Hb from control animals and animal groups exposed to magnetic field as a function of the applied frequency range 100 Hz to 100 kHz. The result indicates that there is a dielectric dispersion for Hb molecule in the low frequency range

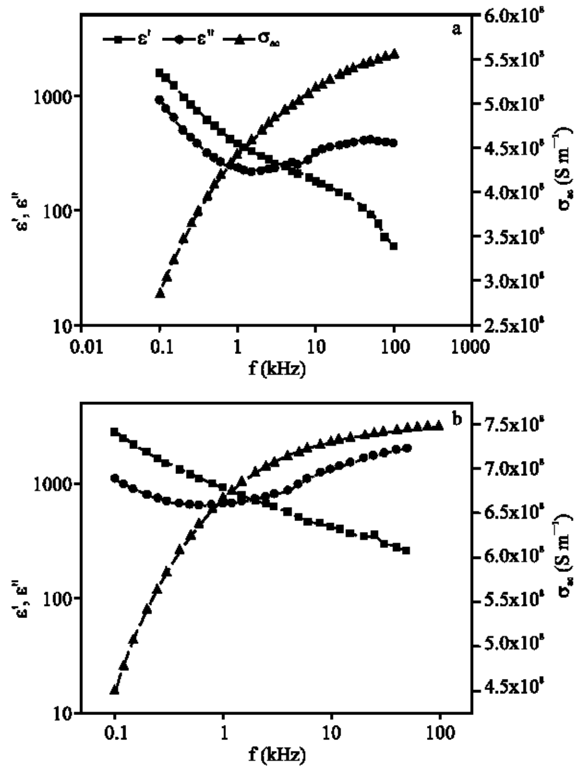

Fig. 1: The variation of relative permitivity ε', dielectric loss ε'' and conductivity σ_{ac} with frequency. (a) Control animals and (b) Exposed to magnetic field

which was known by the α dispersion i.e., α dispersion is related to the whole molecule configuration and agree with the previous findings (Martinsen *et al*., 1998).

From this figure it is clear that ε' decreases slightly with increasing the applied frequency, which shows anomalous dispersion. It is also noticed that the values of ε' for the samples exposed to the magnetic field at the whole range of the tested frequencies are higher than those for the control sample. This increase is related to the induced polarization due to the counter ions around the hemoglobin molecules and that is in accordance with the results reported before (Ali *et al*., 2003) who proved that exposure of animals to magnetic field resulted in decrease in erythrocyte membrane elasticity and permeability and changes in the molecular behavior of hemoglobin molecules.

Figure 1 represents also the dependence of ε'' upon the applied frequency for the above investigated systems. From this figure it is clear that the values of ε' in the lower frequency range seem to be high indicating the presence of dc conductivity. This variation in the dielectric loss and the ionic conductivity from normal levels of dielectric properties leads to structural changes of Hb and hence its physiological functions and molecular behavior (Laogun *et al*., 1997).

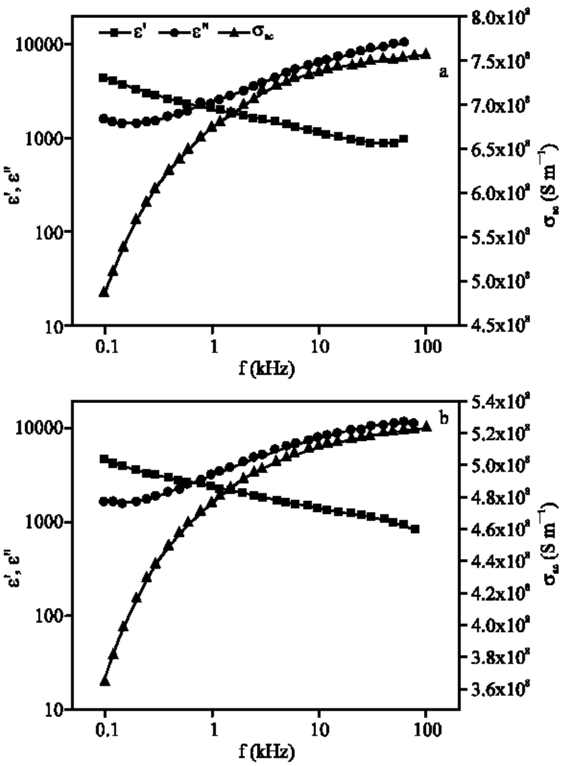

Fig. 2: The variation of relative permitivity ε', dielectric loss ε" and conductivity σ_{ac} with frequency. (a) Treated with 20 mg kg^{-1} ascorbic acid and (b) Treated with 50 mg kg^{-1} ascorbic acid

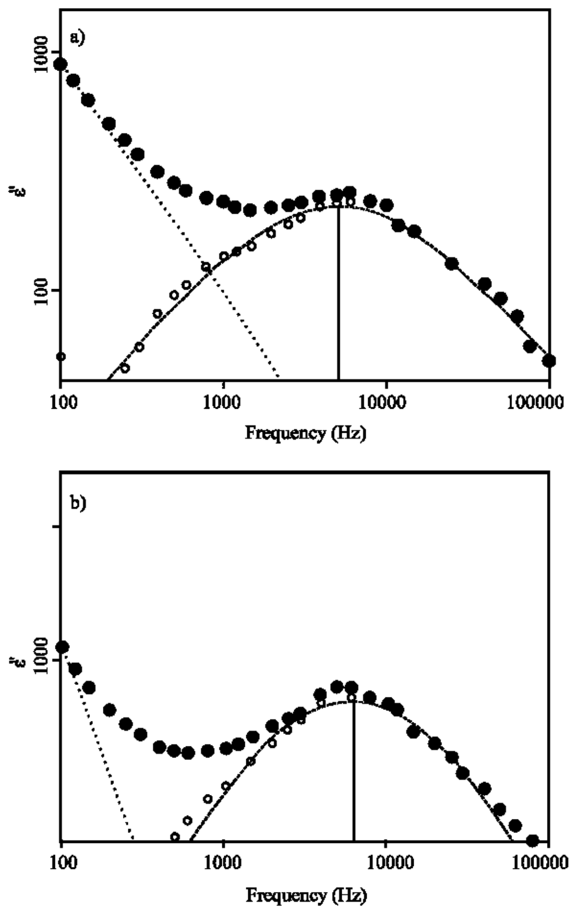

Fig. 3: Absorption curve for the hemoglobin solution. (a) Control and (b) Exposed to magnetic field. The data are fitted by cole-cole and conductivity term

Magnetic field increased the damage in an oxidative stressed rabbit erythrocyte system (Fiorani *et al*., 1997). In addition, magnetic field affects biological systems by prolonging the life of free radicals, excess oxygen free radicals induced lipid peroxidation (Mates, 2000; Hayashi, 2004; Guler *et al*., 2006). Thus one of the defensive systems against free radicals is the supplementation with antioxidant vitamin.

Figure 2a and b shows the variation of the relative permittivity ε', dielectric loss ε" plotted on one scale and electrical conductivity σ_{ac} on another scale for Hb from animal groups treated with ascorbic acid in two different doses (20 and 50 mg kg^{-1}) as a function of the applied frequency range 100 Hz to 100 kHz. The result shows the effect of antioxidant vitamin (ascorbic acid).

Ascorbic acid is known to reduce oxidative radical induced reactions, owing to its high electron-donating power and ready conversion back to the active reduced form and also has a direct O^{2-} and OH^{-} reactive oxygen scavenger action and led to reduced oxidative damage of erythrocyte. Also can protect biomembrane against peroxidative damage (Huange *et al*., 2002). The evidence that ascorbic acid (AA) acts as an important antioxidant in many body tissues is convincing (Jacob and Sotoudeh, 2002).

The conductivity has usually a frequency independent part due to ionic conduction (static conductivity (σ_s) and a frequency dependent part due to dielectric relaxation (σ_{ac}). The conductivity contribution for the dielectric loss was described by:

$$\varepsilon''(\omega) = \frac{\sigma_{dc}}{\varepsilon_0 \omega} \quad (4)$$

A circular arc in the complex plane is also obtained for the Cole-Cole formula

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (\omega\tau)^{1-\alpha}} \qquad 0 \le \alpha \le 1 \quad (5)$$

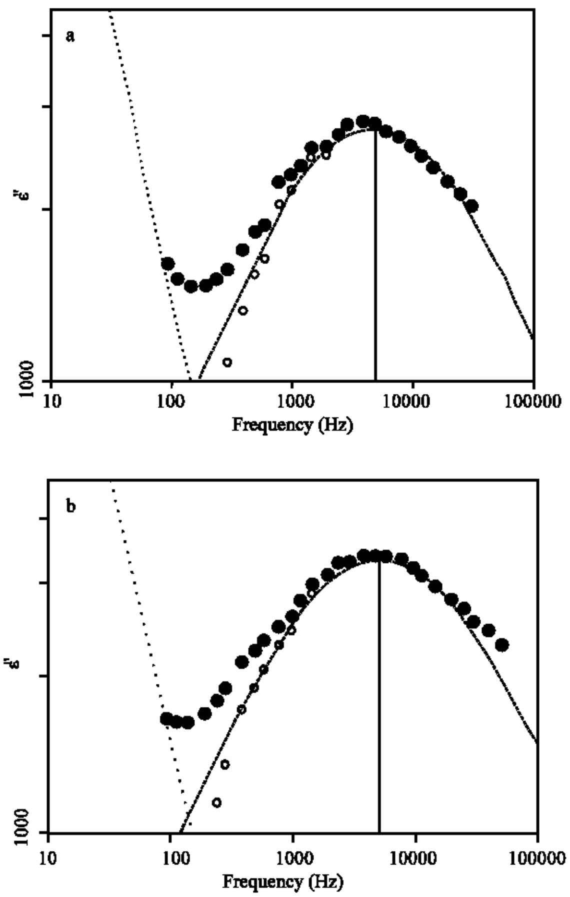

Fig. 4: Absorption curve for the hemoglobin solution. (a) Treated with 20 mg kg^{-1} ascorbic acid and (b) Treated with 50 mg kg^{-1} ascorbic acid. The data are fitted by cole-cole and conductivity term

where ε_∞ is the permittivity at infinite frequency or is the optical permittivity, ε_s is the measured permittivity at low frequency or is the static permittivity ($\Delta\varepsilon = \varepsilon_s - \varepsilon_\infty$) and $\omega = 2\pi f$ (rad sec^{-1}). The relaxation parameter α which describes the shape of the relaxation time distribution function must be in order of $0 \le \alpha \le 1$ (Gross, 1988).

Examples of the analyses are given in Fig. 3 and 4 for the control, exposed animal groups and the two samples treated with ascorbic acid. From both figures it is found that the dielectric spectra give one absorption region which for the applied frequency may be due to the orientation of the whole molecules. The applied spectra were analyzed using a computer program to get the subtraction of the conductivity and to show the pure absorption curves which were fitted by cole- cole arc.

The data of static conductivity σ_s, the relaxation time τ related to the relaxation process and the dielectric increment $\Delta\varepsilon$ and interaction parameter α are given in Table 1. From this table, it is found that the values of relaxation time and interaction parameter decrease while the values of static conductivity and dielectric increment increase with the exposure to magnetic field. These results may be attributed to changes in the size of hemoglobin molecules and the charge distribution affected through the pathway of magnetic field. Moreover the change of the values of the dielectric increments $\Delta\varepsilon$ in Table 1 indicates that there are changes in the dipole moment of the Hb macromolecule. Also it is found that after treatment with ascorbic acid the values are return to the control. The magnitude of returning depends on AA dose treatment. These findings may have important implications for the effect of ascorbic acid on scavenging the free radical species induced by magnetic fields.

Moreover, The radius of the molecule is given by:

$$r^3 = KT\tau/4\pi\dot{\eta} \quad (6)$$

where $\dot{\eta}$ is the viscosity of the sample, K is Boltzman constant, T is temperature in Kelvin and τ is the relaxation time in seconds.

When the electric field is applied, the ions in the system will redistribute under the influence of both the field and the diffusion of ions. The time constant of this dispersion is:

$$\tau = r^2/D \quad (7)$$

where D is the diffusion coefficient of ions.

The results listed in Table 2 showed that the values of molecular radii increase and the values of diffusion coefficient of ions decrease for the exposed sample than the control. Also these values for the treated samples tend to be that for control. These changes indicate that the Hb molecule suffered conformational changes due to the exposure to the magnetic fields and represented in the changes of the molecular volume and shape which may be due to the changes of the molecular radius and/or changes of the charge intensity of the electric dipoles.

Table 1: The data of the analysis, relaxation time τ, cole-cole parameters α, dielectric increments $\Delta\varepsilon$ and conductivity σ_s

Sample	τ×10^-3 (sec)	$\Delta\varepsilon$×10^4	α	σ_s×10^-4 (S/m)
Control	4.45±0.068	1.365±0.028	0.4	60.16±1.04
MF-exposed	1.43±0.043	6.543±0.048	0.3	97.50±2.94
20 mg kg^{-1} ascorbic acid	3.04±0.061	2.230±0.025	0.35	71.83±1.73
50 mg kg^{-1} ascorbic acid	4.12±0.057	1.750±0.039	0.38	66.17±1.70

Table 2: The radius of the molecules r and the diffusion coefficient D

Sample	r(nm)	D×10^-5 = τ r^{-2} sec nm^{-2}
Control	5.10±0.182	8.82±0.063
MF -exposed	10.5±0.29	0.68±0.0061
20 mg kg^{-1} ascorbic acid	8.20±0.28	2.92±0.041
50 mg kg^{-1} ascorbic acid	5.50±0.22	7.27±0.043

Accordingly the results in this study shows a pronounced amelioration in (dielectric parameters which reflect the molecular behavior of the Hb) after treatment with ascorbic acid, its magnitude increases with increasing the dose level of AA. Further, these values return approximately to the normal levels in group (4). This suggests the participation of free radicals which scavenged by Vitamin C dosage, Indicating that oxidative process is one of the main pathways whereby this exposure to magnetic field induces. This leads us to predict that, the presence of ascorbic acid with magnetic field alleviate its harmful effect on molecular behavior of hemoglobin.

CONCLUSION

Dielectric studies have provided a great deal of information on the structure and properties (molecular behavior) of biological molecules. The results of this study suggest that exposure of experimental rats to 0.2 T magnetic field caused changes in the hemoglobin dielectric properties which may be reflected on its molecular behavior and physiological functions. Pre-treatment with ascorbic acid in the recommended dose (50 mg kg^{-1} body weight) may be efficacious and alleviate the harmful effect of magnetic field.

It was concluded that exposure to 0.2 T magnetic field is biologically harmful and occupational exposure to this field should be avoided by vitamin C or other antioxidant supplements. Also coherent studies in this field needed to support or to confirm these results to put guidelines for avoiding harmful effects of long term exposure to MF on biological systems.

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