ELECTRIC FIELD AFFECTED THE MOLECULAR STRUCTURE OF THE TOTAL SERUM PROTEINS OF THE MICE BLOOD

MAGDA SAID HANAFY

Physics Department, Faculty of Science, Zagazig University, Egypt

Abstract. An electric field of 6 kV/m strength and 50 Hz frequency was directed horizontally to three groups of mice for exposure periods 30, 45 and 60 days respectively and for 30 days post exposure. The dielectric properties of the total serum proteins of the exposed mice were studied as an indication of the effect of the electric field on the molecular structure of the serum proteins. The molecular structure of the total serum proteins were studied through measuring their dielectric relaxation and the electric conductivity in the frequency range 0.1 – 5 MHz at 4 ± 0.5 ºC. The absorption spectra of the extracted proteins were also measured in the wavelength range 200 – 600 nm. The results showed that the electric field lowered the permittivity value of the serum proteins and increased its conductivity; a fact that indicates pronounced changes in the molecular structure of the total serum protein of the exposed mice. In addition, the intensity of the absorption spectral bands of the serum proteins of the exposed mice was found to decrease.

Key words: low frequency electric field, total serum proteins, dielectric relaxation.

INTRODUCTION

Possible health effects of exposure to low frequency low intensity electric and magnetic fields are receiving increased interest in the scientific literature. The increasing scientific interest with the effect of electric field on leaving cells during recent decades is mainly attributed to its guide in throwing light on major unsolved biological problems such as irregular cell division Winterhaller [23]

Eman *et al*. [1] showed changes in the dielectric relaxation and electric conductivity of the extracted protein molecules of the exposed mammalian eye (5 kV/m).

Ibrahim [8] stated that application of small d.c. electric field intensities (1–5 V/cm) on erythrocytes increased their electric conductivity.

On the other hand, Walter *et al*. [21], Macginitic *et al*. [12], and Laberge [9] proved that electric field inhibited the biological properties of the cells membrane protein. The Biological effects of such a field (6 kV, 50 Hz) on the bone marrow of

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the mice have been investigated by Fawzia [2]; she showed that the significant frequency of the chromosomal aberrations (CA) and the micronuclei polychromatic erythrocyte (MNPEC) increased by increasing the time of exposure to electric field.

Grota [5] found that serum melatonin levels decreased by electric field exposure, suggesting the possibility that degradation or tissue uptake of melatonin is stimulated by exposure to electric fields.

Tenforde *et al.* [20] mentioned that an extremely low frequency electric field (ELF) induces electrical potentials and resultant current flows in the aqueous medium that surrounds the living cells. Because the membranes of these cells form a dielectric barrier to the passage of current in the ELF frequency range, only a small fraction of the induced current penetrates the cell surface. Yet, it is generally believed that the per-cellular current induced by an ELF field produces electrochemical alterations in the cell membrane surface.

Such induced current, in turn, sends signals across the cell membrane barrier that produces alterations in intracellular biochemical and physiological functions. Saunders *et al.* [19] introduced a mechanism for this biological interaction. They proposed that the electric field induces an electric charge on the surface of conducting bodies such as animals or humans. An electric charge on the surface of the bodies induces electrical potentials within tissue giving rise to a current flow. In the extremely low frequency range < 300 Hz, the electrical impedance of cell membrane is high. The flow of current mainly through the extra cellular fluid of tissue induces changes in the electrical potentials that exist across cell membranes and affects electrically excitable tissue.

In the light of the presence of hundreds of high voltage transmission lines that produce high voltage electric field in the public places, we have undertaken the present study to evaluate the effect of such fields on the dielectric properties of the total serum proteins of the mice exposed to 30, 45, 60 days respectively and for 30 days post exposure. Such a job was achieved through measuring the dielectric relaxation of the serum protein in the frequency range 0.1–5 MHz at 4 ± 0.5 °C. Also, the absorption spectra of total serum protein in the wavelength 200 – 600 nm were measured.

MATERIALS AND METHODS

ANIMALS

In the present study twenty-five male mice were used, aging 3 months and weighing 30 ± 2 g and classified into 5 groups (each of 5 mice). Mice were obtained from Animal House Faculty of Veterinary Medicine, Zagazig University. All animals were kept under the same conditions of nutrition and housing.

ELECTRIC FIELD FACILITY

Extremely low frequency of electric field strength of 6 kV/m and frequency of 50 Hz was generated between two parallel aluminum electrodes of dimensions 60×50×0.2 cm fixed vertically at the two vertical sides of the mice cage (Fig. 1).

The electric field was derived directly from 50 Hz high voltage step-up transformer, manufactured by the Center of Scientific and Electronic Equipment Maintenance, Faculty of Science, Cairo University. The design of the apparatus is shown in Fig. 1.

EXPOSURE PROCESS

Twenty five male mice were exposed as follows: group 1 was used as control, groups 2, 3 and 4 of mice exposed to periods 30, 45 and 60 days respectively. Group 5 was investigated after a period late 30 days from switching off the power supply.

PREPARATION OF TOTAL SERUM PROTEINS

From each mouse, one ml of peripheral blood was obtained by micro-haemotocrite tube from ocular vein of living mice and centrifuged at 3000 rpm for 30 min. After centrifugation the supernatant serum was removed carefully with a micropipette. The total protein in serum was estimated by means of Biuret reaction, according to the technique of Weichselbaum [22] and transferred to new eppendorf tubes and kept in deep freezer until use.

THE DIELECTRIC MEASUREMENTS

The extracted total serum proteins were diluted with bidistilled water at a ratio 1:20 by volume. Loffler *et al.* [10] calculated the dielectric properties of a protein and its solvent and they found that the coupling between the dielectric relaxation of the peptide and that of the water component is particularly important for correctly describing the dielectric constant of the peptide.

The dielectric relaxation of the extracted proteins were measured in the frequency range 100 kHz to 5 MHz using a Loss Factor Meter type 1033, R.F.T., Funkwerk, Erfurt, Germany, and a cell type PW 950/60 manufactured by Philips Holland. The cell has two parallel squared platinum black electrodes of 0.8 cm side each, 64 cm^2 area (A), and 1.0 cm separation distance (d).

Dielectric measurements for the samples were carried out at fixed temperature of 4 ± 0.5 °C using an incubator type 2771 Kattermann, Germany. The relative permittivity (dielectric constant), ε', of the sample is defined as the ratio of

the capacity measured with the sample to that measured by the cell in vacuum. The dielectric loss ε'' is the part of the energy of an electric filed that dissipated irrecoverably as heat in the dielectric. The values of relative permittivity ε' for the samples were calculated at each frequency from the measured value of their capacitance through the relation

$$\varepsilon' = \frac{Cd}{\varepsilon_0 A} \tag{1}$$

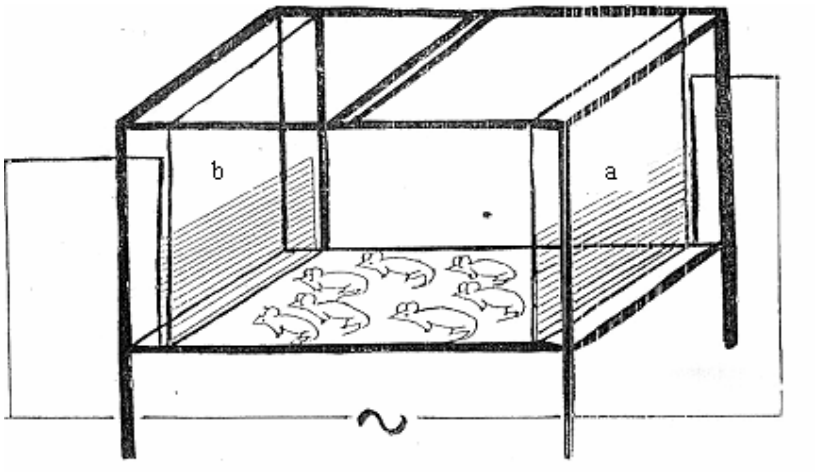

Fig. 1. Electric field facility (a, b aluminum plates).

The dielectric loss, ε'', and the conductivity were calculated from measurements of the sample capacitance and resistance [6].

The difference between the values ε'_s and ε'_∞ at low and high frequency is called the dielectric increment, Δε', i.e. this quantity is a measure for the shape and volume of the nonpolar solution consisting of proteins and bound water [7]. The spectrum of biological macromolecules such as protein at high frequency (hundreds of kHz region) is known as β dispersion, which comes from the polarization of protein and other organic macromolecules [3].

Moreover, the a.c. conductivity S (s^{-1}) was calculated from equation (2) [18].

$$S = \frac{\sigma}{\varepsilon_0} = \omega\varepsilon'' = 2\pi f \varepsilon'' \tag{2}$$

where ε_0 is the permittivity of free space and σ is the real conductivity.

The average molecular radius of the protein molecule was estimated from the relation [18]:

$$r^3 = \frac{kT\tau}{4\pi\eta} \tag{3}$$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the protein solution and τ the relaxation time, namely, the time at which the dielectric molecule has the ability to relax under the effect of the applied field and calculated from the relation:

$$\tau = \frac{1}{2\pi f_c} \tag{4}$$

f_C being the critical frequency corresponding to the mid-point of the dispersion curve (or the frequency at the maximum loss). The accuracy of the experimental set-up was about 1 – 3% in the whole frequency range investigated.

RESULTS

Fig. 2 illustrates the variation of the permittivity (dielectric constant) ε' as a function of the frequency for the total serum protein of five mice groups. It is clear from the figure that the permittivity ε' passed through a dielectric dispersion [4] and the decrease in the values of ε' was accompanied by an increase in the value of conductivity *S*, which we considered as indicating confidence in the measurements. It is clear also that the dielectric increment $\Delta\varepsilon'(=\varepsilon'_s - \varepsilon'_\infty)$ for the exposure periods 30 day is lower than the other periods and increased by increasing it.

The changes in the value of $\Delta\varepsilon'$ were attributed to change in shape and volume of the nonpolar solution consisting of protein molecules [7].

Fig. 3 shows the variation of the dielectric loss ε'' as a function of the frequency for the all the total serum proteins samples.

It is clear from the figure that the middle point of the dispersion curve (at the critical frequency f_c) was changed from one treatment to another as compared with the control sample and then resulted in changes in the relaxation time τ of the samples (Eq. 4). Fig. 4 shows the variation of the conductivity S (s^{-1}) $=\left(\frac{\sigma}{\varepsilon_0}\right)$ for all the groups as a function of the applied frequency. It is clear that the electric conductivity of the total serum proteins molecules of the exposed groups is larger than the control group.

The relaxation time τ (μs), the average molecular radius *R*(nm), the dielectric increment $\Delta\varepsilon'$ and the electric conductivity *S*(s^{-1}) were calculated from the data in the figures and by using the equations (1) – (4) for all the samples as given in Table 1.

Table 1

Values of dielectric increment ($\Delta\varepsilon$), conductivity *S*(s^{-1}), relaxation time τ(μs) and average molecular radius, *R*(nm), for total serum proteins of exposed and unexposed mice

Exposure periods	Dielectric increment $\Delta\varepsilon$	Conductivity *S*(s^{-1}) $\times 10^8$	Relaxation time τ(μs)	Average molecular radius *R*(nm)
Control	6000	15	0.398	2.251
30 days	4800	21	0.454	2.432
45 days	4400	25	0.530	2.510
60 days	3600	35	0.636	2.560
30 days post exposure	5000	17.5	0.353	2.150

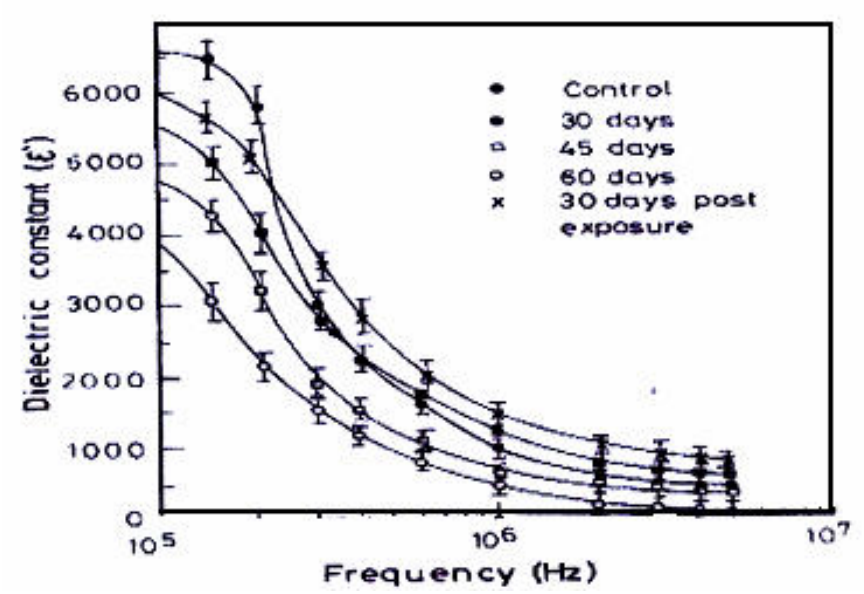

Fig. 2. Variation of the dielectric constant for all the total serum protein groups as a function of the applied frequency.

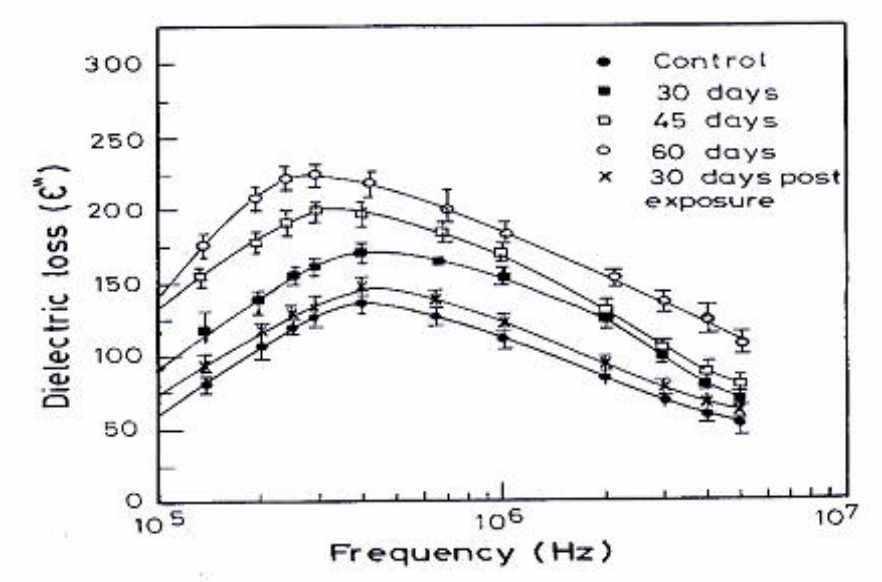

Fig. 3. Variation of the dielectric loss for all the total serum protein groups as a function of the applied frequency.

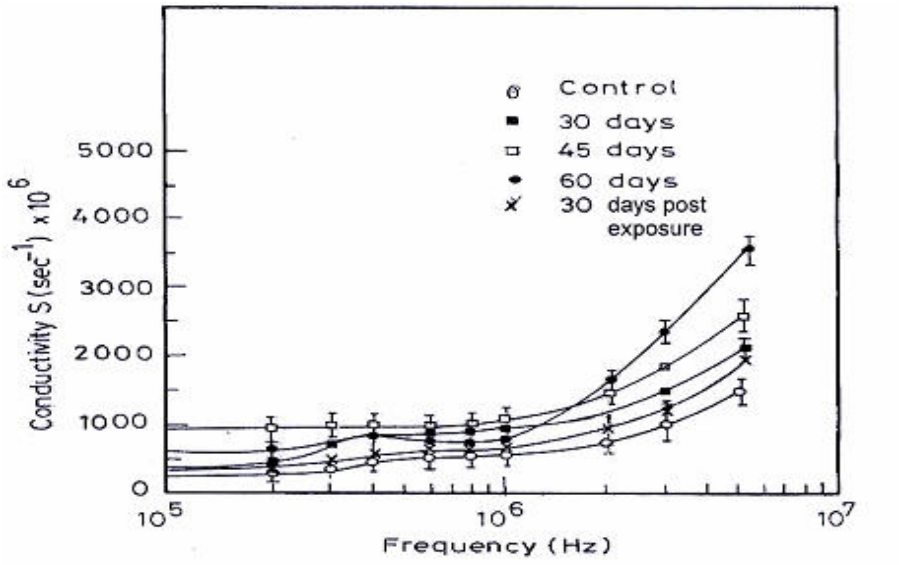

Fig. 4. Variation of the conductivity $S(s^{-1})$ for all the total serum protein groups as a function of the applied frequency.

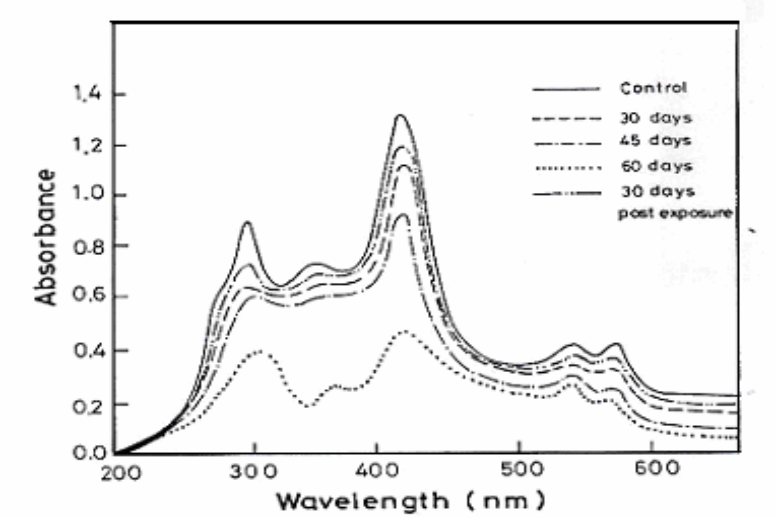

Fig. 5. The absorption spectra of the total serum proteins after different exposure periods to electric fields.

Figure 5 represent the absorption spectra of the total serum protein for all the groups at the result inchoate that the extracted protein from the control group are characterized by absorption bands at 245, 295, 390, 530 and 575 nm and the figure illustrates a continuous decrease in the intensity of each bands for the other groups.

DISCUSSION

The studies presented here on the blood mice are of a great importance, since it provides guidance for the assessment of the hazard extent on the health of occupational and public persons exposed to ELF electric field and, also, to indicate the area of such hazard.

The hazardous effects of the electric field on cell functions may lead to either the change in the character of resonating metabolic processes of the cell involved and/or destroy this process, which may lead finally to the cell death or transmutation [14], also for this reasons they used low beat frequency electric field in treatment of tumor cells.

Therefore, the changes in the molecular structure of the total serum proteins of the animal blood after exposure to different electric field periods may be considered as model examples for the human that may exposed to electric field.

The important property of that biological material is its extremely high dielectric constant ε' (Fig. 1) in comparison with most inanimate matter and the

fact that the dielectric dispersion at low frequency does not follow the typical Deby relaxation curve [17]. It was reported that this magnitude of έ is due to counter-ion polarization.

The presence of a dielectric dispersion in the frequency range $10^5 - 10^7$ for the protein used agrees with the previous finding of Grant [4] for other types of proteins.

Also, the slight increase in the relaxation time and consequently in the average molecular radii of the extracted protein molecules from the exposed groups relative to the unexposed explained that the shape and the volume of the protein molecules are changed [7].

In addition to that, there is a decrease in the values of $\Delta\varepsilon'$ for the blood serum proteins of the exposed mice. Since these changes in $\Delta\varepsilon'$ are functions of changes in the dipole moment of the macromolecules which will consequently depend on the center of mass of the charge distribution and the molecules radius [7], one may conclude that there are some biophysical processes running within the protein molecules resulting from the interactions of the electric field which may cause rearrangement of its charge distribution and hence changing its properties. This result is in a good agreement with. Mccammon [13] who reported that the variation in the surface charge may cause the enzyme receptors to be more sensitive to potential changes.

Also, Pitera *et al.* [16] showed that the behavior of charged residues is the primary determinate of the effective permittivity.

On the other hand, the electric conductivity of the exposed groups became larger than the unexposed. This result has been interpreted before by Sanders *et al.* [19] who suggested that exposure to electric field induces changes in the electrical potential across cell membrane and produce electrically excitable cells.

Also, Ibrahim [8] showed that the increase in the electric conductivity of the exposed cells is due to the changes in the dipoles orientation of their membrane components, which lead to conformational changes in the membrane structure.

The genotoxic effects of extremely low frequency or power (50 – 60 Hz) have been investigated in a variety of systems, most of these studies reported no effects [15]. However, Ma and Chu [11] found an effect on developing embryos of the fruit fly (*Drosophila melanogaster* L.), development as well as survival rates were influenced. Also, Fawzia [3] found that the significant frequency of chromosomal aberrations and the micronuclide in the blood of the mice increased after exposing the mice to the electric field.

CONCLUSION

Exposure to extremely low frequency electric fields should be considered as pollutants to the environment, since it has been shown that it affected the properties of the biological cells. Research studies on the biological effects of such fields encourage helping in finding out means of minimizing their hazardous effects.

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