

***In Vivo* Spectroscopic Study on the Effects of Ionising and Non-Ionising Radiation on Some Biophysical Properties of Rat Blood**

S.H. Allehyani<sup>1</sup>, **H. S. Ibrahim**<sup>2</sup>, F. M. Ali<sup>3</sup>, E. Sayd<sup>4</sup>, and T. Abou Aiad<sup>5</sup>

<sup>1</sup>*Physics Department, College of Applied Sciences, Umm Al-Qura University, 715, Makka, KSA.  
[Saud88882001@yahoo.com](mailto:Saud88882001@yahoo.com).*

<sup>2</sup>*Biophysics Division, Physics Department, Faculty of Science, Mansoura University, 35516, Mansoura  
Egypt, [hsmohamed@yahoo.com](mailto:hsmohamed@yahoo.com).*

<sup>3</sup>*Biophysics Department, Faculty of Science, Cairo University, 12613, Giza, Egypt,  
[Fadel\\_mali@yahoo.com](mailto:Fadel_mali@yahoo.com).*

<sup>4</sup>*Ionising Radiation Department, National Institute for Standards, 12211, Giza, Egypt,  
[Eman\\_sayd@yahoo.com](mailto:Eman_sayd@yahoo.com).*

<sup>5</sup>*Dielectrics Department, National Research Centre, 12622, Cairo, Egypt, [tamany22@yahoo.com](mailto:tamany22@yahoo.com).*

**Abstract**

The present study aimed to analyse the radiation risk associated with the exposure of haemoglobin (Hb) of rat red blood cells (rbc) exposed to a 50-Hz 6-kV/m electric field, a fast neutron dose of 1 mSv, and mixed radiation from fast neutrons and an electric field distributed over a period of three weeks at a rate of 5 days/week and 8 hours/day. The dielectric measurements and the absorption spectra for the haemoglobin molecule in the frequency range of 1 kHz to 5 MHz were measured for all of the samples. The dielectric relaxation results demonstrated an increase in the dielectric increment ( $\Delta\epsilon$ ) for the rbc from all of the irradiated animals, which indicates an increase in the electric dipole. Moreover, the results revealed a decrease in the relaxation time ( $\tau$ ) and the molecular radius ( $r$ ) of the irradiated molecules, which indicates that the increase in  $\Delta\epsilon$  is mainly due to a pronounced increase in the centre of mass of the charge on the electric dipole of the Hb molecule. The results from the absorption spectra indicate that the ratio of met-haemoglobin to oxy-haemoglobin is altered by irradiation. Moreover, the results from the delayed effect studies show that the structure and function of the newly generated Hb molecules are altered and dissimilar to that of healthy Hb.

**Key words:** Rat red blood cell haemoglobin, dielectric properties, absorption spectra, biochemical analysis.

## 1. Introduction

The biological effect of power-frequency fields, which may be due to the magnetic component of the field, was the subject of some debate in the late 1990's [1]. Naturally occurring electric fields in the atmosphere are typically 120–150 V/m, although these can reach 20 kV/m under certain conditions, such as near thunderstorms. Electrostatic fields of up to 20 kV/m may also be found near some equipments that use high voltages, such as television sets and video display units, or can be generated by friction between appropriate materials [2] [3]. A principal mechanism through which radio frequency radiation and microwaves cause biological effects is heating (thermal effects), which can result in cell death. If a sufficient number of cells are killed, burns and other forms of long-term and possibly permanent tissue damage can occur [4].

The effects of exposure to a 50- to 60-Hz weak electric field on cognitive functions, such as memory, attention, and information processing, and time perception were determined by ECG (electroencephalographic) methods and performance measures. It was found that the electric field influences the cognitive processes of the brain (the effect is due to the late compartments of the wave) rather than the perceptive processes of afferent pathways [5]. EMF may alter some of the brain functions, as was determined by the increasing norepinephrine levels that were found in the pineal gland of rats exposed to higher field strength of 100  $\mu$ T to 5 kV/m [6]. Electric and magnetic fields may affect dairy production [7].

The flux density generated from two different transmission lines of 69 kV and 230 kV was evaluated. The flux density of the 69-kV line has decreased to 0.4  $\mu$ T at a distance of 20 m from the line and to 0.2  $\mu$ T at a distance of 30 m from the line. For the 230-kV line, the calculated fields decreased to 0.4  $\mu$ T at a distance of 50 m from the line and to 0.2  $\mu$ T at a distance of 70 m from the line. These results demonstrate that there is a violation of the National American Electrical Code (NAEC) that must be considered for human health and safety [8]. The relative permittivity, dielectric loss, and AC conductivity of diabetic erythrocytes increased significantly compared to the control due to the toxic effects of glucose on erythrocytes, which lead to a restructuring of the erythrocyte membranes [9].

The structural changes in the lipid bilayer exposed to fission neutrons with fast fluences ranging from  $10^4$  to  $10^7$  n  $\text{cm}^{-2}$  were discussed based on the damaging mechanisms induced by fast neutrons on both the hydrophobic and the hydrophilic regions of the lipid bilayer [10]. Richard C. Miller et al. [11] studied both the cell lethality and the neoplastic transformation of fibroblast-clonilised mouse cells that are grown in Eagle's medium after exposure to neutron energies ranging from 0.040 to

13.7 MeV. These researchers found that the irradiation of cells with neutrons, regardless of the energy, resulted in curvilinear functions for both the cell survival and the radiation-induced oncogenic transformation.

## **2. Methodology**

### **2.1. Experimental Animals**

Forty male 2- to 2.5-month-old Albino rats weighing 200–250 g each were divided in four equal groups. Group A was used as the control. The rats in group B were whole-body exposed to a 50-Hz 6-kV/m electric field for a period of three weeks (w). At the end of the exposure period, these animals were equally divided into two subgroups: B<sub>1</sub> and B<sub>2</sub>. The B<sub>1</sub> subgroup was used to study the direct effect of the electric field, and the B<sub>2</sub> subgroup was used to analyze the delayed effects and was thus maintained away from any radiation field (similarly to the control group A) for a period of 45 days. Group C was whole-body exposed to fission neutrons from a <sup>252</sup>Cf source with a present yield of 0.89 × 10<sup>6</sup> n/s. The dose of 1 mSv was distributed over a period of 3 w, 5 days (d)/w, and 8 hours (h)/d at a constant dose rate of 8 μSv/h. At the end of the exposure period, the animals in group C were equally divided into two subgroups: C<sub>1</sub> to study the direct effect and C<sub>2</sub> to study the delayed effect. The animals in group D were exposed to a mixed radiation field of fast neutrons and an electric field for 3 w under the same exposure conditions as those used for groups B and C. At the end of the exposure period, the animals in group D were equally divided into two subgroups: D<sub>1</sub> to study the direct effect and D<sub>2</sub> to study the delayed effect.

The 45-day period was chosen for the delayed effect studies based on the fact that the lifespan of rat erythrocytes is 45 days; thus, the investigation of the delayed effect was conducted on newly generated blood. The animals in all of the groups were maintained under similar environmental conditions of temperature, illumination, and acoustic noise. The animals were maintained in special plastic cages that permit normal ventilation and daylight. The cages were fixed on supports inside the irradiation chamber. Food and water were maintained in special open containers that were fixed on the walls of the cages. The cages were cleaned twice daily, and the water and food were changed twice daily. All of the animals received the same diet throughout the course of the experiment.

### **2.2. Exposure Facility**

The animals were exposed to a 50-Hz 6-kV/m electric field generated between two parallel copper electrodes (fixed at opposite ends of a 1-m-long plastic chamber) with a potential difference of 6 kV supplied from the main supply through a transformer. The plates were covered by a sheet of electric insulating polymer to prevent any electric shock to the animals during the course of the experiment. The animals were exposed to fission neutrons from a Cf<sup>252</sup> point source at a neutron dose rate of 8 μSv/h. The following measurements were performed on all of the animals.

### **2.3. Blood samples**

The animals from the different groups were anaesthetised with ether, and blood samples were then collected from the eye vein using heparinized capillary tubes for subsequent analyses.

### 2.3.1. Haemoglobin Spectrum

A haemoglobin (Hb) solution was prepared according to the method described by Trivelli et al. [12]. The haemoglobin spectrum was generated through the use of a spectrophotometer (Unicam UV/VIS Spectrometer UV<sub>2</sub>, United Kingdom) in the range of 300 nm to 700 nm.

### 2.3.2. Dielectric relaxation

A haemoglobin (Hb) solution was prepared according to the method described by Trivelli et al. [12]. The dielectric measurements were performed in the frequency range of 1 kHz to 10 MHz using a computerised RLC HIOKI 3531 Z Hitester (E. E. Corporation, Japan) at National Research Centre. The sample cell has two (1 x 1)-cm<sup>2</sup> platinum black electrodes with an interelectrode distance of 1 cm. The cell with the Hb samples was maintained at 15±0.1°C in a temperature-controlled incubator (Type 2771, Kottermann, Germany). The value of the dielectric constant ( $\epsilon'$ ) for a sample was calculated at each frequency from the measured values of the capacitance (C) using the following equation:

$$\epsilon' = \frac{C d}{\epsilon_0 A} \quad (1)$$

where C is the capacity of the specimen in farad, d is the interelectrode distance in meters, A is the area of the electrode in squared meters, which is measured from the cell used, and  $\epsilon_0$  is the permittivity of the free space. The loss tangent ( $\tan\delta$ ), the dielectric loss  $\epsilon''$ , and the AC conductivity  $\sigma$  were calculated from the following relationship:

$$\tan \delta = \frac{1}{2\pi fRC} = \frac{\epsilon''}{\epsilon'} \quad (2)$$

$$S = 2\pi f \epsilon'' \epsilon_0 \quad (3)$$

where  $f$  is the frequency applied in Hertz and R is the resistance of the specimen in Ohm. The dielectric constant ( $\epsilon'$ ) decreases from its high value  $\epsilon'_s$  to  $\epsilon'_\infty$  as the frequency increases through the dispersion region, and  $\Delta\epsilon = \epsilon'_s - \epsilon'_\infty$  [13] [14]. It is convenient to the AC conductivity S rather than the actual conductivity  $\sigma$  [15]. The AC conductivity is given by

$$S = \frac{\sigma}{\epsilon_0} \quad (4)$$

and the radius (r) of the molecule is given by

$$r^3 = \frac{KT\tau}{4\pi\eta} \quad (5)$$

where K is the Boltzmann constant, T is the temperature in Kelvin,  $\eta$  is the viscosity of the sample, and  $\tau$  is the relaxation time in seconds.

### 2.3.3. Blood serum tests

Biochemical investigations of the blood serum were also performed. The following four liver enzymes are included on most routine laboratory tests: aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT),

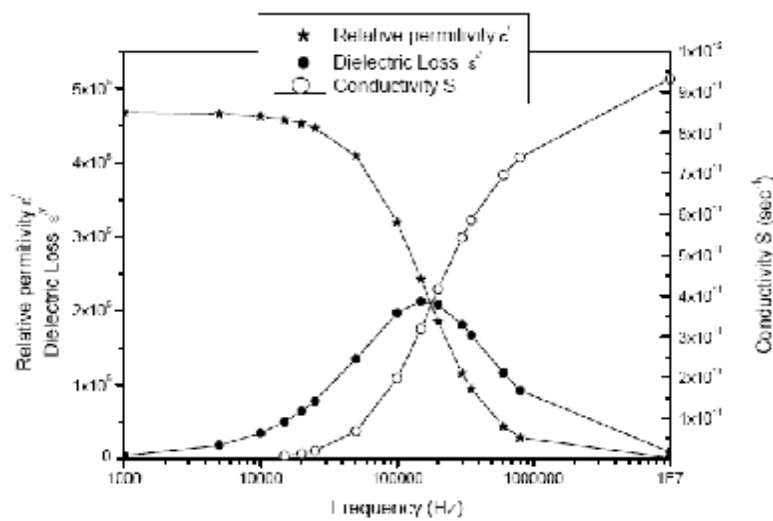
which are known as transaminases, and alkaline phosphatase (AP) and gamma-glutamyl transferase (GGT), which are known as cholestatic liver enzymes [16]. This study focused on the levels of the AST and ALT enzymes and the glucose level in the blood serum. Elevations in the levels of these enzymes can indicate the presence of liver disease.

### 2.3.4. Statistical treatments

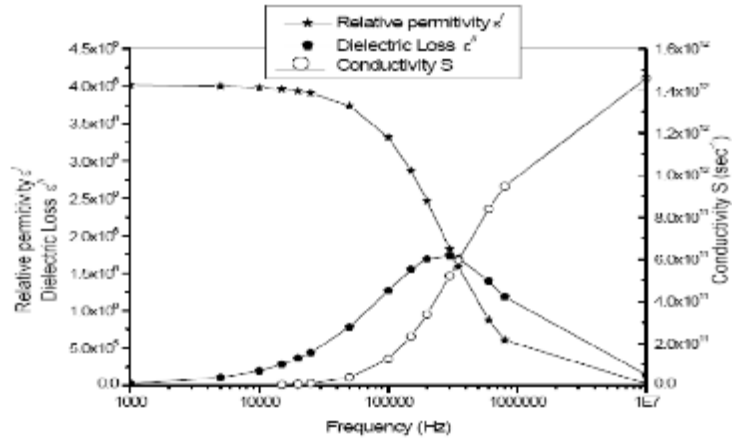
The statistical analyses of the data were used according to Harnet [14] by calculating the arithmetic means and standard deviations for the rbc's dielectric properties, the haemoglobin absorption spectra characteristic peaks, and the biochemical measurements. All of these measurements were conducted for the animals from all of the groups, and the average of five runs was used to calculate the mean and the standard deviations for each group.

### 3. Results and discussion

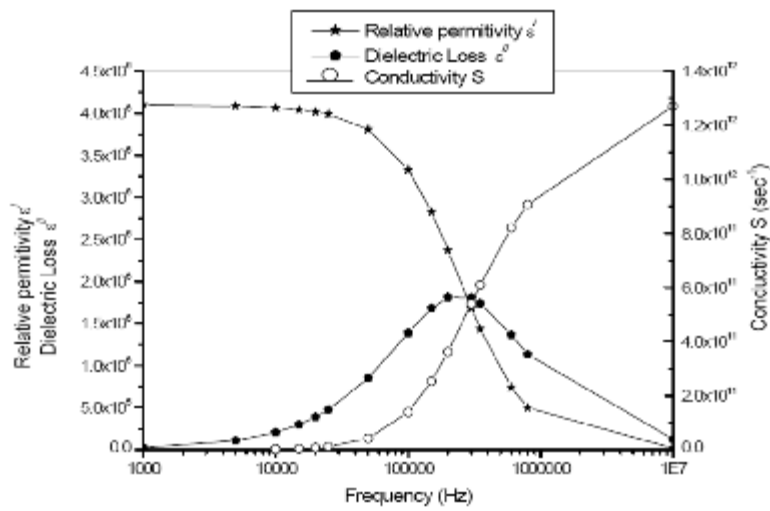
Figures 1a, 1b, 1c, and 1d show the curves of  $\epsilon'$  and  $\epsilon''$  (on one scale) and S (on the other scale) as a function of the applied field frequency on the Hb samples collected from one randomly chosen animal from groups A, B<sub>1</sub>, C<sub>1</sub>, and D<sub>1</sub>, respectively. The amplitude of the dielectric relaxation time ( $\tau$ ), the molecular radius (r), and the Cole-Cole parameter ( $\alpha$ ) for the samples from each group were calculated for each animal from the groups that were used to study the direct effect. The average was then calculated and is shown in Table 1.



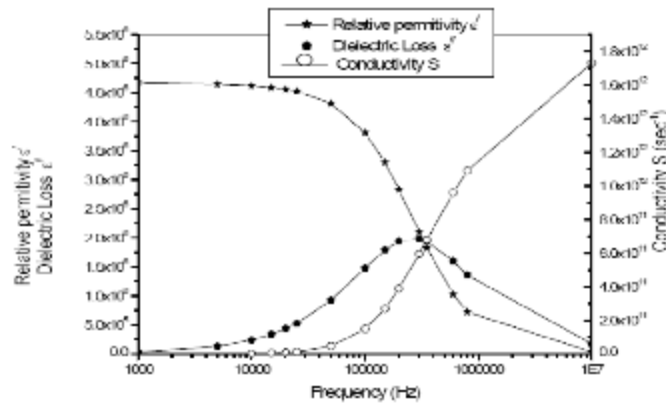
(a)



(b)



(c)



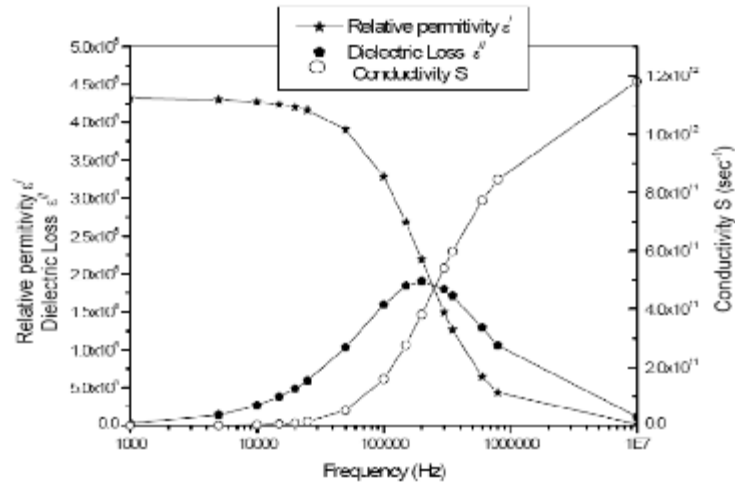
(d)

**Figure 1.** Variation in the dielectric constant, dielectric loss, and the dielectric conductivity as a function of the frequency of the haemoglobin from animals in (a) group A<sub>1</sub>, (b) group B<sub>1</sub>, (c) group C<sub>1</sub>, and (d) group D<sub>1</sub>.

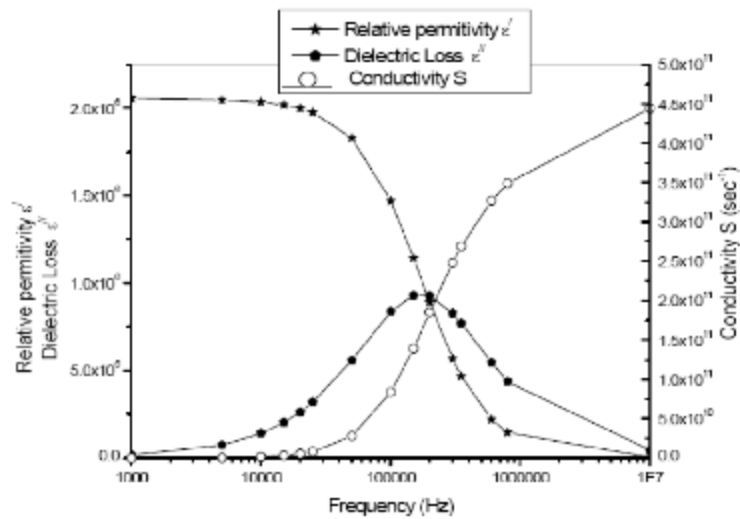
**Table 1.** Average values of the dielectric increment ( $\Delta\epsilon$ ), the relaxation time ( $\tau$ ), the molecular radius ( $r$ ), and the Cole-Cole parameter ( $\alpha$ ) for the haemoglobin samples from subgroups B<sub>1</sub>, C<sub>1</sub>, and D<sub>1</sub> and control group A.

Group	$\Delta\epsilon$	$\tau$ ( $\mu\text{s}$ )	$\alpha$	$r$ (nm)
A <sub>1</sub>	1321 $\pm$ 2.828	6.391 $\pm$ 0.018	0.06	1.264 $\pm$ 0.001
B <sub>1</sub>	1827 $\pm$ 2.500	4.139 $\pm$ 0.003	0.07	1.094 $\pm$ 0.001
C <sub>1</sub>	2005 $\pm$ 2.300	3.739 $\pm$ 0.003	0.09	1.057 $\pm$ 0.001
D <sub>1</sub>	1744 $\pm$ 2.160	3.795 $\pm$ 0.002	0.1	1.062 $\pm$ 0.001

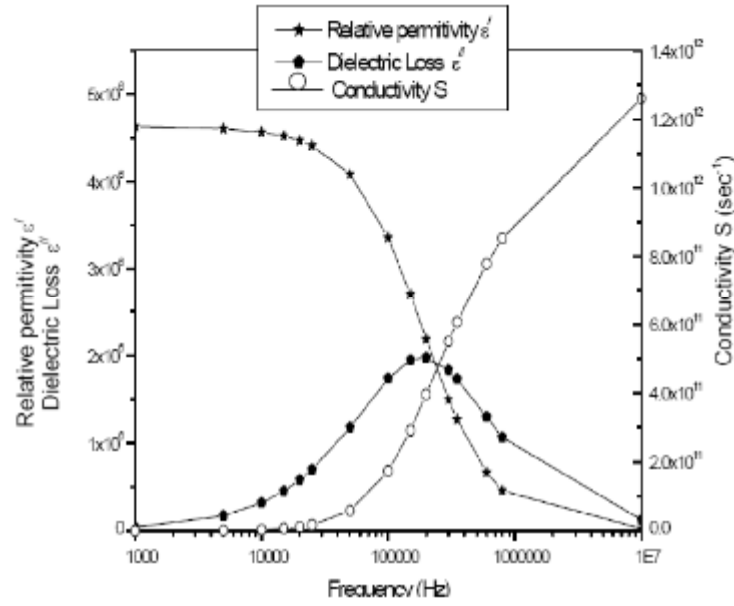
Figures 2a, 2b, and 2c show the curves of  $\epsilon'$  and  $\epsilon''$  (on one scale) and S (on the other scale) as a function of the applied field frequency for the Hb samples collected from groups A, B<sub>2</sub>, C<sub>2</sub>, and D<sub>2</sub>, respectively. The amplitude of the dielectric relaxation time ( $\tau$ ), the molecular radius ( $r$ ), and the Cole-Cole parameter ( $\alpha$ ) for the samples from each of these groups were calculated, and the average values are shown in Table 2.



(a)



(b)



(c)

Figure 2. Variation in the dielectric constant, dielectric loss, and the electric conductivity as a function of the frequency for the haemoglobin from animals in (a) group A<sub>1</sub>, (b) group B<sub>2</sub>, and (c) group C<sub>2</sub>.

Table 2. Average values of the dielectric increment ( $\Delta\epsilon$ ), the relaxation time ( $\tau$ ), the molecular radius ( $r$ ), and the Cole-Cole parameter ( $\alpha$ ) for the haemoglobin samples from subgroups B<sub>2</sub>, C<sub>2</sub>, and D<sub>2</sub> and control group A.

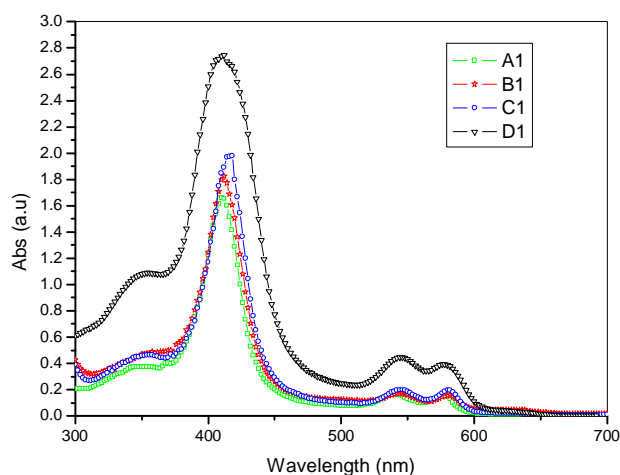
Group	$\Delta\epsilon$	$\tau$ ( $\mu\text{s}$ )	$\alpha$	$r$ (nm)
A <sub>1</sub>	1321±2.828	6.391±0.018	0.06	1.2645±0.001
B <sub>2</sub>	1449±1.699	4.925±0.030	0.08	1.1589±0.008
C <sub>2</sub>	1590±1.24	5.859±0.018	0.06	1.2284±0.009
D <sub>2</sub>	1261±1.000	5.372±0.001	0.1	1.1934±0.001

The results show an increase in the  $\Delta\epsilon$  for all irradiated molecules, which indicates an increase in the electric dipole. Moreover, the results demonstrated a decrease in  $\tau$  and  $r$  for the irradiated molecules, which indicates that the increase in  $\Delta\epsilon$  is mainly due to a pronounced increase in the centre of mass of the charge on the electric dipole of the Hb molecule. This finding is logical because the influence of the electric field on the charge distribution on the macromolecules is expected and can change the centre of mass of the electric dipole and hence  $\Delta\mu$ . In contrast, the effect of fast neutrons on biological macromolecules is the formation of free radicals and highly active charged sites due to the breakage of bonds and the elastic scattering of hydrogen nuclei. Therefore, one may expect the formation of new charges due to neutron irradiation, and the different distributions of these charges (compared with the normal distribution) induce the influence exerted by the electric field.

An additional parameter was used to evaluate the irradiation risk of the haemoglobin structure associated with exposure to the studied fields and was measured through the dielectric relaxation studies. The data indicate that exposure to a fast neutron dose of



1 mSv, a 6-kV/m 50-Hz electric field, or a mixed irradiation field results in an increase in the dielectric increment ( $\Delta\epsilon$ ) and a pronounced decrease in the molecular relaxation time ( $\tau$ ) and the radius ( $r$ ) because changes in  $\Delta\epsilon$  are functions of the change in the dipole moment ( $\Delta\mu$ ) of the Hb molecule, which can increase with an increase in the change density of the electric dipole and with an increase in the molecular diameter.



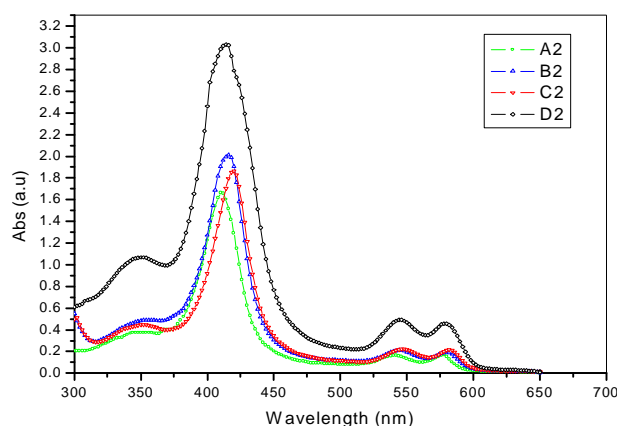
**Figure 3. Spectrum of haemoglobin from control group A, group B<sub>1</sub>, group C<sub>1</sub>, and group D<sub>1</sub> for assessment of the direct effect.**

**Table 3. Average values of the peak height, the position, and the absorption ratios of A<sub>578</sub> / A<sub>540</sub> found for animals from groups A, B<sub>1</sub>, C<sub>1</sub>, and D<sub>1</sub>.**

Group	Peak position (410 nm)	Peak height (410 nm)	A <sub>578</sub> /A <sub>540</sub> ratio
A <sub>1</sub>	410.8±0.748	1.704±0.024	1.024±0.004
B <sub>1</sub>	414±1.632	2.008±0.277	0.977±0.030
C <sub>1</sub>	416±2.000	1.818±0.160	0.979±0.015
D <sub>1</sub>	411.3±0.942	2.483±0.277	0.872±0.013

Figure 3 illustrates the absorption spectra for haemoglobin extracted from animals belonging to the control group A and groups B<sub>1</sub>, C<sub>1</sub>, and D<sub>1</sub>. This figure shows that there are slight changes in both the intensity and the position of the absorption bands that characterise the haemoglobin molecular structure and that the absorption ratios of A<sub>578</sub>/A<sub>540</sub> are less than one [16] [17]. The absorption spectra of the control group show characteristic haemoglobin bands at 578 nm, 540 nm, 410 nm, and 340 nm. The mean ± standard deviation values of the peak height, peak position, and the absorption

ratios  $A_{578}/A_{540}$  obtained from the absorption spectra for haemoglobin extracted from the animals of groups A, B<sub>1</sub>, C<sub>1</sub>, and D<sub>1</sub> were calculated and are given in Table 3.



**Figure 4. Spectrum of haemoglobin from control group A, group B<sub>2</sub>, group C<sub>2</sub>, and group D<sub>2</sub> for assessment of the delayed effect.**

The absorption spectra shown in Figure 4 for the rbc's Hb from groups A, B<sub>2</sub>, C<sub>2</sub>, and D<sub>2</sub> show that there are slight changes in both the intensity and the position of the absorption bands that characterise the haemoglobin molecular structure and that the absorption ratios of  $A_{578}/A_{540}$  are less than one. The absorption spectra of control group A show that the bands that characterise the haemoglobin structure are found at 578 nm, 540 nm, 410 nm, and 340 nm. The mean  $\pm$  standard deviation values of the peak height, the position, and the absorption ratios  $A_{578}/A_{540}$  obtained from the absorption spectra for the haemoglobin samples extracted from the animals in groups A, B<sub>1</sub>, C<sub>1</sub>, and D<sub>1</sub> were calculated and are given in Table 4.

**Table 4. Average values of the peak height, the position, and the absorption ratios  $A_{578}/A_{540}$  obtained for the animals in groups A, B<sub>2</sub>, C<sub>2</sub>, and D<sub>2</sub>.**

Group	Peak position (410 nm)	Peak height (410 nm)	$A_{578}/A_{540}$ ratio
A <sub>2</sub>	410.8 $\pm$ 0.748	1.704 $\pm$ 0.024	1.024 $\pm$ 0.004
B <sub>2</sub>	416.5 $\pm$ 0.500	1.625 $\pm$ 0.375	0.917 $\pm$ 0.005
C <sub>2</sub>	417.3 $\pm$ 0.942	1.900 $\pm$ 0.081	0.971 $\pm$ 0.008
D <sub>2</sub>	415 $\pm$ 1.000	2.762 $\pm$ 0.264	0.958 $\pm$ 0.024

All of the abovementioned effects may result in the deterioration of the molecular function of Hb, and the absorption spectra obtained indicate that the ratio of met-haemoglobin to oxy-haemoglobin is altered by irradiation. The delayed effect studied showed that the structure and function of the newly generated Hb are altered and dissimilar to that of healthy Hb. Table 5 shows the average  $\pm$  standard deviation of the SGPT, SGOT, and glucose levels in the blood serum of animals from all of the groups.

**Table 5. Biochemical analysis of the blood serum collected from animals from all of the groups.**

Group	ALT			AST			Glucose		
	Average	min	max	Average	min	max	Average	min	max
A	41±1.72	39	44	24±2.31	22	29	84±3.52	81	90
B <sub>1</sub>	17±4.33	17	30	72±5.07	60	74	113±7.02	95	113
C <sub>1</sub>	30±4.74	28	40	89±6.79	70	89	110±5.0	100	115
D <sub>1</sub>	40±4.11	32	44	76±4.74	70	82	120±5.25	105	120
B <sub>2</sub>	26±2.15	24	30	88±4.2	85	90	80±2.56	78	85
C <sub>2</sub>	12±1.01	11	14	74±2.15	70	76	88±2.16	87	92
D <sub>2</sub>	14±1.72	12	17	103±2.48	98	<b>105</b>	130±2.33	128	133

It is known that an increase in the SGOT level in blood serum is a marker of cellular membrane damage (80% from whole-body cells and 20% from liver cells). Therefore, one may state that the increase in the SGOT level in the blood serum from animals exposed to radiation is mainly due to two effects: direct and rbc feedback. The direct effect is induced by the radiation, whereas the rbc feedback is due to the increasing toxicity in different organs due to anaemic diseases.

As shown in the present study, the direct effect of the exposure of animals to an electric field, fast neutrons, or mixed irradiation fields is reflected in the changes in the structural properties of the rbc membrane. Therefore, the direct effect of irradiation is a change in the structural properties of the cellular membranes in the different organs of the body. In contrast, the deterioration in the rbc, which is associated with a deterioration in their metabolic processes, will result in an increasing toxicity in the cells of the different organs. Therefore, both parameters may be causal factors involved in the pronounced increase in the SGOT level in the blood. However, the second influencing parameter is the main factor responsible for this phenomenon. This analysis is based on the data obtained from the delayed effect studies, which indicated a continuous increase in the SGOT level in the blood for at least 45 days after the end of the exposure period. The data (from the delayed effect

studies) also show that the rbc's generated after irradiation are abnormal (unhealthy) cells that will cause further toxicity increases in the different organs [18] [19].

#### 4. Conclusion

The results obtained in this study show that there is an increase in the  $\Delta\epsilon$  for all the irradiated molecules, which indicates an increase in the electric dipole due to a pronounced increase in the centre of mass of the charge on the electric dipole of the Hb molecule. In addition, the absorption spectra indicate that the ratio of met-haemoglobin to oxy-haemoglobin is altered by irradiation. The results of the measurements of the liver enzymes and the glucose level in the serum indicate that the observed increase of the SGOT level in the blood serum of animals exposed to radiation is mainly due to two effects: direct and rbc's feedback.

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