DIELECTRIC PROPERTIES OF RED BLOOD CORPUSCLES OF WORKERS CHRONICALLY EXPOSED TO BENZENE IN WORKPLACE

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Abstract

Studying the dielectric properties of cells and of structural parts of the cell (membrane, cytoplasm, etc.) is an important way to obtain valuable information about cell structures and their functions and metabolic mechanisms. In this work, the RBCs human blood of control and benzene exposed workers were separated by centrifugation and washed with phosphate buffer and adjusted to pH = 7.4, 290$\pm$ 3 mOsm/Kg and 50% hematocrit value. The relative permittivity ε', dielectric loss ε'', dielectric strength Δε', relaxation time τ and the ac conductivity σ a-cof these cells were studied using an a-c voltage in the frequency range 50Hz -1MHz. The results obtained indicate that all the studied electric parameters, including real conductivity, real and imaginary relative permittivity in addition to the relaxation time, dielectric loss and the dissipation factor suffered significant changes in the RBCs of the benzene exposed workers than that of the control subjects.

Keywords: Benzene, Dielectric properties, Human erythrocytes, MDA, Total-antioxidant

Introduction

Benzene, an important starting material for the manufacture of many industrial commodities, is a highly occur naturally as a component of petroleum, or may be manufactured synthetically. It is found in the environment as a contaminant from both human activities and natural processes posing serious bio-hazards from exposure (Glass et al, 2003;Kirkeleit et al, 2006). The reported most serious health hazards appear to be of hematologic, immunologic, genetic and malignant nature. Developmental and reproductive effects have also been noted (Garte et al, 2000;Gardner, 2003). Benzene causes harmful effects on the bone marrow leading to aplastic anemia, agranulocytosis and myelodysplastic syndromes. It can also cause thrombocytopenia leading to tendency for excessive bleeding and can cause leukemia. The inhibitory effect on the immune system increases the chance for infection (Huff, 2007;Dougherty et al, 2008).

The mature human erythrocyte is one of the most highly specialized of cells, consisting of plasma membrane surrounding a solution of protein and electrolytes. More than 95% of the cytoplasm protein is hemoglobin. The remainder includes the enzymes required for energy production and for the maintenance of hemoglobin in a functional, reduced state (Wintrobe et al, 2000; Uzoigwe, 2006; Desouky, 2009). The normal human erythrocyte is shaped like biconcave disk, which is well suited to its function. The ratio of surface to volume approaches the maximum possible value in such a shape, thereby facilitating gas transfer. Furthermore, the biconcave disk is more deformable than a sphere and can undergo the changes in shape necessary for optimal movement within the microvasculature (Bull& railsford, 1989).

Certain carbohydrates on the plasma membrane have numerous negative charges and as a result affect the interaction of regulatory molecules with the membrane. The negative charges at the surface also affect interaction between cells; they help keep blood cells apart. Removal of the carbohydrates from the outer surface of the RBCs results in more rapid destruction by the liver, spleen and bone marrow (Nishiguchi et al, 1998).

In the present study some electrical properties of RBCs from normal were investigated in workers exposed to benzene during their daily activity. Deviation from values obtained from unexposed subjects were assessed using an LCR bridge in the frequency range 50 Hz to 1M Hz

Subjects, materials and methods

Subjects

An all-male cohort of 270 non-smoker individuals was recruited to participate in the present study. This cohort included 90 healthy individuals (Group I, age range: 25-58 year, and mean age ± SD: 35.45 years) that served as a control group and 180 individuals (Group II, age range: 23-60 year and mean age ± SD: 38.05 years) occupationally exposed to benzene in their daily activity during routine work as printers, painters, autoworkers and in benzene car wash stations.

All participants were instructed about the study aim, procedures and benefits and provided a signed informed consent prior to their inclusion in the study protocol. The conditions of the work environment, working time and work shift were also recorded. The Ethics Committee of the Medical Research Institute, Alexandria University; the Scientific Technology Development Fund (STD) committee approved the study protocol and all experimental procedures are in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Materials

From each individual, ten milliliter blood sample was withdrawn from each individual by qualified personnel. The plasma and Buffy coat were removed by centrifugation and the RBCs were washed three times with phosphate-buffered saline (PBS, pH 7.4 and 290 ± 3 mOsm/Kg). The RBCs were then diluted with PBS to 50% hematocrit in a home-made electric cell of a total volume of 1ml.

Methods

- *Dielectric Measurements*

A homemade electric cell takes the shape of a parallel-plate capacitor made of silver containing the sample material (plate distance 0.0129 m and plate area 1.07 E^{-4} m^2). The electrodes were connected to LCR meter (Model Instek 819, Good Will Instrument Co., LTD, Hsin-Tien City, Taipei Hsein,Taiwan) , that measures the capacitance and resistance with accuracy ± 0.05%, over a frequency range from 1 Hz- 1 MHz**.**

Capacitance C, and conductance G, were used to calculate: the relative permittivity, real (ε') and imaginary (ε'') and real conductivity (σ') of RBCs, using the equations (Polk and Postow, 1996).

$$C = \varepsilon' \varepsilon_0 A/d \qquad (1)$$

$$G = \sigma A / d \qquad (2)$$

where C (Farad) and R (Ohm), are the capacitance and resistance of the capacitor between the two measuring electrodes, A (m^2) is the surface area of the electrode, d (m) the separation distance between the two electrodes, ε' the relative permittivity (Farad/meter), ε_0 the permittivity of vacuum (8.85 * 10^{-12} F/m), and σ is the electrical conductivity (Siemens / m).

The imaginary part of complex permittivity ε'' and conductivity σ'' were calculated according to the relation (Irimajiri et al, 1987; Asamiero et al, 1988).

$$\varepsilon'' = (\sigma - \sigma_L) / 2\pi f \varepsilon_0 \qquad (3)$$

Where σ_L is the low frequency limiting conductivity taken at 50 Hz, and ε_h is the high frequency limiting permittivity taken at 1 MHz.

Results

- *Real Conductivity*

The variation of the real conductivity, σ', with frequency, f, for both the RBCs of the control and benzene exposed workers, are illustrated in Fig.1.

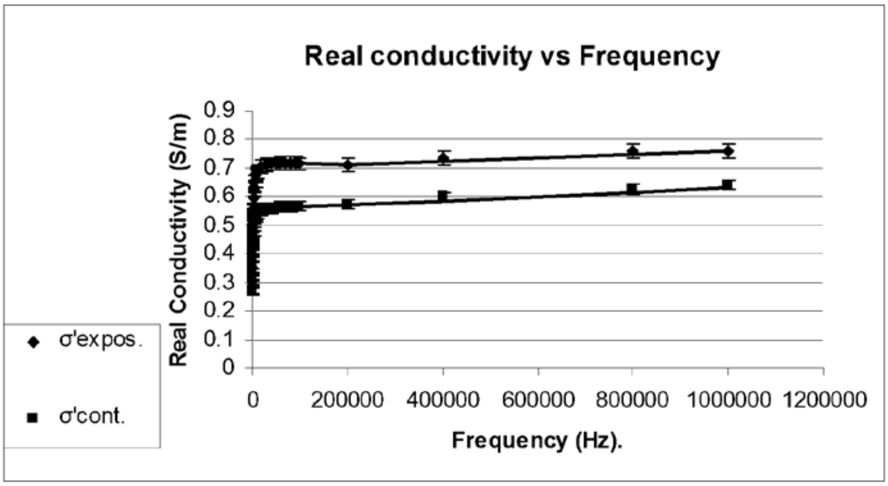

Fig. 1: Variation of the real conductivity σ' (S/m) with frequency F(Hz) ,in the frequency range (50Hz – 1 M Hz).

Each point in Fig. 1, represents the mean ± SD of 180 RBCs sample of benzene exposed workers and 90 RBCs sample of control subjects.

It is clear from Fig. 1, that the real conductivity of either the RBCs of the control subjects or benzene exposed workers increases with increasing the frequency of the applied electric field. The variation of the conductivity with frequency after about 500 Hz, increases upwards with very small rate of increasing. The difference between each similar points on the two curves corresponding to the same frequency value is significant at level $p < 0.05$.

Variation of real conductivity in the frequency range 50 – 400 H

The increase of the real conductivity σ' (S/m) with frequency in the low frequency range (50 - 400 Hz) , as described in Fig. 2, reveals that the variation is exponential and could be described by the exponential relations ; $Y = 0.1056 X^{0.2574}$, and $Y = 0.124 X^{0.2}$ for the RBCs of the exposed and the control subjects. Figures 1& 2, also indicate that the level of the conductivity of the RBCs of the benzene exposed workers is higher than that of the control subjects and the separation between the behaviors of the two curves begins at the minimum frequency used, 50 Hz.

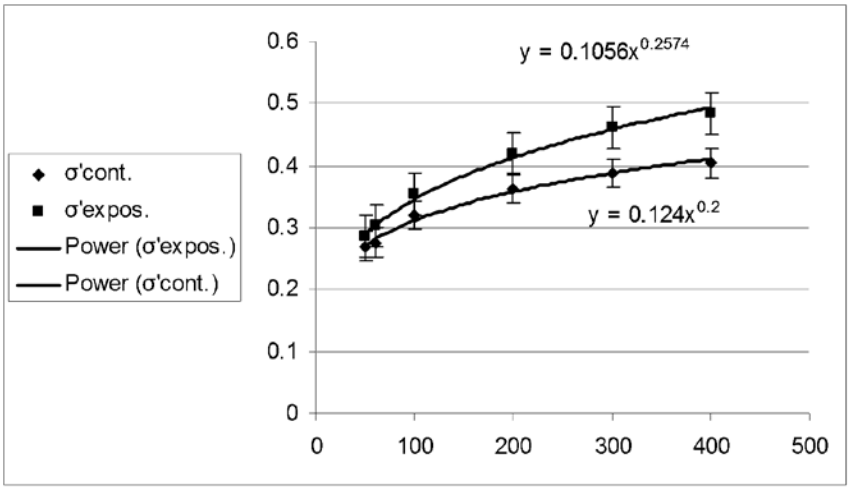

Fig. 2: Variation of the real conductivity σ' (S/m) with frequency f(Hz) , in the frequency range (50 – 400 Hz).

- *Real and Imaginary Permittivity*

The variation of real part of permittivity *ε′* and imaginary permittivity ε” *as* a function of frequency are calculated and represented graphically in Figs. 3 & 4, respectively.

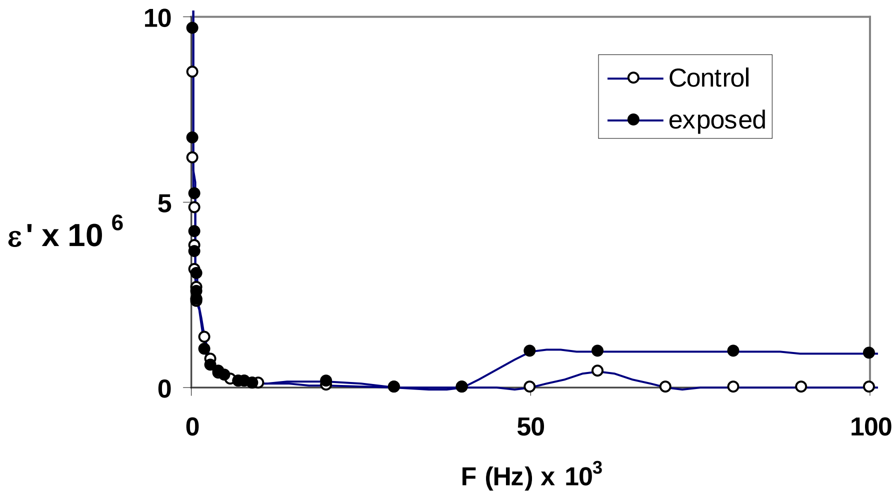

Fig. 3: Variation of the relative permittivity ε' (F/m) with frequency F (Hz), in the frequency range (50Hz – 100kHz).

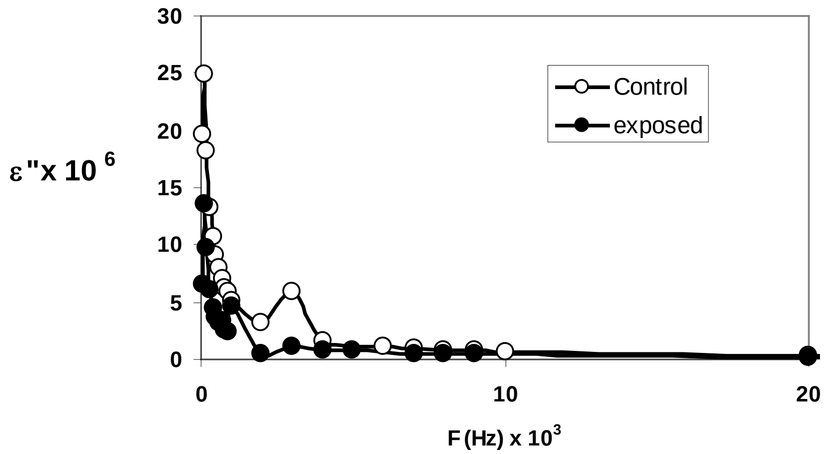

Fig. 4: Variation of the imaginary permittivity ε"(F/m) with frequency F(Hz) ,in the frequency range (50Hz – 20 kHz).

- *Impedance, Z:*

  The electrical impedance, Z, was calculated using the relation:

$$Z = [R^2 + (Xc)^2]^{0.5}. \text{ Where } X_C = 1/ 2\pi f C \qquad (4)$$

The variation of Z with the applied frequency is illustrated in Fig. 5.

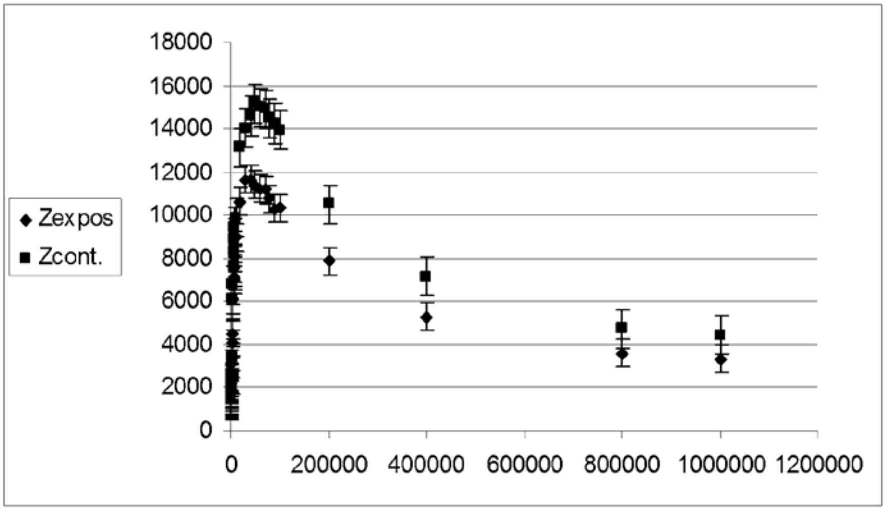

Fig. 5: Variation of the RBCs impedance Z (Ohm) with frequency F(Hz) , in the frequency range (50Hz – 1 M Hz); (▪ Control),(♦ benzene exposed subjects).

However, the variation in the electric impedance of the RBCs of the control and/or the exposed workers decreases exponentially with frequency in the low frequency range from 50 kHz to 400 Hz, Fig. 6. The curve

describing the change in the RBCs impedance is exponential and could be described by the relations:
$Y = 2316.2\ x^{-0.0369}$, and $Y = 7339.6\ x^{-0.2205}$ for the benzene exposed workers and controls, respectively.

Beginning from 500 Hz, the impedance increases with increasing frequency, Fig. 7, to reach maximum values of $\approx$ 16 kΩ and 12 kΩ for the control and the benzene exposed workers respectively. In this frequency range 50 – 60 kHz, the increase in impedance with frequency for the control and benzene exposed workers overlapped then began to separate starting from 10 kHz after wards, as described in Fig. 7.

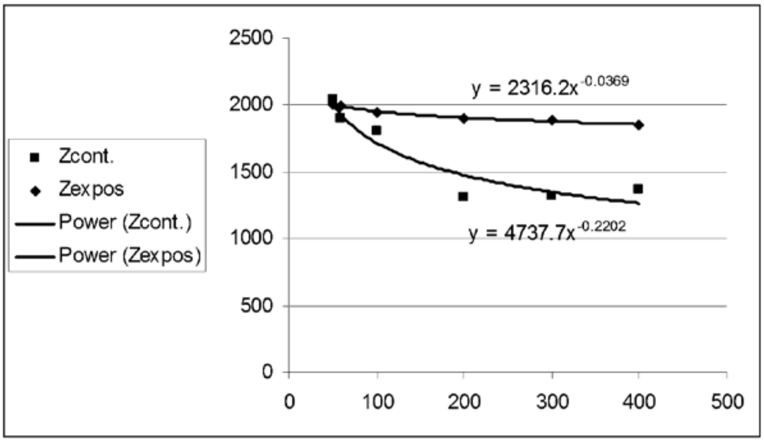

Fig. 6: Variation of impedance Z with frequency f in the frequency range 50 – 400 Hz.

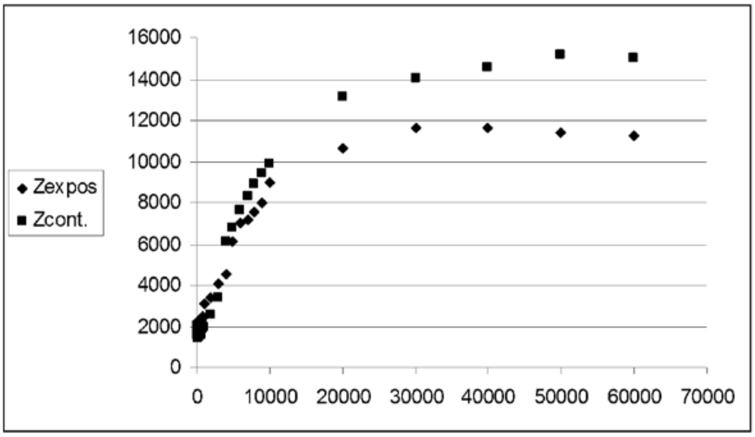

Fig. 7: Variation of the RBCs impedance Z (Ohm) with frequency F(Hz) , in the frequency range 500Hz – 60 kHz ; (▪ Control),(♦ benzene exposed subjects).

The impedance after that describes an exponential decrease to reach minimum values at the end of the frequency range, 1 MHz.

Fig. 8, describes the correlation between the dielectric increment, Δε', and the real conductivity, σ', of the RBCs of the benzene exposed workers.

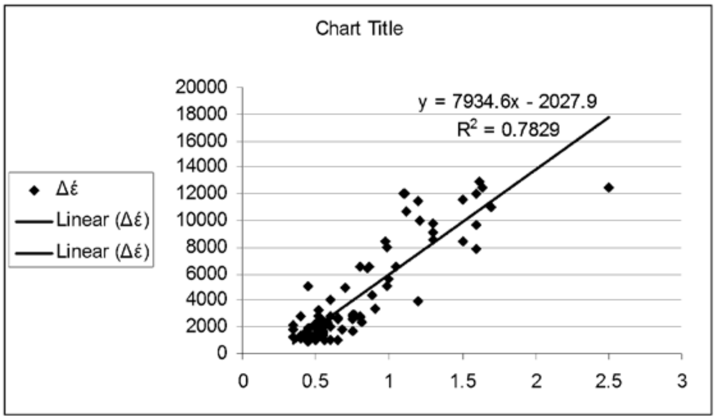

Fig. 8: Correlation between the dielectric increment, expressed as Δέ and the real conductivity σ'.

This graph indicates a significant correlation between these two parameters as expected theoretically.

- *The loss tangent ε'' / ε', (tan δ)*

The dissipation factor or loss tangent, tan δ, is often used to characterize the dielectric loss of a dielectric material. This dissipation factor was calculated using the relation: tan δ = 1/ ω R C (5)

Fig. 9, illustrates the variation of tan δ with frequency, F(Hz), for the RBCs of the control subjects and benzene exposed workers.

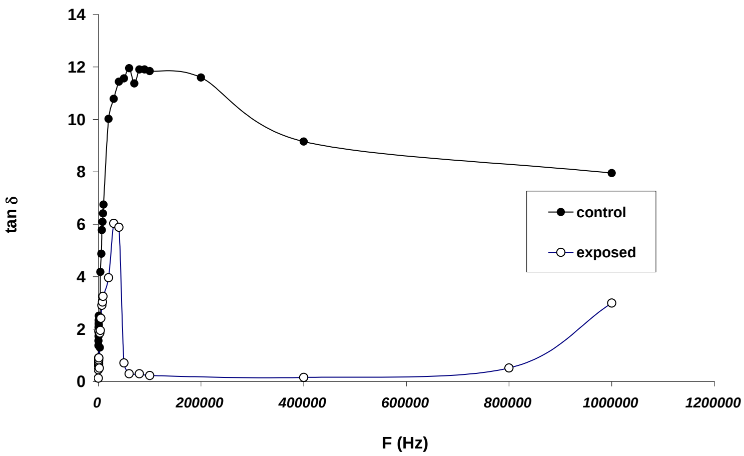

Fig. 9: Variation of the dissipation factor, tan δ,with frequency, F(Hz), for the RBCs of the benzene exposed workers (○) and control subjects (●).

The Fig., shows that there is a sharp peak and a broad peak for the RBCs of the benzene exposed workers and the control subjects respectively.

The decline in the value of this factor is very sharp in case of the exposed benzene workers, while that of the control subjects is of lower decline rate.

- *Cole – Cole diagram*

The Cole – Cole diagrams relating ε’ and ε" are illustrated in Fig. 10, for the case of the control subjects and benzene exposed workers, respectively.s

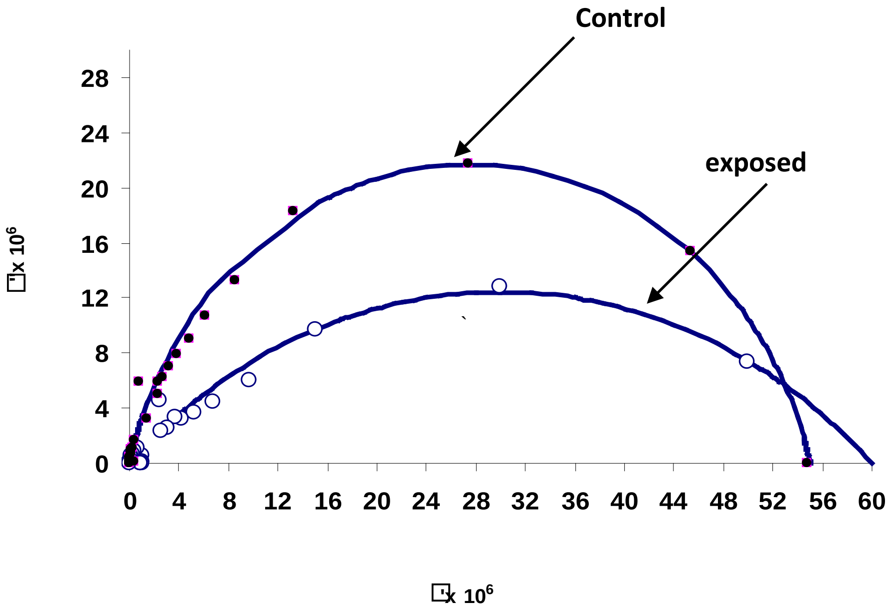

Fig. 10: Cole – Cole diagram between the relative permittivity ε'(F/m and the dielectric loss ε’’ (F/m). Control (●): Exposed (○).

It is clear from the two diagrams that the Cole – Cole diagram of benzene exposed workers has a maximum of $(\varepsilon'')_{max} \approx 13*10^{6}$ (F/m) at $\varepsilon' = 30*10^{6}$ F/m, which is much lower than that of the control subjects, $(\varepsilon'')_{max} = 27 * 10^{6}$(F/m) at the same value of ε', and is, also, shifted towards higher value of ε' on the horizontal axis.

The calculated relaxation time ţ, tangent angle α, dielectric increment **Δέ,** andconductivityσ́, are illustrated in Table 1.

Table 1: The calculated and measured parameters.

Sample	sec μτ	α	$\Delta\acute{\varepsilon} = \varepsilon'_0 - \varepsilon'_\infty$ F/m	Conductivity S sec^{-1} at 1M Hz
Control	$2x10^{-5}$	0.15	$55x10^{6}$	0.76 ± 0.15
exposed	$5x10^{-5}$	0.5	$60\ x10^{6}$	0.62 ± 0.09

Data of the malonialdehyde (MDA) , total anti-oxidant of the blood serum, the aggregation shape parameter, and Form Factor of the RBCs of control and benzene exposed subjects, are illustrated in Table 2.

Table 2: Data of the malonialdehyde (MDA), total anti-oxidant of the blood serum,the aggregation shape parameter (ASP), and Form Factor (FF) of the RBCsof control and benzene exposed subjects,

	MDA m mole/ml	Total anti-oxidant m mole/l	(FF)	(ASP)
Control	1.13 ±1.02	1.38±0.52	159.28±15.66	0.65±0.1
Exposed	2.35±1.4	0.98±0.46	171.36±	0.73±0.1
P Value	< 0.05	< 0.05	< 0.05	< 0.05

- Values are expressed as mean ± SD.

Discussion

When a particle is placed in an electric field, it becomes electrically polarized as a result of particle charge separations induced at boundaries defining its structure. This polarization can be represented as induced dipole moment (Wai-Kai, 2005; Raymond et al, 2009).

On the other hand, studying dielectric properties of biological materials are based on quantifying the response of a material to an applied electric field. The response is typically described by the material's conductivity and permittivity. Electrical conductivity (σ), measured in S/m, is the reciprocal quantity of resistivity, and measures a material's ability to conduct an electric current. Permittivity, ε, measured in F/m, is the amount of charge that is stored by the material due to the polarization of its components. Permittivity of the material is often expressed as relative to the permittivity of vacuum ($\varepsilon_o = 8.854 \times 10^{-12}$ F/m), giving the dimensionless relative permittivity, which is also called dielectric constant, $\varepsilon' = \varepsilon / \varepsilon_o$.

Early measurements of the electrical properties of blood contributed significantly to unravel the constitution of red blood cells (RBC). The results started by Höber (Höber, 1912) provided the first indications of a dispersion (i.e. frequency dependence), caused by the membrane of RBCs, in the radio frequency (RF) spectrum of the dielectric properties of blood.

In the present work, the obtained results indicate that the real conductivity of the RBCs increases with increasing frequency with that of the benzene exposed workers is higher than that of the control subjects through the whole frequency range of 50 – 1MHz. The value of the real conductivity at the end of experimental point, i.e., 1 MHz, is 0.76 ±0.15 and 0.62 ±0.09 S/m for the RBCs of the control and benzene exposed workers, respectively. The difference is statistically significant at the level $p < 0.05$. Also, the difference is statistically significant between any two corresponding values of conductivity for the control subjects and benzene exposed workers through the range from 2 KHz – 1 MHz.

The increase in real conductivity with frequency is probably due to the change in the surface charges of the RBCs membrane and/or the increasing penetration of the applied electric field through the plasma membrane as previously described by electro rotation. (Gismo et al, 1996; Hölzel, 1997).

This means that exposure to benzene, even at low level of exposure, .i.e., < 100 μg/l, as detected in this work (Mohamed et al, 2013) causes changes in the charge distribution or RBCs membrane permeability. The evidence of this changes is confirmed by the elevation of the malondialdehyde (MDA) level and the lowering of the total anti-oxidant level in the blood serum of the benzene exposed workers, in addition to elevation of the aggregation shape parameter (ASP) and RBCs form factor (FF), as illustrated in Table 2. The two later factors indicate that the charges around the RBCs membrane decreased causing more aggregation due to the decreasing repulsive forces responsible for making neighboring RBCs quite apart.

The dielectric constant, ε', contains useful information on the energy storage capacity, the electric dipole arrangement and the ionic migration mechanism. According to the Debye equation (Debye, 1913), ε' varies with the frequency of an applied electric field as follows:

$\varepsilon' = \varepsilon_\infty + [(\varepsilon_s - \varepsilon_\infty) / (1 + \omega\tau)^2]$, where ε_∞ corresponds to the instanteous electric polarization ($\omega \rightarrow \infty$) , ε_s is the static dielectric constant ($\omega = 0$), and τ is the relaxation time corresponding to the frequency at which the ionic and dipole configuration is changed to another equilibrium configuration. At this frequency the rate of power loss exhibits a maximum value. Debye's equation is, however, only applicable to the ideal case.

The results of the present work, Fig. 5, indicate that within the chosen frequency range, ε', goes through a transition from one level to another. The variation could be described by a two kinetic behavior, ie., $\varepsilon' = 2898\, e^{-21.929F} + 1800e^{-7.5F}$ and $\varepsilon' = 4032e^{-28.57F} + 1400e^{-4.5F}$, for the control RBCs and that of the benzene exposed workers, respectively, with tendency to relatively higher values of , ε', in the RBCs of the benzene exposed workers.

The dielectric loss, ε'', in dielectrics may result from one or more of three primary processes, namely; ionic migration, electron polarization and finally ionic vibration and deformation**.** The first process manifests its existence in the dc conductivity, the ionic jump and in the dipole relaxation while the other two processes usually produce insignificant effect at frequencies less than 10^{10} Hz. Besides, the dc conductivity loss is very small at frequencies greater than 100 Hz. Thus, within the frequency range studied the possible energy loss mechanism would be ionic mobility and dipole relaxation. This process of molecular rearrangement is expressed by the Debye equation as follows : $\varepsilon'' = [(\varepsilon'_s - \varepsilon'_\infty) \omega\tau / (1 +(\omega\tau)^2)]$, which assumes the existence of a single relaxation mechanism leading to a peak in the value of ε'' at a frequency $f_c = 1/\tau$.

It is clear from Fig. 12, that this situation is non-ideal Debye model, and may follow a non- ideal Debye model given by the non-ideal relation:

$$[\varepsilon'' (\omega) - \varepsilon'_\infty]/ [\varepsilon'_s - \varepsilon'_\infty] = 1 / [1 + i \omega \tau]^{1-\alpha} \qquad (6)$$

Where α is an empirical parameter ($0 < \alpha < 1$) that measures the degree of departure from the ideal Debye model. Equation (6) could be solved by origin drawing.

The analysis of the dielectric results could reveal interesting parameters such as the distribution parameter α,static dielectric constant ε'_o and the optical dielectric constant ε'_∞, the molecular relaxation time τ and the oscillating strength of the dipoles $L = \varepsilon'_o - \varepsilon'_\infty$ are all illustrated in Table 1.

The overall results of this work indicate that both the dielectric increment ($\Delta\varepsilon$) and conductivity (σ) have much higher values for exposed RBCs samples as compared to the control. It is known that the dielectric increment ($\Delta\varepsilon$) is a parameter directly proportional to the electric dipole moment (μ) of the molecule which is given by: (Polk and Postow, 1996)$\mu^2 = [(2 \varepsilon_o MKT \boldsymbol{(\Delta\varepsilon)}/ NC]$ (7)

Where ε_o = permittivity of the air, M = molecular weight, K =Boltzmann constant, T = temperature in Kelvin=Avogadro's number and C = Concentration. Since the parameters ε_0 , M, K, T, N and C are kept constants for both control and exposed samples, the variation in $\Delta\varepsilon$ is proportional to the square of the electric dipole moment. The electric dipole is a resultant of the center of mass of the electric charge multiplied by its distance from the axis of rotation, so the increase of the value of the electric charge of the dipole causes the increase of the dipole moment .This finding is supported by the high remarkable of the electrical conductivity of the RBCs of the exposed benzene workers as shown in Fig. 1. It is clear from Table 1, that the relaxation time τ increased from $\mathbf{2x10^{-5}}$Secfor unexposed to $\mathbf{5x10^{-5}}$Sec for exposed samples. This increase in relaxation time and the change of the Cole -Cole parameter α, from **0.15** to **0.5** indicate that the shape of the molecule is changed.

Conclusion

The dielectric changes found in the present study indicate that exposure, even to low variable levels of benzene, may be detrimental to the health and well-being of exposed workers. The changes in the conductivity and the dielectric properties of the RBCs of the benzene exposed workers indicate that the RBCs membrane permeability suffer certain changes in thickness and/or shape which are manifested by the results of the oxidative stress markers (elevation of MDA and reduced levels of total antioxidant) and the increased RBCs form factor and aggregation shape parameter.

Acknowledgement

This work was supported in part by Grant ID: 963 from the Science and Technology Development Fund, Egyptian Ministry of Higher Education and Scientific Research.

The authors would like to extend their special thanks to Mrs. Mahrvan Ragab, Senior contracting and Technical follow-up, Science & Technology Development Fund (STDF), and her staff for their help and co-operation throughout the course of the present study.

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