COMPARATIVE STUDY ON THE EFFECT OF RADIATION ON WHOLE BLOOD AND ISOLATED RED BLOOD CELLS

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Abstract. Assessment of the dielectric properties of red blood cells requires several steps for preparation and isolation from whole blood. These steps may result in changes in the cells properties, and they are time consuming. The present study aims to compare the properties of both whole blood and isolated red blood cells and the effect of gamma radiation on these properties. Adult male rats were exposed to 1, 3.5 and 7 Gy as single dose, from Cs-137 source. The dielectric properties studies, in the frequency range 40 kHz to 5 MHz, and light scattering studies for suspensions of whole blood and isolated red blood cells from the same groups were performed. The obtained results showed that whole blood and red blood cells suspensions followed the same trend in their response to radiation, which suggests the possibility of using whole blood suspension for the evaluation of the red blood cells properties.

Key words: Blood, red blood cells, gamma radiation, dielectric properties.

INTRODUCTION

Radiation applications are indispensable in many fields nowadays. Hence, the monitoring of the radiation effects on workers is important in order to get maximum benefit and to reduce its drawbacks. Blood is the most accessible sample one can obtain for analysis. It consists of 45% cells and 55% plasma. Red blood cells (RBC) constitute 99% of its cellular components. They mainly govern the blood behavior either rheologically [11] or electrically [4]. RBC is a biconcave anucleated cell containing hemoglobin molecules. Several types of analysis can be performed to investigate the effect of radiation on blood. Among these analyses are measuring the dielectric properties of cell suspensions. They provide suitable parameters characterizing cell states and dynamics which are related to different physical, chemical or biological interaction [7]. They are expressed as conductivity and permittivity, taking into account both the dynamic and steric properties of cell

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membrane structure. These parameters can be considered as a significant tool to investigate modifications in the overall biological membrane structure [3]. However, the preparation of red blood cells for measurement involves several steps of centrifugation and washing in normal saline, in order to separate plasma and white blood cells. These steps are time consuming and the centrifugation process may affect the structure of the cell membrane and its conductivity [14]. Besides, there is a continuous change in both the conductivity and the permittivity with time for the prepared samples. It was reported that the conductivity, of isolated tissues, increases and permittivity decreases with time [18].

From the electric point of view, blood is considered as a heterogeneous medium essentially constituted of red blood cells in the plasma. The latter can be considered as a homogeneous medium with an electrical behavior close to a physiological solution [8]. When it is exposed to an alternating electric field various mechanisms of polarization such as dipole, Maxwell-Wagner's, electrochemical, etc. can be realized [4]. The Maxwell-Wagner's effect occurs at the interface between the intra or extracellular solution and the phospholipids membrane. It manifests itself as a β-dispersion, which is observed in the frequency range of 100 kHz to 10 MHz, and can be effectively measured and monitored using the dielectric spectroscopy [9]. The contribution of other elements of blood in comparison with that of red blood cells is much less. For example, leukocytes contribute around 1% and thrombocytes around 0.5% of the dielectric increment. Contribution of dipole polarization of hemoglobin molecules is also smaller in comparison with the permittivity of the whole blood. Hence, the contribution of the red blood cell membrane is a dominant one [4]. So, measuring whole fresh blood has the benefit of measuring viable cells close to its physiological state, and to avoid any induced changes in the sample during preparation or rouleaux formation during settling in the measuring tubes. However, blood as a whole is a viscous fluid, which can be difficult to apply in routine analysis. Hence, the search for a suitable condition is worthwhile.

In this work a comparative study between the dielectric properties of both whole blood and separated red blood cells was performed. Both blood and red blood cells are diluted in buffered saline at the same volume fraction. The effect of whole body gamma irradiation on these parameters is monitored to check the validity of this procedure.

MATERIALS AND METHODS

GAMMA IRRADIATION

Adult male Albino rats weighing 200 g were used. The irradiation process was carried out in the National Center for Radiation Research and Technology using Cs-137 source designed for animals' irradiation. The animals were divided

into groups of five animals each. The first group was kept as control and the others were exposed to 1, 3.5 and 7 Gy single doses, respectively. The animals were dissected 24 hours after exposure. The blood samples were withdrawn from the left ventricle of the heart using heparinized needles.

NORMAL RED BLOOD CELLS HEMOLYSIS

Normal red blood cells hemolysis was determined by measurement of hemoglobin released from the cells relative to the total cellular hemoglobin content. Ten μL of whole fresh blood was incubating in 5 mL normal saline for 30 min. The samples were centrifuged at 3000 rpm for 10 min, and the supernatant was measured spectrophotometrically at 540 nm. The percentage of hemolysis was taken against complete blood hemolysis [6].

DIELECTRIC MEASUREMENTS

The dielectric measurements were performed using LCR meter HIOKI 3531, manufactured in Japan, in the frequency range 40 kHz to 5 MHz. The measuring cell is a parallel plate conductivity cell with platinum electrodes. The blood samples were diluted immediately after withdrawal in buffered saline (pH 7.4 and conductivity 0.627 S/m), to avoid any changes in the cell membrane structures. While the red blood cells were prepared by centrifugation at 3000 rpm for 5 minutes. The plasma and buffy coat were removed by aspiration. They were washed twice in buffered saline and separated by centrifugation at 3000 rpm for 10 minutes. The red blood cells were resuspended in buffered saline, and the hematocrit was adjusted at 3%. The samples were incubated in water bath at 37^{o}C during the measurement. The measured parameters were the capacitance C (F) and resistance R (ohm), from which the relative permittivity ε' (dimensionless) and ac conductivity σ (S/m) can be calculated as follows [12-15].

$$C = A\varepsilon'\varepsilon_0 / d \tag{1}$$

where A is the area of the electrode, d is the distance between the two electrodes, and ε_0 is the vacuum permittivity (F/m)**.** The dissipation factor tan δ (dimensionless) is given by:

$$\tan\delta = 2\pi fRC \tag{2}$$

It is related to the dielectric loss ε'' by:

$$\varepsilon'' = \varepsilon'\tan\delta \tag{3}$$

$$\sigma = \omega\varepsilon_o\varepsilon'' \tag{4}$$

The permittivity can be expressed in a complex quantity as:

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \tag{5}$$

The real part ε' represents the permittivity constant and is given by:

$$\varepsilon' = \varepsilon_\infty + \frac{\Delta\varepsilon'}{(1+\omega^2\tau^2)} \tag{6}$$

where $\Delta\varepsilon'$ is the relaxation strength. It is the difference between ε_s (the limiting low frequency permittivity), and $\varepsilon\infty$ (the permittivity value at the end of the dispersion), τ is the relaxation time. And the imaginary part ε'' (the dielectric loss):

$$\varepsilon'' = \frac{\Delta\varepsilon'\ \omega\tau}{\left(1+\omega^2\tau^2\right)} \tag{7}$$

The total area under the loss curve is proportional to the total concentration of dipoles in the material and their dipole moment, irrespective of their distribution of relaxation times [13]. It is related to the relaxation strength by:

$$area = \pi\frac{\Delta\varepsilon'}{2} \tag{8}$$

Simple relaxation process is characterized by single relaxation time. Induced heterogeneity in the material results in several relaxation processes with distribution of relaxation times. To check up for relaxation time distribution, the factor α' is calculated:

$$\alpha' = \pi\varepsilon''_{max}/\text{area under loss peak} \tag{9}$$

The value of α' falls from unity and tends to zero as the distribution tends to an infinite one [13].

LIGHT SCATTERING

Light scattering gives information about size and shape of the cell suspension. When monochromatic light passes through cell suspension, a part of the incident light is scattered. This scattered part of incident light increases as the number and size of the particles in the suspension increase. It can be defined in terms of the turbidity, which is the absorption coefficient due to scattering after subtracting the scattering due to solvent alone. The turbidity (T) is given by:

$$I = I_0 e^{-Tl} \tag{10}$$

where I_0 and I are the incident and transmitted light respectively and l is the length of the light path through the scattering solution [5]. The transmittance was measured at 600 nm using a UV-visible spectrophotometer CECIL-3041. The samples were prepared as for dielectric measurements.

HEMOGLOBIN CONCENTRATION

The hemoglobin concentration was evaluated using Drabkin's reagent and hemoglobin standard, obtained from EAGLE Diagnostics, USA.

RESULTS AND DISCUSSION

The RBC's membrane consists of two interrelated parts: outer lipid bilayer with integral embedded proteins, and underlying protein membrane (cytoskeleton). The latter is responsible for the shape, structure and deformability of the RBC and contains the pumps and channels for movement of ions and metabolites between the RBC's interior and the blood plasma. Also, the proteins in the membrane act as receptors, RBC antigens and enzymes. The insoluble lipid outer membrane provides a barrier to separate the different ions and metabolite concentrations of the interior of the RBC from the external environment of the blood plasma. Several studies have been performed on the effect of gamma radiation on blood and red blood cells. They showed that the exposure to gamma radiation produces lipid peroxidation, cross linking in membrane proteins and induces change in the membrane permeability [17]. Other investigators reported gain of sodium [10] and calcium [15], and loss of potassium [17] by the RBCs as a general effect of exposure to ionizing radiation. They stated that radiation can alter the metabolism or active transport (inhibition of ATP-ase activity) and also may lead to loss of membrane sulphydryl groups.

The dielectric properties measurements of RBCs, after exposure to gamma radiation, in the frequency range 40 kHz to 5 MHz reveals the induced damage in the cell membrane polar groups. The relative permittivity or dielectric constant is a measure of its polarizability in the electric field. It is related with the structural arrangement of the lipid bilayer and with the conformation and localization of proteins in the membrane, consequently with the spatial distribution of charge and dipolar groups at the hydrophobic interface [3]. The polarized part in the membrane is the protein either integral or embedded in the hydrophobic lipid part. The change in both relative permittivity and dielectric loss reflects the change in the protein part of the cell membrane. Both parameters showed a marked decrease after exposure to gamma radiation as the dose increased. Although the value of ε' and ε'' reported for the blood suspension differed from that for the RBCs suspension, both of them followed the same trend (Figs. 1 and 2).

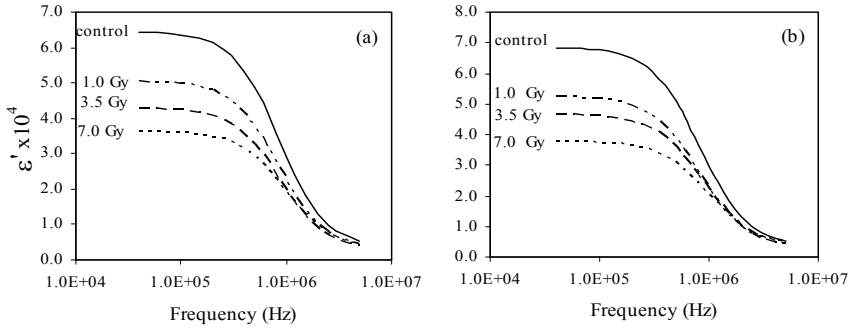

Fig. 1. The relative permittivity for control and irradiated groups of (a) blood and (b) RBCs.

The conductivity depends on the dynamical ionic transport through the membrane [2]. The conductivity shows a decrease with dose increase for both blood and RBCs suspension (Fig. 3).In order to show the radiation induced change in the RBCs, normal hemolysis test was performed, in parallel with the determination of the hemoglobin concentration.

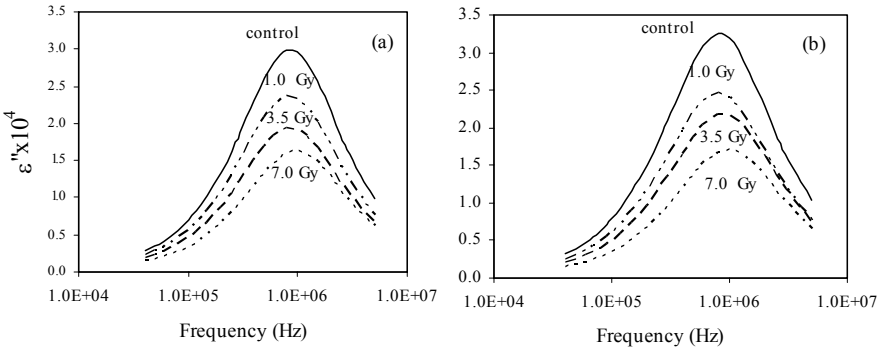

Fig. 2. The dielectric loss for control and irradiated groups of (a) blood and (b)RBCs.

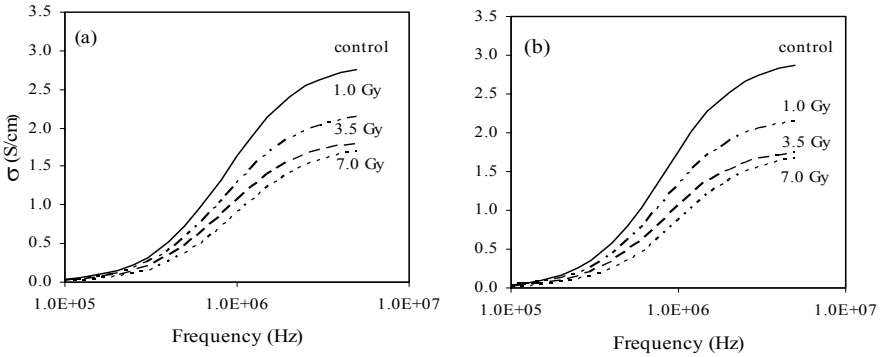

Fig. 3. AC conductivity for control and irradiated groups of (a) blood and (b) RBCs.

In this study, the hemolysis test was also performed using whole blood, thus avoiding any mechanical stress on the RBCs' membrane. Figure (4) shows the increase in the hemoglobin release from the RBCs with a concomitant decrease in the hemoglobin concentration as the dose increases. It illustrates the increasing damage in the cell membrane with dose. Hence, the radiation-induced permeability impairment can be the major cause of loss of the intracellular ions and electrolytes.

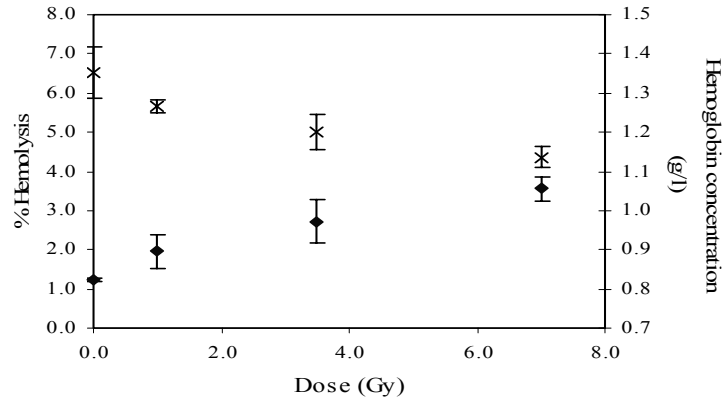

Fig. 4. The percentage of hemolysis (♦) and the hemoglobin concentration (x) versus dose for control and irradiated groups.

The turbidity test reflects the radiation induced changes in the over all RBCs structure, since it depends on the size and shape of the scattering object. Both blood and RBCs suspensions showed the same trend in the turbidity behavior with dose. But, the blood suspension showed higher values than that of RBCs (Fig. 5).

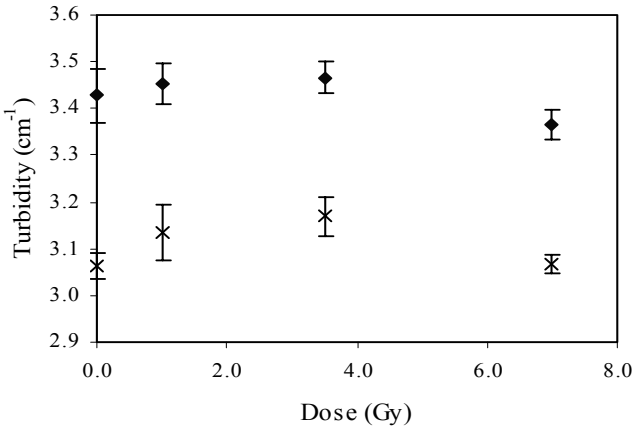

Fig. 5. The turbidity for control and irradiated groups of blood (♦) and RBCs (x).

The relaxation time depends on several factors. The two factors related to this study are the size and charge. The relaxation time was found to increase slightly after exposure to 1 Gy and then decreases as the dose increases (Fig. 6), where both the whole blood and RBCs' suspensions exhibit the same trend. The α' factor was found to be around unity except for the highest dose (7 Gy) for both blood and RBCs samples (Table 1). It was reported that exposure to 6 Gy gamma radiation produced deformation of the RBCs structure and the appearance of echinocyte. This transformation was determined by the progressive appearance of regularly spaced spicules on the surface of the RBCs membrane with the gradual transformation to ovoid shape [17]. The radiation-induced irregularity in the membrane may result in the decrease of the relaxation time distribution from unity.

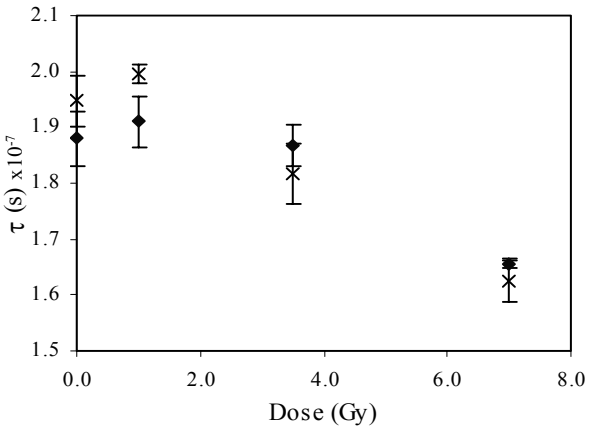

Fig. 6. The relaxation time for control and irradiated groups of blood (♦) and RBCs (x).

Table 1

The α' factor, the AC conductivity at 1 MHz and the area under loss peak for control and irradiated groups

Dose (Gy)	α'		$\sigma_{1\ MHz}$ (S/m)		Area under loss peak	
	Blood	RBCs	Blood	RBCs	Blood	RBCs
control	1.007	1.005	1.644	1.766	9.54×10^4	1.02×10^5
	± 0.034	± 0.048	± 0.072	± 0.067	$\pm 4.52\times10^3$	$\pm 5.61\times10^3$
1.0	0.998	1.002	1.293	1.334	7.43×10^4	7.71×10^4
	± 0.06	± 0.099	± 0.102	± 0.072	$\pm3.88\times10^3$	$\pm 4.87\times10^3$
3.5	0.975	1.017	1.065	1.053	6.26×10^4	5.36×10^4
	± 0.088	± 0.120	± 0.080	± 0.061	$\pm 2.83\times10^3$	$\pm1.59\times10^3$
7.0	0.959	0.936	0.905	0.870	5.24×10^4	4.92×10^4
	± 0.001	± 0.068	± 0.051	± 0.051	$\pm 2.96\times10^3$	$\pm 2.91\times10^3$

The decrease in AC conductivity, at 1 MHz, with doses, can be attributed to the radiation-induced impairment in the membrane permeability. The area under the loss peaks for whole blood and RBCs decreases in a similar manner as the dose increases, reflecting the partial degradation of the protein part, as well as the changes in the membrane conformation and spatial distribution of the lipid protein bilayer.

The chosen irradiation doses started with a relatively low dose (1 Gy), which is known as the general characteristic of the radiation sensitivity of the bone marrow cells and ended with sub-lethal dose (7 Gy) [1], with the aim to perform the comparison over a wide range of effects. However, both blood and isolated RBCs showed the same trend.

CONCLUSION

In the present study, the effects of radiation on RBCs properties were monitored from measuring whole blood and isolated RBCs. Both blood and RBCs were suspended in isotonic buffered saline. The considered sample dilution (3%) has the advantage of measuring small sample volumes, and to avoid intercellular interaction during measurements. The obtained results showed that both whole blood and RBCs suspensions followed the same trend in their response to the radiation damage, although they showed different values. The preparation of whole blood suspension in isotonic buffer has the advantages of measuring fresh viable cells, in a condition similar to their physiological condition, preventing settling and rouleaux formation and avoiding any delay time before measurements, with the subsequent changes in the cells properties. The study shows that the freshly prepared suspension of whole blood can be applied for different measurements such as red blood cells hemolysis, turbidity test and dielectric properties of RBCs.

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