

Rheological properties of blood after whole body gamma-irradiation

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Background: The study of rheological properties of blood has special interest; since it is a circulating fluid exposed to shear rates during its life time. This work aims to investigate the influence of whole body gamma irradiation on the rheological properties of rat's blood. The applied shear rate was from 12 to 375 s⁻¹. Low shear viscosity (up to 100 s⁻¹) depends mainly on the erythrocytes aggregation while the high shear viscosity depends on the erythrocytes deformability. **Materials and Methods:** Adult male rats were exposed to 1, 2.5, 3.5, 5, 7 and 9 Gy single doses. The consistency index, apparent viscosity, yield stress and aggregation index were increased after exposure to gamma radiation. The dielectric properties of the erythrocytes, in the low frequency range (60 Hz to 40 kHz), were measured in order to investigate the changes in the membrane surface charge. **Results:** The results obtained indicate that the viscosity, consistency index and yield stress increased after the exposure to the lowest dose taken; 1 Gy, and continued to increase as the exposure dose increased up to dose 7 Gy and then decrease after exposure to 9 Gy. The relative permittivity and relaxation time showed significant decrease after exposure to the lowest dose and continue to decrease as the dose increased. **Conclusion:** The obtained results can be attributed to the decrease of membrane surface charge after exposure to gamma radiation. The decrease in the membrane surface charge is known to decrease the repulsion between the cells and increase blood viscosity. *Iran. J. Radiat. Res.*, 2009; 7 (1): 11-17

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INTRODUCTION

Blood is formed of 45% cells and 55% plasma. Erythrocytes constitute 99% of its cellular components, so they govern its rheological properties ⁽¹⁾. The rheological properties deal with the relation between the viscosity at different shear rates. It is generally accepted that at constant hematocrit and temperature, low shear

blood viscosity is primarily determined by erythrocyte aggregation, while high shear viscosity is dependent on erythrocytes deformability ⁽²⁾. Aggregation is the tendency of erythrocytes to align themselves into linear arrays, termed rouleaux, in which they are arranged like stacks or coins. This aggregation is dependent on the magnitude of shearing forces acting on the cells, and it is determined by both the properties of plasma, fibrinogen concentration, hematocrit and erythrocytes characteristics such as surface electric charges and shape ⁽³⁾. Erythrocyte aggregation has become an issue of increasing interest because of its pathogenic implications in thrombus formation, both at the venous and arterial levels. Deformability depends on fluid-electrolyte balance and hence its volume and cytoplasmic viscosity, and the mechanical properties of the cell membrane. Maintenance of normal erythrocyte deformability depends on the availability of metabolic energy in the form of ATP (Na⁺-K⁺-ATPase and Ca⁺-ATPase) that serve to regulate intracellular cation and water content thereby maintaining cell volume and thus the cell's surface to volume ratio. Calcium accumulation causes massive ions and water losses, and it has been shown that extensive dehydration causes an increase in intracellular viscosity with attendant loss of whole cell deformability ⁽⁴⁾.

The study of the effects of radiation on the rheological properties of blood reveals

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important information about the interaction of radiation with biological cells. This information is useful in the determination of the degree of radiation damage, and can help one to find correlation between the dose and the radiation effects. Also, the determination of the precise reasons for changes in the blood rheological properties can open the door to many research works to find the suitable radioprotectors and the convenient therapy for many cases of radiation exposure.

The dielectric properties of erythrocytes deal with the structural arrangement of the lipid bilayer and with the conformation and localization of proteins in the membrane, and consequently with the spatial distribution of charges and dipolar groups at the surface of cell membrane ⁽⁵⁾. The process of erythrocyte aggregation can be considered the result of a balance between aggregating and disaggregating forces; disaggregating forces include fluid shear forces, electrostatic repulsion between cells and the elastic energy of the cell membrane ⁽⁶⁾.

This work aims to study the effect of gamma radiation on the different parameters of erythrocytes rheological properties in correlation to the relative permittivity, which depends on the surface charge of the erythrocyte membrane.

MATERIALS AND METHODS

Gamma Irradiation

Adult male Wister rats weighing 200 gm were used. During the experiments they received a standard rodent pellet feed and water. They were divided into 7 groups of 6 animals each. The irradiation process was carried out in the National Center for Radiation Research and Technology using Cs-137 source for animals manufactured in Canada. The dose rate was (0.883 cG/sec) at the beginning of the experiment. The animals were exposed to 1, 2.5, 3.5, 5, 7 and 9 Gy single doses. They were dissected 24 hours after exposure. The blood samples were withdrawn from the left ventricle of

the heart using heparinized needles.

Rheological Measurements

The rheological properties of whole blood were measured using cone-plate Brookfield DV-III rheometer manufactured in USA. The blood samples were measured immediately after withdrawal from the heart, to avoid any aggregation or sedimentation or erythrocytes rouleaux formation. The applied shear rate was 12 to 375 s⁻¹, and the measurements were carried out at temperature 25°C. Practically, the low shear region can be characterized by the consistency index (low shear viscosity) and flow index, which can be calculated from the power fit of this range of the flow curve (equation 1).

$$F = \eta S^f \quad (1)$$

where F is the shear stress, η the viscosity, S shear rate and f is the flow index. The later is less than unity for non-Newtonian fluid. The high shear region can be characterized by the apparent viscosity and yield stress. Different models have been proposed to explain the blood flow curve. From these models the Casson model was shown to be the more reliable ⁽⁷⁾ because it incorporates both shear-thinning and yield stress characteristics (equation 2).

$$\sqrt{F} = \sqrt{F_0} + \sqrt{\eta} \sqrt{S} \quad (2)$$

where F₀ is the yield stress.

Dielectric Measurement

The dielectric measurements were performed using LCR meter type HIOKI 3531, Japan, in the frequency range 60 Hz to 40 KHz. The measuring cell is a parallel plate conductivity cell with platinum black electrodes with area 4 cm² and separating distance 2 cm. The blood samples were centrifuged at 3000 rpm for 5 minutes. The plasma and buffy coat were removed by aspiration. The erythrocytes were washed twice in buffered saline and separated by

centrifugation at 3000 rpm for 10 minutes. The erythrocytes were resuspended in isotonic buffered sucrose (0.3 M sucrose in phosphate buffer pH 7.4, and conductivity 0.223 S/m), and the hematocrit was adjusted at 3%. The samples were incubated in water bath at 37°C during measurement. The measured parameters are the capacitance C and conductance G , from which the permittivity ε' and conductivity ε'' can be calculated as follows:

$$C = A \varepsilon' \varepsilon_0 / d \quad (3)$$

$$G = \sigma A / d \quad (4)$$

where A is the area of the electrode, d is the distance between the two electrodes and ε_0 is vacuum permittivity. The permittivity can be expressed in complex quantity as:

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \quad (5)$$

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

$$\varepsilon' = \varepsilon'_\infty + (\varepsilon'_s - \varepsilon'_\infty) / (1 + \omega^2 \tau^2) \quad (6)$$

where τ is the relaxation time (s), and the imaginary part ε'' (the dielectric loss):

$$\varepsilon'' = (\varepsilon'_s - \varepsilon'_\infty) (\omega \tau / (1 + \omega^2 \tau^2)) \quad (7)$$

By mathematical manipulation of equations 6 and 7 can be modified to give an equation representing straight line in the form:

$$\varepsilon'' / \omega = (\varepsilon'_s - \varepsilon'_\infty) \tau \quad (8)$$

A plot of $\log (\varepsilon''/\omega)$ versus $\log (\varepsilon'_s - \varepsilon'_\infty)$ yields a straight line from which one can calculate the relaxation time τ (8). Once the relaxation time is calculated, we can obtain ε'_s from the Kramers-Kronig equation (9):

$$\sigma_\infty - \sigma_s = \Delta \varepsilon / \tau \quad (9)$$

where $\Delta \varepsilon$ is the relaxation strength. It is the difference between the limits of ε'_s and ε'_∞ . This equation is applicable in the α -dispersion since the investigations of relaxation phenomena at low frequencies often involve very small changes of the resistance of the cell suspensions, and very large changes in capacitance (10).

Hemoglobin concentration and hematocrit

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

Turbidity test

The samples were prepared as for dielectric measurements. The turbidity (T) is given by:

$$I = I_0 e^{-Tl} \quad (10)$$

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 (11). The transmittance was measured at (600 nm) using UV-visible spectrophotometer CECIL-3041 manufactured in England.

RESULTS

Rheological properties

The rheological properties of blood study the change of the viscosity with shear rate. Blood is non-Newtonian, shear-thinning fluid, its viscosity decreases as the shear rate increases. Blood flow curves for control and irradiated groups (up to 7 Gy) are shown in figure 1. The flow curve is characterized by two regions: in the low shear rate up to 100 s⁻¹, and high shear region; from 100 s⁻¹ up to the shear rate at which no change in viscosity is obtained. The ratio of the viscosities at 20 and 100 s⁻¹ can be regarded as quantitative characteristic of erythrocytes aggregation efficiency (aggregation index) (12).

The results obtained in this study indicate that the viscosity, consistency index and yield stress increased after the exposure to the lowest dose taken; 1 Gy, and continued to increase as the exposure dose increased up to dose 7 Gy and then decrease after exposure to 9 Gy (table 1). The value of the flow index remains below unity for the entire dose taken, indicating the non-Newtonian behavior of the blood.

There are several probable reasons for increasing blood viscosity and aggregation

index. The hematocrit is a major determinant of blood viscosity. However, it showed significant decrease in the irradiated groups (figure 2a). The decrease in hematocrit at the same time of the increase in viscosity can reflect the radiation-induced damage in the erythrocyte membrane. At the same time, the mean corpuscular volume (MCV) showed significant increase as the dose increased (figure 2b).

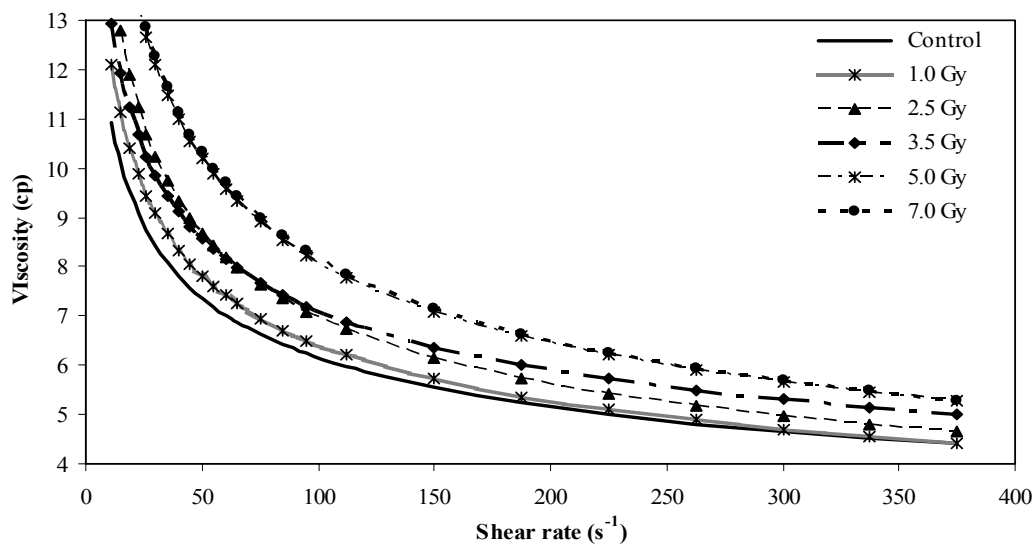


Figure 1. Blood flow curves (viscosity –shear rate curves) for control and irradiated groups (up to 7 Gy). The applied shear rate was 12 to 375 s⁻¹, and the measurements were carried out at temperature 25°C. The low shear region can be characterized by the consistency index (low shear viscosity) and flow index (calculated using power law model), The high shear region can be characterized by the apparent viscosity and yield stress (calculated using Cassion model)

Table 1. Viscosity, yield stress, consistency, flow and aggregation indices for control and irradiated groups (mean ± S.D.).

Dose	Viscosity (cp)	Yield Stress (D/cm ²)	Consistency Index (cp)	Flow Index	Aggregation Index
Control	2.68 ± 0.14	0.47 ± 0.04	20.14 ± 1.92	0.74 ± 0.02	1.58 ± 0.05
1.0 Gy	2.70 ± 0.21	0.56 ± 0.04	26.37 ± 1.62	0.68 ± 0.04	1.77 ± 0.06
2.5 Gy	2.85 ± 0.15	0.64 ± 0.04	27.32 ± 2.92	0.69 ± 0.03	1.71 ± 0.10
3.5 Gy	2.88 ± 0.17	0.68 ± 0.07	28.41 ± 2.54	0.61 ± 0.02	1.80 ± 0.04
5.0 Gy	3.00 ± 0.14	0.75 ± 0.05	29.65 ± 2.81	0.69 ± 0.03	1.74 ± 0.07
7.0 Gy	3.14 ± 0.08	0.80 ± 0.07	29.21 ± 2.02	0.66 ± 0.06	1.88 ± 0.12
9.0 Gy	2.89 ± 0.27	0.68 ± 0.04	26.69 ± 1.53	0.69 ± 0.05	1.80 ± 0.08

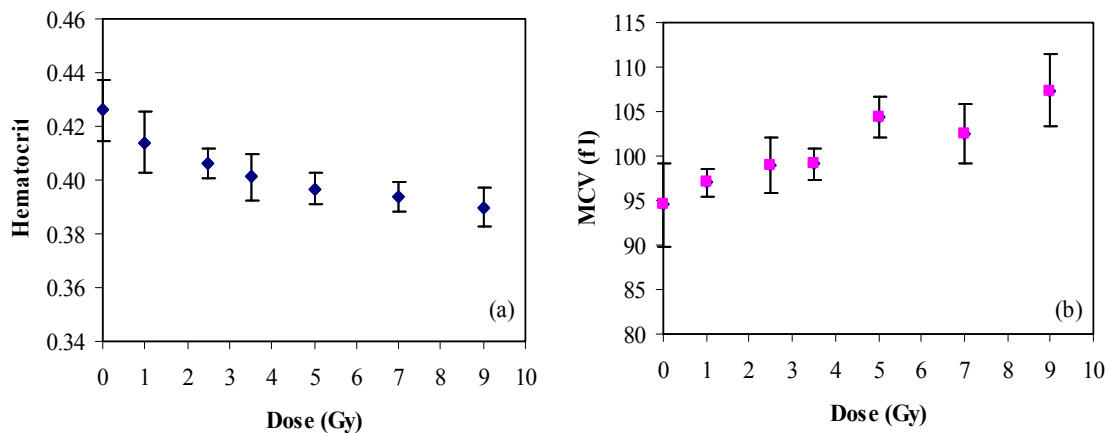


Figure 2. (a) The hematocrit versus dose and (b) The mean corpuscular volume (MCV) versus dose for control and irradiated groups.

Dielectric measurements

The relative permittivity ϵ'_s , relaxation strength $\Delta\epsilon'$ and relaxation time τ (s) calculated in this study showed significant decrease after exposure to the lowest dose and continued to decrease as the dose increased (table 2).

To elucidate the decrease in the surface charge density of the erythrocytes membrane, a comparison between the

variation in relaxation time and turbidity with dose has been performed. The relaxation time depends on both the charge and size on the cell, while the turbidity depends on their size only. Figure 3 shows a decrease in the relaxation time as the dose increased, however, the turbidity increased up to 3.5 Gy, and then decreased as the dose increased.

Table 2. Relative permittivity, relaxation strength and relaxation time for control and irradiated groups (mean \pm S.D.).

Dose	ϵ'	$\Delta\epsilon'$	τ (s)
Control	$7.20 \times 10^6 \pm 1.37 \times 10^6$	$10.6 \times 10^6 \pm 1.38 \times 10^6$	$7.23 \times 10^{-3} \pm 8.76 \times 10^{-4}$
1.0 Gy	$5.71 \times 10^6 \pm 1.76 \times 10^6$	$7.80 \times 10^6 \pm 1.76 \times 10^6$	$4.94 \times 10^{-3} \pm 1.80 \times 10^{-3}$
2.5 Gy	$5.35 \times 10^6 \pm 5.25 \times 10^6$	$5.33 \times 10^6 \pm 5.27 \times 10^5$	$4.08 \times 10^{-3} \pm 1.40 \times 10^{-3}$
3.5 Gy	$4.65 \times 10^6 \pm 1.42 \times 10^6$	$4.62 \times 10^6 \pm 1.41 \times 10^6$	$3.98 \times 10^{-3} \pm 7.76 \times 10^{-4}$
5.0 Gy	$4.17 \times 10^6 \pm 7.99 \times 10^5$	$4.36 \times 10^6 \pm 7.99 \times 10^5$	$3.07 \times 10^{-3} \pm 6.21 \times 10^{-4}$
7.0 Gy	$4.10 \times 10^6 \pm 1.38 \times 10^6$	$4.08 \times 10^6 \pm 1.38 \times 10^6$	$2.58 \times 10^{-3} \pm 7.91 \times 10^{-4}$
9.0 Gy	$3.05 \times 10^6 \pm 1.23 \times 10^5$	$3.03 \times 10^6 \pm 1.21 \times 10^5$	$1.97 \times 10^{-3} \pm 7.28 \times 10^{-4}$

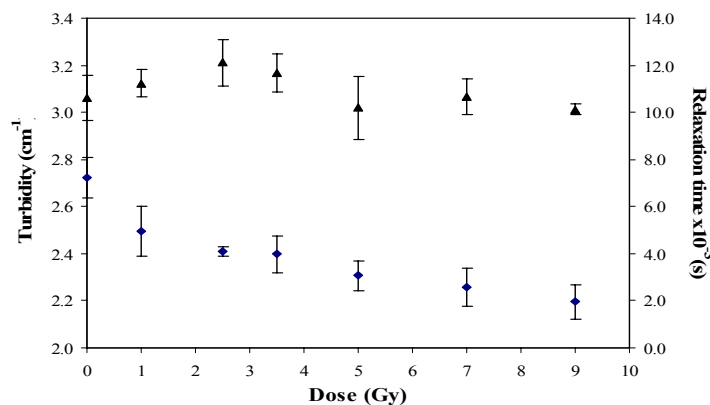


Figure 3. The relaxation time (♦) and turbidity (▲) versus dose, for control and irradiated groups.

DISCUSSION

The rheological properties of whole blood study the change of the viscosity with shear rate. Because blood is a non-Newtonian suspension, its fluidity cannot be described by a single value of viscosity. Rotational viscometers allow the measurement of viscosity over a range of shear rates, yielding a flow curve for a blood sample. Low shear viscosity depends mainly on the erythrocytes aggregation while the high shear viscosity depends on the erythrocytes deformability. This deformability is responsible for the low viscosity at higher shear rates⁽³⁾. The results described in this study indicated that the exposure to different doses of gamma radiation resulted in increase in the blood viscosity, consistency index and the value of the yield stress (defined as the minimum shear stress required to start the flow). The value of the flow index remains below unity for the entire dose taken, indicating the non-Newtonian behavior of the blood. The increase in blood viscosity may result in tissue damage by slowing the circulation of blood, and thus reducing the supply of oxygen and nutrient to the tissue cells. Reports have been shown that several alterations of hemorheological properties may take place as a result of free radicals generated during exposure to ionizing radiation, such as lipid peroxidation. For instance, lipid peroxidation may lead to decrease in the deformability of red cells and increase of aggregation⁽¹³⁾. Direct radiation attack on cell membrane may induce membrane abnormality. Among these abnormalities, are a loss of lipid, increase in rigidity of the lipid bilayer and aggregation of membrane proteins⁽⁴⁾.

The dielectric properties in the α -dispersion were measured in this study in the range of 60 Hz–40 kHz. In this frequency range, the dielectric permittivity of the living cells is due to the reorientation of the dielectric dipoles of the individual cells and the polarization of the surface

charges accumulated on the cell membrane⁽¹⁴⁾. The calculated relative permittivity in this study showed significant decrease after exposure to the lowest dose and continued to decrease as the dose increased (table 2). The electrical surface properties of the cell membrane play a critical role in the whole cell adhesion process and in the resulting alteration in the membrane structure and function. The electrostatic repulsion between red blood cells reduces erythrocyte aggregation⁽⁶⁾. Increased erythrocytes aggregability in this study is most likely related to decrease in the surface charge density and a shift of the balance toward aggregating forces because of decreased electrostatic repulsion among adjacent erythrocytes. The decrease in the surface charge density of the red blood cells can be explained through the reported effects of gamma radiation. It has been reported that the free radicals formed during radiolysis of water can cause a variety of membrane changes including lipid peroxidation, hydrolysis of phospholipids head groups, lipid-lipid crosslinks, disulfide bridge formation and amino acid residue damages in membrane protein⁽¹⁵⁾. The significant decrease in the relaxation time with dose reflects the decrease in the membrane surface charge. However, the increase in turbidity, which depends on the cell size only, emphasize the deformation in the membrane structure to yield larger size, as appeared from the increase in the mean corpuscular volume with the irradiation dose. Gamma radiation was shown to induce deformation in the cell membrane structure and change the cell shape from discoid to echinocyte. This transformation was determined by the progressive appearance of regularly spaced spicules on the surface of the erythrocytes membrane with the gradual transformation to ovoid shape⁽¹⁶⁾.

CONCLUSION

The present work studies the effect of gamma radiation on the rheological

properties of blood. The results showed increase in the blood viscosity as a function of dose, which can be attributed to several reasons. In this study we tested the change in the erythrocyte's membrane surface charge by measuring the relative permittivity and relaxation time at the low frequency range. Both factors revealed the decrease in the membrane surface charge density as a result of exposure to radiation. The decrease in the membrane surface charge is known to decrease the repulsion between the cells and increase blood viscosity. The turbidity test and mean corpuscular volume reflect the radiation-induced increase in the erythrocyte' membrane, a factor that can also, increase blood viscosity.

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