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Full Length Research Paper

Effect of gamma irradiation on biophysical and protection properties of melanin

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Melanins are natural pigments distributed in living organisms and they are responsible for pigmentation of surface structure. In the present work, gel exclusion chromatography, spectrophotometric, and dielectric relaxation techniques were used to characterize DOPA melanin before and after irradiation with ^{60}Co gamma rays in the range of 5 to 50 Gy. The results show that the studied melanin is composed of two structural groups. The extinction absorption coefficient and relative permittivity and conductivity of melanin sample increased after irradiation with gamma rays and were dose dependent in manner. These increases were attributed to the formation of melanin aggregation and crosslinks which result from the growing number of the formed free radicals by radiation. It was concluded from the results that melanin goes through some structural changes after irradiation with the gamma doses demonstrated. Further studies were recommended to investigate and evaluate whether these changes could affect its efficiency as a radio protector against gamma radiation.

Keywords: Melanin structure, gamma irradiation, gel chromatography absorption spectra, relative permittivity and conductivity.

INTRODUCTION

Melanins are amorphous, irregular polymeric pigments distributed in living organisms and they are responsible for the pigmentation of surface structure (Crippa and Michelini, 1999; Mosca et al., 1998; Riley, 1997; Bilinsk, 1996; Rosei and Mosca, 1996; Prota, 1992; Crippa et al., 1989; Miyake et al., 1986). They are insoluble in aqueous solution and organic solvents, and sometimes soluble in alkalis (Bilinsk, 1996; Harki et al., 1997). The basic chemical structure of melanin is not well defined but it is usually represented by covalently linked models. Melanin synthesis is produced either by auto-oxidation of catechola or by the tyrosin action on the enzyme tyrosinase (Rile, 1997; Rosei and Mosc, 1996; Blarzinoet al., 1999; Rosei and Mosca, 1995).

Several investigations have been devoted to characterize their physical and chemical properties, such as its higher molecular weight, insolubility in water and common organic solvents, specific heat, carrier mobility, electrical conductivity, redox, chelating, and photo protective action (Crippa and Michelini, 1999; Mosca et al., 1998; Riley, 1997; Bilinsk, 1996; Rosei and Mosca, 1996; Prota, 1992; Crippa et al., 1989; Miyake et al., 1986; Harki et al., 1997; Blarzino et al., 1999; Rosei and Mosca, 1995; kollias et al., 1991; Saran, 1992; Krol and Liebler, 1998; Yong-gang et al., 2009; Hengshan et al., 2006). However, the molecular mechanism of melanin at cellular and sub-cellular level is not yet fully explained by the biophysical and biochemical studies (Crippa and Michelini, 1999; kollias et al., 1991; Saran, 1992).

Dielectric properties of biological materials have been previously investigated (Ghannam et al., 2002; Foster and Schwan, 1995; Subrata, 1992; Grant et al., 1978). It has been established that the direct current (DC) conductivity of 3,4-dihydroxyphenylalanine (DOPA) melanin is strongly dependent on the water content in the polymer structure (Jastrzebsk et al., 2002, 1995). In vacuum, where the humidity of melanin reaches a minimum value,

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the conductivity is of the order 10^{-13} S/cm and increases as the temperature increases (Jastrzebsk et al., 2002). Kirkpatrick et al. (1999) concluded that the high dielectric constant of melanin was due to a collective interaction between melanin, water and ions.

The increase in the level of the environmental contaminations accelerates the urgent need to have a proper radio protector material. In this regards, melanin as an important natural radio protector material against radiation hazards is growing considerably. Mosse et al. (2000) found out that melanin decreases the clastogenic effect of ionizing radiation in human and mouse somatic cells and modifies the radio adaptive response. They concluded that melanin is capable of completely removing low-dose radiation effects. In another study on the effects of γ-irradiation on the physiological-biochemical properties of strains of *Cladosporium cladoporiodes* de Veries, Vember et al. (1999) found a correlation between the presence of melanin pigment in the cell wall of the studied strain and the activity of glutathione transferees. Moreover, Melanin has a photo-protection contribution against ultraviolet radiation by absorbing or scattering the incident light and by scavenging reactive free radicals and other oxidants (Prota, 1992; Krol and Liebler, 1998; Kvam and Tyrrell, 1999; Bustamant et al., 1993; Cedekel and Zeise, 1988).

The aim of the present work is to study the effect of different gamma irradiation doses on some biophysical properties of melanin as a step forward to investigate its usage as a radio-protector against gamma irradiation doses.

MATERIALS AND METHODS

Synthetic melanin (DOPA), hybrid electrical power sources (HEPS) buffer, Sephadex beads G-200 and standard molecular weight markers kit MW-GF-1000 were purchased from Sigma (St. Louis, MO, USA) and were used without further purification. Melanin solutions were obtained by dissolving melanin in 1 M NaOH (20 mg/ml) at pH = 13. After complete melanin solubility, the solution pH was adjusted at 7.4.

Gel chromatography and melanin sizing

For measuring the molecular weight distribution and the size of melanin molecule, 2 ml of melanin solution (0.2 mg/ml, pH = 7.4) was carefully applied on a Sephadex G-200 gel column surface. The homogeny of the gel beds was tested by filtration of blue dextran 2000 and the elution volume of which was considered as a void volume, V_o. The column was then calibrated for the molecular weight estimation by a well-characterized globular protein standard kit model MW-GF-1000 containing: carbonic anhydride, albumin bovine serum, alcohol dehydration, β-amylase and thyroglobulin B. The absorption of proteins and melanin fractions were measured at 380 nm. The column parameter (distribution coefficient) K_d was calculated from the relation:

$$K_d = (V_e - V_o) / (V_t - V_o) \quad (1)$$

where V_o, V_t, and V_e are the effective exclusion (void volume), the total and the sample elution volumes, respectively. The K_d values of the studied samples is related to the molecule size by the following relation (Paternostre et al., 1995; Lesieur et al., 1991):

$$\text{Log (MD)} = 3.03 - 4.43\ K_d + 9.63\ (K_d)^2 - 8.85\ (K_d)^3 \quad (2)$$

where MD is the mean molecule diameter in nanometer (nm).

Spectrophotometric measurements

Melanin absorption spectra were recorded by ultraviolet-visible (UV-VIS) double beam spectrophotometer type 1601 PC (manufactured by Shimatzu, Japan).

Dielectric measurements

Dielectric measurements were taken in the frequency range from 20 Hz up to 3 MHz using a Wayne Kerr precision component analyzer, model 6440 B (UK) together with a conductivity cell, model 19250, manufactured by Cole Palmer Co. The sample cell has two squared platinum black electrodes with a cell constant of 1 cm^{-1}. The measurements were performed for diluted melanin solution in distilled water at a concentration of 0.1 µg/ml and were measured at 22°C.

For a dielectric material placed between two parallel plates capacitor, the measured values of the capacitance, C and resistance, R, were used to calculate the conductivity, σ, as well as the real, ε′, and imaginary, ε″, parts of the complex permittivity, $\varepsilon^* = \varepsilon' - j\varepsilon''$, using the following equations:

$$\varepsilon' = Cd/\varepsilon_o A\ ,\ \varepsilon'' = \varepsilon' \tan\delta \text{ and } \sigma = G\ (d/A) = d/\ RA$$

where d is the cell inter electrode distance (m), A is the electrode area (m^2), so d/A is the cell constant = 1 cm^{-1}, G is the conductance (Ω^{-1}), ε_o is the permittivity of free space and tanδ is the loss tangent factor, $\tan\delta = 1/\omega RC$.

Gamma irradiation facility

The studied samples were irradiated by ^{60}Co gamma rays presented at King Saud University, College of Science, Kingdom of Saudi Arabia with 87 Gy/h dose rate at the center of the chamber. In the present work, melanin samples received irradiation doses in the range of 5 to 50 Gy.

RESULTS AND DISCUSSION

Figure 1 shows the elution pattern of DOPA melanin exposed to gamma rays in the range of 5 to 50 Gy when compared with the unexposed one (control). It is clear from the figure that the melanin eluted into two fractions. The elution volume V_e for each fraction was obtained and the distribution coefficient K_d of the eluted volume was calculated by means of using Equation 1. Consequently, the mean diameter of the melanin molecule was calculated through the use of Equation 2 and the corresponding molecular weights were obtained using the column calibration curve.

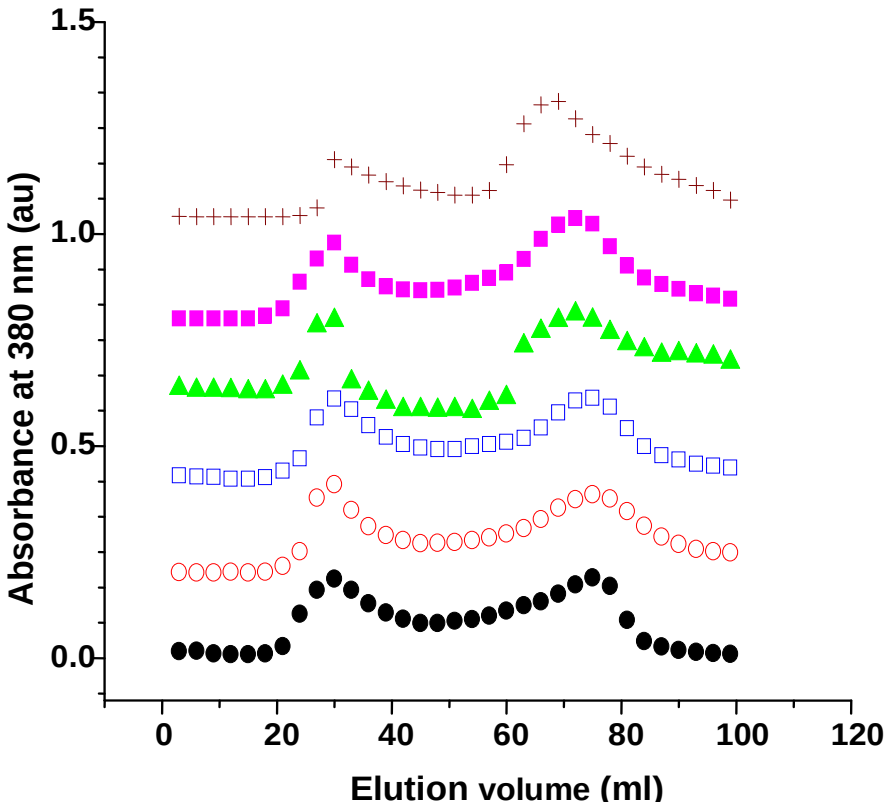

Figure 1. Elution pattern of DOPA melanin for unexposed and exposed to different gamma irradiation doses at 380 nm: (●) Unexposed (Control), (○) 5 Gy, (□) 10 Gy, (▲) 20 Gy, (■) 30 Gy, and (+) 50 Gy.

The results indicate that normal melanin consist of two groups; the first one is the higher molecular weight of approximately 1,000 kD and 3 nm mean diameter, and the second one is the smaller one of 28 kD and 0.3 nm mean diameter. Melanin exposed to gamma rays show an increase in the molecular weight up to 15 kD for 50 Gy exposure dose of the second group. This may be attributed to formation of some melanin aggregation which results from the free radicals produced by irradiation.

Figure 2a shows the absorption spectrum of melanin at pH = 7.4 in the visible range before and after gamma irradiation in the dose range of 5 to 50 Gy. It is clear from the figure that there is no characteristic absorption band for melanin in the visible range. However, the absorption of visible light increases as the irradiation dose increases. The plots of log absorbance versus wavelength (Figure 2b) gave a linear relation with negative slope of 0.0031 for all studied samples. The slope is often used to characterize and compare the different types of melanin. This obtained value agrees with the previous findings of Harki et al. (1997).

Figure 3a shows the relation between melanin concentration (µg/ml) at pH = 7.4 and absorbance measured at 380 nm for the control and those exposed to gamma rays in the range of 5 to 50 Gy. A linear relation was obtained in the concentration range used. The slope of this relation gives the value of the extinction coefficient (ε) at constant light path length. The values of ε corresponding to the melanin irradiation dose are indicated in Table 1 and drawn in Figure 3b. It is clear from the table and figure that as the exposure dose increase, the melanin absorption coefficient ε gradually increases. This data confirm the results obtained from Figure 2, which show that as the exposure dose increases, melanin absorbance to electromagnetic radiation increases.

Figure 4 illustrates the variation of relative permittivity ε' (left y-axis) and conductivity σ (right y-axis), as a function of frequency in the range of 20 to 3×10^6 Hz for control and melanin exposed to 50 Gy (two graphs are given only for comparison). The results indicate a strong dielectric dispersion for the studied samples. This behavior was identified as anomalous frequency dispersion and it was found for different biological materials (Ghannam et al., 2002; Foster and Schwan, 1995; Subrata, 1992; Grant et al., 1978). The results also indicate remarkable increase in the electrical conductivity (σ) of the samples after irradiation with all gamma doses demonstrated. The variation of σ as a function of the irradiation dose is shown in Table 2 and represented in

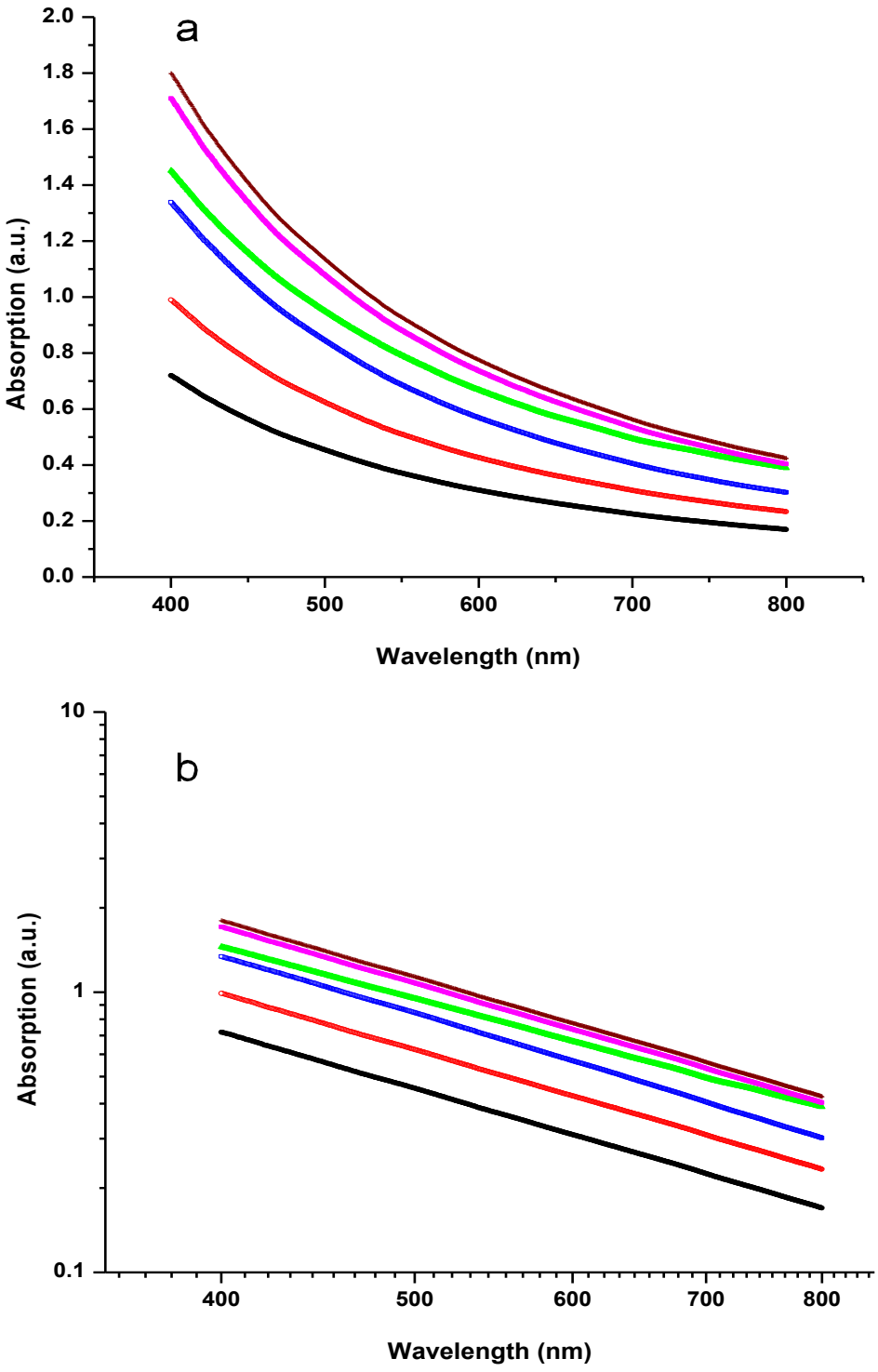

Figure 2. (a) The absorption spectrum of melanin solution of unexposed and exposed to different gamma doses. (b) The plot of relation between log absorbance and wavelength in the visible range: (●) Unexposed (Control), (○) 5 Gy, (□) 10 Gy, (▲) 20 Gy, (■) 30 Gy, and (+) 50 Gy.

Figure 5. It is clear from the figure that σ increases linearly with the irradiation dose up to 30 Gy. This charge density of the melanin macromolecules which resulted from breakage of chemical bonds by irradiation and formation of highly active molecular species which recombine, at random, forming new macromolecular

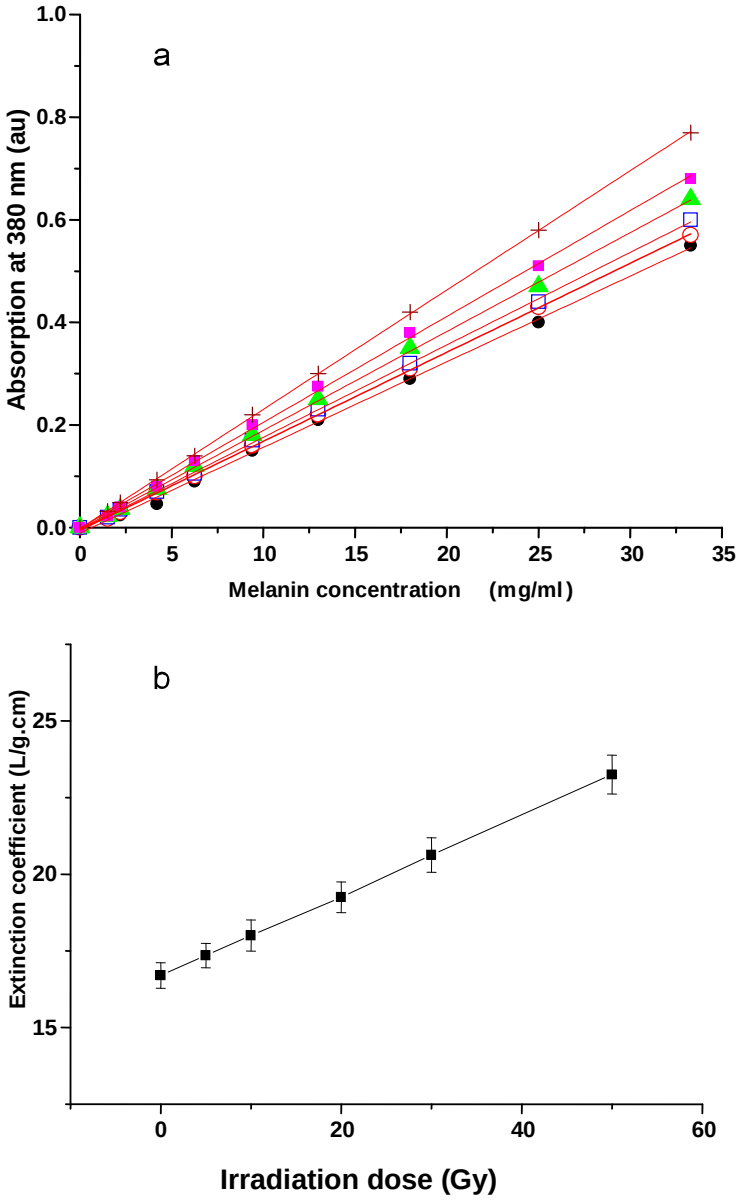

Figure 3. (a) The relation between absorbance measured at 380 nm and melanin concentration (mg/ml) for unexposed and exposed samples to different gamma irradiation doses: (●) Unexposed (Control), (○) 5 Gy, (□) 10 Gy, (▲) 20 Gy, (■) 30 Gy and (+) 50 Gy.(b) Melanin extinction coefficient as a function of gamma irradiation doses.

Table 1. Extinction coefficient (ε) of melanin solution at the different irradiation doses.

Irradiation dose (Gy)	Extinction coefficient ε ± SD (L/g cm)
Unexposed	16.8 ± 0.19
5	17.5 ± 0.19
10	18.1 ± 0.2
20	19.4 ± 0.21
30	20.6 ± 0.22
50	23.3 ± 0.24

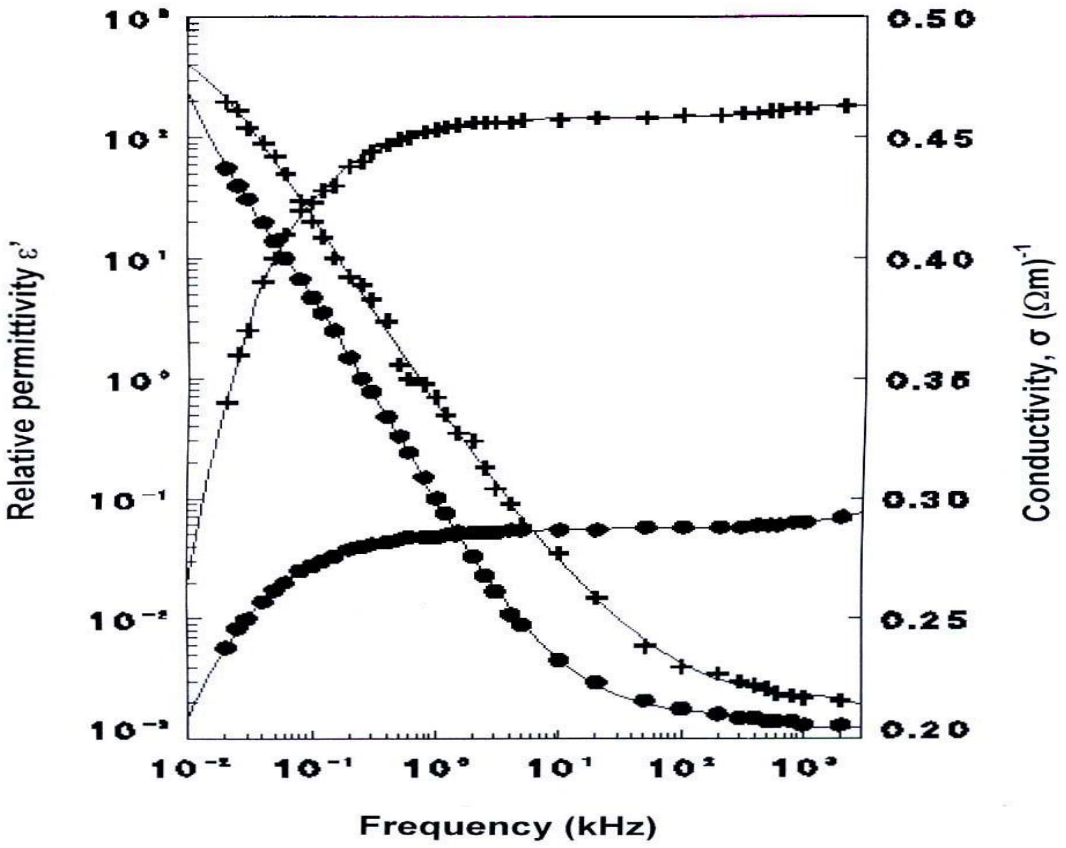

Figure 4. Variation of relative permittivity ε′ (left y-axis) and conductivity σ (right y-axis), as a function of frequency in the range of 20 to 3 × 10^6 Hz for unexposed and exposed melanin to different gamma doses: (●) Unexposed (Control), and (+) 50 Gy.

Table 2. The value of the electrical conductivity at 3 MHz of melanin solution corresponding to the different irradiation doses.

Irradiation dose (Gy)	Electrical conductivity at 3 MHz $(\Omega m)^{-1}$
Unexposed	0.294 ± 0.009
5	0.320 ± 0.009
10	0.350 ± 0.012
20	0.395 ± 0.012
30	0.425 ± 0.014
50	0.464 ± 0.014

forms. These results are supported by the gel filtration data presented in Figure 1.

Since melanin eluted into two fractions at 28 kD and 1,000 kD, the dielectric relaxation curve will represent the behavior of both groups in addition to the counter ion molecules. Therefore, the phenomena will be complicated, because of the different partners involved in the process of dielectric relaxation and it will be difficult to get some calculations for the dipole moment, relaxation time and molecular diameter from the present dielectric data.

Conclusion

It may be concluded from the present data that when melanin molecules are irradiated with ^{60}Co gamma doses in the range of 5 to 50 Gy, some structural changes occur in the macromolecules forming melanin. The efficiency of

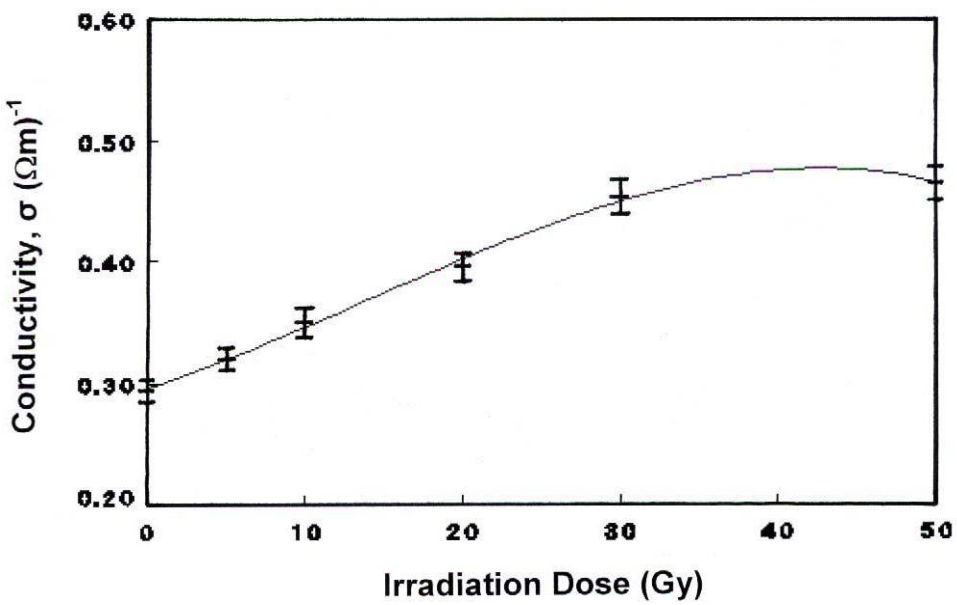

Figure 5. Variation of the conductivity measured at 3 MHz with the irradiation doses (Gy).

melanin as a radio protector may be affected by irradiation with the gamma doses demonstrated. However, more work is needed to decide this speculation, such as, dielectric relaxation for each group of melanin molecules and *in vivo* studies for unirradiated and irradiated melanin to be used as radio protector.

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