

## THE DIELECTRIC BEHAVIOUR OF $\gamma$ -IRRADIATED KERATIN

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Dielectric measurements as a function of temperature and frequency are reported for non-irradiated and  $\gamma$ -irradiation keratin with doses of 5 and 50 kGy. The effect of  $\gamma$ -irradiation on the dielectric permittivity of keratin is not observed up to 190°C. In this temperature range, the values of the relaxation time and dipole moment are similar for non-irradiated and irradiated keratin at the same temperature. The influence of irradiation is manifested as a shift of the peak parameter ( $\epsilon_r - \epsilon_\infty$ ) associated with denaturation process towards lower temperatures. This finding is supported by a lower values of the dipole moment for irradiated than for non-irradiated keratin, as a result of a decrease in the number of polar groups in the side and main chains of macromolecule in the irradiated samples.

### INTRODUCTION

Water states and thermal denaturation of keratin has been studied by electric methods. The hydration dependence of electric resistance of natural hair (Leveque, 1994), has emphasised the role of water in stabilisation of the structure of the keratin macromolecule. Martinsen, Grimnes and Konghaug (1997) measured the conductance and susceptance of the hair fibres as a function of frequency established their water dependence. The temperature dependence of the dielectric constants of horn (Maeda, 1989; Marzec & Kubisz, 1997) reveals a maximum due to water molecules. The studies suggested electrode polarisation and the Maxwell-Wagner effect as possible mechanisms of polarisation in keratin. In an earlier paper (Marzec, 1998) a maximum was observed in the temperature dependence of the dielectric constants of keratin near 240°C, which was interpreted as corresponding to denaturation of the keratin macromolecule. The occurrence of denaturation phase transition of keratin at a high temperature was confirmed by differential scanning calorimetry (DSC) method (Milczarek, Zieliński & Garcia, 1992). According to the results of the studies, water significantly affects the temperature of the appearance of the endothermic maximum.

The main purpose of this study was to obtain information on the behaviour of  $\gamma$ -irradiated keratin in a wide temperature range. To the best of our knowledge, literature does not report results concerning the electric behaviour of  $\gamma$ -irradiated keratin in the high temperature denaturation phase.

Studies of the dielectric properties of other gamma irradiated proteins (Jaroszyk & Marzec, 1994) revealed a decrease in the denaturation temperature with increasing doses of irradiation, which indicated a degradation of the protein structure. In the present paper the temperature dependence of the dielectric permittivity of non-irradiated and irradiated keratin was analysed taking into account the values of molecular dipole moment and relaxation time of the  $\alpha$ -dispersion.

### MATERIALS AND METHODS

The keratin samples used in this study were obtained from calf horn taken from 3–4-year-old animals. The procedure used to obtain the samples was described in a previous paper (Marzec & Kubisz, 1997). The samples were approximately rectangular slabs of the size  $6 \times 4 \times 1$  mm. A certain number of samples were subjected to  $\gamma$ -irradiation. Irradiation was performed at room temperature at a relative humidity of 70% using a  $^{60}\text{Co}$  source. The irradiation doses were 5 and 50 kGy.

In the present work, the components of the complex permittivity of keratin were performed by the bridge method (Jaroszyk & Marzec, 1991) in temperatures from 22 to 260°C and for the electric field frequency ranging from 10 Hz to 100 kHz. Prior to the measurement the samples were covered with silver paste electrodes and subjected to elimination of loosely bound water by keeping them in the measuring cell at constant temperature of 120°C for 2 h (Marzec & Kubisz, 1997). As a

result of this procedure samples lost about 8% of their mass, which corresponds the water content absorbed by these samples at room temperature at relative humidity (RH) of 60–70%. Following this procedure, the samples were cooled to room temperature and subjected to dielectric measurements in the cycle of heating up to 260°C.

The values of  $\epsilon'$  and  $\epsilon''$  which are the real and imaginary parts the of complex permittivity for non-irradiated and irradiated keratin are given as average from four to six samples in this study.

## RESULTS AND DISCUSSION

The temperature dependencies of the parameter  $(\epsilon_s - \epsilon_\infty)$  for the keratin samples non-irradiated and irradiated with doses of 5 and 50 kGy, where  $\epsilon_s$  and  $\epsilon_\infty$  are the low-frequency ("static") and high-frequency permittivities, are presented in Fig. 1. In the case considered  $\epsilon_s$  and  $\epsilon_\infty$  is determined at 100 Hz and 100 kHz, respectively. Up to 190°C, the differences between the values of  $(\epsilon_s - \epsilon_\infty)$  are small for all samples. Most probably, in this temperature range a similar molecular process occurs in non-irradiated and irradiated keratin. However, for these curves recorded above 190°C, the effect of irradiation is manifested as a shift of  $(\epsilon_s - \epsilon_\infty)$  of peaks associated with denaturation process towards lower temperatures and a decrease in their amplitude. In an earlier paper (Jaroszyk & Marzec, 1994), a similar behaviour of the dielectric constant  $\epsilon'$  below 200°C was observed for collagen  $\gamma$ -irradiated with doses up to 300 kGy. The frequency dependencies of the average values and standard deviations of  $\epsilon''$  for all keratin samples, non-irradiated and irradiated, are presented in Fig. 2. Similarly, as in the case of  $(\epsilon_s - \epsilon_\infty)$ , the values of

$\epsilon''$  show a small scatter (less than 9%) between samples at the same temperature. For these curves dielectric absorption peaks do not appear due to dipolar rotations. The behaviour observed for  $\epsilon''$  is similar to that of other proteins (Kakivaya & Hoeve, 1977; Sasaki, 1984) and is a consequence of interfacial polarization induced by the mobile protons ( $H^+$ ) present in keratin. The curves shown in Fig. 2 are determined by the release of water molecules as a result of breaking intra- and inter-microfibril hydrogen bonds. Changes in the values of  $\epsilon''$  are proportional to the number of jumps performed by protons between sites formed by water molecules during the period of oscillation of the electric field. The fact that the slope of the curves recorded up to 120°C is significantly lower than that for higher temperatures indicates the absence of loosely bound water molecules in these samples as a consequence of their heating prior to the measurements. The character of the curves recorded in the range 140–190°C implies the release of the remaining strongly bound water from the samples, which is accompanied by about 3% mass loss (Marzec & Kubisz, 1997). On the basis of the dielectric results shown in Figs. 1 and 3 we can determine the total moment  $m$  and relaxation time  $\tau$  of the dipoles for keratin up to 190°C. The dipole moment  $m$  can be obtained from the dependence Hawkes and Pethig (1988):

$$(\epsilon_s - \epsilon_\infty)(2\epsilon_s + \epsilon_\infty)/\epsilon_0(\epsilon_\infty + 2)^2 = Nm^2/9\epsilon_0kT, \quad (1)$$

where  $kT$  is the Boltzmann energy,  $\epsilon_0$  is the permittivity of vacuum. Furthermore,  $N$  is the number density of the dipoles responsible for dispersion. Keratin has mean molecular weight of  $7 \times 10^4$  g/mol, so in the case considered for the specific density samples of  $1.5 \times 10^6$  g/m<sup>3</sup>, the number

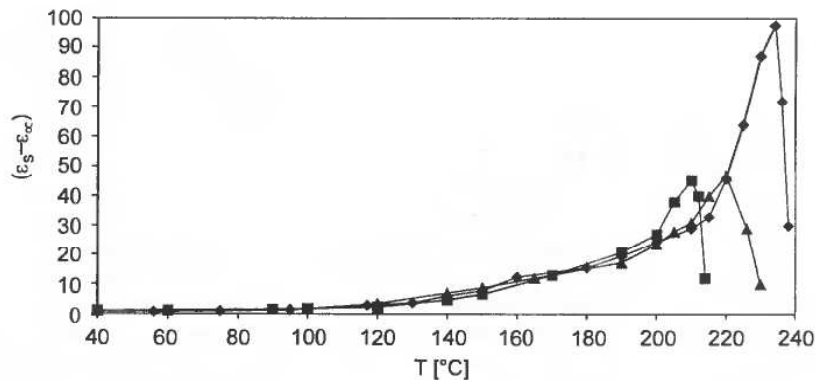


Fig. 1. Temperature dependencies of the parameter  $(\epsilon_s - \epsilon_\infty)$  for the keratin non-irradiated ( $\blacklozenge$ ) and irradiated with doses 5 kGy ( $\blacktriangle$ ) and 50 kGy ( $\blacksquare$ )

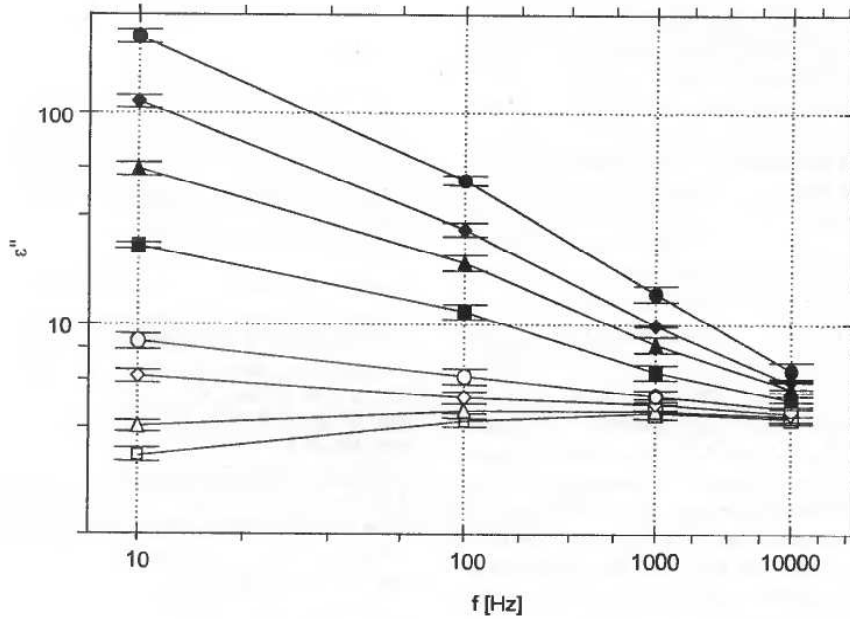


Fig. 2. Frequency dependencies of the average values and standard deviations of  $\epsilon''$  for keratin non-irradiated and irradiated with doses of 5 and 50 kGy at temperatures of 40°C ( $\square$ ); 80°C ( $\Delta$ ); 100°C ( $\circ$ ); 120°C ( $\circ$ ); 140°C ( $\blacksquare$ ); 160°C ( $\blacktriangle$ ); 180°C ( $\blacklozenge$ ); 190°C ( $\bullet$ )

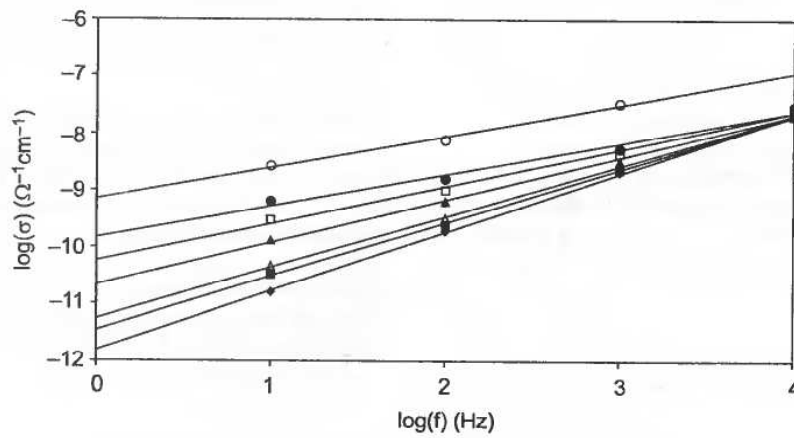


Fig. 3. Frequency dependencies of the average values of conductivity  $\sigma$  for keratin non-irradiated and irradiated with doses of 5 and 50 kGy at temperatures of 40°C ( $\blacklozenge$ ); 100°C ( $\blacksquare$ ); 120°C ( $\Delta$ ); 140°C ( $\blacktriangle$ ); 160°C ( $\square$ ); 180°C ( $\bullet$ ); 190°C ( $\circ$ )

density  $N$  is  $1.29 \times 10^{25} \text{ m}^{-3}$ . The left hand side of Eq (1) implies that  $m$  changes with temperature. A similar behaviour can be observed for the relaxation time  $\tau$  given by Pethig (1992):

$$\tau = \epsilon_0 \epsilon_\infty / \sigma, \quad (2)$$

where, in the case considered,  $\sigma$  is the conductivity at 1 Hz. Numerical values of  $\sigma$  for 1 Hz were extrapolated from the frequency dependencies of the

average values of conductivity for all keratin samples, presented in Fig. 3. As follows from these curves, the conductivity at 1 Hz varies from  $1.5 \times 10^{-12}$  to  $7.8 \times 10^{-10} (\Omega\text{cm})^{-1}$  in the temperature range of 40–190°C. Table 1 gives the average values and standard deviation of  $\tau$  and  $m$  of keratin non-irradiated and irradiated with doses of 5 and 50 kGy. It is observed that  $m$  increases and  $\tau$  decreases with increasing temperature. Probably, the obtained data can be explained by an increase in

the number and mobility of protons between sites made by bound water and an increase in the rotation of polar groups in the main chain and the side chains. In fact, Table 1 indicates that, both  $\tau$  and  $m$  change up to 120°C and in the range 140–190°C, by 65 and 95% on the average, respectively. The influence of ionizing radiation on the character of the curves above 190°C in Fig. 1, is reflected by lower values of  $m$  for irradiated keratin than for non-irradiated one at the temperatures at which the maxima ( $\epsilon_s - \epsilon_\infty$ ) occur for all samples. These values of  $m$  for keratin irradiated with doses of 0, 5 and 50 kGy are of about  $2.21 \times 10^{-28}$ ,  $1.39 \times 10^{-28}$  and  $1.30 \times 10^{-28}$  Cm, respectively. Most probably, in the irradiated samples the number of such polar groups as OH, CO and NH in the side and main chains, which are responsible for the total dipole moment of keratin, decrease as a result of the macromolecule degradation. This process involves damage to peptide and hydrogen bonds and disulfide bridge which are responsible for the strength of the keratin molecules.

Table 1. Average values and standard deviation of the relaxation time  $\tau$  and dipole moment  $m$  of keratin non-irradiated and irradiated with doses of 5 and 50 kGy

T [°C]	$m$ [Cm]	$\tau$ [ms]
40	$(0.26 \pm 0.02) \times 10^{-28}$	$418 \pm 7$
100	$(0.32 \pm 0.02) \times 10^{-28}$	$251 \pm 1$
120	$(0.44 \pm 0.04) \times 10^{-28}$	$155 \pm 1$
140	$(0.56 \pm 0.03) \times 10^{-28}$	$43.5 \pm 0.7$
160	$(0.81 \pm 0.05) \times 10^{-28}$	$18.5 \pm 1.3$
180	$(0.94 \pm 0.05) \times 10^{-28}$	$6.7 \pm 0.4$
190	$(1.11 \pm 0.06) \times 10^{-28}$	$2.9 \pm 0.3$

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