JOURNAL OF MATERIALS SCIENCE 29 (1994) 5353–5356

The effect of ionizing radiation on dielectric properties of bovine achilles tendon collagen in the temperature range of thermal denaturation

F. JAROSZYK, E. MARZEC*
Department of Biophysics, Academy of Medicine, Fredry 10, 61-701 Poznań, Poland

The effect of γ-irradiation, with doses from 10^2–2×10^3 kGy, on the dielectric properties of solid-state collagen was studied. The temperature dependence of the constants ε′ and ε″ revealed a decrease in the denaturation temperature with increasing dose of irradiation. Dielectric dispersion observed in the frequency range 10 Hz to 10 kHz was suggested to be due to Maxwell–Wagner–Sillars polarization. In addition, an increase in the irradiation dose resulted in increasing activation energy of bovine achilles tendon collagen.

1. Introduction

In order to recognize the mechanism of radiation-induced changes in collagen fibres of tendons and skin, many physico-chemical measurements have been made [1–9]. For example, reports on the investigation of solubility [1, 3], shrinkage temperature [4–7], tensile strength [3, 6] and aminoacid analysis of collagen [1, 5, 9] may be found in the literature. The results of these studies prove that γ-irradiation initiates two important processes: main-chain degradation and cross-linking. These processes depend first of all on the irradiation dose and water content in the collagen. The conditions of irradiation have only a slight effect on the physical and chemical properties of collagen. Degradation of collagen structure manifests itself, among others, by a decrease in the temperature of shrinkage and a reduction in the tensile strength. The increase in these parameters, together with a reduction in collagen solubility observed as a result of irradiation, indicate the formation of additional cross-linking bonds. Both processes are accompanied by a reduction in the total number of aminoacids in collagen. The studies reported so far were performed in the temperature range up to 373 K. No data on physical properties of γ-irradiated collagen at higher temperatures are available.

The aim of the present work was to study the complex permittivity components ε′ and ε″ of γ-irradiated collagen over a wide temperature range. Dielectric methods were chosen because they have not as yet been applied extensively to study the thermal denaturation of γ-irradiated bovine achilles tendon collagen.

2. Materials and methods

Prior to irradiation, bovine achilles tendon (BAT) collagen samples were prepared as reported elsewhere [10] and then placed for 2 wk at 298 K in an desiccator containing appropriate saturated salt solution. After this time the samples contained 0.05 g H_2O/g dry collagen. The water content of the samples was determined from the loss of weight after drying to constant weight over P_2O_5 at 368 K for 12 h. Irradiation was performed at room temperature from ^{60}Co source. The mean quantum energy of the γ-rays used was 1.25 MeV. Doses of γ-irradiation varied from 10^2–2×10^3 kGy. The BAT samples to be studied were covered with silver paste electrodes. The samples were cylindrical in shape, 1 cm diameter and 4×10^{-2} cm high. Prior to measurement, the samples were again placed in a desiccator containing the saturated salt solution to gain the same water content as at the moment of irradiation. In the present work, the measurements of dielectric properties of collagen in the solid state were made over a frequency range of electric field from 10 Hz to 10 kHz and at temperatures from 298–503 K with a heating rate of 1 K min^{-1}. The experiments were performed under normal pressure (in air). The measuring apparatus was designed on the basis of the bridge solution applied in earlier papers of Jaroszyk and Marzec [11].

3. Results

Figs 1 and 2 show the temperature dependences of dielectric constant, ε′, and dielectric loss factor, ε″, obtained for γ-irradiated and non-irradiated (0 kGy) collagen at an electric field frequency of 10^3 Hz. As can be seen, the values of ε′ and ε″ components increase significantly for temperatures above 373 K for all irradiation doses applied. In this range, the temperature dependences of ε′ obtained for collagen irradiated with the doses 0, 10^2 and 3×10^2 kGy reveal

* Author to whom all correspondence should be addressed.

0022–2461

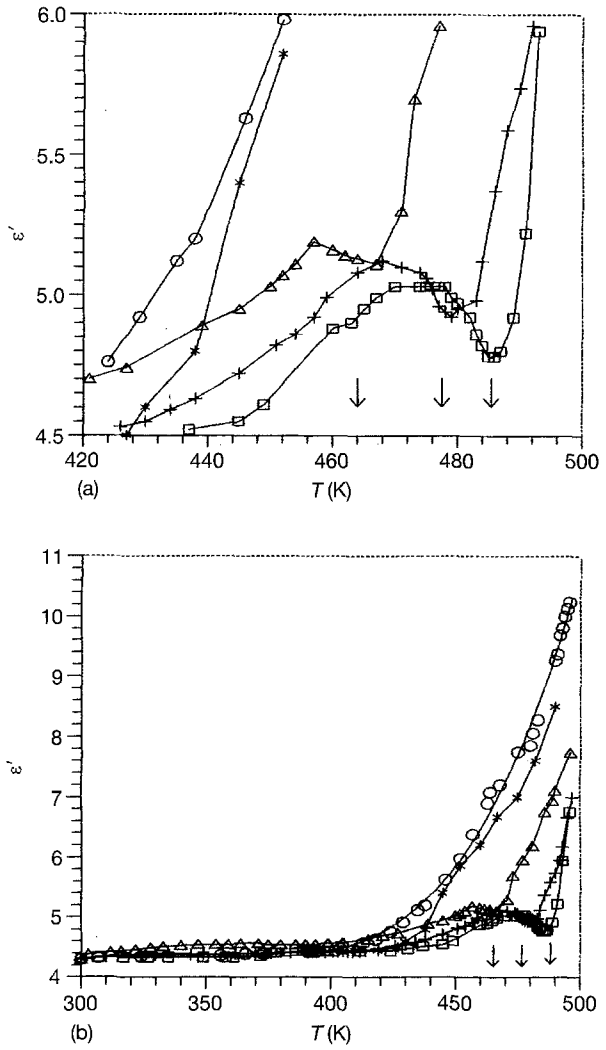


Figure 1 Temperature dependence of the dielectric constant, ε′, for collagen irradiated with (□) 0 kGy, (+) 10^2 kGy, (△) 3×10^2 kGy, (*) 5×10^2 kGy, (○) 2×10^3 kGy: (a) in over wide temperature range; (b) in the region of the denaturation temperature.

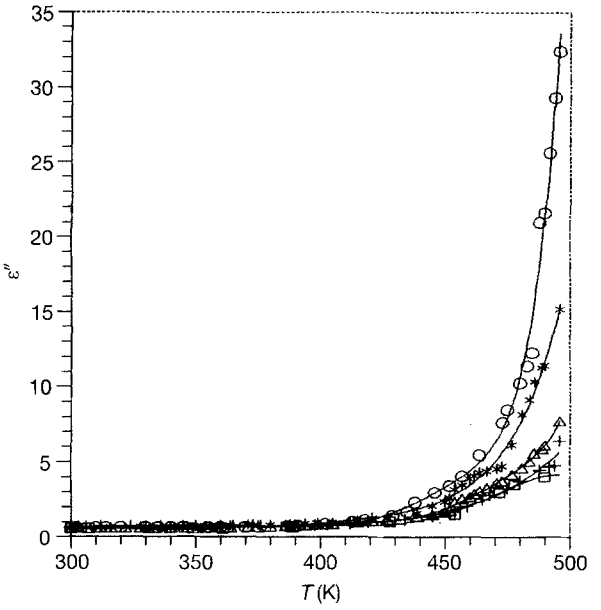


Figure 2 Temperature dependence of the dielectric loss factor, ε″, for collagen irradiated with (□) 0 kGy, (+) 10^2 kGy, (△) 3×10^2 kGy, (*) 5×10^2 kGy, (○) 2×10^3 kGy.

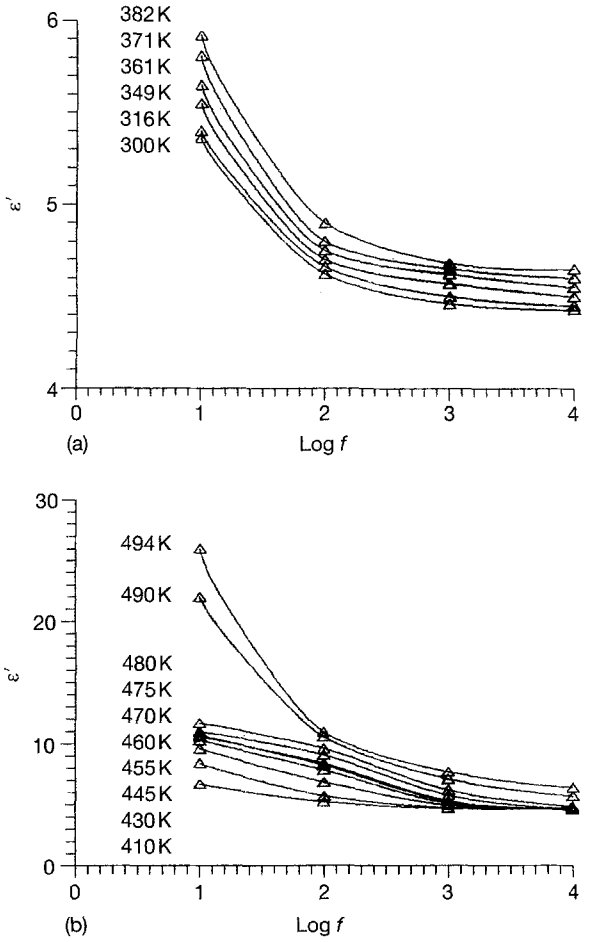


Figure 3 (a, b) Frequency dependence of the dielectric constant, ε′, for collagen irradiated with 3×10^2 kGy, at various temperatures.

maxima ε'_{max} and minima ε'_{min} which shift towards lower temperatures with increasing doses of irradiation. For the irradiation doses of 5×10^2 and 2×10^3 kGy, the local maxima and minima in ε′ disappear. The temperature dependence of ε″ increases with increasing irradiation dose.

The frequency dependence of the dielectric constant ε′ and loss factor ε″ are shown in Figs 3 and 4, respectively, for collagen irradiated with 3×10^2 kGy. The curves presented in the figures were taken for a few temperatures in the range 298–503 K. Numerical values of ε′ and ε″ decrease with increasing frequency at each temperature studied. From the obtained dispersion curves (Fig. 3) the activation energy, ΔH, of irradiated collagen could be determined using the method of time–temperature superposition [10, 12]. The frequency dependences of ε′ for different temperatures were shifted by log$a$ along log f to give a continuous master curve of ε′ at 316 and 480 K, assuming a to be a shift factor. Fig. 5 shows the dependence of $\log a = F(T^{-1})$ for collagen irradiated with a dose of 3×10^2 kGy obtained according to the above procedure, as well as curves for other doses determined in a similar manner. From the slopes of these curves, the numerical values of the activation energy, ΔH, given in Table I, were found for different temperature ranges. Table I indicates significant changes of ΔH occur with irradiation dose in the temperature range 410–494 K.

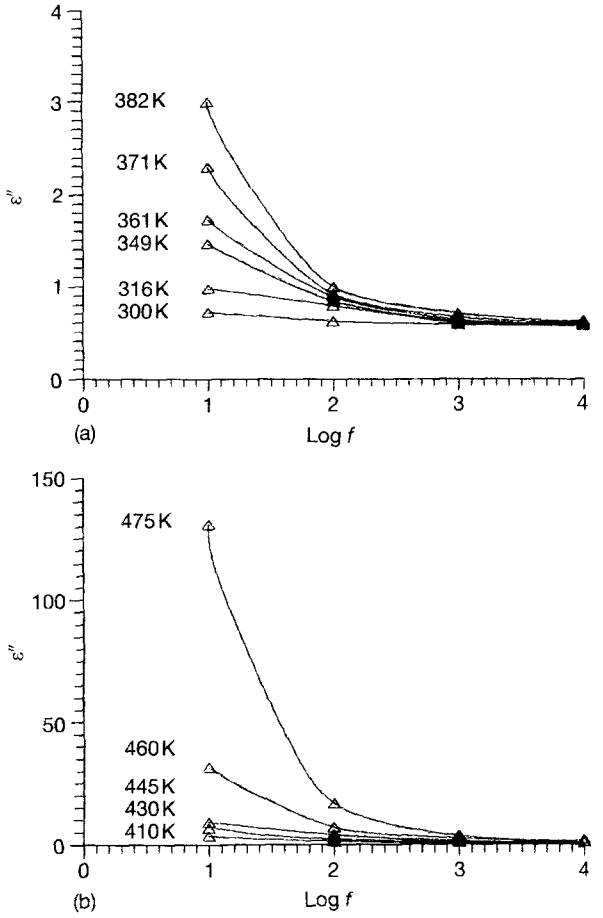


Figure 4 (a, b) Frequency dependence of the dielectric loss factor, ε″, for collagen irradiated with 3×10^2 kGy, at various temperatures.

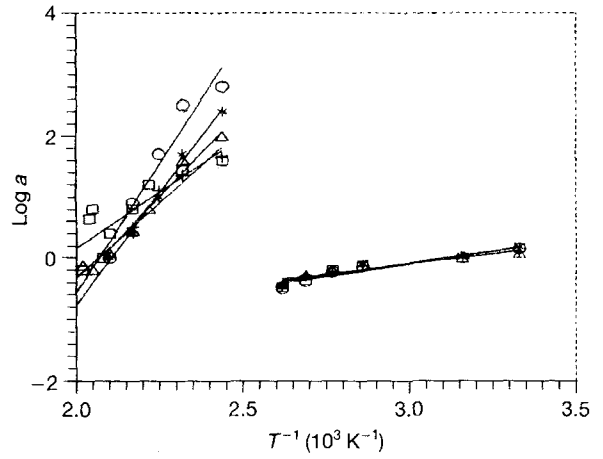


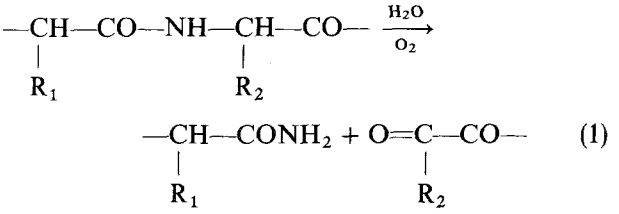
Figure 5 Dependence $\log a = F(T^{-1})$ for collagen irradiated with (□) 0 kGy, (+) 10^2 kGy, (△) 3×10^2 kGy, (*) 5×10^2 kGy, (○) 2×10^3 kGy.

TABLE I Activation energy, ΔH, of collagen irradiated in the dose range $0-2 \times 10^3$ kGy

Dose (KGy)	ΔH (Kcal mol^{-1})	
	300–382 K	410–494 K
0	3.6	22
100	3.1	22
300	3.6	25
500	3.6	33
2000	2.9	41

4. Discussion

The obtained temperature and frequency dependences of the components ε′ and ε″ reflect the structural changes which take place upon irradiation in a collagen–water system. The character of these changes can be interpreted on the basis of the mechanism of dielectric polarization of the Maxwell–Wagner–Sillars (MWS) type. The MWS polarization occurring in a heterogeneous medium, such as a collagen–water system, is caused by the localized hopping of charge carriers (free protons) between neighbouring sites [13–16]. In the studied system of non-irradiated collagen (0 kGy), sites are produced by structural water molecules forming strong hydrogen bonds with the main chain of the macromolecule [17–19]. In the case of irradiated collagen, the number of such sites is increased by the products of water radiolysis. Such radicals as OH°, H° and their molecular products H_2, H_2O and H_2O_2 produce hydrogen bonds with newly formed carbonyl and amide groups. The latter are formed in the process of degradation which, for a polypeptide chain of collagen, can be written as [5]



(1)

It follows from the temperature dependences of ε′ and ε″ of collagen presented in Figs 1 and 2, that the influence of the sites on MWS polarization is revealed at certain temperatures depending on the irradiation dose. Small changes in ε′ and ε″ for each curve up to a temperature of about 373 K indicate that, in this temperature range, MWS polarization is a consequence of limited mobility of protons between the sites strongly bound to a macromolecule of collagen. Confirmation of the nature of the changes in ε′ and ε″ is obtained from the low values of the activation energy in the temperature range 300–382 K (Table I). For temperatures above 373 K, when the thermal energy is still being supplied to the studied sample, hydrogen bonds within the collagen macromolecule are gradually broken, causing a release of water molecules and the products of water radiolysis. As a consequence of the increased number of sites and proton mobility, the MWS polarization increases. Most probably the process of liberation of the structural water from collagen irradiated with 0 kGy is completed at about 480 K, at which ε'_{max} is observed [10]. In the case of irradiated collagen, the release of structural water and products of its radiolysis, takes place at temperatures lower than 480 K, which is manifested as a shift of ε'_{max} towards lower temperatures with increasing irradiation dose. The minimum value, ε'_{min}, observed at about 490 K for a dose of 0 kGy and appropriately shifted towards lower temperatures for doses of up to 3×10^2 kGy, indicates the completion of the diffusion of water molecules and products of its radiolysis, from the collagen–water system. Further increase in ε′ and ε″, after they have reached ε'_{min}, is a consequence of

polarization originating from polar groups of aminoacid residues located in the main and side chains of a melting (denatured) macromolecule. The decrease in the temperature of denaturation observed for irradiated collagen as a result of macromolecule degradation, confirms the results obtained for this protein by other physical methods [4–7]. The temperature dependence of ε' for doses 5×10^2 kGy and 2×10^3 kGy, presented in Fig. 1, does not reveal maximum or minimum values. Most probably, denaturation of the samples irradiated with these doses had already occurred at the moment of irradiation at an ambient temperature. Confirmation of this supposition is the rapid increase in the values of ε' and ε'' above 373 K for the samples irradiated with 5×10^2 and 2×10^3 kGy, when compared with the dependences obtained for the samples irradiated with lower doses, see Figs 1 and 2. Such characteristic changes in the permittivity components are due to MWS polarization whose sites are formed not only by structural water molecules and the products of its radiolysis, but also by elements of damaged first-order structure of the collagen macromolecule. As a result of the formation of a greater number of sites for mobile protons, the activation energy increases for doses greater than 3×10^2 kGy in the temperature range 410–494 K (Table I).

References

1. J. H. CASSEL, *J. Am. Leather Chem. Assoc.* **54** (1959) 432.
2. A. J. BAILEY and W. J. TROMANS, *Radiat. Res.* **23** (1964) 145.
3. A. J. BAILEY, D. N. RHODES and C. W. CARTER,, *ibid.* **22** (1964) 606.
4. A. J. BAILEY, R. J. BENDALL and N. D. RHODES, *Int. J. Appl. Radiat. Isotopes* **13** (1962) 131.
5. J. H. BOWES and J. A. MOSS, *Radiat. Res.* **16** (1962) 211.
6. N. RAMANATHAN, V. MOHANARADHAKRISHNAN and Y. NAYUDAMMA, *Biochim. Biophys. Acta* **102** (1965) 533.
7. K. PIETRUCHA, *Polym. Med.* **XIX** (1–2) (1989) 3.
8. J. MONBOISSE, M. GARDES-ALBERT, A. RANDOUX, J. BOREL and C. FERRADINI, *Biochim. Biophys. Acta* **965** (1988) 29.
9. A. CHARLESBY, *Polym. J.* **19** (1987) 649.
10. F. JAROSZYK and E. MARZEC, *Ber. Bunsenges. Phys. Chem.* **97** (1993) 868.
11. *Idem, Polish J. Chem.* **65** (1991) 123.
12. E. P. CHANG and J. C. W. CHIEN, *J. Polym. Sci. Phys.* **11** (1973) 737.
13. R. PETHIG, "Dielectric and Electronic Properties of Biological Materials" (Wiley, Chichester, 1979).
14. H. MAEDA, *Biophys. J.* **56** (1989) 861.
15. R. PETHIG, *Ann. Rev. Phys. Chem.* **43** (1992) 177.
16. N. SASAKI, *Biopolymers* **23** (1984) 1725.
17. J. R. GRIGERA and H. J. C. BERENDSEN, *ibid.* **18** (1979) 47.
18. P. L. PRIVALOV, *Adv. Protein Chem.* **35** (1982) 1.
19. G. N. RAMACHANDRAN, *Int. J. Peptide Protein Res.* **31** (1988) 1.

Received 23 September 1993
and accepted 28 April 1994