JOURNAL OF MATERIALS SCIENCE 30 (1995) 5237–5240

The dielectric relaxation time of collagen over a wide temperature range

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

The dielectric properties of collagen were measured at two hydration levels over a frequency range of 10^1–10^5 Hz and at temperatures from 298–470 K. In the low-frequency range, dielectric dispersion was observed which results from Maxwell–Wagner–Sillars polarization. The relaxation times for a collagen sample containing loosely bound water were shorter than for a collagen sample containing only structural water in the temperature range 298–390 K. Above 390 K, continuous decrease in the relaxation times was observed for both samples.

1. Introduction

Measurements of dielectric properties of collagen in the solid state over a wide range of frequency, temperature and hydration are reported. The observed dielectric dispersion originates from several polarization mechanisms.

According to Lim and Shamos [1] and Tomaselli and Shamos [2], the frequency and temperature dependences of the dielectric constant, ε', and the loss factor, ε'', of collagen measured in the frequency range 10^2–10^5 Hz and for temperatures 300–450 K, appear as a consequence of dipolar orientation of water molecules. A similar molecular mechanism has been proposed by Hoeve and Lue [3] who studied the effect of water molecules on dielectric properties of collagen in the frequency range 10^2–10^5 Hz at room temperature. Chang and Chien [4] and Nguyen *et al.* [5] have investigated the temperature dependence of the complex dielectric constant of collagen in the range 113–433 K and found two kinds of relaxation, both of which depended on the hydration level and frequency. The first relaxation around 193 K was associated with the side-chain rotations or localized movements in the main chain. The second relaxation, around 373 K, which shifts towards lower temperatures as the hydration level increases, was attributed to Maxwell–Wagner–Sillars polarization. In addition, many authors [6–9] observed a remarkable dispersion in the frequency range 10^{-4} Hz to 1 MHz for various proteins, and attributed it to proton transport processes. Grigera *et al.* [10] carried out dielectric measurements on partially hydrated collagen in the frequency ranges 100 kHz–5 MHz, 100 MHz–1 GHz and 8–23 GHz. The obtained data indicated a dielectric dispersion around 100 kHz, 0.3 and 10 GHz. These three relaxation types result from the Maxwell–Wagner–Sillars effect, motion of polar side chains and rotation of water molecules, respectively.

The main purpose of this study was to obtain information on the dielectric relaxation time of the collagen–water system. It is a heterogeneous material whose large main chains and side chains of very low conductivity as well as water molecules of high conductivity, determine the temperature dependence of the complex dielectric constant. The reported results of the studies of collagen in two states of hydration allow one to conclude which of the phases (the non-conducting core or the conducting layer) decide the properties in different temperature ranges. In this kind of study it is essential to know the exact water content in the sample before the measurements and the mass of the sample after them. The appropriate procedure was applied in this work. Analysis of physical and chemical properties of collagen is based on the theoretical stereochemical model [11]. According to the hydration model, water molecules in collagen are classified into three categories [12–14]: structurally bound (0.0–0.1 g H_2O/g collagen), loosely bound (0.1 to 0.3–0.5 g H_2O/g collagen) and free (> 0.5 g H_2O/g collagen). The paper reports the results of dielectric measurements carried out for two samples:

(i) sample A containing structurally and loosely bound water (0.22 g H_2O/g collagen);

(ii) sample B containing only structurally bound water (0.06 g H_2O/g collagen) at room temperature.

2. Experimental procedure

The collagen used in this study was obtained from bovine Achilles tendon (BAT). The method of preparation of the collagen samples was described in a previous paper [15]. The tendons were subjected to fat elimination procedure in ethyl ether, then they were washed in distilled water and in 0.1 *n* NaCl solution, dried at room temperature, powdered and formed into pellets. The samples were finally shaped into cylinders 1 cm diameter and 0.04 cm height. Samples were equilibrated to constant weight for 2 weeks at 298 K in a desiccator containing different saturated

0022–2461

salt solutions. As a result of this treatment, two sets of collagen samples A and B, containing 0.22 and 0.06 g H_2O/g dry collagen, respectively, were obtained. 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 [10].

In the present work, the complex dielectric constant ($\varepsilon^* = \varepsilon' - j\varepsilon''$; j is $-1^{1/2}$) of collagen in the solid state was measured by the bridge method described elsewhere [16]. Measurements were made over the electric field frequencies from 10 Hz to 100 kHz and at temperatures from 298–470 K at a heating rate of 1 K min^{-1}. The experiments were performed under normal pressure (in air).

3. Results

The dependences of ε' and ε'' components on frequency are shown in Figs 1, 3 and 2, 4 for collagen samples A and B, respectively. The curves presented in these figures were taken for temperatures from the range 298–470 K. These curves indicate that at each temperature studied the numerical values of ε' and ε'' components are higher for sample A than for sample B. As can be seen, both ε' and ε'' for samples A and B show remarkable dispersion in the low-frequency region. These results are very similar to those found by Tomaselli and Shamos [2] for collagen. From the obtained frequency dependences of ε' and ε'' for collagen we could determine the dielectric relaxation time, τ, of this dispersion, by the relationship [9, 17]

$$\tau = \frac{\varepsilon_0 \varepsilon_\infty}{\delta} \quad (1)$$

where ε_0 is the permittivity of free space, ε_∞ is the permittivity at the high-frequency limit of the dispersion (in this study we can take a value of 100 kHz) and δ is the electric conductivity at the low-frequency limit of this dispersion, which is 10 Hz. The value of δ is associated with proton transport along pathways interconnecting neighbouring sites. In this study, δ was obtained from the dependence

$$\delta = 2\pi f \varepsilon_0 \varepsilon'' \quad (2)$$

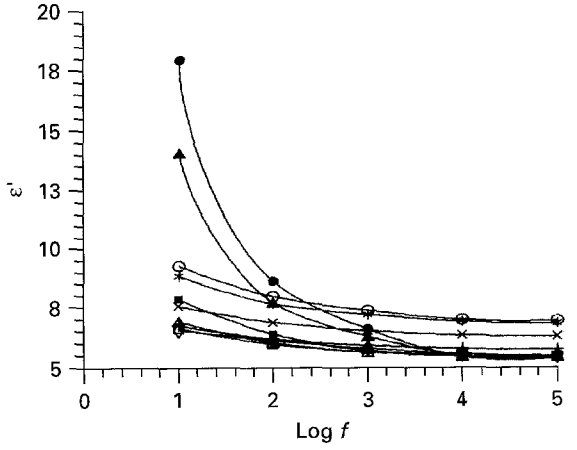

Figure 1 Frequency dependence of the dielectric constant, ε', for collagen sample A. (*) 300 K, (○) 316 K, (×) 330 K, (+) 350 K, (◇) 370 K, (□) 390 K, (△) 410 K, (■) 430 K, (▲) 460 K, (●) 470 K.

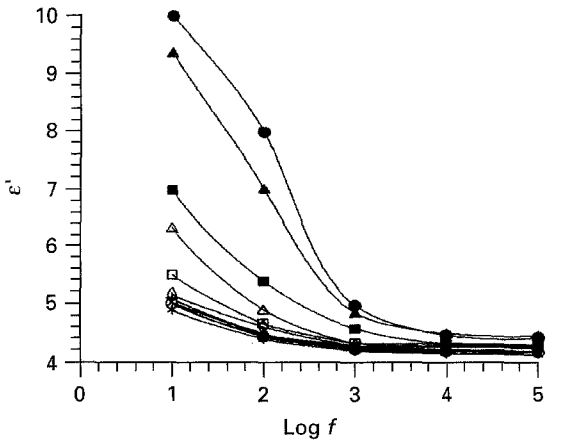

Figure 2 Frequency dependence of the dielectric constant, ε', for collagen sample B. For key, see Fig. 1.

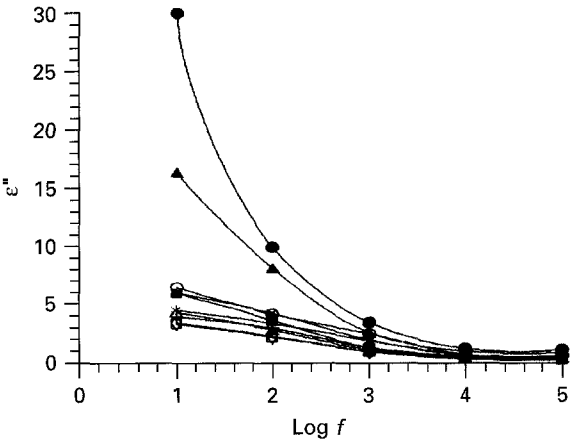

Figure 3 Frequency dependence of the dielectric loss factor, ε'', for collagen sample A. For key, see Fig. 1.

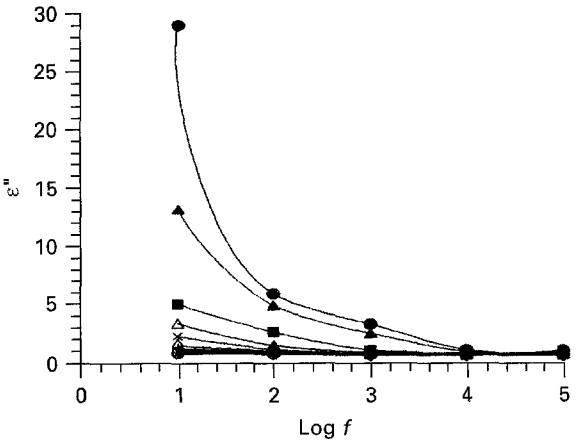

Figure 4 Frequency dependence of the dielectric loss factor, ε'', for collagen sample B. For key, see Fig. 1.

The value of ε'' was determined for 10 Hz from the curves presented in Figs 3 and 4 for samples A and B, respectively. From Equation 2, the calculated conductivity in the temperature ranges 298–470 K varies from 2×10^{-11} to $8.8 \times 10^{-11}\ \Omega^{-1}\,m^{-1}$ for sample A and from 4×10^{-12} to $7 \times 10^{-11}\ \Omega^{-1}\,m^{-1}$ for sample B. Fig. 5 shows the temperature dependence of the relaxation time, τ, for collagen. As follows from these curve in the temperature range 298–390 K, the

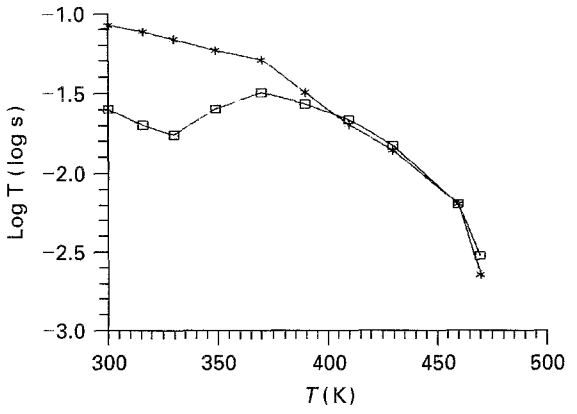

Figure 5 Temperature dependence of the relaxation time, τ, for collagen samples (□) A and (*) B.

relaxation times for sample A are shorter than for sample B. Above 390 K, numerical values of the relaxation times for both samples are considerably reduced when compared to those in the range 298–390 K. These curves are qualitatively similar to the results presented by Pethig [17] for other proteins. He investigated the variation of the relaxation time of the α-dispersion for bovine serum albumin and lysozyme as a function of protein hydration at room temperature. This dependence implies that the values of the relaxation time decrease with increasing hydration.

4. Discussion

Dielectric dispersion observed in collagen in the studied range of frequencies 10^1–10^5 Hz and shown in Figs 1–4, has been interpreted as appearing due to Maxwell–Wagner–Sillars (MWS) polarization and, to a lesser degree, the dipolar polarization of water bound to collagen molecules. In the frequency range investigated, no effect of electrode polarization was observed for A and B collagen samples. For samples in the solid state, the electrode effect is usually significant only at very high hydration (> 0.5 g H_2O/g collagen) and low frequencies, so for samples whose conductivity is greater than $10^{-8}\,\Omega^{-1}\,m^{-1}$ [18]. Only then can the electrode capacitance exceed the sample capacitance. The conductivities of the studied samples A and B are lower than $10^{-8}\,\Omega^{-1}m^{-1}$. In the following part of the discussion we analyse the effect of the field frequency and sample temperature on the behaviour of the ε′ and ε″ components assuming the mechanisms of MWS polarization. The appearance of MWS is induced by discontinuities in the ratio of permittivity to conductivity as collagen is a heterogeneous biological system.

A difference in potentials appears on the border of phases of different conductivity (water molecules and polypeptide chains). MWS polarization in the collagen–water system arises from the localized hopping of free protons between sites formed by water molecules bound to collagen molecules. In the case of the studied collagen systems, such sites are made by loosely bound water molecules occurring in sample A and structural water molecules present both in sample A and B.

Changes in MWS polarization of the collagen are manifested as changes in the values of ε′ and ε″ components which are proportional to the number of jumps performed by protons in the period of the electric field applied to the sample [6]. On the other hand, the number of those jumps is determined by the degree of collagen hydration and the sample temperature. The curves shown in Figs 1 and 3 recorded for sample A at temperatures from 298–390 K, illustrate the polarization due to protons moving freely between sites made by the loosely bound water molecules. The corresponding curves recorded for the same temperature range for sample B, shown in Figs 2 and 4, reflect small changes in the sample polarization, being a consequence of the lack of the loosely bound water in this sample. Most probably the observed behaviour of the ε′ and ε″ components for sample B is a consequence of limited mobility of protons between molecules of structural water forming strong hydrogen bonds with a macromolecule of collagen [11].

The remaining frequency dependences of ε′ and ε″ obtained for samples A and B in the temperatures ranging from 390–470 K, shown in Figs 1–4, illustrate the polarization appearing due to proton hopping between sites made by structural water molecules gradually released as a result of breaking of the hydrogen bonds with a collagen macromolecule. However, numerical values of ε′ and ε″ are higher for sample A than for sample B. Probably the reason for this is a greater number of sites in sample A, increased by that number of loosely bound water molecules which have not undergone diffusion from the collagen–water system.

The presented frequency dependences of ε′ and ε″ imply an increase in polarization with decreasing field frequencies as the number of jumps which protons can perform with the period of the field ($1/f$) increases. The above-discussed dielectric dispersion in collagen is reflected in the temperature dependences of the relaxation times τ, obtained for samples A and B and shown in Fig. 5. The relaxation times for sample A containing loosely bound water molecules are shorter than for sample B in the temperature range 298–390 K. Most probably, thermal energy supplied to sample A causes an increase of the mobility of protons between the liberated molecules of loosely bound water whose total number remains the same up to about 316 K, at which point the relaxation time reaches a minimum. Above 316 K the process of diffusion of water molecules from sample A begins, which is manifested by the relaxation time increasing up to a temperature of about 390 K. The lack of a minimum value in the relaxation time, τ, below 390 K for B, indicates the absence of the loosely bound water in this sample. The significant decrease in τ for both samples above 390 K is a consequence of greater mobility of protons between sites made by the liberated molecules of structurally bound water which, supposedly, do not diffuse from the collagen–water system before a temperature of 470 K is reached [15, 19, 20].

References

1. J. J. LIM and M.H. SHAMOS, *Biophys. J.* **11** (1971) 648.
2. V. P. TOMASELLI and M. H. SHAMOS, *Biopolymers* **12** (1973) 353.
3. C. A. J. HOEVE and P. C. LUE, *ibid.* **13** (1974) 1661.
4. E. P. CHANG and J. C. W. CHIEN, *J. Polym. Sci.* **11** (1973) 737.
5. A. NGUYEN, B. T. VU and G. L. WILKES, *Bipolymers* **13** (1974) 1023.
6. N. SASAKI, *ibid.* **23** (1984) 1725.
7. S. BONE and R. PETHIG, *J. Molec. Biol.* **181** (1985) 323.
8. G. CARERI, M. GERACI, A. GIANSANTI and J. A. RUPLEY, *Proc. Nat. Acad. Sci. USA* **82** (1985) 5342.
9. R. PETHIG, *Ferroelectrics* **86** (1988) 31.
10. J.R. GRIGERA, F. VERICAT, K. HALLENGA and H.J.C. BERENDSEN, *Biopolymers* **18** (1979) 35.
11. G.N. RAMACHANDRAN, *Int. J. Peptide Protein Res.* **31** (1988) 1.
12. S. NOMURA, A. HILTNER, J. B. LANDO and E. BAER, *Biopolymers* **16** (1977) 231.
13. M. LÜSCHER-MATTLI and M. RÜEGG, *ibid.* **21** (1982) 403.
14. M. H. PINERI, M. ESCOUBES and G. ROCHE, *ibid.* **17** (1978) 2799.
15. F. JAROSZYK and E. MARZEC, *Ber. Bunsenges. Phys. Chem.* **97** (1993) 868.
16. F. JAROSZYK and E. MARZEC, *Polish J. Chem.* **65** (1991) 123.
17. R. PETHIG, *Ann. Rev. Phys. Chem.* **43** (1992) 177.
18. N. E. HILL, W. E. VAUGHAM, A. H. PRICE and M. DAVIES, "Dielectric properties and molecular behaviour" (Van Nostrand Reinhold, London, 1969).
19. F. JAROSZYK and E. MARZEC, *J. Mater. Sci.*, **29** (1994) 5333
20. A. BIGI, A. M. FISCHERA, N. ROVERI and M.H.J. KOCH, *Int. J. Biol. Macromol.* **9** (1987) 176.

Received 7 July 1994
and accepted 2 May 1995