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Dielectric properties of a protein–water system in selected animal tissues

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Abstract

Dielectric spectroscopy has been applied to study aspects of the organization of water in selected animal tissues (tendon, bone and horn). The measurements of the relative permittivity ϵ' and the dielectric loss ϵ'' were carried over the frequency range of 10–100 kHz and at temperatures from 22 to 240 °C. The water content was 10% for bone and horn, and 22% for tendon by mass at room temperature at a relative humidity of 70%. The temperature dependencies of ϵ' and ϵ'' reveal distinctively the temperature ranges corresponding to the release of water in temperatures up to about 200 °C for all tissues and the melting of the crystalline structure only for tendon and horn, above this temperature. The frequency dependencies of ϵ' and ϵ'' show a remarkable dispersion in the low-frequency at selected temperatures up to 200 °C for all tissues due to the release of the loosely and strongly bound water. The results were discussed in terms of the interfacial (Maxwell–Wagner) polarization and polarization mechanism involving hopping charge carriers interacting with the bound water molecules. The information on the effect of temperature, water content and frequency of the electromagnetic field on the dielectric behaviour of the tissues studied is of importance in the design and construction of medical diagnostic or therapeutic instruments based on the use of electric signals.

Keywords: Dielectric properties; Protein–water system; α-dispersion; Hydrogen bonds; Activation energy

1. Introduction

The tissues of the bone, horn and tendon have been treated as composites of hydroxyapatite–collagen–water, matrix–keratin–water and collagen–water, respectively. Thermal properties of these materials have been studied by differential scanning calorimetry (DSC) [1–3]. The results performed up to 250 °C revealed the occurrence of phase transitions related to the release of water and the melting of the crystalline structure, such as collagen and keratin. The experimental methods used to recognise the physicochemical properties of these tissues also include various dielectric spectroscopy techniques [2–8]. These studies report investigation of the effects of water, electric field frequencies and ionising radiation on the dielectric properties of constituent phases of these tissues.

The main aim of this work was to compare dielectric relaxation behaviour in three types of tissues, being composites of different phase composition, different volume ratio of the phases and similar way of the phases connection. This paper extends earlier dielectric studies of solid animal tissues in the α-dispersion electric field region reported by other authors. In biological materials, the α-dispersion occurs at audio frequencies and is manifested by the very large increase in permittivity up to 10^6 below 100 Hz, while the conductivity exhibits small changes [9]. However, as follows from literature data, determination of the accurate range of frequencies and the relaxation frequency of the α-dispersion for biological tissues is difficult because these materials have inhomogeneous structure and are of different origin. Moreover, the measurements of dielectric properties of tissues at low frequencies can be masked by the electrode polarization effects, despite the use of many techniques to overcome them. So, the available literature gives the results of the dielectric properties of human and animal tissues in incomplete α-dispersion region, i.e., starting from 10 Hz [10–16]. More information on the dielectric behaviour of biological materials in the α-dispersion has been collected for proteins [3,17–21]. The results collected for proteins indicate that the dispersion in them is influenced by the water content of the

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samples, which is manifested by a shift of the characteristic dispersion frequency towards higher frequencies. The authors of the papers [18–20] have observed the α-dispersion for proteins is in the range of 10^{-4}–10^{6} Hz, as a result of effective restriction of the artefacts due to the electrode polarization.

In this paper, analysis of the temperature and frequency dependencies of the complex permittivity of animal tissues in the wet and dry states was performed on the basis of proton transport processes. The proton transfer in hydrogen-bonded networks in the solid and liquid states has been earlier discussed by other authors [5,22–24]. In recent years, the electrical conduction properties of biopolymers are considered to arise from proton [5,25] and also electron [26] transport.

The results of this study using the dielectric spectroscopy over a wide range of temperature provide new information on molecular interactions in complex biological tissues. Recognition of the dielectric behaviour of these tissues can be biomedical helpful because of the use of animal tissues as medical implants as well as because of the occasional use of low-intensity electric fields for treatment (e.g., electro-therapy of bone fractures and osteoporosis).

2. Materials and method

The tissues studied were bovine cortical bone, calf horn and bovine Achilles' tendon. Samples from tissues were obtained as follows: tissues were cleaned mechanically, immersed in ethyl ether to remove fat, washed in distilled water and in 0.1 N NaCl solution, dried at room temperature, cut perpendicular to the fiber axis and formed into rectangular samples of a typical size, 6×4×1 mm. Two sets of samples were studied: air-dried at room temperature of relative humidity of ~70% termed 'wet' and devoid of loosely bound water at room temperature termed 'dry'. In order to obtain the dry state of samples, prior to the measurements, these samples were kept at temperature about 120 °C until their dielectric parameters reached constant values. Then these samples were cooled to room temperature.

The water content in wet state, determined from the loss of mass of samples weighed at 200 °C relative to their mass at room temperature, was 10% for bone and horn, and 22% for tendon. The water content in dry state, determined from the loss of mass of samples weighed at 200 °C relative to their mass at 120 °C, was 4% for bone and horn, and 6% for tendon.

The experiments were carried out using an AC bridge method [27] over the frequency range of 10 Hz–100 kHz, belonging to the α-dispersion electric field region and at temperatures from 22 to 240 °C. The measurements were performed in the direction parallel to the tissues fibers. Prior to the measurements, the two largest surfaces of each sample were covered with silver paste electrodes. Each sample was subjected to the cycle of measurements only once. In our experiments we assumed that electrode polarization effects are negligible, as the charge that could be collected at the interfaces between the electrodes and the sample would have to come from the water in the tissues and the solvent present in silver paste. The electrode effects have been taken into account in the materials with very high water contents, whose conductivity at low frequencies is greater than 10^{-8} $(\Omega cm)^{-1}$ [28]. In this study, the conductivities at room temperature and at 10 Hz for wet bone, horn and tendon are 2.7×10^{-9}, 1.3×10^{-10} and 1.6×10^{-9} $(\Omega cm)^{-1}$, respectively. These values are lower than 10^{-8} $(\Omega cm)^{-1}$, so electrode polarization effects from the water in tissues should not occur. To make sure if the solvent can diffuse in the wet and dry samples from the moment the electrodes are deposited, we determined the time in which the resistance of the electrode measured at different points on its surface reached very small and comparable values. This time, irrespective of the hydration state, was nearly 8 min. Thus, we could conclude that, in this time, the tissues could not have been affected by the solvent, but the solvent must have evaporated from the paste, which was favoured by its low thickness.

The dielectric properties of tissues are described by the measured components of the relative permittivity ϵ' and the dielectric loss ϵ'', which are the real and imaginary parts the complex permittivity ϵ^* ($\epsilon^*=\epsilon'-j\epsilon''$). The values of ϵ' and ϵ'' for each tissue in the whole temperature range are given as the average from four to six samples. The resulting standard deviation in both parameters is less than 9%.

3. Results

Figs. 1–3 present the temperature dependencies of the logarithm of the relative permittivity ϵ' and dielectric loss ϵ'' for wet and dry tendon, bone and horn at a chosen frequency of 1 kHz, respectively. The curves in each figure reveal distinctively the temperature ranges corresponding to the two

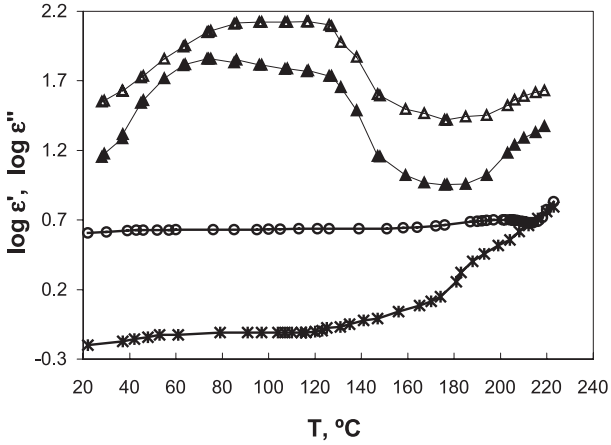

Fig. 1. Temperature dependencies of ϵ' (△) and ϵ'' (▲) for wet tendon and ϵ' (○) and ϵ'' (*) for dry tendon.

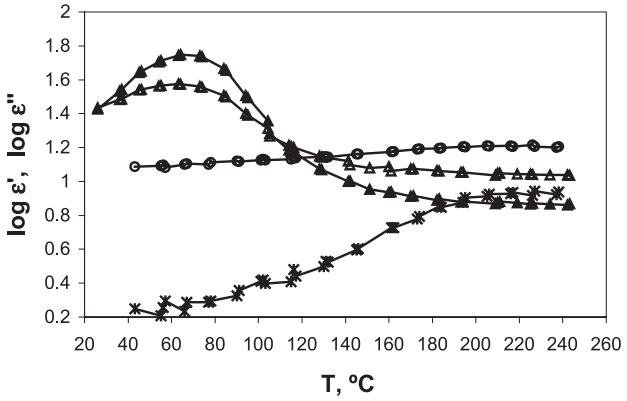

Fig. 2. Temperature dependencies of ϵ' (△) and ϵ'' (▲) for wet bone and ϵ' (○) and ϵ'' (*) for dry bone.

molecular processes: the release of water in temperatures up to 200 °C for all tissues and the melting of the crystalline structure only for tendon and horn, above this temperature. The release of water from the tissues is a continuous process involving breaking up of hydrogen bonds formed by loosely and strongly bound water and also water diffusion out of the biological system. The liberation of loosely bound water in wet tissues is manifested by the ϵ' and ϵ'' maxima occurring up to 140 °C. For different types of tissues, the maxima appear at different temperatures, have different amplitude and shape, which is determined by the water content absorbed by the substance studied at room temperature before the measurements. For dry tissues that do not contain loosely bound water, the peaks in ϵ' and ϵ'' do not occur in this temperature range, and these parameters show only small changes. The temperature range above 200 °C, in which ϵ' and ϵ'' increase for tendon and horn, and reach significant maxima near 240 °C for horn, corresponds to the process of melting of the collagen and keratin macromolecules. In the same temperature range, ϵ' and ϵ'' for bone slowly increase, which would indicate that, in this tissue, the process of collagen melting has not started yet. It has been generally accepted for many years that the melting of the ordered

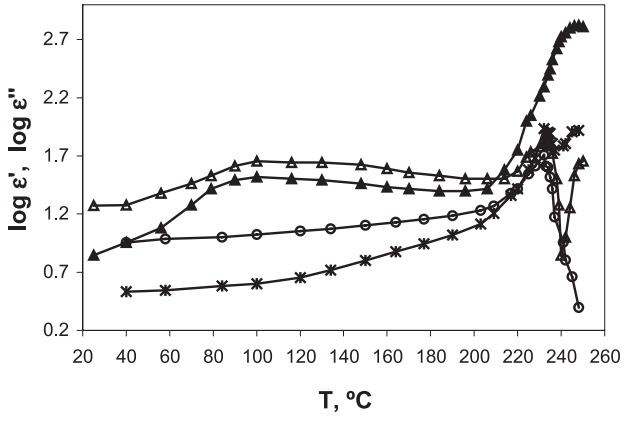

Fig. 3. Temperature dependencies of ϵ' (△) and ϵ'' (▲) for wet horn and ϵ' (○) and ϵ'' (*) for dry horn.

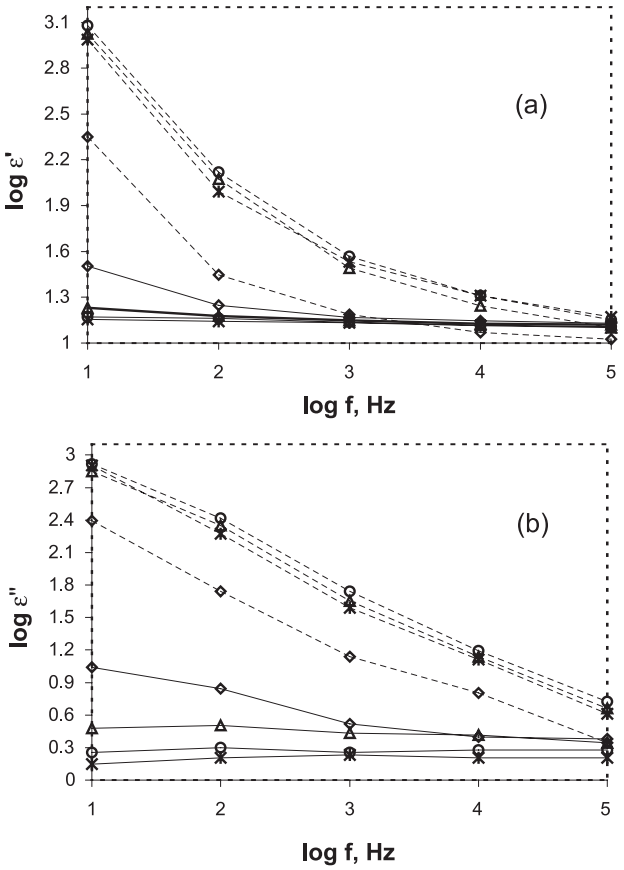

Fig. 4. The variation of ϵ (a)′ and ϵ'' (b) vs. frequency for dry (—) and wet (- - - -) bone at selected temperatures of 40 (*), 60 (○), 80 (△) and 120 °C (◇).

crystalline structure includes rupture of hydrogen bonds and a rearrangement of the triple helix into a random configuration.

The frequency dependencies of ϵ' and ϵ'' for bone at selected temperatures up to 120 °C and above this temperature are shown in Fig. 4a and b, and Fig. 5a and b, respectively. At each temperature, two curves corresponding to the dry and wet state were recorded. Only the curves for bone are shown since the character of the dependencies for all the tissues under study is similar. For the wet tissues, the plots of ϵ' and ϵ up to 120 °C show a remarkable dispersion in the low frequency as a consequence of the release of loosely bound water. For the dry tissues, small changes in ϵ' and ϵ'' with increasing frequency at the same temperature range are observed. Above 120 °C, the curves of both parameters show a significant dispersion for wet and dry bones due to the release of the strongly bound water.

4. Discussion

The dielectric results presented in the figures for the tissues studied are determined probably by the interfacial (Maxwell–Wagner) polarization which appears on the border of phases of different dielectric properties and by the polarization due to protons hoping between neighbour-

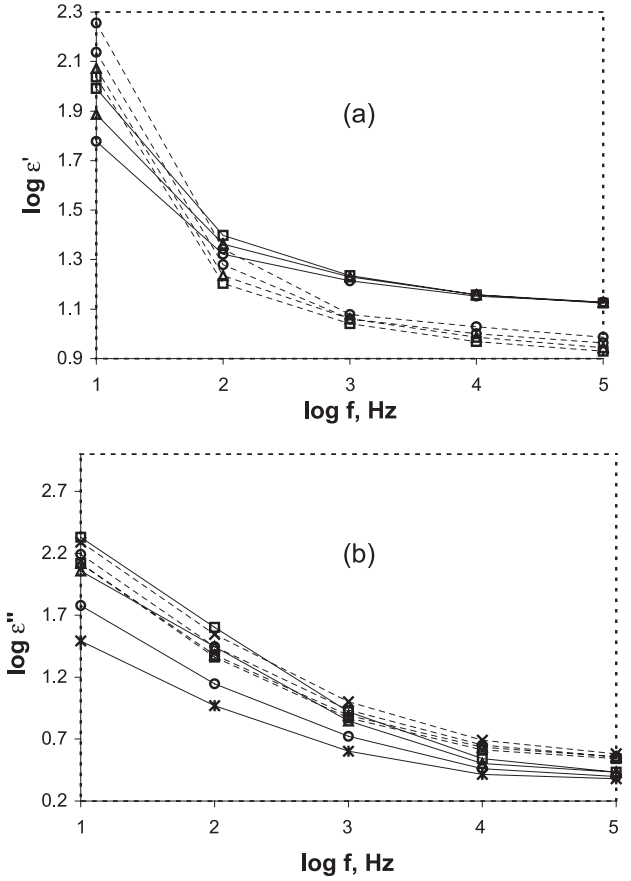

Fig. 5. The variation of ϵ' (a) and ϵ'' (b) vs. frequency for dry (—) and wet (- - - -) bone at selected temperatures of 140 (*), 160 (○), 180 (△) and 200 °C (□).

ing sites. The appearance of Maxwell–Wagner polarisation in the bone, tendon and horn is probable, as the high conductivity phases are composed of water molecules and the very low conductivity ones are collagen, hydroxyapatite, keratin and matrix. The difference in the conductivity of the phases restricts the flow of electric current in these tissues, on applied voltage. This situation causes charge accumulation at the interface, i.e., Maxwell–Wagner polarization, which is manifested as an increase in ϵ' at low frequencies (Figs. 4a and 5a). On the other hand, the free motion of the charge in the conduction phase (water) results in an increase in ϵ'' with decreasing frequency (Figs. 4b and 5b). The interfacial polarization becomes more important with increasing temperature due to an increasing number of carriers and their increasing mobility. In the tissues studied, we considered protons as charge carriers. Recently, H^+ and OH^- ions were considered as the conducting charge carriers in natural calcium phosphate hydroxyapatite (Hap) [5], studied by means of the thermally stimulated depolarisation current (TSDC) technique in the temperature range of 50–340 K. In hydrated biological materials, the polarization mechanisms are a result of not only the accumulation of protons at the phase interface but also of hopping of protons between localized sites. In this study, we also include this second possible mechanism for the observed dielectric dispersion. Based on our results, changes in ϵ' and ϵ'' of the tissues would depend on the number of jumps performed by protons (H^+) between sites formed by water molecules bound to protein molecules and also to hydroxyapatite and matrix. Results of these studies and our earlier papers [29,30] suggest that the influence of hydroxyapatite and matrix on the mechanisms of polarization and conduction in bone and horn is negligible in the studied temperature range, but it arises from protein–water systems. We obtained the relaxation time of collagen–water and keratin–water systems for tendon and bone, and horn, respectively, from the dependence [20]

$$\tau = \varepsilon_o \varepsilon_\infty / \sigma \tag{1}$$

where ϵ_o is the permittivity of free space, σ is the steady-state conductivity and the ϵ_∞ is the relative permittivity at the high-frequency limit of α-dispersion. As the α-dispersion is affected by water in the proteins, the high-frequency limit of α-dispersion can change [19]. To be able to apply Eq. (1) for our calculations, we have assumed 100 kHz as a high-frequency limit of α-dispersion.

As seen in Figs. 4a and 5a at this frequency, the relative permittivity ϵ', which corresponds to ϵ_∞, takes the smallest value and undergoes small changes with temperature and hydration. As a steady-state conductivity, we have assumed the value of conductivity at 10 Hz (low-frequency limit in this study), taking into regard the fact that the conductivity in the α-dispersion for biological materials is almost independent of frequency [9,13], and therefore, this parameter can be treated as a steady-state conductivity. The steady-state conductivity was calculated from the relation

$$\sigma = 2\pi f \varepsilon_o \varepsilon'' \tag{2}$$

for the numerical values of ϵ'' at 10 Hz from the curves shown in Figs. 4b and 5b. By combining Eqs. (1) and (2), the relaxation time is expressed in the form:

$$\tau = \varepsilon_\infty / 2\pi f \varepsilon''. \tag{3}$$

Fig. 6 presents the plots of log τ against the inverse temperature T for wet and dry tendon, bone and horn. The numerical values of τ for these tissues in the chosen temperatures are given in Table 1.

The values of τ for wet and dry states of tissues change with increasing temperature, depending on the number of sites among which protons can jump and the mobility of these protons. In temperatures up to about 160 °C, the relaxation times are considerably longer for dry than for wet samples. This suggests that the wet tissues must contain a greater number of sites available for mobile protons as a result of the presence of loosely bound water. Above 160 °C, the relaxation times of protons jumping between the sites made by the released water strongly bound in the proteins are similar for all tissues.

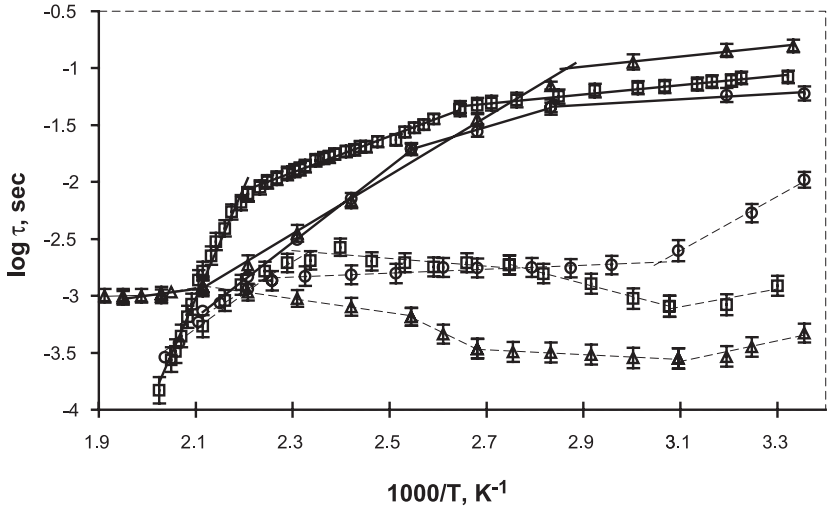

Fig. 6. Temperature dependencies of the relaxation time τ for dry (—) and wet (- - - -) tendon (□), bone (△) and horn (○).

From the slope of the straight lines of the dependencies shown in Fig. 6, we can obtain the activation energy ΔH for water release at appropriate temperature ranges, according to the Arrhenius temperature law

$$\tau = \tau_o \exp(\Delta H/RT), \quad (4)$$

where τ_o is a constant, and R is the gas constant.

For wet tissues, the activation energy needed for liberation of loosely bound water is comparable with that of hydrogen bonds formed among the water molecules or between water molecules and protein macromolecules. The occurrence of this process up to 50 °C is supported by positive values of ΔH of about 15, 17 and 36 kJ/mol for tendon, bone and horn, respectively. The greater the amount of the loosely bound water in a tissue, the lower the energy needed to its release because water molecules form hydrogen bonds mainly among themselves. Above 50 °C, the Arrhenius plots, except for this curve for horn, show negative slopes, probably as a result of diffusion of water out of the wet samples. As a consequence, the number of sites and jumps performed by protons is expected to decrease. The rate of diffusion is highest up to 90 and 120 °C for tendon and bone, respectively, and is reflected by negative values of the activation energy ΔH of −21 and −39 kJ/mol. In the case of wet horn, the low positive value of the activation energy ΔH of about 4.4 kJ/mol up to 170 °C suggests that the process of removal of the strongly bound water, which includes breaking of hydrogen bonds, is more intensive than the diffusion process. Above 150 and 170 °C, the values of ΔH for wet tendon and horn are 48 and 59 kJ/mol, respectively. These positive values indicate that the activation energy is needed to break up the double and triple hydrogen bonds formed by water inside the protein molecules. However, the negative value of ΔH of−12 kJ/mol for wet bone up to 200 °C suggests that the process of release of the strongly bound water is masked by the diffusion of water out of this system.

Table 1
Relaxation time τ for wet and dry tendon, bone and horn for chosen temperatures

T, °C	τ, ms					
	Wet			Dry		
	Tendon	Bone	Horn	Tendon	Bone	Horn
40	0.8	0.3	5	77	143	58
80	1.6	0.3	1.7	56	70	45
100	1.9	0.3	1.8	47	36	28
140	2.7	0.8	1.5	19	7	7
160	1.9	0.9	1.5	14	3	3
180	1.2	1.0	1.1	7	2	1
200	0.5	1.2	0.6	2	1	1

The Arrhenius plots for dry tissues shown in Fig. 6 differ in character from those for wet tissues. The activation energy ΔH responsible for the water release takes a positive value for all dry tissues up to 200 °C. Probably, the lack of the loosely bound water in dry samples is reflected by a low value of ΔH of about 0.8 kJ/mol up to near 100 °C. Above this temperature, high values of ΔH for all tissues indicate that the process of liberation of the strongly bound water already began. Up to 200 °C, the values of ΔH are about 30, 50 and 56 kJ/mol for tendon, bone and horn, respectively.

5. Conclusions

The data obtained in this paper supported the earlier dielectric results for various proteins [17–21], which indicate that water significantly affects the α-dispersion region. The temperature dependencies of the complex permittivity of the tissues studied suggest the important role of water in the stabilisation of the collagen and keratin macromolecule structure. In fact, the process of water release includes the breaking up of hydrogen bonds, and also, diffusion of water out of the biological system demands a wide temperature range up to 200 °C. The occurrence of this process for these tissues is supported by

positive and negative values of the activation energy. The results of this study suggest that the influence of hydroxyapatite and matrix on the dielectric properties of bone and horn is negligible in the studied temperature range, but it arises from protein–water systems. Analysis of polarization and conduction mechanisms for the tissues studied is interpreted on the basis of proton transport. The polarization mechanisms are a result of not only the accumulation of protons at the phase interface (Maxwell–Wagner polarization) but also of the hopping of protons between bound water molecules. The conduction mechanisms arise from proton transfer through the intra- and intermolecular hydrogen bonds in collagen–water and keratin–water systems for tendon and bone, and horn, respectively. This paper is an extension of earlier dielectric studies of complex biological tissues reported by the literature.

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