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On the piezoelectricity of anionic collagen films

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Abstract

In this paper, we report results of a study of the physicochemical, dielectric and piezoelectric properties of anionic and native collagen films, considering the development of new biomaterials which have potential applications in coating of cardiovascular prostheses, support for cellular growth and in systems for controlled drug delivery. The piezoelectric strain tensor element d_{14} , the elastic constant s_{55} , and the dielectric permittivity ϵ_{11} were measured for the anionic and native collagen films. It was observed that the collagen samples submitted to the alkaline treatment present lower thermal stability than those made from native collagen. However, the treated samples present a higher piezoelectricity compared with the native collagen. The frequency constant fL and the piezoelectric strain element d_{14} , obtained for the alkaline-treated sample (72 h), present the highest values for the samples under study (444.1 kHz m and 0.079 pC/N, respectively). We believe that the alkaline treatment led to an increase of the organization of the microscopic fiber structure of the sample, which could result in an increase of the piezoelectricity. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Some biological materials and biopolymers are found to exhibit the polar uniaxial orientation of molecular dipoles in their structure and can be considered as bioelectret. Such materials show pyroelectricity and piezoelectricity. Biocompatible polymeric materials are now used extensively after proper polarization treatment for biomedical applications such as antithrombogenic surfaces and artificial membranes [1]. Pyro- and piezoelectric studies in various types of biological systems show the presence of natural polarity in the structure of various parts of animals and plants. In many natural structures, polar molecules such as proteins are aligned in parallel with a preferred direction of the polar axis to form crystalline structures. Therefore, such structures can be regarded as natural electrets. Because of this intrinsic polarization, pyroelectricity and piezoelectricity in the axial direction can be observed [2,3].

Collagen, the most abundant protein of the animal kingdom, has a long history as a biomaterial. We can find it in prostheses

of heart valves, in artificial skins, in contact lenses and in injectable gels for soft tissue augmentation [4–6]. Collagen molecules (molecular weight 300,000) are rod-like triple helices, which are 300 nm in length and 1.5 nm in diameter. Collagen fibers possess a high degree of axial alignment of collagen molecules and are characterized by a regular stagger of approximately 1/4 of a rod length between each molecule and its axially aligned neighbor [7,8].

The piezoelectric properties of collagen have been investigated in complex biological systems such as bone and tendon. The major difference between them is the structural organization level over the tissue. For different tissues, one has different organization of the protein. However, the chemical composition is similar. The piezoelectric properties of bone are of interest in view of their role in bone remodeling [9]. The magnitude of the piezoelectric sensitivity coefficients of bone depends on frequency, on direction of load, and on relative humidity. Values up 0.7 pC/N have been observed in bone [10]. Marino et al. [11] examined the relation between collagen's film piezoelectricity and its electron microscopic appearance, in films with different degree of organization, and suggested that the piezoelectric effect originates at the level of tropocollagen.

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In this work, we studied the thermal, dielectric and piezoelectric properties of anionic and native collagen films, considering the development of new biomaterials which have potential applications in coating of cardiovascular prostheses, support for cellular growth and in systems for controlled drug delivery.

2. Experimental methods

The anionic collagen was prepared by solubilization of collagen from bovine sera after different periods (24, 72 and 168 h) of treatment under alkaline conditions in the presence of salts, followed by homogenization in acetic acid solution, at pH 3.5 [12]. The native collagen was prepared by solubilization of collagen from bovine sera after treatment with acetic acid under low pH, followed by homogenization in acetic acid solution, at pH 3.5. All samples were dialyzed against acetic acid solution, at pH 3.5, and brought to a final concentration of 1%, determined by hydroxyproline [13].

The membranes, cast in acrylic molds from a collagen solution, were equilibrated in phosphate buffer, 0.13 mol/l, pH 7.4 for 24 h, followed by extensive water washes and dried in laminar flow of air. The membranes were prepared from collagen with 0, 24, 72 and 168 h under alkaline treatment (samples S0, S1, S2 and S3, respectively).

The thermal stability of the collagen was determined by measures of its denaturation temperature, using a Shimadzu DSC-50 equipment, membrane samples were sealed in aluminum cells and heated at a rate of 5°C/min with N₂ atmosphere.

The complex dielectric function measurements were obtained from a HP 4291A material impedance analyzer in conjunction with a HP 4194 impedance analyzer, which jointly covered the region of 100 Hz–1.8 GHz. In Fig. 2 one has the sample geometry we used for the dielectric and piezoelectric measurements. Rectangular coordinates are assigned to the samples as shown in Fig. 2. The 2–3 plane is the sample plane, and the 1 axis is perpendicular to the plane of the sample. The flat faces of the samples are painted with a silver electrode. The thickness and the diameter of each sample are found in Table 1. For each the sample, we took around 100 measurements of the thickness, using a digital paquimeter, and calculated the average value (see Table 1). For all the studied samples, the diameter ‘*L*’ varied between 1.5 and 3.2 cm, and

the average thickness ‘*e*’ was 85, 51, 67 and 54 μm for samples S0, S1, S2 and S3, respectively.

3. Piezoelectric measurements

The coupled electromechanical equations for our system are:

$$S_I = s_{IJ}^E T_J + d_{IJ} E_j \quad (1)$$

$$D_i = \epsilon_{ij}^T + d_{ij} T_j \quad (2)$$

$S_I (S_1, \dots, S_6)$ is the strain and $T_J (T_1, \dots, T_6)$ is the stress, E_j is the electric field and D_i is the displacement vector. With constants s_{IJ}^E (compliance for constant electric field), ϵ_{ij}^T (dielectric permittivity for constant stress) and the piezoelectric strain element d_{ij} . Similarly, the coefficients relating the displacement vector (D_i) to the strain vector (S_j) are called the piezoelectric stress elements e_{ij} . Piezoelectricity may exist for certain symmetries of crystalline structures. In general anisotropic crystals have the piezoelectric effect and their piezoelectric constants can be represented by the matrix: d_{ij} [14,15]:

$$\begin{pmatrix} d_{11} & d_{12} & d_{13} & d_{14} & d_{15} & d_{16} \\ d_{21} & d_{22} & d_{23} & d_{24} & d_{25} & d_{26} \\ d_{31} & d_{32} & d_{33} & d_{34} & d_{35} & d_{36} \end{pmatrix}$$

d_{ij} are called the piezoelectric strain constants. Some of them are null, according to the symmetry of the material [14,15]. In the case of natural biopolymers, which show only shear piezoelectricity, the symmetry observed is $D_\infty (\infty 2)$. In this case, $d_{25} = -d_{14}$. In the case of bone, the piezoelectricity appears only when the shearing force acts on the oriented collagen fibers so that they slip past one another [2,3]. Shear piezoelectricity is observed almost universally for the oriented textures of biopolymers [2]. For this symmetry, the tensor d_{ij} is given by [2,3,14,15]:

$$\begin{pmatrix} 0 & 0 & 0 & d_{14} & 0 & 0 \\ 0 & 0 & 0 & 0 & -d_{14} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

In this notation, i, j take values 1, 2, and 3. When indices i

Table 1

Alkaline treatment time (AT), denaturation endotherm (DE), density (ρ), thickness (e), dielectric permittivity (ϵ), frequency constant (fL) and piezoelectricity (d_{14}) of samples S0, S1, S2 and S3

Sample	Alkaline treatment AT (h)	Denaturation endotherm DE (°C)	ρ (kg/m ³)	e (μm)	1 MHz $\epsilon_{11}^T/\epsilon_0$	1GHz $\epsilon_{11}^S/\epsilon_0$	fL (kHz m)	d_{14} (10 ⁻¹² C)
S0	0	78.33	998.47	85.45	2.56	2.23	437.69	0.066
S1	24	71.05	936.10	51.66	2.49	2.18	386.12	0.074
S2	72	57.38	1020.62	67.5	3.15	2.66	444.11	0.079
S3	168	58.35	986.2	54.1	2.21	1.88	404.85	0.072

and j are interchangeable, (ij) is abbreviated to I or J , so that: $11 \rightarrow 1, 22 \rightarrow 2, 33 \rightarrow 3, 23 \rightarrow 4, 31 \rightarrow 5, 12 \rightarrow 6$.

Shear piezoelectricity is also observed for the oriented films of a variety of synthetic polymers with optical activity. For the same symmetry group, the dielectric tensor ϵ_{ij} is given by:

$$\begin{pmatrix} \epsilon_{11} & 0 & 0 \\ 0 & \epsilon_{11} & 0 \\ 0 & 0 & \epsilon_{11} \end{pmatrix}$$

For this symmetry, the elastic compliance tensor s_{ij} is given by [14,15]:

$$\begin{pmatrix} s_{11} & s_{12} & s_{13} & 0 & 0 & 0 \\ s_{12} & s_{11} & s_{13} & 0 & 0 & 0 \\ s_{13} & s_{13} & s_{33} & 0 & 0 & 0 \\ 0 & 0 & 0 & s_{55} & 0 & 0 \\ 0 & 0 & 0 & 0 & s_{55} & 0 \\ 0 & 0 & 0 & 0 & 0 & s_{66} \end{pmatrix}$$

Rectangular coordinates are assigned to the film sample. The 1 axis is perpendicular to the plane of the sample, plane 2–3 (Fig. 2).

Using the tensors defined in Eqs. (1) and (2), and taking into account that the electric field is applied in direction 1 ($E_2 = E_3 = 0$), one has:

$$D_1 = \epsilon_{11}^T E_1 + d_{14} T_4 \tag{3}$$

$$S_4 = s_{55}^E T_4 + d_{14} E_1 \tag{4}$$

From Eqs. (3) and (4) one can see the electro-mechanical coupling through the d_{14} piezoelectric tensor element. In this geometry, one has a mechanical wave propagating in the plane of the sample ($S_4 \leftrightarrow S_{23}$) coupled with the electric field in the ‘1’ axis.

After an easy manipulation one has for the D_1 vector:

$$D_1 = \epsilon_{11}^T \left(1 - \frac{d_{14}^2}{s_{55}^E \epsilon_{11}^T} \right) E_1 + \frac{d_{14}}{s_{55}^E} S_4 \tag{5}$$

One can now define k_{14} , which is the piezoelectric coupling factor:

$$k_{14}^2 = \frac{d_{14}^2}{s_{55}^E \epsilon_{11}^T} \tag{6}$$

From the definition of k_{14} , one can define the relations of elastic compliance and electric permittivity measured under different conditions:

$$\frac{s_{55}^D}{s_{55}^E} = \frac{\epsilon_{11}^S}{\epsilon_{11}^T} = 1 - k_{14}^2 \tag{7}$$

The piezoelectric strain element d_{14} for the shear piezoelectric

is given by Eq. (6) [14,15]:

$$d_{14} = k_{14} \sqrt{\epsilon_{11}^T s_{55}^E} \tag{8}$$

with Eq. (7):

$$k_{14} = \sqrt{1 - \frac{\epsilon_{11}^S}{\epsilon_{11}^T}} \tag{9}$$

where k_{14} is the piezoelectric coupling factor, ϵ_{11}^T is the dielectric permittivity obtained by a measurement of the capacitance at a frequency below the fundamental resonance, ϵ_{11}^S is obtained by measurement of the capacitance at a frequency above the resonance mode. The measurement of the dielectric permittivity was done in the range of 1 MHz–1 GHz in the geometry of Fig. 2. The obtained values for 1 MHz, and 1 GHz can be found in Table 1. In our calculations, we assume ϵ_{11}^T at 1 MHz (below the piezoelectric resonance frequency) and ϵ_{11}^S at 1 GHz (above resonance).

From Eqs. (3) and (4), one has for T_4 :

$$T_4 = \frac{1}{s_{55}^D} S_4 - \frac{d_{14}}{s_{55}^E \epsilon_{11}^S} D_1 \tag{10}$$

Assuming that $\partial D_1 / \partial x_4 = 0$ we can obtain the equation of motion:

$$\frac{\partial^2 u_4}{\partial t^2} = \frac{1}{\rho s_{55}^D} \frac{\partial^2 u_4}{\partial x_4^2} \tag{11}$$

where u_4 is the displacement on the plane of the sample (plane perpendicular to electric field, see Fig. 2), and ρ is the density.

The obtained velocity is:

$$v = \frac{1}{\sqrt{\rho s_{55}^D}} = \frac{\omega}{k} \tag{12}$$

where ω is the frequency and k is the wavenumber ($k = 2\pi/\lambda$).

The elastic compliance s_{55}^D , which is determined from the successive resonance frequencies of the shear mode can be calculated from Eq. (12), assuming that the most pronounced resonances are those where the body can accommodate one half wavelength of the standing elastic wave ($L = \lambda/2$). In this case, one can obtain s_{55}^D from Eq. (12):

$$s_{55}^D = \frac{1}{4\rho(fL)^2} \tag{13}$$

where fL is the frequency constant which is the product of the resonance frequency and the diameter of the sample (controlling dimension of the sample, $L \gg e$, see Fig. 2) and ρ is the sample density. From the experimental measurement of ϵ_{11}^T , ϵ_{11}^S , fL and ρ one can obtain s_{55}^D (Eq. (13)), k_{14} (Eq. (9)) and d_{14} (Eq. (8)). Table 1 shows the experimental values of these constants under study.

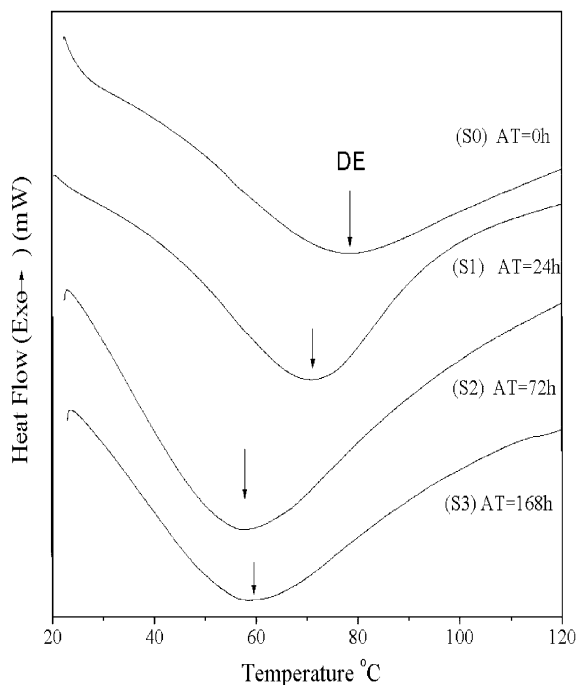


Fig. 1. DSC curves of the samples S0, S1, S2 and S3.

4. Results and discussion

Fig. 1 shows the DSC curves of collagen films. The main feature in the DSC curves of collagen are the denaturation endotherm, at 78.33, 71.05, 57.38 and 58.35°C for samples S0, S1, S2 and S3, respectively (see Table 1). These results show that films made from anionic collagen have lower thermal stability than those made from native collagen. We can explain these results by the fact that alkaline treatment of the protein may cleave the esterlike bonds present in tropocollagen [16,17]. Otherwise, it is known that the alkaline treatment hydrolyzes the carboxiamide residues of asparagine and glutamine [18]. This hydrolysis results in a net negative charge of collagen molecules at neutral pH. The thermodynamics and kinetics of the collagen precipitation have been extensively studied [19,20]. Temperature, pH and ionic strength all exert profound effects on fibril precipitation. Primarily electrostatic and hydrophobic interactions between amino acid side chains of neighboring collagen triple helices stabilize the fibers. Attractive interactions result from pairing of oppositely charged amino acids and repulsive interactions result from pairing of like charged amino acids. The changes in net charge and number of ionized groups as pH varies would be expected to exert a significant effect on forces between molecules in fibers and, hence, on fiber physical properties.

Fig. 3 shows the frequency dependence of the absolute value $|Y|$ of the admittance of sample S0. The measurement was done with the sample in disk type geometry as shown in

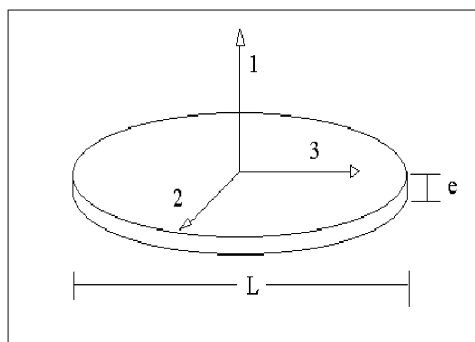


Fig. 2. A rectangular coordinate assigned to the sample for the measurement of piezoelectric and dielectric constants.

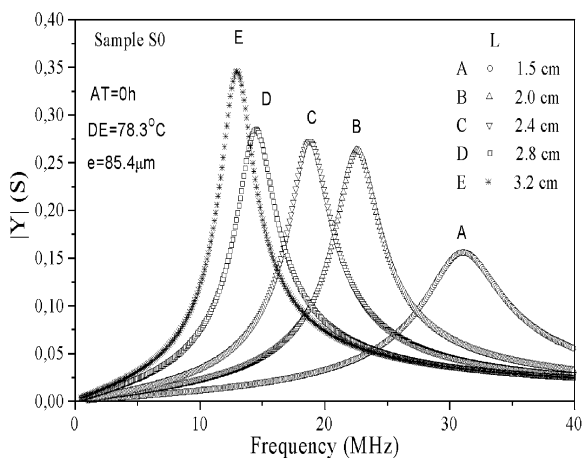


Fig. 3. Electrical admittance as a function of external frequency and the disk diameter (L) for sample S0.

Fig. 2. It is quite clear that the electro-mechanical resonance is associated to the piezoelectricity of the sample. From Fig. 3 one can confirm the reduction of the resonance frequency associated to the increase of the electrode diameter (L). This is an expected result in view of Eq. (13) associated to the main characteristic of the acoustic resonator. We also did measurements in piezoelectric ceramic PZT to obtain the resonances for this material, to use as a calibration of our measurements.

Fig. 3 shows five measurements for five different diameters of the disk, which is indicated in the figure. From Fig. 3, the frequency constant, fL associated to the shear piezoelectric mode of this sample was obtained. The average value obtained for the shear mode was $fL \sim (437.69 \text{ kHz m})$, which is a value about two orders of magnitude higher compared with PZT [14,15]. Figs. 4–6 show the admittance of samples S1, S2 and S3. We also did the measurement of the dielectric permittivity ϵ_{11}^T at 1 MHz (which is the dielectric permittivity obtained by a measurement of the capacitance at a frequency below the

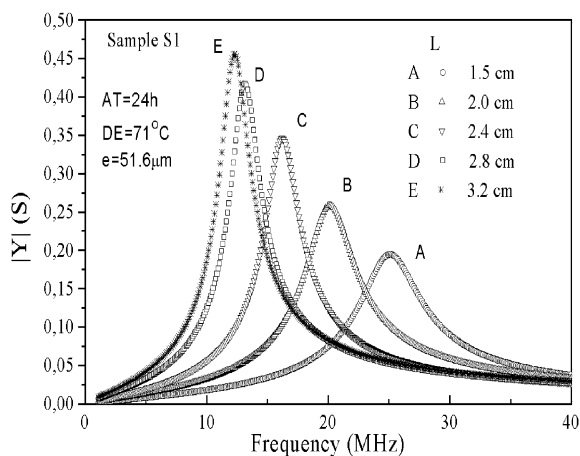


Fig. 4. Electrical admittance as a function of external frequency and the disk diameter (L) for sample S1.

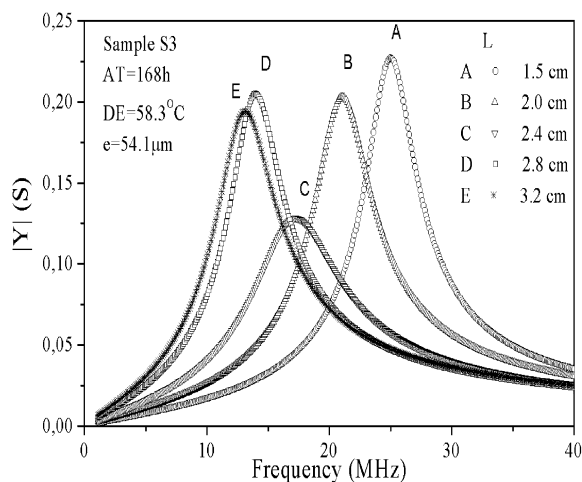


Fig. 6. Electrical admittance as a function of external frequency and the disk diameter (L) for sample S3.

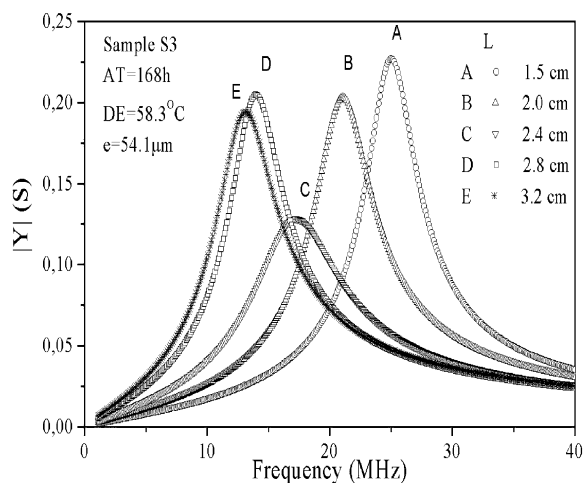


Fig. 5. Electrical admittance as a function of external frequency and the disk diameter (L) for sample S2.

fundamental resonance) and ϵ_{11}^S at 1 GHz (which is the dielectric permittivity obtained by measurement of the capacitance at a frequency above the resonance mode). With the experimental measurements of ϵ_{11} , fL and ρ and using Eqs. (8), (9) and (13) one can obtain the piezoelectric strain element d_{14} for the shear piezoelectricity (see Table 1). The piezoelectric strain tensor element d_{14} obtained for the natural collagen was around 0.066 pC/N, which is in good agreement compared with values reported in the literature obtained with other techniques [2,3,11,16]. In Ref. [11] collagen films was prepared by evaporation and electro-deposition from solution. It was observed the electro-deposited films were more organized presenting higher piezoelectric coefficients than the evaporated films, with values around 0.076 pC/N. In Figs. 4–6 one has the reson-

ances associated to samples S1, S2 and S3. For sample S2, a slight increase of the value of the piezoelectricity (0.079 pC/N) was observed. The increase of the piezoelectricity for the S2 sample was not well understood up to this point in this study. From Table 1, one can see that sample S2 presents the highest density, dielectric permittivity and frequency constant fL . We believe that the alkaline treatment, of the native collagen results in lower thermal stability than those made from native collagen, which was observed with the decrease of the denaturation endotherm (see Table 1). However, the treatment led to an increase of the organization of the microscopic fiber structure of the sample, which could explain the increase of the piezoelectricity.

5. Conclusions

In this paper we did a study of the physicochemical, dielectric and piezoelectric properties of anionic and native collagen films, considering the development of new biomaterials which have potential applications in coating of cardiovascular prostheses, support for cellular growth and in systems for controlled drug delivery. The piezoelectric strain tensor element d_{14} , the elastic constant s_{55} , and the dielectric permittivity ϵ_{11} were measured for the anionic and native collagen films. It was observed that the collagen samples submitted to the alkaline treatment presents, exhibited lower thermal stability than those made from native collagen. However, the treated samples present a higher piezoelectricity compared with the native collagen. The frequency constant fL and the piezoelectric tensor element d_{14} obtained for the heat-treated sample (72 h) present the highest values for the samples under study (444.1 kHz m and 0.079 pC/N, respectively). We believe that the alkaline treatment led to an increase of the organization of the microscopic structure of the sample, which could result in an

increase of the piezoelectricity. This high value for the frequency constant and piezoelectricity opens the possibility of using this film in electronic devices based on acoustic waves (such as surface acoustic wave devices, SAW) which operate in the MHz frequency range using piezoelectric substrates. We are studying in our laboratory a SAW filter prepared over the surface of a collagen film to be used as a pressure sensor in biological applications.

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