DIELECTRIC STUDIES IN-VITRO OF ISCHAEMIC AND HEALTHY HUMAN TISSUES

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Abstract: Measurements of the dielectric properties of healthy and ischaemic human artery tissues were made in the frequency range of 100Hz – 100kHz and temperatures from 22 to 260°C. The temperature dependencies of the relative permittivity and conductivity for healthy tissues reveal distinctively the temperature ranges corresponding to the release of water up to 200°C and the glass transition of elastin and collagen melting, above this temperature. The influence of ischaemic on the dielectric parameters of artery tissues is significant in the whole temperature range. Up to 200°C, the relative permittivity and conductivity for ischeamic tissues at the same temperature is much lower and higher above 80°C than for the healthy tissues, respectively. This suggests, that the polarization and conduction in these tissues due to protons hoping between a smaller number of sites as a results of collagen-water degeneration and higher proton transport than in healthy tissues. The data obtained above 200°C indicate that the ischaemic induces the higher physico-chemical changes in the collagen when compared to elastin.

Introduction

The human arteries in vitro have been studied by different methods, based on measurements of different parameters: mechanical, optical or electric [1-7]. Results of such measurements provide information on the primary and secondary structures of elastin and collagen – the basic proteins of the arteries tissue. At higher temperatures, both elastin and collagen reveal the glass transition around 200°C and the melting process near 230°C, respectively.
Results of the studies imply that different diseases of the human artery tissues e.g. arteriosclerosis, are reflected by changes in the physical properties of the proteins, such as the molecules vibrations – detectable in the IR and Raman spectra, elasticity, tensile strength, tenacity, conductivity and permittivity. Moreover, thanks to the in vitro studies, the physical properties of these proteins can be measured as functions of temperature, humidity, pressure or ionising and non-ionising radiation. In this study dielectric spectroscopy was applied to get the information on healthy and ischaemic human artery tissues. As water is an integral component of the proteins in the artery tissues, it seems necessary to establish dielectric properties of the systems elastin–water and collagen-water.

Materials and methods

The measurements were performed for the artery tissues collected from a group of 8 patients, operated on because of chronic ischaemia of legs, and the control artery tissues from two healthy men. The ischaemic changes were classified as 2nd and 3rd degree according to the R. Fontaine classification. Prior to the operation, the patients were subjected to digital subtraction angiography (DSA), which provided evidence of occlusion or critical narrowing of the terminal fragment of the abdominal aorta and the hip arteries. The aorta section taken was 0.5 cm in diameter. Prior to measurements by the dielectric spectroscopy method, the healthy and ischaemic tissues were immersed in a solution of 0.1*M* NaCl to remove fat. In order to obtain the tissues containing only elastin, some portions of these materials were subjected to collagen elimination procedure in 0.1*M* NaOH and at temperature about 98°C for 1h [7,8]. However, this procedure was used only to the healthy tissues because the ischaemic tissues were too brittle and crumbled. Then all tissues were washed in distilled water, dried in air at room temperature, cut into rectangular samples of the size 5x3x1mm and on two sides covered with silver paste electrodes. Two sets of samples were studied: those air-dried at room temperature of relative humidity of ~70% called 'wet' and those devoid of loosely bound water at room temperature, called 'dry'. In order to obtain the dry state of samples, prior to the measurements these samples were kept at a temperature of about 160°C until their dielectric parameters reached constant values. Then these samples were cooled to room temperature and subjected to the cycle of heating up to 260°C.The following notation of samples studied was introduced:

ISSN: 1727-1983

A, B and C, D - wet and dry healthy tissues and healthy tissues treated in 0.1 *M* NaOH, respectively, and E - dry ischaemic tissues.
The measurements were performed by an LCR bridge made by HIOKI over the frequency range of 100Hz – 100kHz and temperatures from 25 to 260°C.The parameters measured were relative permittivity ε', dielectric loss ε" and conductivity σ ($\sigma = 2\pi f \varepsilon_o \varepsilon''$).

Results and discussion

Fig. 1a and b show the plots of the relative permittivity ε' and conductivity σ for artery tissues samples A, B, C, D and E versus the temperature at a chosen frequency of 10kHz.

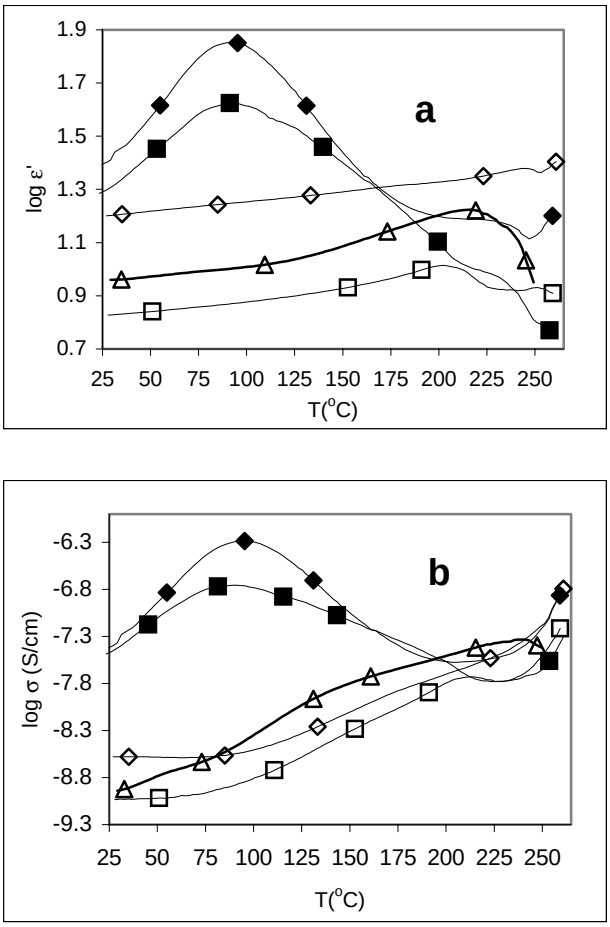

Figure 1: The variation of ε' (a) and σ (b) as a function of temperature for samples A (♦), B (■), C (◊), D (□) and E (Δ) at 10kHz.

The character of the curves is determined by the release of water in temperatures up to 200°C and the processes of glass transition of the elastin and collagen melting, above this temperature. These results obtained for all samples up to about 200°C support the mechanism of the release of water from the tissues studied involving braking up of hydrogen bonds formed among the water molecules or between water molecules and macromolecules of collagen and elastin. The liberation of loosely bound water in wet samples A and B is manifested by the ε' and σ maxima occurring near 80°C. This process is completed near 200°C, in which the minimum values of ε' and σ are observed. For dry samples C, D and E that do not contain loosely bound water, the peaks in ε' and σ do not occur in this temperature range and these parameters increase with increasing temperature as a result of the release of strongly bound water. In addition, at each temperature, ε' and σ are higher in sample C than in samples D and E. Only, for sample D above 80°C the plot of σ is significantly higher than for the samples C and D. On the basis of our earlier results for other tissues in the temperature range corresponding to the release of water [9], changes in the relative permittivity ε' of the artery tissues depend on the number of jumps performed by protons (H^+) between sites formed by water molecules bound to collagen and elastin molecules and also on the density of charge accumulation on the border of the protein-water phases. The conductivity σ for these tissues is probably dependent on proton transfer through the intra- and intermolecular hydrogen bonds in collagen-water and elastin-water systems**.** For sample C, ε' and σ arise from the mechanisms of polarization and conduction in both systems. However, for sample D these parameters are much lower than for sample C as a consequence of the presence only the elastin-water system. Because the ε' spectra for sample E is between the plots of ε' for samples C and D, this suggests that the ischaemic artery tissues must contain a smaller number of sites available for mobile protons as a results of collagen-water system degeneration. In contrast, the sharp increase in σ for sample E above 80°C can indicate that proton transport in ischaemic tissues is higher than in healthy tissues**.**
Above 200°C, small differences have been observed in ε' and σ between wet samples A and B, and dry samples C and D, respectively. This suggests, that in this temperature range the mechanisms of polarization and conduction in these tissues involve elastin and collagen. In addition, the higher values of ε' and σ for samples A and C than for B and D indicate the presence of both proteins in the former. However, the plots of ε' for samples A and C show significant maxima around 230-240°C corresponding to the process of melting of collagen. For these curves the peaks assigned to the glass transition of elastin are obscured, as probably they are masked by the peaks appearing at higher T and assigned to collagen. The transition for elastin around 210°C is well observed for samples B and D, because these tissues do not contain collagen. The plots of ε' and σ for sample E above 200°C differ in character from those for samples C and D. These curves for sample E show significant peaks assigned to elastin, but slightly shifted to higher temperatures than for sample D. However, the peak associated with collagen does not appear because ε' sharply decreases. This suggests that the ischaemic induces the higher physico-chemical changes in the collagen when compared to elastin.

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Fig. 2 compares the relative permittivity ε' (a) and conductivity σ (b) spectra at chosen temperatures of 60°C and 210°C for the dry tissues. The plots of ε' for each sample at 60°C show a weak dispersion and the ε' values of sample C are much higher than those of samples D and E. At 210°C, the curves of ε' for all samples show a remarkable dispersion over the entire frequency range. In addition, little difference is observed in ε' below 1kHz for samples C and E and the plots for these samples lay significantly higher than that for sample D.

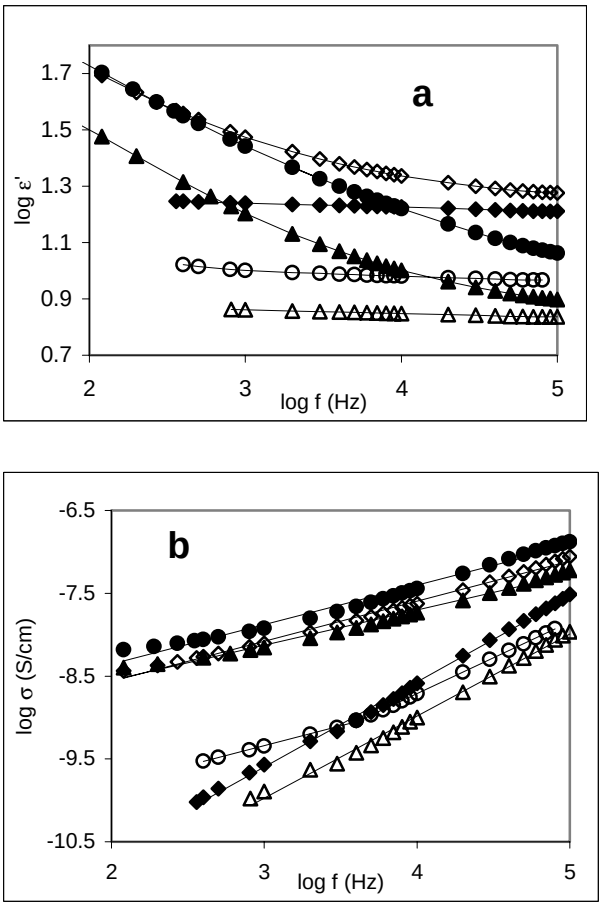

Figure 2: The variation of ε' (a) and σ (b) as a function of frequency for samples C (♦ 60°C, ◊ 210°C), D (Δ 60°C, ▲ 210°C) and E (○ 60°C, ● 210°C).

The plot of conductivity σ at 60°C for ischaemic tissues differs in character from those for healthy tissues at the same temperature. The curves for samples C and D in the whole frequency range show a linear behaviour with the slope value about 1.00 for the coefficient of determination, r^2, greater than 0.9959. However, the curve for sample E can be divided into two sections of linear character but of different slope, corresponding to two ranges of frequency values. Hence, for this spectra below 4kHz and in the range 4-100kHz, the slope values are 0.48 (r^2=0.9981) and 0.86 (r^2= 0.9998), respectively. The slope from the range 0.8-1.0 indicates that the dielectric results presented in Fig. 1 and 2 for all samples can be interpreted as related to the proton conduction processes. As for low temperatures, the conductivity curves at 210°C for all samples exhibit a linear behaviour over the entire frequency range. For r^2 greater than 0.9846 the slope values are similar for all materials and are about 0.42-0.48. These values lie below the range 0.8-1.0, which suggests that for healthy and ischaemic tissues the process of proton conduction in higher temperatures does not occur. In order to compare the dynamics of the processes taking place up to 200°C and above this temperature for all dry tissues, Fig. 3 presents the Arrhenius plots of the conductivity σ against T^{-1} at a chosen frequency of 1kHz and 100kHz.

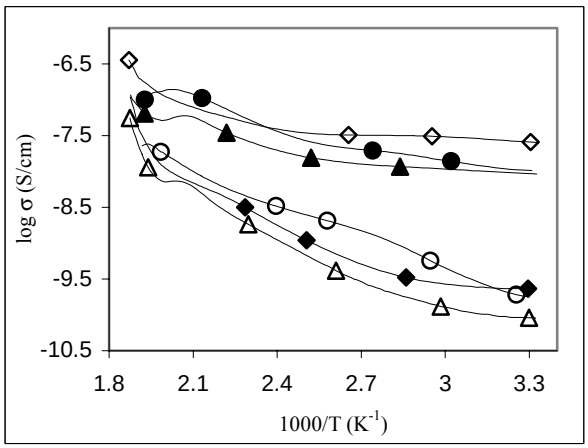

Figure 3: Plots of log σ vs. 1/T for samples C (♦1kHz, ◊ 100kHz), D (Δ 1kHz, ▲ 100kHz) and E (○ 1kHz, ● 100kHz).

From these dependencies we can obtain the activation energy ΔH of conductivity responsible for the water release and the processes of glass transition in elastin and melting of collagen. The ΔH needed for liberation of water decreases from 8, 13 and 32kJ/mol at 1kHz to 2, 6 and 10kJ/mol at 100kHz for samples C, D and E, respectively. The values of ΔH for the phase transition in the elastin and collagen are about 36, 41 and 23kJ/mol at 1kHz and 19, 29 and 32kJ/mol at 100kHz for samples C, D and E, respectively. The ΔH for healthy tissues decreases but for ischaemic tissues increases with increasing frequency.

Conclusions

The aim of this work was to compare dielectric relaxation behaviour in healthy and ischaemic human artery tissues, being composites of water, collagen and elastin. The temperature dependencies of the dielectric parameters of the tissues studied suggest the important role of water in stabilisation of the collagen and elastin macromolecule structure. In fact, the process of water release includes the breaking up of hydrogen bonds, and also, diffusion of water out of the tissues in a wide

ISSN: 1727-1983

temperature range up to 200°C. The results of this paper indicate that ischaemic affects the dielectric properties of collagen-water and elastin-water systems in the artery tissues. This fact is supported by higher values of the activation energy responsible for the water release for the ischaemic tissues than for the healthy tissues.

References

[1] LEE T.C., MIDURA R.J., HASCALL V. C., and VESELY I.. (2001): 'The effect of elastin damage on mechanics of the aortic valve', *Journal of Biomechanics*, 24, pp. 201-210

[2] VESELY I.. (1998): 'The role of elastin in aortic valve mechanics', *Journal of Biomechanics*, 31, pp. 115-123

[3] LILLIE M. A., GOSLINE J. M. (2002): 'The viscoelastic basis for the tensile strength of elastin', *Int. J. Biol. Macromol*., 30, pp. 119-127.

[4] BUSCHMAN H.P., DEINUM G., Motz J. T., FITZMAURICE M., KRAMER J. R., van der LAARSE A.,. BRUSCHE A. V., and FELD M S. (2001): 'Raman microspectroscopy of human coronary atherosclerosis: biochemical assessment of cellular and extracellular morphologic structures in situ', *Cardiovascular Pathology*, 10, pp. 69-82

[6] SAMOUILLAN V., LAMURE A., MAUREL E., DANDURAND J., LACABANNE C., and SPINA M. (2000): 'Dielectric characterization of collagen, elastin, and aortic valves in the low temperature range', *J. Biomater. Sci. Polymer Edn*, 11, pp. 583-598

[7] PEZZIN G., and SCANDOLA M. (1976): 'The low-temperature mechanical relaxation of elastin. I. The dry protein', *Biopolymers*, 15, pp. 283-292

[8] ROSENBLOOM J., ABRAMS W. R., and MECHAM R. (1993): 'Extracellular matrix 4: The elastic fiber', *FASEB J*., 7, pp. 1208-1218

[9] MARZEC E., and W. WARCHOŁ W. (2005): 'Dielectric properties of a protein-water system in selected animal tissues', *Bioelectrochemistry*, 65, pp. 89-94

ISSN: 1727-1983