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In vitro evaluation of the effect of tobacco smoke on rat cornea function

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Abstract

The influence of tobacco smoke on the dielectric properties of rat cornea were measured *in vitro* over the frequency range of the electric field of 500Hz–100kHz and in temperatures of the air from 25 to 150 °C. The temperature dependencies of the loss tangent for both healthy and smoky cornea represent the relation between the energy lost and the energy stored in the epithelium-stromal-endothelium systems of the cornea. The differences between the healthy and the smoky cornea concerned the temperature ranges in which there appeared the decomposition of loosely-bound water and β-relaxation associated with polar side-chains relaxations on protein molecules of this tissue. The effect of smoke is manifested as a shift of the loss tangent peaks of these two processes towards higher temperatures, when compared with the control. The results are interpreted as caused by the toxic compounds of the tobacco smoke leading to higher ion transport in the nonhomogeneous structure of the cornea when compared to that of the control tissue. The activation energy of conductivity were similar for the healthy and smoky cornea as a consequence of the braking of hydrogen and Van der Waals bonds between loosely bound water, and the proteins of channels in the epithelium and endothelium. Recognition of the effect of frequency and temperature on the dielectric behaviour of the smoky cornea may be of interest for disease characterization of this tissue.

Key words

cornea; tobacco smoke; loss tangent; water; tear film; activation energy

INTRODUCTION

Dielectric spectroscopy is one of the methods used to study the thermodynamic first- and second-order transition of solid biopolymers. In the case of protein molecules, such as collagen and keratin, the appearance of first- and second-order transition is associated with the main-chain movements above 200°C, and the polar side-chains rotations below the temperature of the decomposition of loosely bound water, respectively [1, 2, 3]. Investigation of the effect of many external factors, such as moisture, ionizing radiation and noxious gases, on the thermal phase transition of human and animal tissues is especially suitable in the production of biomedical materials [4, 5].

The literature reported data concerning the electrical properties of the cornea provide information about the structure and functions of this tissue [6, 7, 8], and also possibilities of its clinical application [9, 10]. Unfortunately, no *in vitro* studies related to the dielectric behaviour of the human or animal cornea exposed to the toxic compounds of tobacco smoke have been performed. In the presented study, dielectric research was undertaken to determine the effect of tobacco smoke on the thermal processes of rat cornea. The results obtained were analysed on the basis of data concerning the effect of tobacco smoke exposure on the ocular surface, tear film and visual functions obtained using other techniques [11, 12, 13, 14, 15]. The results of these measurements indicated that exposure to tobacco smoke leads to a decrease in tear film stability, damage to the ocular surface epithelia, as well as biomechanical changes associated with collagen cross-links in cornea.

MATERIALS AND METHODS

The study was conducted on a healthy group of 12 rats and an experimental group of 12 rats exposed to tobacco smoke. Male, Wistar rats weighing about 422g were used. All investigations were approved by the Local Ethics Commission for Animal Studies in Poznań (Approval No. 26/2010). The rats were kept at room temperature within the range of 20-22 °C and humidity between 50-60%. The experimental group of rats was exposed to passive tobacco smoke twice in a period of 16 weeks in a controlled smoke chamber (during the 8th and 16th weeks they were exposed for 6 hours, 5 days a week). The chamber air carbon monoxide (CO) concentration was 1,500mg/m^3. All the rats were then anaesthetized by an intramuscular injection of 5mg/kg ketamine and 40mg/kg xylazine, and sacrificed by bleeding out through the right ventricular puncture. The corneas were immediately excised and immersed in a solution of 0.9% NaCl.

Prior to the dielectric measurements, the corneas were washed in distilled water, dried at room temperature, and

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formed into rectangular samples of a typical size of 3.0mm × 4.0mm × 0.1mm. The samples were covered with silver paste electrodes. Dielectric measurements were performed in air for 2 sets of corneas. The first set, termed 'wet', included the corneas that had been air-dried at room temperature with a relative humidity of 65-70%. The second set, termed 'dry', consisted of the corneas from the samples in which the water was removed after having been annealed for 1 hour at 150 °C [1], and then cooled to room temperature. Following the water removal procedure, the mass loss in the cornea equaled about 12% of its original mass at room temperature before the measurement corresponding to the content of loosely bound water in the wet sample.

The measurements of the relative permittivity ε', dielectric loss ε'', loss tangent, tan δ, and conductivity σ ($\sigma = 2\pi f \varepsilon_o \varepsilon''$) were carried out using an impedance analyser HIOKI 3522-50 LCR over the frequency, f, range of 500Hz-100kHz and in temperatures, T, from 25-150 °C. The values of the dielectric parameters for each group of healthy and experimental samples were given as the average from 6-8 measurements.

RESULTS AND DISCUSSION

Fig. 1 presents the effect of water and tobacco smoke on the temperature dependence of the loss tangent, tan δ, for the cornea at 16 and 100kHz. The curves in this figure represent the relation between the energy lost and the energy stored in the epithelium-stromal-endothelium systems of the cornea. An earlier paper by the authors of the presented study [16] revealed the relationship between the electric properties of cornea, which involve the toxic elements of the tobacco smoke, and cornea structure. The measurements performed at the temperature of 35 °C indicate that the lost energy appears as a results of current movements through the 3 layers of the cornea. While the energy stored in the cornea is the effect of charges accumulation between the layers of the cornea and those inside. Further, as follows from the curves in Figure1, the differences between the healthy and the smoky cornea concern the temperature ranges in which there appears the decomposition of loosely bound water and β-relaxation associated with polar side-chains relaxations on the protein molecules of this tissue. These 2 processes for the smoky cornea are shifted by about 10 °C to higher temperatures when compared to those for the healthy one. This behaviour observed in the loss tangent for the smoky samples is a consequence of the chemical cross-linking of collagen with the toxic compounds that become incorporated into the epithelium from the tear film [13] following exposure of the rats to the tobacco smoke. Earlier biomechanical studies of the human cornea have also revealed the influence of cigarette smoke on the cross-linking of collagen [12, 15]. In addition, for the wet healthy and smoky cornea (Fig. 1a) the peaks in tan δ around 55-65 °C, respectively, are nearly independent of frequency; this behavior therefore indicates the thermodynamic first-order transition of this tissue. For the dry healthy and smoky cornea (Fig.1b), the frequency affected the temperature of the tan d peaks, which were shifted about 30 °C in the range of 16-100kHz for both samples. This is the result of the second-order transition (referred to as β-relaxation) of cornea.

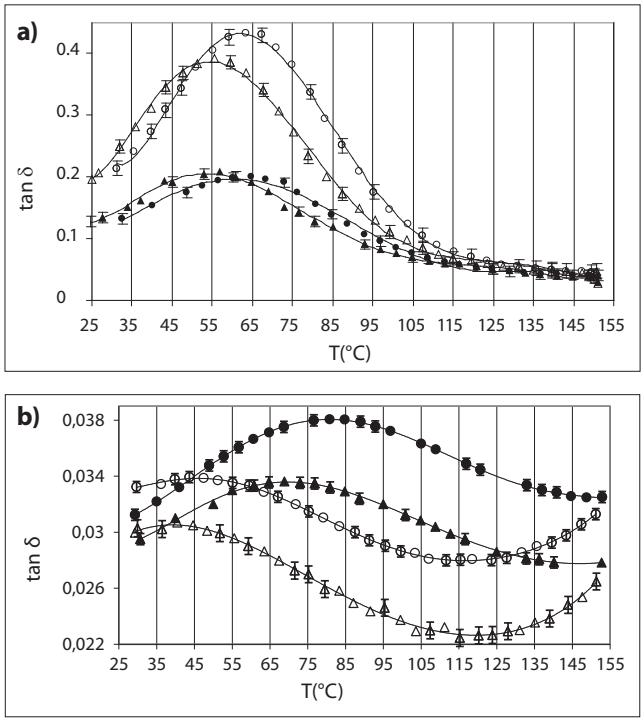

Figure 1. Variation of tan δ versus temperature for wet (a) and dry (b) healthy (Δ – 16kHz; ▲ – 100kHz) and smoky (○ – 16kHz; ● – 100kHz) cornea. Vertical bars – ±SD

In order to compare the mechanism of ionic conduction associated with these 2 phase transitions in the healthy and the smoky cornea, the plots of logarithm of conductivity against the reciprocal of temperature for 100kHz are presented in Figure 2. The activation energy ΔH of conductivity for each curve was obtained from the slope of the straight line up to the temperature peak of log σ [1].

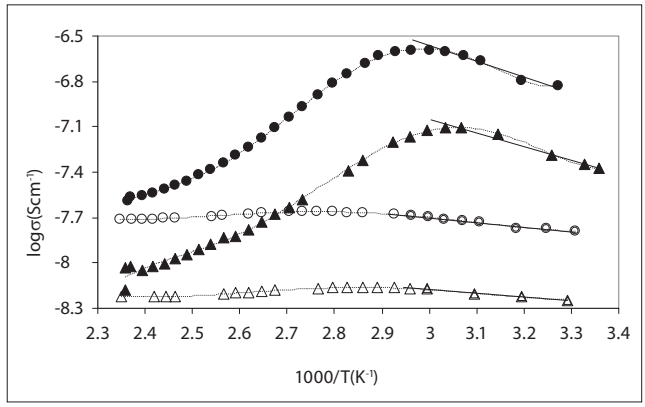

Figure 2. Plots of log σ versus $(T)^{-1}$ for dry (Δ – healthy; ○ – smoky) and wet (▲ – healthy; ● – smoky) cornea at 100 kHz

For the wet samples, the activation energy of about 18kJ/mol was consistent with the kind of relaxation motion of the hydrogen bonds. In these materials, the conduction of protons and other ions with increasing temperature is a consequence of the breaking of hydrogen bonds between loosely bound water and the proteins of channels in the epithelium and endothelium. In the case of the dry healthy and smoky samples, the value of ΔH (about 5kJ/mol) was significantly lower than that of the wet samples. This indicates that in the dry cornea the conduction process appeared as a result of the breaking of other important bonds, such as Van der Waals. These values of ΔH are similar for the healthy and smoky cornea, which would indicate on the

same conduction mechanism, although differences in the values of conductivity between both these materials are shown in Figure 2. This suggests that in the smoky cornea the intensity of the conduction mechanism is higher than that of the healthy cornea. Most probably, the conductivity of the smoky sample is due to a large density of ion and the formation of toxic-induced crosslinks in the 3 structural layers of the cornea.

CONCLUSIONS

The differences between the healthy and the smoky corneas concern the temperature ranges in which there appears the decomposition of loosely bound water and β-relaxation associated with polar side-chains relaxations on protein molecules of this tissue. These 2 processes for the smoky cornea are shifted by about 10 °C to higher temperatures when compared to those for the healthy one. The toxic compounds of the tobacco smoke gave rise to higher ion transport in the epithelium-stromal-endothelium systems of the cornea, when compared to that of the healthy tissue. Knowledge of the dielectric behaviour of the smoky rat cornea may facilitate early detection of non-beneficial changes in the human cornea after exposure to passive tobacco smoke. The results of this study performed by dielectric spectroscopy provide new information on molecular interactions in the healthy and smoky cornea.

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