Thermochimica Acta 506 (2010) 73–76

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Dielectric analysis of the thermal processes of human nail

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ARTICLE INFO

Article history:
Received 21 October 2009
Received in revised form 17 February 2010
Accepted 26 April 2010
Available online 26 May 2010

Keywords:
Nail keratin
Dielectric properties
Activation energy
Keratin melting
Biomaterials

ABSTRACT

The temperature and frequency dependencies of the dielectric parameters for the human fingernail are used to analyse keratin degradation in high temperatures. Measurements were performed over the frequency range of 100 Hz to 100 kHz and at temperatures from 22 to 260 °C. The nail samples contained 11% water by mass at room temperature at a relative humidity of 70%. The results were discussed in terms of the distribution of relaxation frequencies and the activation energy for the conduction and polarization mechanisms in the keratin–water system. The information on the dielectric behavior of the keratin degradation might become handy in course of future research on biomaterials obtained from nail keratin.

1. Introduction

Obtaining the biomaterials for different purposes requires performance of such preliminary processes as extraction from tissues, purification, crosslinking and sterilization. Such a procedure is vital to obtain the required biochemical properties of these biomaterials, e.g. biocompatibility, weak antigenicity and biodegradability. Recently, by applying the required processing technology, it has become possible to obtain keratin-based materials [1–4]. Yet, finding information on advantageous properties of these new substances demands application of appropriate physico-chemical research methods. Among these, thermal methods such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) appear quite important since they allow α-helical structure thermal stability analysis. Previous research on different tissues containing keratin as well as those keratin regenerated ones performed by means of these methods, supply enough information on thermal behavior thereof on the basis of such parameters as heat capacity or weight measurement [5–10]. These thermal methods are still applied to research physico-chemical properties of keratin-based biomaterials [11–13]. Electric methods are rarely used to analyse protein melting in high temperatures [14–16]. Neither there is any information available on the methods applied to test thermal stability of keratin-based materials. In the past, to study the dielectric behavior of the human nail [17], as the source of keratin, we applied dielectric spectroscopy below the melting temperature thereof. The research was performed on healthy and diabetic nail specimens.

The present study broadens the area of research into the dielectric properties of the human nail up to 260 °C. According to literatures [10,13], keratin may have a denatured melting behavior around 200–230 °C in an inert atmosphere. However, the TGA [18,19] results reveal, that this process is accompanied by the loss of a small mass (∼5%). Thus, it should not be considered the pure melting of keratin, because the physical properties of this transition reflect as well a non-oxidative chemical decomposition. As follows from the DSC and TGA analysis of keratin [7,20], if samples were highly hydrated with water molecule or were in the environment of silicone oil, the melting points may decrease below 200 °C. In fact, this is a safe range of temperature, since there are no losses of mass resulting from the degradation of keratin. However, the thermal degradation of keratin [19–22] can easily occur at above 230 °C regardless of the environment surrounding this substance. Ėhen et al. [23] indicated that histidine revealed the least and tyrosine the highest thermal stability which was supported by different mass losses.

In the present paper we analyse the dielectric properties of the crystalline keratin up to 200 °C and above this temperature during its degradation. Since the measurements were carried out under normal pressure in air, there was intense oxidation of the samples even at above 200 °C with the loss of mass about 19% up to 260 °C. Recently [24–26] the degradation process of collagen, a protein of an alike melting temperature as the one typical of keratin, has been

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doi:10.1016/j.tca.2010.04.018

also examined in an air atmosphere using other method than the dielectric spectroscopy.

2. Experimental

2.1. Materials

The nails of the middle fingers of the upper limbs, 3 mm in width, were obtained from the group of 12 normal individuals (44–80 years old). Prior to the dielectric measurements these nails were immersed in a solution of 0.1 M NaCl to remove fat, washed in distilled water, dried at room temperature and formed into rectangular sample of a typical size, 2 mm × 4 mm × 0.3 mm. Then the sample was covered with silver paste electrodes. Dielectric measurements were performed in air for two sets of nails. The first set termed 'wet' included nails air-dried at room temperature of relative humidity of 70%. The set was subject to continuous heating up to 260 °C. The second set, termed 'dry', included the nails after removing of water from the samples [18]. Following the water removal procedure, the nail samples were heated from room temperature (RT) to 150 °C, annealed for 1 h in this temperature and then cooled to RT. The set was immediately subjected to continuous heating up to about 260 °C. The sample mass loss due to heating was estimated at the same heating rate as the one in the dielectric study and in the same measuring cell. The mass loss in the wet nail at 260 °C equaled about 30% of its mass at room temperature before the measurement and this sample contained about 11% water. In the dry nail there was no mass loss in the range from RT to 200 °C, only above this temperature and up to 260 °C the loss of mass amounted to 19% just as in the case of the wet nail.

2.2. Dielectric measurements

Measurements of the complex permittivity ε^* ($\varepsilon^* = \varepsilon' - j\varepsilon''$) and conductivity σ ($\sigma = 2\pi f \varepsilon_0 \varepsilon''$) were carried out using an impedance analyser HIOKI 3522-50 LCR over the frequency, f, range of 100 Hz to 100 kHz and in temperatures, T, from 22 to 260 °C. The values of the dielectric parameters for nail samples are given as the average ranging from 6 to 10 measurements.

3. Results and discussion

Fig. 1 presents the temperature dependencies of the relative permittivity ε' and dielectric loss ε'' for the wet and the dry nail at 80 kHz, and up to about 260 °C. The release of 11% water from the wet nail is manifested by ε' and ε'' maxima near 80 °C. The peaks in ε' and ε'' for the wet nail occurring at around 230–260 °C correspond to the polarization and conduction mechanisms of the degraded keratin. In the case of the dry nail that does not contain water, the maxima of both parameters do not occur. Only the plot of ε' for the dry nail shows a broad peak around 230 °C due to keratin degradation. As shown in Fig. 2 for the degradation temperature range of the wet nail, the peaks in ε' and ε'' are nearly independent of frequency, therefore this behavior indicates on the thermodynamic first-order transition [27] of this substance. In this temperature range the frequency only affects the values of ε' and ε''. Similar effect of frequency on the dielectric parameters of the studied nail appears for the decomposition of water near 80 °C (not presented). The occurrence of the degradation process in keratin above 230 °C has been verified by DSC and TGA analysis [19,21]. In addition, the thermal stability studies of such amino acids as aspartic acid (Asp), glutamic acid (Glu), histidine (His) and tyrosine (Tyr) also confirmed that keratin related proteins were not stable above this temperature [23]. Thus, the amount and the mobility of such polar groups as OH, CO and NH in the side chains of these

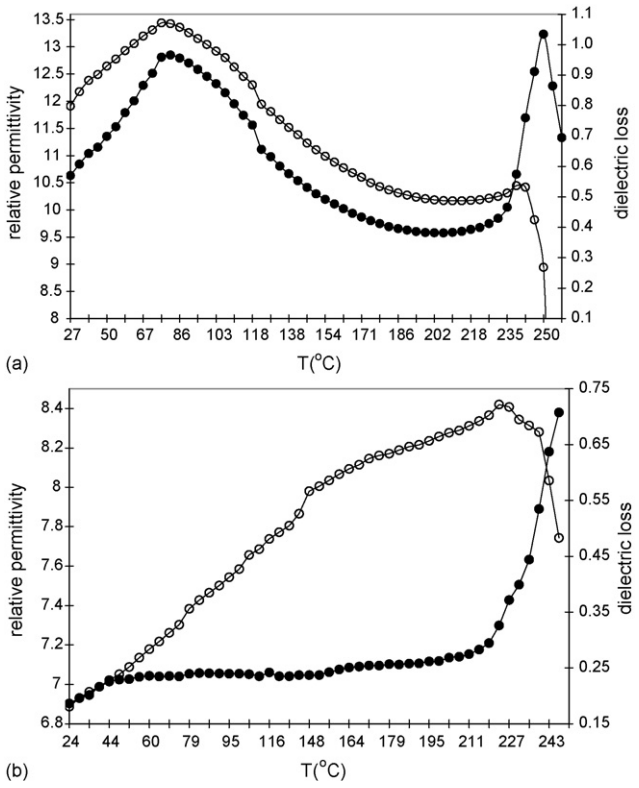

Fig. 1. The variation of (○) ε' and (●) ε'' versus temperature for the wet (a) and dry (b) nail at 80 kHz. Solid lines represent experimental results.

amino acids determine the dielectric stability of keratin over a wide range of temperature. Further, Fig. 3 presents the frequency dependence of ε' and σ near 190 and 240 °C, so for the temperatures from the range of the non-degradation and the degradation processes of keratin, respectively. These data of nail can be described by the Cole–Cole relation [28] with the two-dispersion terms above 2 kHz as follows:

$$\varepsilon* = \varepsilon'_h + \frac{\Delta\varepsilon'_1}{1 + (jf/f_1)^{1-\alpha_1}} + \frac{\Delta\varepsilon'_2}{1 + (jf/f_2)^{1-\alpha_2}} + \frac{\sigma_L}{j2\pi f\varepsilon_0} \quad (1)$$

where $\Delta\varepsilon'_1$ and $\Delta\varepsilon'_2$ are the permittivities decrement, f_1 and f_2 are relaxation frequencies, α_1 and α_2 are the Cole–Cole parameters of the dispersion 1 and 2, respectively, σ_L and $\Delta\varepsilon'_h$ are the limiting low- and high-frequency conductivity and permittivity around 2 and 100 kHz, respectively. The α_1 and α_2 values which reflect the spread of relaxation frequencies were estimated from

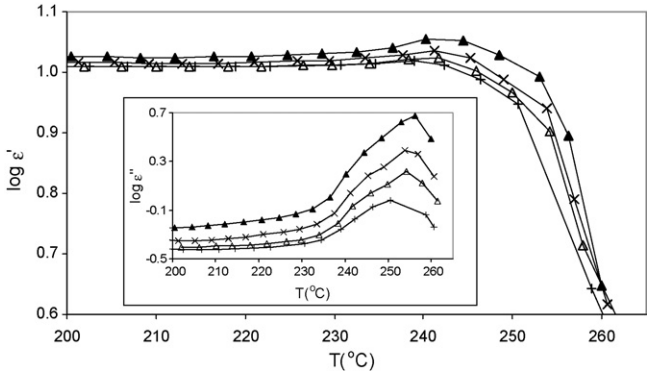

Fig. 2. Temperature dependencies of ε' and ε'' for the wet nail above 200 °C at several frequencies of (▲) 10 kHz, (×) 25 kHz, (△) 50 kHz, (+) 100 kHz. Solid lines represent experimental results.

Table 1
Dielectric parameters (mean ± SD) of wet and dry nail.

Nail		$\Delta\varepsilon'_1$	α_1	f_1 (kHz)	$\Delta\varepsilon'_2$	α_2	f_2 (kHz)
Wet	190 °C	0.56 ± 0.03	0.25	5.0 ± 1.0	0.34 ± 0.03	0.37	24 ± 2
	240 °C	1.27 ± 0.08	0.27	5.0 ± 1.0	0.67 ± 0.05	0.44	24 ± 2
Dry	190 °C	0.36 ± 0.02	0.35	4.5 ± 1.5	0.20 ± 0.01	0.53	23 ± 3

the Cole–Cole plots of ε'' against ε', as shown in Fig. 4 for the dry nail at 190 °C. Table 1 compares the dielectric parameters of the wet and the dry nail obtained from the fitted curves to Eq. (1) (Figs. 3 and 4). This table shows two significant relaxation frequencies of about 5 and 25 kHz, which are similar below and above 200 °C for both sets of the nail. However, the distribution parameters α_1 and α_2 are in the range of 0.3–0.5 for both sets of the nail. These obtained values of α are characteristics for many tissues [29,30], being composites of different phase composition and different volume ratio of the phases. Furthermore, a distribution of relaxation frequencies for these two dispersions is accompanied by the conductivity power-law relation of the form $\sigma \sim f^p$, with the exponent p varied from 0.6 to 1, as shown in Fig. 5. In addition, the linear fits of these curves below 2 kHz yield p values between 0.4 and 0.6 for two sets of nails. The obtained values of p suggest that below

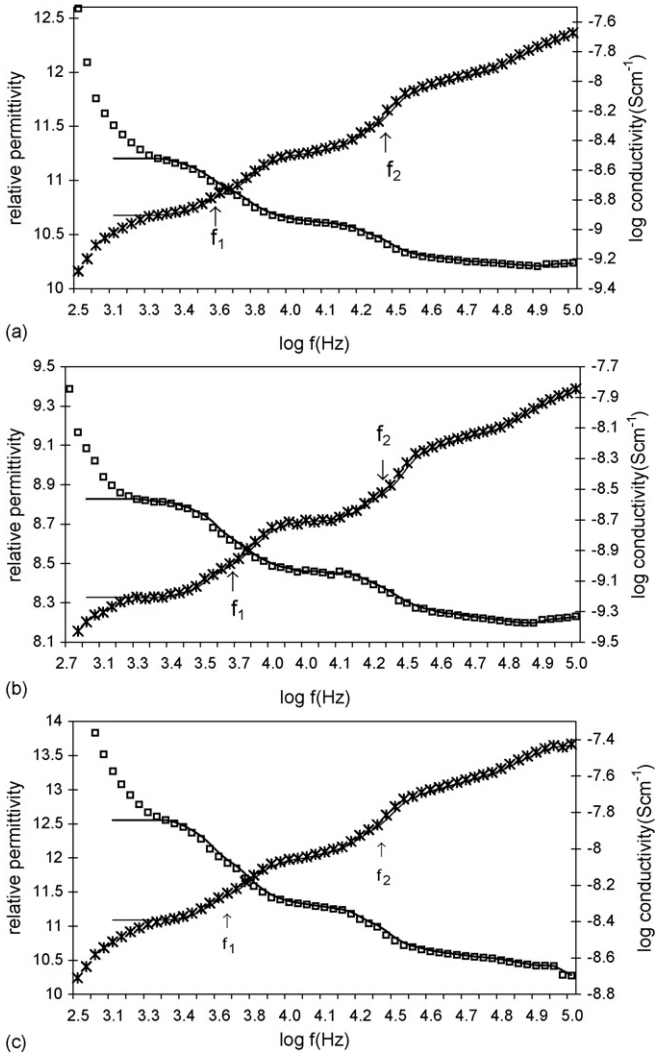

Fig. 3. Plots of (□) ε' and (*) $\log\sigma$ versus frequency for the wet nail at 190 °C (a) and 240 °C (c), and the dry nail at 190 °C (b). Solid lines are the fits to the Cole–Cole equation.

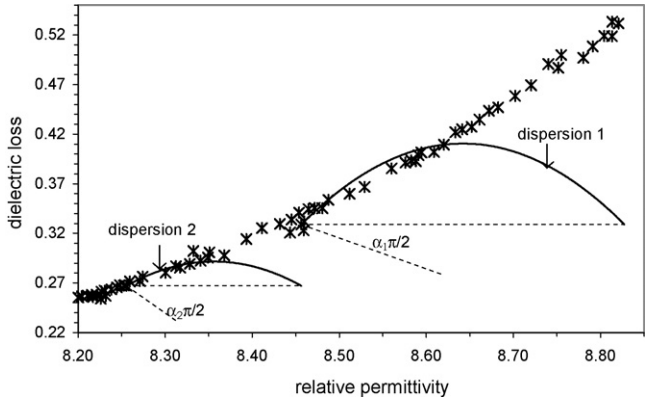

Fig. 4. The variation of the dielectric loss as a function of the relative permittivity for the dry nail at 190 °C. Solid and dashed lines indicate the two dispersions and the distribution parameters α, respectively, calculated from Eq. (1).

2 kHz the electrode polarization effects have no influence on the dielectric behavior of nail. Thus, the dielectric properties of this material in the studied temperature and frequency range might be due to the polar sites and the relaxation of surrounding free ions of keratin. Most probably, above 200 °C as a consequence of the degradation process the amount of such relaxing sites as the side chains, inter-chain hydrogen bonds, and also disulphide bridges decrease but the mobility of the remaining sites and ions in the keratin increase. This degradation behavior of the nail is visible in the magnitude of the $\Delta\varepsilon'_1$ and $\Delta\varepsilon'_2$, which are significantly higher at 240 °C than those of the one at 190 °C. From the plots of $\log\sigma$ against T^{-1} depicted in Fig. 6 was obtained the activation energy ΔH (Table 2) of conductivity for 5 and 25 kHz and at temperatures from 3.35 K^{-1}(25 °C) to 1.87 K^{-1}(260 °C) for both wet and dry nails. Table 2 shows that the values of ΔH in the two temperature regions differ by a factor of about 9. In fact, the strengths of the hydrogen bonds are much weaker when compared to the covalent disulphide bridges. Therefore, below and above 200 °C, the ΔH is needed for the conduction of protons and other ions during the break up of inter- and intra-molecular hydrogen bonds and the degradation of keratin, respectively. In addition, at the frequency of 5 kHz, the ΔH

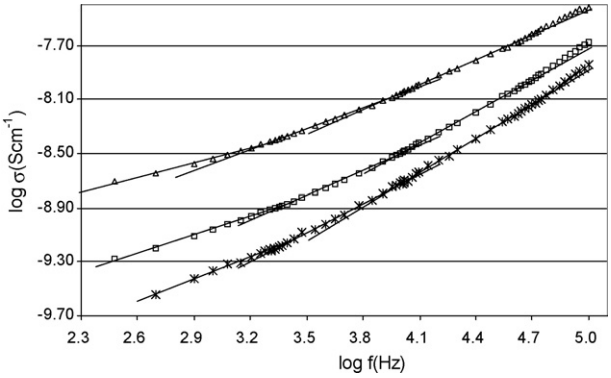

Fig. 5. Frequency dependencies of the conductivity for the wet (□, at 190 °C; △, at 240 °C) and the dry (*, at 190 °C) nail. Solid lines are the linear fits to the power-low relation.

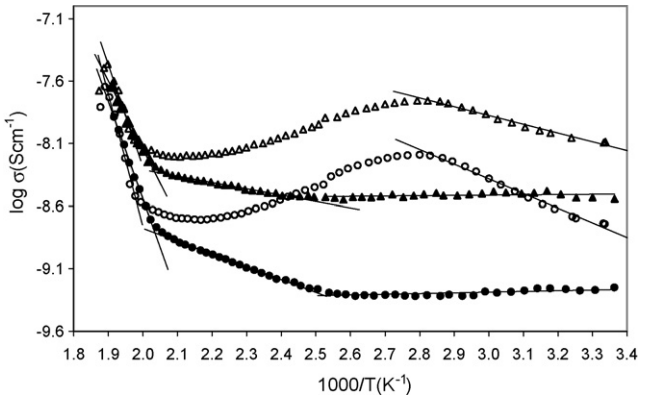

Fig. 6. The variation of $\log\sigma$ versus $(T)^{-1}$ for the wet (○, 5 kHz; △, 25 kHz) and dry (●, 5 kHz; ▲, 25 kHz) nail. Solid lines are the fits to the Arrhenius equation: $\sigma = \sigma_0 \exp(-\Delta H/RT)$, where σ_0 is the pre-exponential factor, R is the gas constant.

Table 2
Activation energy ΔH (mean ± SD) of conductivity for the wet and the dry nail at 5 and 25 kHz.

Nail	f (kHz)	ΔH (kJ/mol) *below* 200 °C	ΔH (kJ/mol) *above* 200 °C
Wet	5	22 ± 2	190 ± 10
	25	13 ± 2	153 ± 5
Dry	5	19 ± 2	149 ± 5
	25	10 ± 1	103 ± 5

of conductivity is higher than the one for 25 kHz, since the hopping distance for charge carriers between their transport sites increases.

4. Conclusions

The temperature dependencies of the dielectric parameters for the wet and dry nail show the peaks around 230–260 °C correspond to the process of the keratin degradation. The origin of the broad two relaxations of the nail at 190 and 240 °C might be due to the polar sites of the non-degraded and degraded keratin, and the relaxation of surrounding free ions. The degradation behavior of the nail is supported by high values of the activation energy of conductivity above 200 °C than those of the one below this temperature. The results of this study indicate that the dielectric spectroscopy is useful in observing the degradation process of the nail, similarly as DSC or the thermal chemiluminescence (TCL) [22] techniques.

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