Journal of Thermal Analysis and Calorimetry, Vol. 79 (2005) 289–293

THERMAL ANALYSIS OF CAUCASIAN HUMAN HAIR

Valéria F. Monteiro[*]*, A. P. Maciel and E. Longo*

CMDMC - LIEC - UFSCAR, Rod. Washington Luís, km 235, C. P. 676 - CEP 13565-905 São Carlos, SP, Brazil

TG and DSC analyses were carried out in this work to evaluate the changes in the denaturation of human hair keratin submitted to different chemical effects. Hair bleaching and chlorinating treatments caused changes in the denaturation temperatures and denaturation enthalpies of hair keratin. Bleached hair and hair kept in a chlorinated solution presented a lower denaturation enthalpy and a higher denaturation temperature compared to the control hair sample. The TG and DSC analyses allowed to quantify the degradation level of hair fibers after the chemical treatments. AFM was also utilized to characterize the morphological alterations in the hair fiber surfaces caused by the chlorinating and bleaching treatments.

Keywords: *AFM, DSC, hair fibers, keratin denaturation*

Introduction

Biomaterials such as α-keratin, the main component of human hair, exhibit a complex morphological structure. The human hair presents three principal components: cuticle, cortex and medulla. The cortex is organized in macrofibrils, microfibrils and cell membrane complexes. Swift proposed that microfibrils were axially oriented as crystalline filaments, and embedded within an amorphous matrix in the hair cortex [1]. Studies on the α-keratin hair conformation have been performed by thermoanalytical techniques [2, 3].

Thermoanalytical studies of human hair were previously carried out by Humphries *et al*. [2]. They executed thermogravimetric (TG) and differential scanning calorimetry (DSC) analyses of hair with the purpose of verifying keratin structural changes.

At room conditions, keratin retains remarkable amounts of water, which is an integral part of the keration structure. The thermal transitions in the keratin are strongly affected by the amount of water. At temperatures about 100°C, a broad endothermic signal is related to the removal of loosely bond water. The liberation of strongly bound water is observed at temperatures above 140°C [2]. Studies of thermal expansion of hair indicate a process characterized by a maximum temperature at the 210–250°C interval. It is referred to the denaturation of the helical keratin fraction. This process is strongly affected by water and chemical treatments. The area of the peaks related to the keratin denaturation is a good measurement of the α-helix content [4].

Spein and Holzen [5] describe that the cortical cells of the wool, as well as the hair, possess a structure composed of two phases, consisting of intermediate filaments with a low sulfur content (microfibrils) and inside an amorphous matrix with a high sulfur content. Investigations of hair samples extended between 10 and 80% show that the intensity of the microfibrillar peaks decreased continuously with the stretching ratio, whereas the matrix peak remained unchanged, showing that this first keratin denaturation peak can be attributed to the loss of α-keratin conformation.

Milczareck *et al*. [3] verified that water stabilizes the crystalline structure of keratin while the partial removal of water disorganizes the crystalline phase. Hair samples kept in increasing relative humidities exhibited an increase in the enthalpy of water evaporation.

The microfibrils and matrix consist the cortical cells that are packed within the hair fiber. Wortmann and Deutz [6] verified two types, ortho- and para-cortical cells, which were separated and identified according to methodology to differentiate the cells to their coloration. The ortho- and para-cortical cells present a different structure for the two components, which are arranged bilaterally in the crossed section. The ortho-cortex contains a smaller concentration of disulfide linkages than the para-cortex. They considered that the occurrence of double denaturation endotherms of keratin of the wool is probable due to the cystine content and disulfide linkages, which are large enough and makes possible to separate the peaks. The isolation of ortho- and para-cortical cells was verified by their respective denaturation temperatures. The values of denaturation temperatures, obtained in DSC analyses performed in an aqueous medium, is about 138°C for the isolated ortho-cortical cells and for the para-cortical cells 144°C was obtained. These values were in good agreement with the ones obtained for the whole

* Author for correspondence: valeria@liec.ufscar.br

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

wool structure. The DSC studies were done in a dry environment, yielded keratin denaturation peaks at 230 and 240°C, referring also to the ortho- and para-cortical cells, respectively [6].

Studies accomplished by Tonin *et al.* using wool keratin, confirm the existence of these endotherm double peak in this temperatures interval. They also verified the decrease of denaturation the first peak referring to the ortho cells for the wool treated without changes in the second endotherm peak [7].

Belletti *et al*. [8] also applied the DSC technique to study hair fibers. They investigated the effect of the hydration on hair using DSC and GC techniques, verifying that some cosmetic products applied on hair formed films on the hair surface. Hair treated with a cosmetic product showed an increase in the enthalpy of water vaporization confirmed by DSC.

Wortmann *et al*. [9] verified that the effect of bleaching and perm-waving changes the denaturation temperatures and denaturation enthalpies. The increase of perm-waving treatments decreased the denaturation temperature of keratin. These results indicate that the enthalpy probably depends on the structural integrity of the α-helical material in intermediate filaments, while denaturation temperature is kinetically controlled by the density of cross linkages of the matrix, in which the intermediate filaments are embedded.

Atomic force microscopy, (AFM), was found to be useful for imaging nonconductive substrates. The attractive or repulsive forces that arise from the interactions between the surface of the sample and tip generate the digital images. The forces involved are in the order of 10^{-9} N. AFM has two modes of applied force, contacting and non-contacting modes. In the contacting mode, the force that the cantilever exerts on the sample, caused by displacements in the *z*-axis of the piezoelectric sensor, allows the quantification of surface properties of the material being analyzed. In non-contacting mode the image is obtained by tapping the surface with an oscillating tip that measures the van der Waals interactions [10].

The present work aims to investigate the influence of bleaching and chorinating solution on the hair fiber keratin denaturation using TG and DSC. The AFM technique was also used to verify the level of damage in hair fibers. These investigations allow to verify the structural changes and degree of degradation of hair keratin based on the enthalpy data and denaturation temperature of keratin.

Experimental

The hair fibers used were classified as untreated brown Caucasian hair. The samples were stored at selected levels of humidity (RH of 45%) and temperature (20–22°C) for 24 h prior to the analyses. For the bleaching treatment, it was utilized a Caucasian hair bleached immersed to a 2:1:0.5 solution containing 20% *v*/*v* of hydrogen peroxide, ammonium hydroxide and ammonium persulfate, respectively, for 1 and 2 h. For the chlorinating treatment, the tresses were immersed in a chlorinated solution for 96, 120 and 168 h in bath, simulating swimming pool conditions. The sodium hypochloride solution contained 3 ppm of free chlorine.

Thermogravimetric analysis (TG) (Netzsch, STA 440), under synthetic air was used with a flow rate of 100 mL min^{-1} and at a heating rate of 3 K min^{-1}. The tests were conducted from 25 to 720°C. The DSC measuements have been performed on Netzsch, 204 TASC 414/3A under synthetic air with a flow rate of 30 mL min^{-1} and at a heating rate of 20 K min^{-1}. The tests were conducted from –10 to 500°C.

AFM can be used for imaging of non-conductive surfaces. The equipment consists of a probe, a laser beam and a photodiode used to detect the movement of the probe. The probe, in the form of a sharp tip attached to a cantilever, scans the surface by using a force in the order of 10 to 20 nN in the contact mode, and 0.1 nN in the tapping mode. The Digital Instruments model Nanoscope IIIa® equipment was used for these experiments.

Results and discussion

Figures 1a–c show the TG curves for control, bleached and hair kept for 168 h in chlorinated solution, respectively. In the hair control sample the first mass loss stage observed can be attributed to the water release in the range of 25–131°C. The second and third mass loss stages are related to the denaturation of hair keratin, with organic degradation of hair microfibrils and matrix, at around 280, 320 and 350°C. In the temperature range of 350–550°C the complete degradation of the hair keratin carbonic chains takes place (Fig. 1a). For the samples of hair treated with chlorinated solution and bleached hair, the mass loss profiles are different compared to the control hair. The loss of water occurs up to 131°C, the degradation of the organized structures completes at about 400°C and the subsequent complete degradation of keratin and the hair structure completes up to 700°C.

It was observed that untreated control hair presents more loss steps and lower temperature values for keratin degradation (Figs 1b and c). For bleached and chlorinated hair fibers, less number of mass loss stages related to the hair structure degradation were found as they were compared to the untreated hair that exhibited more degradation stages.

This fact indicates that these treatments influence the mass loss process suggesting that the hair keratin becomes more disorganized after these treatments,

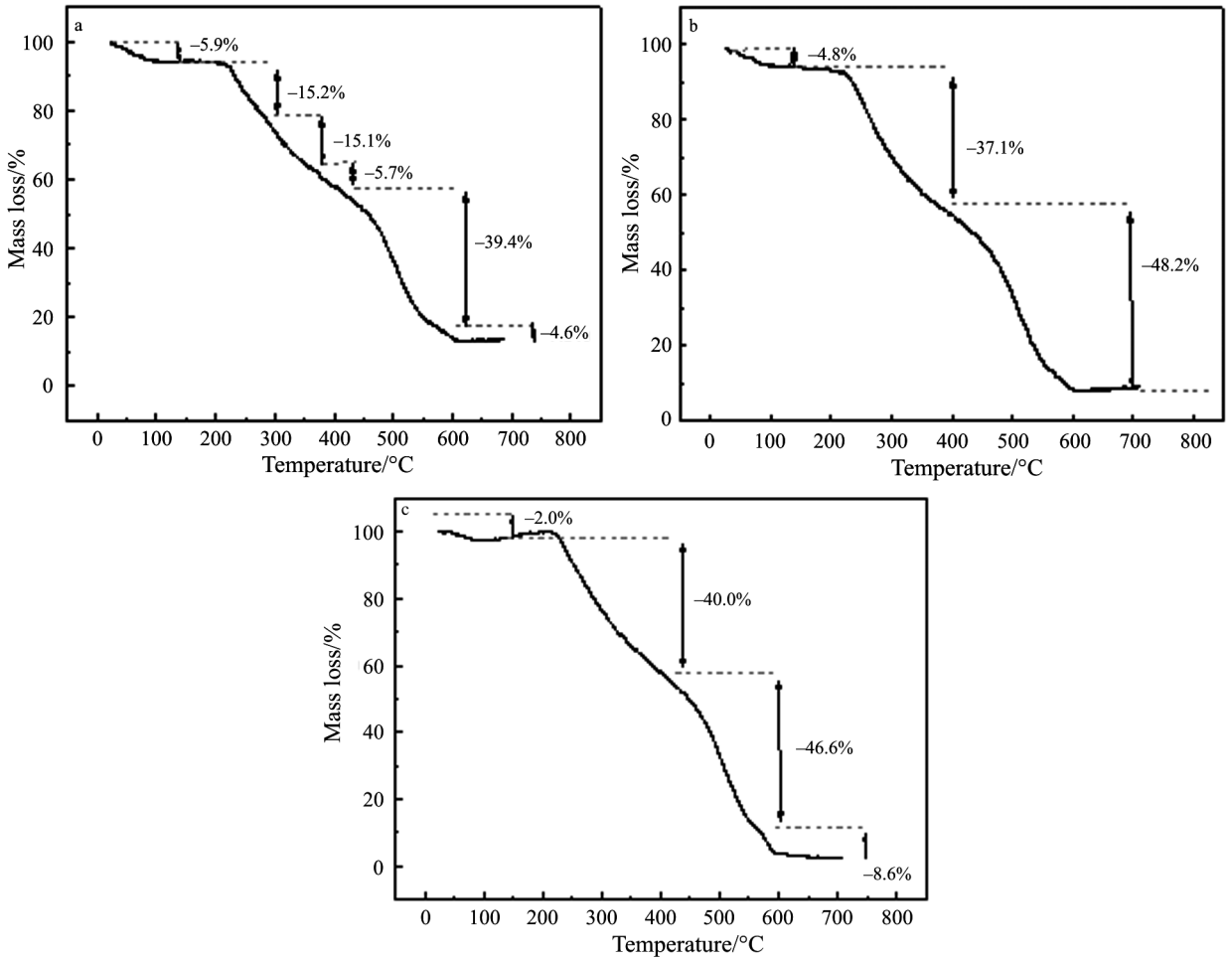

Fig. 1 TG curves of a – Caucasian hair control, b – bleached hair and c – hair treated in chlorinated solution

yielding a less number of mass loss stages (Figs 1b and c). The amount of water and each mass loss stage related to the hair keratin degradation appear at higher temperatures for the untreated hair sample (Fig. 1a) than for the bleached and chlorinated hair samples (Figs 1b and c).

Figures 2a and b show the DSC curves of native and treated hair samples respectively, indicating endotherm processes. The first one is corresponding to the water evaporation. The second and third peaks denote endothermic reactions of keratin polypeptide chain denaturation. Figure 2a shows the broadening and the decrease in its height of the endothermic denaturation peaks. This profile change is due to the damages caused to hair keratin structure, mainly to the cystine disulfide linkages which maintain the α-keratin conformation. The DSC curves of hairs kept in a chlorine solution for 96, 120 and 168 h are also different of the control hair (Fig. 2b). The peak area attributed to the keratin denaturation of the chlorinated hair is obviously smaller than the area related to the control hair. Chlorinated hair also presents a large degradation of the cystine disulfide bonds inside and between the chains indicating the presence and a remarkable content of cysteic acid, and therefore exhibiting higher denaturation temperatures compared to the control hair.

Chlorine can interact with the organic content of hair, producing new structures at temperatures above 270°C. The exothermic peaks refer to the reorganization of the carbonic chains, with the peak at 280°C. For the hair, chlorinated for 168 h an exothermic peak was observed at 321°C. This probably refers to the oxidative degradation of the organic material (Fig. 2b).

It was observed that the denaturation temperature increases for the hair fibers after bleaching and chlorination (Table 1). In dry environment (45% RH), bleaching, chlorine treatments promote the increase of the ionic interactions, increasing the stability of the structure of the keratin and shifting the denaturation temperature toward higher temperatures. The probable explanation for these observations is, the analyses carried out in reactive environment increased the cysteic acid concentration, produced by the cystine oxidation. Therefore the denaturation temperatures shifted towards the higher values. The denaturation enthalpy de-

Table 1 Enthalpies and denaturation temperatures obtained from the DSC curves of hair fibers

Treatment	Denaturation temperature/°C		Denaturation enthalpy between 220–280°C/J g^{-1}	Fourth peak temperature/°C
	first peak	second peak		
Control	237.4	250.9	405.1	318.8
Chlorine solution for 96 h	237.6	251.5	395.9	319.2
Chlorine solution for 120 h	237.2	251.8	389.2	312.5
Chlorine solution for 168 h	238.1	251.9	371.7	321.1
Bleached for 1 h	240.4	256.3	291.9	–
Bleached for 2 h	240.9	256.2	269.7	–

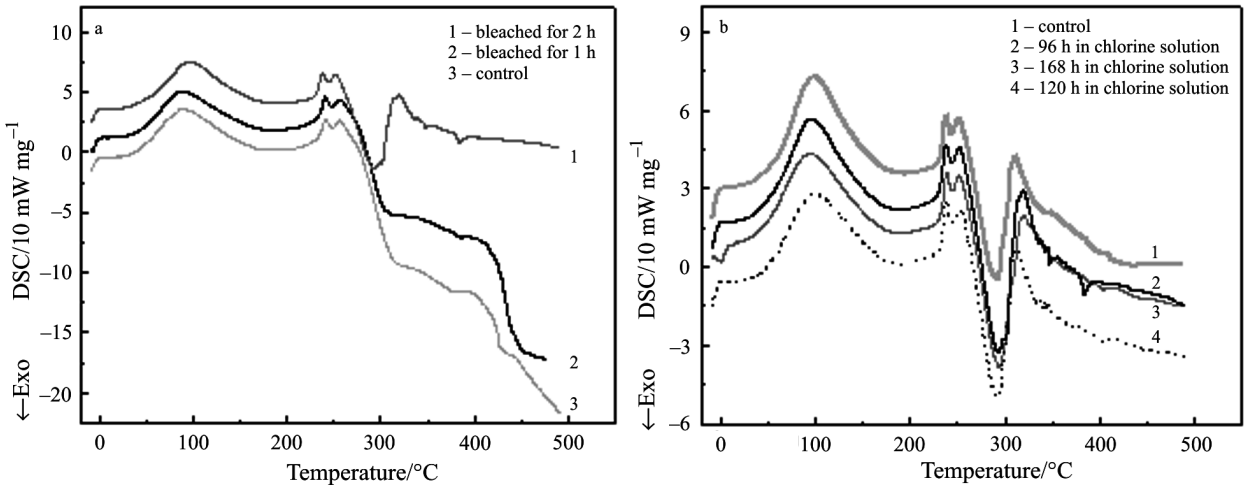

Fig. 2 DSC curves of a – control and bleached hair and b – control and chlorinated solution hair samples

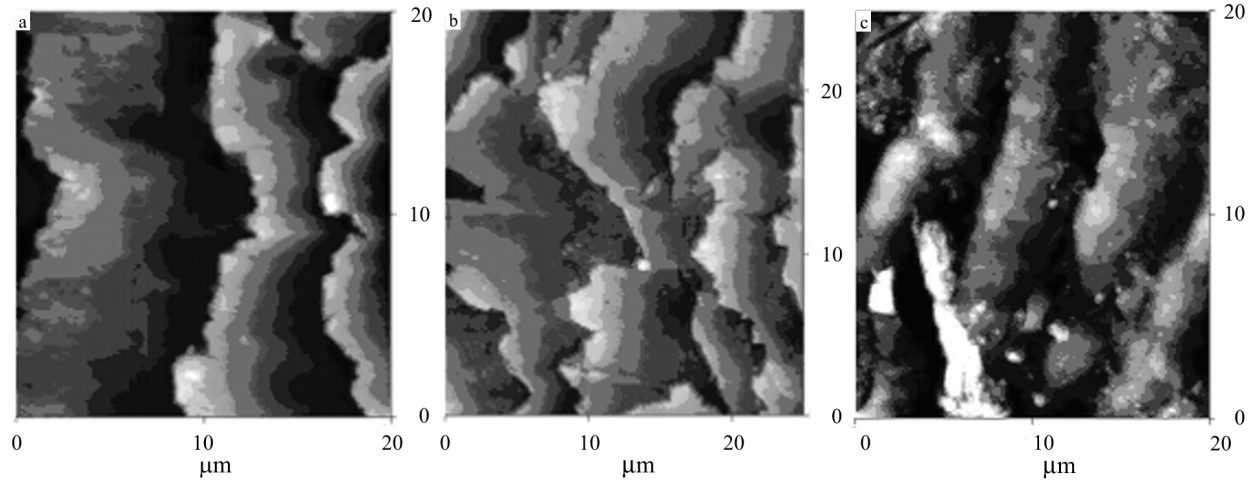

Fig. 3 AFM images of cuticle fibers of a – control, b – bleached and c – chlorinated hair

creased about 33% for the higher soaking times of hair fibers within the bleaching solution (2 h). The endotherm peak regarding the organic material degradation that occurs upon the keratin denaturation is not observed in the DSC curves of bleached hair. Table 1 shows the temperatures and enthalpy values for the keratin denaturation, for untreated (control), bleached and chlorinated hair samples. It is evident that more energy is spent to disorganize the α-keratin structure of untreated hair then to disorganize the chlorinated hair. This fact is evidenced by the decrease in the keratin denaturation enthalpy and the increase of the denaturation temperature.

AFM images show the changes in hair cuticle surface (Fig. 3). It was possible to verify that the degradation in the hair fibers submitted to the bleaching

(Fig. 3b) and chlorination (Fig. 3c). It can be seen that the treated hair samples show degraded cuticles with chemical modification in the cortex hair, confirmed by opening and damaging of cuticles and the cortex degradation confirmed by DSC analysis.

Conclusions

The results obtained by the TG analyses show that hair fibers treated with chlorination and bleaching solutions have a small water content and a subsequent keratin degradation exhibiting less degradation stages than the control untreated hair sample. The results indicate that these treatments initially cause a transformation in the keratin structure. Subsequently, a higher degradation of the hair fiber keratin is favoured by such previous oxidative treatments. DSC analyses confirm the effect of bleaching and chlorinating treatments on the damage, showing a lower keratin denaturation enthalpy, which is due to the increase in the disorganization of the keratin structure. On the other hand, the higher denaturation temperature is owned to reorganization of organic chains after the denaturation. AFM images confirm the complete fiber degradation in hair cuticle caused by bleaching and chlorinated agents.

Acknowledgments

The authors acknowledge the following Brazilian funding support agencies: CNPq, CAPES and FAPESP.

References

1 J. A. Swift, Fundamentals of Human Hair Science, Micelle Press Ed., 1997.
2 W. T. Humphries, D. L. Miller and R. H. Wildnauer, J. Soc. Cosmet. Chem., 23 (1972) 359.
3 Zs. Éhen, Cs. Novák, J. Sztatisz and O. Bene, J. Therm. Anal. Cal., 78 (2004) 427.
4 P. Milczarek, M. Zielinski and M. L. Garcia, Colloid Polym. Sci., 270 (1992) 1106.
5 M. Spei and R. Holzen, Colloid Polym. Sci., 265 (1987) 965.
6 F. J. Wortmann and H. Deutz, J. Appl. Polym. Sci., 68 (1998) 1991.
7 C. Tonin, A. Aluigi, M. Bianchetto Songia, C. D'Arrigo, M. Mormino and C. Vineis, J. Therm. Anal. Cal., 77 (2004) 987.
8 K. M. S. Belletti, I. H. Ferferman, T. R. O. Mendes, A. D. Piaceski, V. F. Monteiro, N. V. Carreño, A. Valentini, E. R. Leite and E. Longo, J. Cosmet. Sci., 54 (2003) 527.
9 F. J. Wortmann, C. Springob and G. Sendelbach, J. Cosmet. Sci., 53 (2002) 219.
10 J. A. Swift and J. R. Smith, Scanning, 22 (2000) 310.