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Investigations of cosmetically treated human hair by differential scanning calorimetry in water

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Synopsis

By applying differential scanning calorimetry (DSC) on human hair in water, the thermal stability of hair's major morphological components is determined. Against the background of the two-phase model for α-keratins, these components are identified as the partially helical, fibrous intermediate filaments (IF) and the intermediate filament associated-proteins (IFAP) as a cross-linked, amorphous matrix. DSC yields the denaturation enthalpy ΔH_D, which depends on the amount and structural integrity of the α-helical material, and the temperature T_D, which is kinetically controlled by the cross-link density of the matrix. To assess the effects of cosmetic treatments, hairs were investigated that had undergone either multiple bleaching or perm-waving treatments. The respective dependencies between denaturation temperature and enthalpy show that both morphological components are similarly affected by bleaching, while reductive damage, in comparison, is more pronounced in the IFs. For both types of treatments, changes in enthalpy follow apparent first-order kinetics with respect to the number of treatments as well as treatment time (perm-waving), yielding characteristic reaction rate constants. It appears that DSC in water is an especially suitable method to determine the kinetics of damage formation in human hair resulting from cosmetic treatments.

INTRODUCTION

Materials made from hard α-keratin, including human hair, exhibit a complex, morphologically fine structure (1). The study of the mechanical properties (2) has led to the two-phase filament-matrix model for α-keratins, originally proposed by Feughelman (3). In this model, axially oriented, crystalline filaments, traditionally called *microfibrils* are embedded in an amorphous *matrix.*

One main component of the model can be identified as being comprised of the partly α-helical intermediate filaments (IF), with their helical fraction as the crystalline filamentous phase. The matrix in consequence comprises summarily the rest of the mor-

Dedicated to Prof. Dr. H. Zahn on the occasion of his 85th birthday.

phological components, with the intermediate filament associated proteins (IFAP) as the major component, but also including minor morphological components such as cuticle, cell membrane complex, nuclear remnants, etc. (4).

The two dominant components, IFs and IFAPs, largely determine the mechanical properties of human hair (2) and, according to their molecular structure, play specific roles for the performance and effects of hair cosmetic treatments (5).

The thermal analysis of keratins in water by applying differential thermal analysis (DTA) (6–8) or differential scanning calorimetry (DSC) (9–11) was found to be especially suited to investigate the denaturation performance of the α-helical IF structures, the role of the cystine cross-linked IFAPs, and the effects of physical and chemical treatments.

Spei and Holzem (12) and others (13) have shown that the denaturation peak can usually be detected adequately and evaluated also for dry fibers (approx. 240°C). It is obvious from their DSC curves, however, that the effect is always secondary in size compared to a large background peak, due to general keratin pyrolysis (14). By measuring in water, the helix denaturation peak shifts for human hair down to around 150°C and exhibits no background effects (9).

This paper deals specifically with the effects that bleaching and permanent waving leave in the DSC curves of hair fibers measured in water. These effects, as reflected in changes in denaturation temperatures and enthalpies, are discussed in terms of the thermal properties of filaments and matrix and lead to a kinetic approach for the description of the influences of the cosmetic treatments.

EXPERIMENTAL

All investigations were conducted on a power-compensated DSC instrument (DSC-7, Perkin Elmer) using pressure-resistant (25-bar), stainless steel, large-volume capsules in the temperature range of 50°–190°C (heating rate: 10°C/min; sample weight: 4–7 mg). The samples consisted of short fiber snippets (approx. 2 mm in length) cut from the middle sections of hair swatches. Prior to measurement the samples were stored under standard room conditions (20°C, 65% RH) to ensure invariant water content. Under these conditions a given material was weighed into the sample container, 50 µl of water was added, and the container was sealed and stored over night to achieve equilibrium water content and distribution.

Caucasian mixed hair, untreated, medium brown, from a commercial source (Kerling, Backnang) was used in the form of swatches (16 cm long, 100 hairs).

Bleaching was done with a commercial preparation (Wella) based on an alkaline solution (pH 8.3) of hydrogen peroxide (9%) and ammonium persulfate applied for 30 min and at room temperature. Eight hair swatches were prepared, namely the unbleached start material and swatches bleached repeatedly and at intervals of 24 hr up to seven times. DSC tests on these samples were conducted five times.

Perm-waving was done on hair swatches at room temperature with a commercial preparation (Wella) based on thioglycolate (8%) as reducing agent at pH 8, adjusted with ammonia, for various times between 10 and 30 min. Hydrogen peroxide solution (2.5%) (pH 3) was used as neutralizer. This process was repeated up to five times at 24-hr

intervals, yielding eleven hair swatches with different chemical histories. DSC tests on the samples were conducted three times.

DATA ANALYSIS

Figure 1 shows a DSC curve typical for untreated human hair in water. In accordance with conventional practice in calorimetry, endothermic effects, i.e., heat absorption by the sample, are represented by an increase in the ordinate value from the baseline.

The DSC peak is characterized by its position (peak temperature), which is taken as the denaturation temperature T_D of the helical material in the IFs. The area of the peak with respect to its baseline yields the denaturation enthalpy ΔH_D, which is the energy required for the helix denaturation.

The enthalpy depends on the amount and the structural integrity of the α-helical material in the intermediate filaments of human hair. There is good evidence to suggest (9,10), that T_D is kinetically controlled by the cross-link density of the matrix (IFAPs) in which the IFs are embedded. The higher the cross-link density in the IFAPs, the higher their viscosity and the more hindered is the helix/coil transition in the IFs and vice versa.

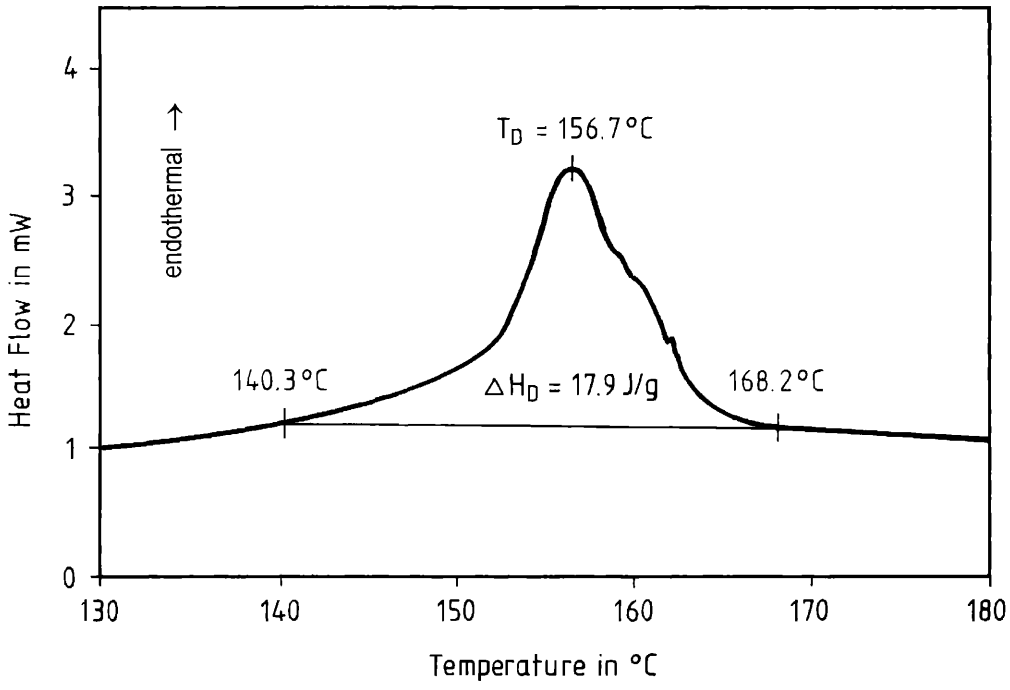

Figure 1. Typical DSC curve for untreated human hair in water. The location of the peak gives the denaturation temperature T_D, and its area, with respect to the baseline, gives the denaturation enthalpy ΔH_D. The start and end temperature of the baseline, defining the peak, are marked.

On the assumption that ΔH_D is a measure of the amount of native, α-helical material, the relative helix content HX_{rel} is given by:

$$HX_{rel} = \Delta H_D / \Delta H_D^{\,0} \quad (1)$$

$\Delta H_D^{\,0}$ is the arithmetic mean denaturation enthalpy for the start material for each specific part of the study.

As a first approach and against the background of a previous investigation (11), first-order kinetics are assumed for the treatment-induced decrease of the helix content in terms of time:

$$-dHX_{rel}/dt = k_t HX_{rel} \quad (2)$$

as well as of the number of treatments

$$-dHX_{rel}/dn = k_n HX_{rel} \quad (3)$$

where k_t is the reaction rate constant related to treatment time and k_n the *apparent* reaction rate constant with respect to the number of treatments, respectively.

In view of the properties of equations 2 and 3 after integration, plotting $ln(HX_{rel})$ vs t or vs n should yield a straight line with the slopes $-k_t$ and $-k_n$, respectively.

RESULTS AND DISCUSSION

Bleaching and perm-waving lead to unique changes in the DSC curves of the treated hair. In this paper, results for the changes in the principal parameters, namely denaturation temperature and enthalpy are presented and discussed. Tables I and II summarize the results for T_D and ΔH_D for the bleached and perm-waved samples, respectively, in the form of the arithmetic means and standard deviations.

With values for the standard deviation for T_D of generally around or less than 1°C and of around 1 J/g or less for the denaturation enthalpy, the method shows good precision. The values for T_D for the untreated samples (bleach: 158°C; perm-wave: 155°C) as well as for ΔH_D (bleach: 19.3 J/g; perm-wave: 15.3 J/g) in both sets of experiments are in the usual range for α-keratins (9).

Table I
Denaturation Temperatures T_D and Enthalpies ΔH_D for Hair Samples Bleached Multiple Times

Number of bleachings	$T_D \pm s$ (°)	$\Delta H_D \pm s$ (J/g)
0	158.3 ± 0.34	19.3 ± 0.41
1	157.8 ± 0.18	18.0 ± 0.21
2	152.6 ± 0.47	15.6 ± 1.13
3	145.3 ± 0.39	13.7 ± 0.81
4	140.9 ± 0.36	12.4 ± 0.41
5	141.4 ± 0.32	12.9 ± 0.44
6	139.6 ± 0.23	13.0 ± 0.44
7	138.4 ± 0.44	11.7 ± 0.25

s: standard deviation for fivefold measurements.
0: untreated hair material.

Table II
Denaturation Temperatures T_D and Enthalpies ΔH_D for Hair Samples Perm-Waved Multiple Times

Number of treatments	Time of treatment (min)	$T_D \pm s$ (°)	$\Delta H_D \pm s$ (J/g)
Start	—	155.2 ± 1.03	15.3 ± 0.32
Bleach	—	152.5 ± 0.57	14.9 ± 0.40
1	10	152.9 ± 0.58	11.3 ± 0.36
	20	150.3 ± 0.36	11.5 ± 1.05
	30	148.7 ± 1.08	10.2 ± 0.56
3	10	146.4 ± 1.07	8.5 ± 0.97
	20	144.0 ± 0.86	7.4 ± 0.68
	30	143.5 ± 0.57	7.5 ± 1.04
5	10	136.7 ± 0.71	3.2 ± 0.97
	20	134.6 ± 0.74	1.9 ± 0.36
	30	121.3 ± 2.01	1.0 ± 0.33

s: standard deviation for threefold measurements.
Start: untreated reference hair material.
Bleach: once-bleached reference material, on which the perm-wave treatments were performed for different time periods.

The variability of the locations and shapes of the peaks for individual samples can be attributed to general, inherent, natural inhomogeneities of the samples as well as to variations induced by the chemical and physical history of the samples beyond the applied treatments.

BLEACHING EFFECTS

Figure 2 summarizes the results for peak temperatures and denaturation enthalpies, respectively, for the bleached samples, in the form of a multiple "box & whisker" plot.

The results show that the first four bleaches lead to a roughly linear decrease for both parameters. Beyond, T_D levels off at a temperature that is 20°C lower than for the untreated material. In parallel, ΔH_D decreases by 40% from 19 to 12 J/g.

The plot of denaturation enthalpy vs temperature (Figure 3) reveals a strong linear relationship, showing the similarity in the course with which both parameters decrease. Taking their relationship to specific morphological components into account, as discussed above, it can be concluded that bleaching leads to largely homogeneous damage in IFs and IFAPs.

Assuming first-order kinetics and consequently plotting $ln(HX_{rel})$ vs the number of bleachings, as realized in Figure 4, yields a well-defined straight line from which the apparent reaction rate constant (k_n = 0.069 ± 0.0103; 95% confidence limits) is deduced.

Leroy *et al.* (13) investigated virgin, bleached, and permwaved hair by DSC in the dry state. For the virgin hair they observed a strong bimodality of the curves, which is related to fractions of cortical cells differing in cystine content (10), rather than to a filament/matrix structure (12,15).

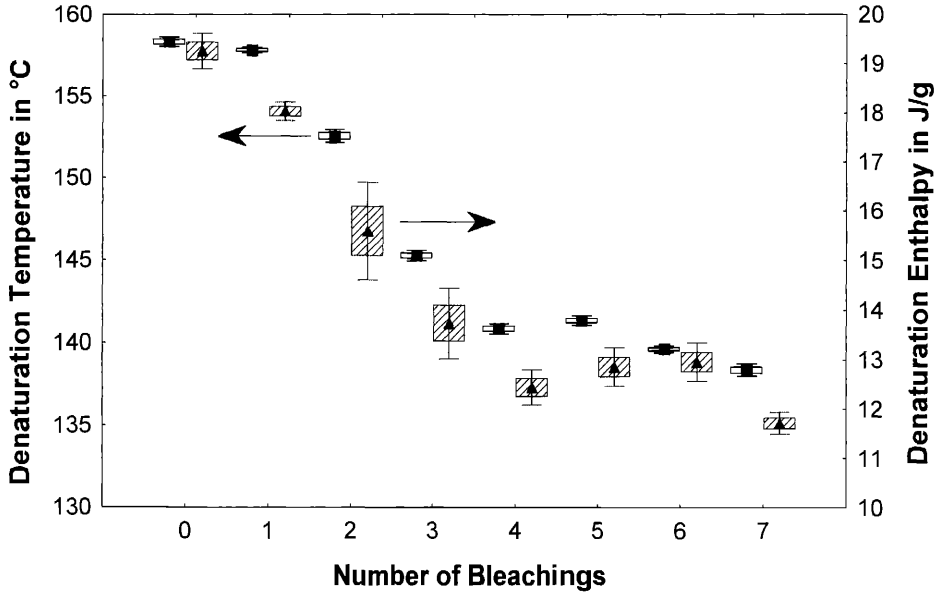

Figure 2. Denaturation temperatures T_D (left y-axis) and enthalpies ΔH_D (right y-axis) of the bleached samples as multiple "box & whisker" plot, characterized by the arithmetic means (symbol), the standard errors (box), and the expectation ranges for the 95% confidence limits (whisker). *0* refers to the untreated material.

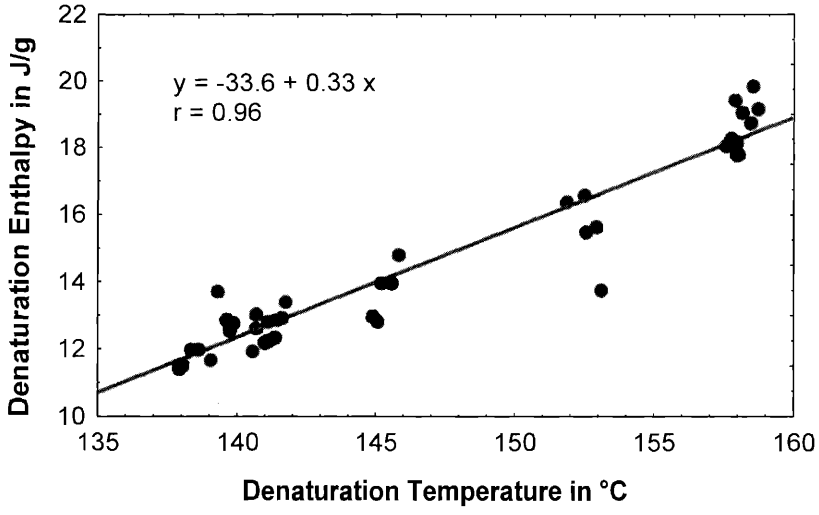

Figure 3. Individual values for denaturation enthalpy ΔH_D plotted versus temperature T_D for the bleached hair samples. The linear regression line through the data and its description are given. r is the correlation coefficient.

They observed that with bleaching the DSC peaks for dry fibers shift to higher temperatures. This is in contrast to our results for wet fibers. Bleaching leads to increased concentrations of cysteic acid and thus of ionic interactions. It can be assumed that these will be effective in the dry fiber but will be broken in the wet state, thus leading to different shift directions of the denaturation peaks through a bleaching treatment.

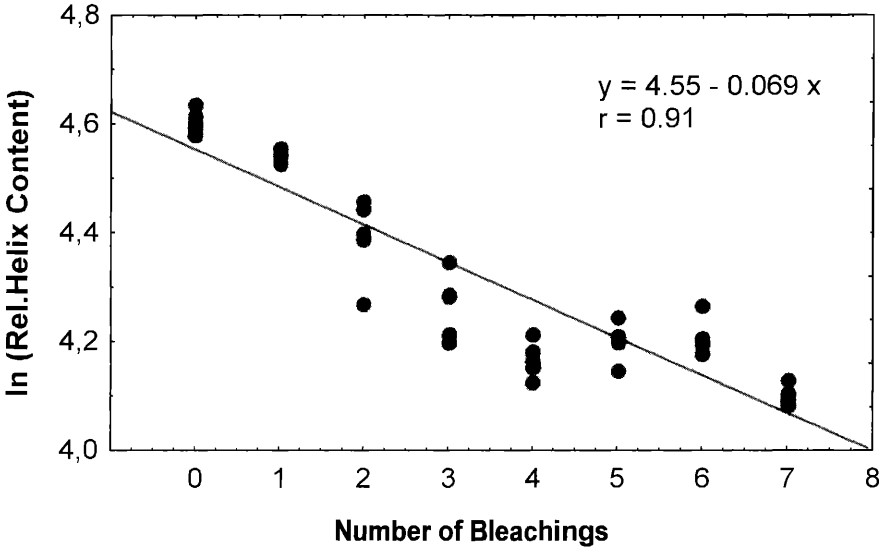

Figure 4. Plot of $ln(HX_{rel})$ vs number of bleachings, according to the assumption of first-order denaturation kinetics. The linear regression line is shown as described by the equation on the graph.

In the wet state, furthermore, the increase in the content of anionic groups induces an increase in water content. This leads to a continuous decrease of matrix viscosity and thus of T_D, in contrast to a less oxidative treatment such as perm-waving, where an exponential relationship is observed, as discussed below.

In agreement with our results, Leroy *et al.* (13) observed a decrease in denaturation peak area through bleaching. This can be attributed to a loss of crystalline material, as observed by SAXS (small angle X-ray scattering) (16) or to a general decrease in native α-helical material, which can be denatured (11).

PERM-WAVING EFFECTS

Figure 5 summarizes the results for the peak temperature and the denaturation enthalpy for the perm-waved samples in the form of a "box & whisker" plot. Data are given for the *start* material for this study and the once-*bleached* material. The latter material, considered as representing an average type of common hair damage, was submitted to the perm-waving treatments. With respect to the scaling of the x-axis of Figure 5, the first number, characterizing a treatment, gives the number of treatments and the second one the duration in minutes. *3 × 30* is thus a sample perm-waved three times for 30 min each.

One notes a consistent decrease in T_D as well as for ΔH_D with increasing numbers of treatments. This is in agreement with common hairdressers' knowledge, that multiple permanent waving treatments are more effective than a single treatment of equivalent duration.

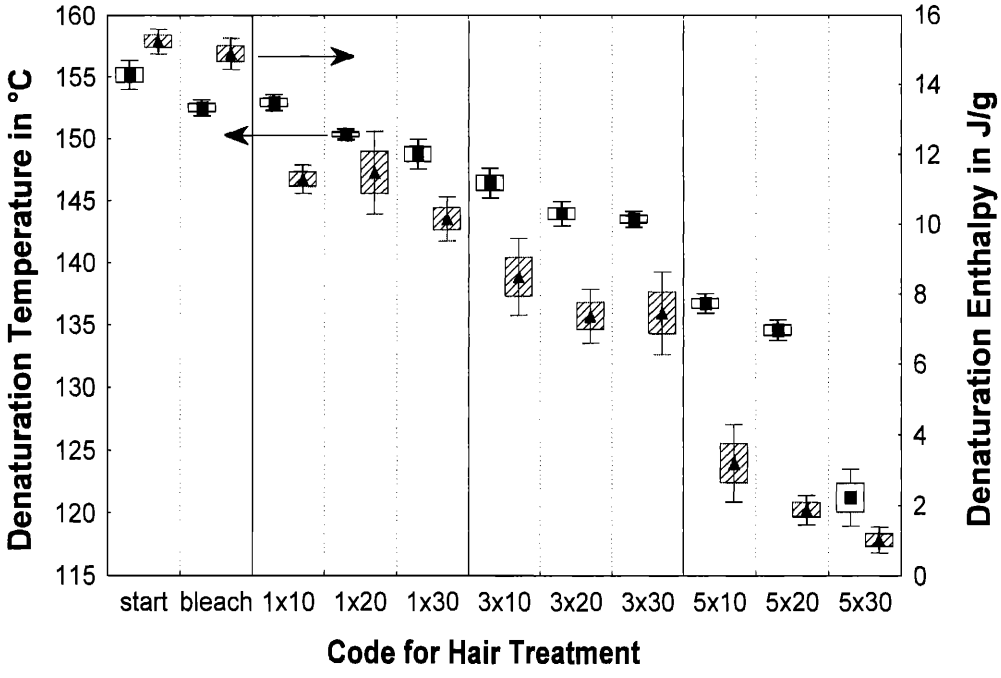

Figure 5. Denaturation temperatures and enthalpies of the perm-waved samples as multiple "box & whisker" plot (see Figure 2). For the definition of codes see text. Vertical lines separate data groups that have undergone the same number of treatments.

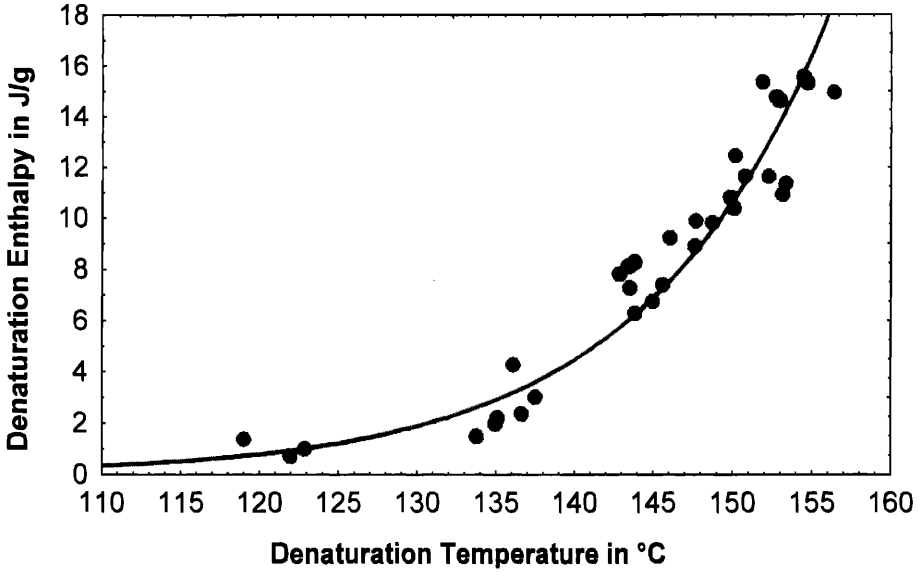

Figure 6. Plot of ΔH_D vs T_D for the perm-waved samples. As a guide for the eye, an exponential fit (solid line) is drawn through the data.

Plotting all individual values of ΔH_D vs T_D for the perm-waved samples, as realized in Figure 6, shows a heuristic, exponential relationship. The decrease in enthalpy occurs much faster than that of the peak temperature.

In view of the morphological interpretation of the parameters, it can be concluded that perm-waving damage is much more pronounced in the helical segments of the intermediate filaments than in the surrounding, highly sulfur cross-linked matrix. Perm-waving greatly reduces the amount of native, α-helical material in hair.

Leroy *et al.* (13) in their DSC studies on dry hair also found the progressive decrease of the peak area through perming. This is in agreement with ^{13}C CP/MAS NMR studies by Nishikawa *et al.* (17), showing a decrease in the amount of α-helical material in Asian hair through perm-waving. Similar effects have been observed by Ogawa *et al.* (18), upon submitting hair to strong heat/reduction conditions.

In contrast to our results, however, Leroy *et al.* (13), when testing dry hair, found no change in the peak temperature. This shows the overriding importance of hydrogen bonds in the dry state. Once these are broken through the presence of water, the effects of perming on the disulfide bonds can be detected by thermal analysis.

Calculating HX_{rel} on the basis of ΔH_D = 14.9 J/g for the bleached material (bleach) and plotting the results as $ln(HX_{rel})$ vs time as well as against the number of treatments, yields in the 3D-plot of Figure 7 a well-defined plane (r = 0.89), which supports the assumption of additive first-order kinetics in both parameters.

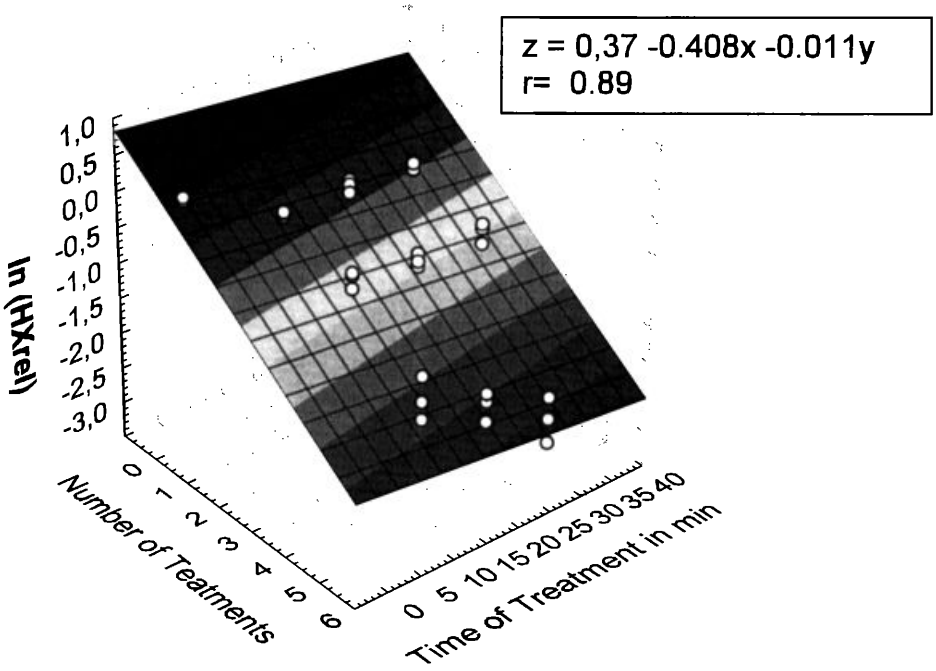

Figure 7. Plot of $ln(HX_{rel})$ vs time (y) and number (x) of perm-waving treatments. Based on the assumption of first-order kinetics for both parameters, a plane, for which the equation is given, is fitted through the data.

From the slopes of the plane in both directions, the related rate constants for the reactions (95% confidence limits) were determined:

$$k_t = 0.011 \pm 0.0164 \text{ min}^{-1} \ (1.83 \pm 2.73 * 10^{-4} \text{ s}^{-1})$$

and

$$k_n = 0.408 \pm 0.0896$$

CONCLUSIONS

DSC analysis of human hair in water yields results for the denaturation temperature T_D and the related enthalpy ΔH_D. The enthalpy depends on the structural integrity of the α-helical material in the intermediate filaments (IF), while T_D is kinetically controlled by the cross-link density of the matrix (IFAPs) in which the IFs are embedded.

Against the background of this view, a detailed description and interpretation of the changes, or rather the structural damage bleaching and permanent waving impart to human hair, can be given, including kinetic considerations.

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