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Investigations of cosmetic treatments on high-pressure differential scanning calorimetry

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Synopsis

High Pressure Differential Scanning Calorimetry (HPDSC) can be used to gain information on both the degree of crystallinity in the intermediate filaments (IFs) and the structural rigidity of the surrounding matrix or intermediate filament associated proteins (IFAP) of the hair cortex. We have used HPDSC to measure changes in denaturation temperature (T_D) and enthalpy (δH_D) of the crystalline components after treatment with bleach products. Literature reports suggest that a decrease in peak denaturation temperature is indicative of permanent damage to the hair. However, changing the rigidity of the matrix surrounding the IFs, by temporarily changing electrostatic interactions, should also result in a similar decrease in peak temperature.

The complex nature of bleach formulations including oxidants, alkalizers and salts suggests that several of the components could have a non-permanent affect on salt bridges and hydrogen bonds and hence rigidity or viscosity of the matrix. We have compared the denaturation temperature with levels of lightening (dL) and tensile properties of the fiber after treatment both before and after removal of actives from the fiber. It is evident that the HPDSC results are strongly influenced by formulation components and that these changes are reversible with extensive washing or dialysis. Combined with tensile data, it is proposed that a decrease in T_D and δH_D following treatment with bleach products can be due to both permanent and reversible changes to either the intermediate filaments or intermediate filament associated proteins of the hair fiber.

INTRODUCTION

Cosmetic treatments such as bleaching, perming and the use of permanent colorants have been shown to cause changes to the fibre structure of the hair (1–4). These changes are often seen by the consumer as damage such as increased hair breakage, reduced shine and a poor hair feel in both the wet and dry state. It is important to relate these consumer noticeable effects to measurable changes in the fibre structure.

One technique that has been used to measure structural changes to hair is High Pressure Differential Scanning Calorimetry (HPDSC) (5–7). The technique records the thermal behaviour of a sample, such as hair, under controlled heating and in a sealed vessel with a known amount of water. The thermal transition observed is in the range 130–180°C and has been related to the denaturation of the hair's keratin structure. The hair is a

complex morphological structure composed of three components: the cuticle, the cortex, and the medulla (8). It is proposed that it is the cortex that is the significant contributor to the thermal stability of the fiber. The cortical structure has been described as being composed of the intermediate filaments (IFs), which have an α-helical structure, embedded inside an amorphous matrix containing the intermediate filament associated proteins (IFAPs) (9,10). As measured by the HPDSC technique, the denaturation enthalpy (δH_D) is proposed by Wortmann *et al.* (11) as being dependent on the structural rigidity of the α-helical material in the intermediate filaments. The peak temperature (T_D) is controlled by the cross-link density and viscosity of the matrix in which the intermediate filaments are embedded.

Several authors have specifically investigated the effect of multiple bleaching treatments on the HPDSC peak temperature and enthalpy. Wortmann *et al.* (12) showed a steady decrease in both δH_D and T_D with an increasing number of treatment cycles. Monteiro *et al.* (13) observed a similar trend. In both cases the decrease in δH_D and T_D was linked to the oxidative cleavage of the cystine protein to form cysteic acid, with a decrease in inter-protein cross-linkage.

In this present study we also look at the effect of multiple bleaching treatments on the HPDSC peak temperature and enthalpy, but we have also considered the role of the chassis formulation and its components on influencing the HPDSC results. Specifically, we studied treatments with the alkalizer alone (either ammonia or ethanolamine) buffered at pH 10, the same pH as a typical bleach product. The results from the HPDSC experiment were also compared to tensile strength measures such as plateau load and break force. The tensile strength method has also been reported to indicate the oxidative damage caused by bleach products (14–16).

EXPERIMENTAL

Caucasian untreated mixed hair (medium brown), obtained from a commercial source (IHIP, New York), was formed into swatches (16 cm, 1.5 g). The hair swatches were subjected to a number of repeat wash cycles between each treatment. One wash cycle consisted of two shampoo plus rinses in tap water with a commercial clarifying shampoo.

The following products were used:

- *Bleach product A*—alkaline solution of hydrogen peroxide, ammonium persulfate and ethanolamine (pH 10) in a gel formulation
- *Bleach product B*—alkaline solution of hydrogen peroxide, ammonium persulfate and ammonium hydroxide (pH 10) in a gel formulation
- *Bleach product C*—alkaline solution of hydrogen peroxide, ammonium persulfate and ammonium hydroxide (pH 10) in a cream formulation

Dialysis of the hair was achieved by soaking in repeated changes of 1liter deionised water for a total of 25 liters. The pH of the dialysed water was monitored for each change of deionised water. For treatment with the alkalizers two swatches were soaked in 100 ml of the alkalizer solution (1.27M) for 30 minutes at 30°C. The hair was washed twice with a clarifying shampoo before HPDSC analysis.

HPDSC investigations were carried out using either:

- A Dynamic Difference Calorimeter DSC 204 Phoenix supplied by the Netzsch Company. The heating range was 90–170°C at a rate of 5°C / minute. Hair tresses were

cut into 2–3 mm lengths and conditioned overnight. The next day 10–12 mg of hair was placed under climate controlled conditions into a medium pressure 120 µl volume, steel crucible (Netzsch) with a lid and sealing elastomer, max pressure 20 bar; max temperature 200°C. 50 µl of distilled water was added to the crucible which was then sealed. 4 replicates are used for each determination.

- A Dynamic Difference Calorimeter DSC7 calibrated with indium supplied by Perkin Elmer. The heating range was 70–170°C at a rate of 10°C / minute. Hair tresses were conditioned for 24 hours at RH 55% and 22°C before samples were removed and cut into 0.5mm lengths. 4–7mg of hair was placed into a Perkin Elmer pan. 50µl of distilled water was added to the crucible which is then sealed. At least 3 replicates were used for each determination.

The tensile properties of the fibres were measured using a Diastron Miniature tensile tester (MTT 675) equipped with laser micrometer.

The color of the hair was measured with a bench top Minolta CM3600D spectrophotometer. Lab values were calculated under D65 illuminant, 10° observer, specular included. The lightening (dL) was calculated as the difference in L value between the final color and the starting color on the untreated hair.

RESULTS AND DISCUSSION

The results detail the effect on the HPDSC denaturation temperature and enthalpy following treatments with commercial retail bleach products A and B. Both products have three components: the alkalizer, the hydrogen peroxide, and the persulfate salt powder. These two products have the same level of oxidant (hydrogen peroxide and ammonium persulfate) and the formulations of these two components are very similar. However the formulations of the alkalizer components are different. Product A has a gel alkalizer formulation with ethanolamine as the alkalizer; Product B has a liquid alkalizer formulation with ammonium hydroxide as the alkalizer.

Three repeat treatments were performed with five wash cycles in between each treatment. After this treatment protocol the swatches were analysed for lightness vs. the starting substrate (dL), tensile strength and HPDSC peak temperature and enthalpy. Table I summarises the results of these analyses.

For the HPDSC determination, the results show that both of the bleach products induce a decrease in peak temperature vs. the untreated hair as one would expect from previ-

Table I
The Effect of Treatments of Bleach Formulations on HPDSC and Tensile Strength

		Tensile strength measurements				
Product	dL after 3 cycles	Plateau load Gmf/sq.micron ($\times 10^3$)	Load @ 25% Gmf/sq.micron ($\times 10^3$)	Break load Gmf/sq.micron ($\times 10^3$)	Peak temp. $T_D \pm s$ (°C)	Enthalpy $\Delta H_D \pm s$ (J/g)
Untreated hair	0	6.47 ± 0.23	7.52 ± 0.23	20.9 ± 1.9	148.3 ± 0.1	6.7 ± 0.5
Bleach A	48 ± 0.6	4.00 ± 0.27	4.81 ± 0.34	16.5 ± 1.5	143.8 ± 0.8	5.8 ± 0.5
Bleach B	49 ± 0.5	4.12 ± 0.38	4.70 ± 0.45	16.5 ± 3.7	138.8 ± 0.3	5.1 ± 0.3

ously published work. However, there is a significant difference of 5.1°C in denaturation peak temperature between the two products. Further, for the treated switches, there is no significant difference between the lightening (dL) values and the tensile parameters which are also indicators of oxidative covalent bond cleavage. In addition, the amount of oxidant in the systems is the same so the proposed explanation for this data was that there were other factors that were contributing to the decrease in peak temperature.

It was hypothesized that these two sets of results as described above are due, in part, to the incorporation of different actives into the fibre such as salts, alkalizers and formulation actives during the treatment process. For these two products tested, the formulations were different and contain different alkalizers, salts and formulation actives. A possible mechanism is that these product components are able to penetrate inside the fibre and change the stability and viscosity of the IFAP proteins by changing the arrangement of the electrostatic and hydrogen bonds. These changes could affect the immediate environment of the intermediate filaments and in turn their denaturation temperature and enthalpy. To test this hypothesis we would predict that either on dialysis of the hair or repeated washing cycle, these components would slowly diffuse out of the hair and the peak temperature would increase back toward the untreated hair values. This means that the changes in peak temperature would be reversible.

The starting untreated hair was treated with just one component of the colorant and bleach formulations; the alkalizer. All bleach products contain an alkalizer to ensure the product is at the pH required for effective lightening and in the majority of colorant and bleach products this alkalizer is either ammonia, ethanolamine or silicate. Two swatches of untreated hair were soaked in the alkalizer (1.27M) for 30 minutes, the same time as the bleach treatment. The swatches were then rinsed in tap water for one minute and then treated with one wash cycle (i.e. 2 shampoos). The HPDSC peak temperature and denaturation enthalpies were measured both after treatment and after dialysis of the hair in deionised water. To perform the dialysis the hair was first soaked in 50ml of deionised water followed by 100ml of deionised water followed by 1L of deionised water. The pH was measured at all three stages. The hair was then soaked in 20L of deionised water over a 24 hour period where the water was replenished six times (i.e. swatch exposed to 120L of deionised water).

The tensile strength of the hair was also measured before dialysis. There was no expected or observed lightening so dL is not reported.

Table II summarises the results of the HPDSC and tensile strength.

Table III summarises the pH measurements on dialysis for the ammonium hydroxide alkalizer.

The results support the hypothesis that selected components of the bleach products can lower the denaturation peak temperature and enthalpy and that this effect is at least partially reversible on dialysis. The lightening and tensile strength data confirm that the drop in HPDSC peak temperature and enthalpy is not due to oxidative covalent bond cleavage as expected as we have no oxidant present. In addition, the pH data indicates that even after rinsing and two shampoos there is still residual alkalinity in the hair that is only gradually removed by the dialysis. It is hypothesised that the residual alkalinity is participating in electrostatic and hydrogen bonding interactions with the IFAPs which will change the viscosity of the matrix and cause a change in the HPDSC peak

Table II
The Effect of Alkalizers on HPDSC and Tensile Strength

Product	Post-treatment	Tensile strength measurements: Plateau load Gmf/sq.micron (× 10^3)	Tensile strength measurements: Load @ 25% Gmf/sq.micron (× 10^3)	Tensile strength measurements: Break load Gmf/sq.micron (× 10^3)	Peak temp $T_D \pm s$ (°C)	Enthalpy $\Delta H_D \pm s$ (J/g)
Untreated hair	None	5.73 ± 0.35	6.81 ± 0.52	20.59 ± 1.6	149.0 ± 0.1	11.0 ± 0.4
Untreated hair	Dialysed				147.9 ± 0.2	8.6 ± 0.9
Ammonium hydroxide	None	5.17 ± 0.40	6.06 ± 0.39	18.86 ± 3.1	142.8 ± 0.5	9.1 ± 0.8
Ammonium hydroxide	Dialysed				150.7 ± 0.4	10.1 ± 0.1
Ethanolamine	None	5.15 ± 0.38	5.96 ± 0.38	18.91 ± 2.6	147.5 ± 0.1	8.3 ± 0.2
Ethanolamine	Dialysed				150.1 ± 0.1	9.1 ± 0.3

Table III
The pH Measurements During Dialysis

Ammonium hydroxide soak	DI water control	1 L	2 L	3 L	4 L	5 L	6 L
pH	6.67	9.10	8.30	7.33	6.74	6.68	6.69

temperature and enthalpy. As the alkalizer can removed on dialysis, or by multiple washing cycles, the effect of these alkalizer components is not linked to permanent oxidative cleavage of the covalent bonds (e.g. cystine) but to temporary effects that are reversible and not damaging to the fiber.

Extending this hypothesis to full formulations of hair colorants and bleaches, we may expect to see the combination of the two effects on the denaturation peak temperature and enthalpy: a change due to the irreversible oxidation of the covalent bonds, and a reversible effect due to the incorporation of components such as salts, alkalizers and formulation ingredients. To test this hypothesis the untreated hair was treated with five repeat cycles of product C. After cycles 1, 3 and 5 two swatches were removed and dialysed. In addition the tensile strength properties of the hair were measured at the 1, 3 and 5 cycles. The results are summarised in Table IV.

The data in Table IV show that for one cycle of treatment the effect of the product, as measured by T_d is reversible. Three cycles of treatment shows incomplete recovery of the peak temperature. At cycle 5, the peak temperature remains depressed indicating permanent damage to the fibre. This result correlates with both visual observations of the hair tresses and the measured tensile strength properties. The tensile properties significantly decrease on repeat treatment and observationally the hair breaks easily on combing and brushing.

The reversible effects observed in the tables above are likely to represent a change in the environment of the intermediate filaments rather than a change in either the crystallinity or amount of crystalline material in the IFs. It is predicted that other product components can display this reversible effect on the HPDSC data, and specifically product components that can readily penetrate into the fiber and interact with the IFAPs via electrostatic interactions. Such product components include salts such as sodium, potassium, ammonium carbonate or persulfate salts and surfactants such as alkyl and alkoxy sulfates and sulfonates. In addition, we may expect to see some changes due to the increased uptake of calcium and magnesium ions as the hair is treated with multiple cycles of bleach treatments. Figure 1 sets out conceptually a proposed mechanism of these reversible and irreversible effects.

The findings in this study demonstrate the need for careful interpretation of HPDSC data in the context of formulations that are designed to change morphological components within the hair cortex e.g. bleaches, perms, colors etc. Where the site of product action is known to be the cortex (intermediate filaments, matrix, pigment) one should also expect some ingredients to remain in the fibre pending removal by repeated washing, as is the practice by consumers. In this case it is essential to separate permanent and temporary changes to the cortex when employing HPDSC methods.

Table IV
The Effect of Dialysis on Denaturation Peak Temperature and Enthalpy and Tensile Strength Properties after Multiple Treatments with a Professional Bleach Product

Cycle # untreated hair	Post-treatment	dL	Tensile strength measurements: Plateau load Gmf/sq.micron ($\times 10^3$)	Tensile strength measurements: Load @ 25% Gmf/sq.micron ($\times 10^3$)	Tensile strength measurements: Break load Gmf/sq.micron ($\times 10^3$)	Peak temp $T_d \pm s$ (°C)	Enthalpy $\Delta H_D \pm s$ (J/g)
0	Untreated					149.7 ± 0.2	13.7 ± 0.4
1	None	46.5 ± 0.8	6.16 ± 0.98	8.71 ± 1.6	19.7 ± 2.9	143.5 ± 1.0	13.0 ± 0.4
1	Dialysed					149.1 ± 0.3	14.6 ± 0.2
3	None	53.8 ± 0.2	5.09 ± 1.69	7.47 ± 2.3	16.5 ± 4.3	137.9 ± 0.2	9.1 ± 0.2
3	Dialysed					145.5 ± 0.7	10.6 ± 0.2
5	None	57.1 ± 0.1	2.79 ± 0.79	3.04 ± 0.8	10.4 ± 4.4	134.5 ± 1.7	5.9 ± 0.03
5	Dialysed					135.1 ± 0.1	3.0 ± 1.4

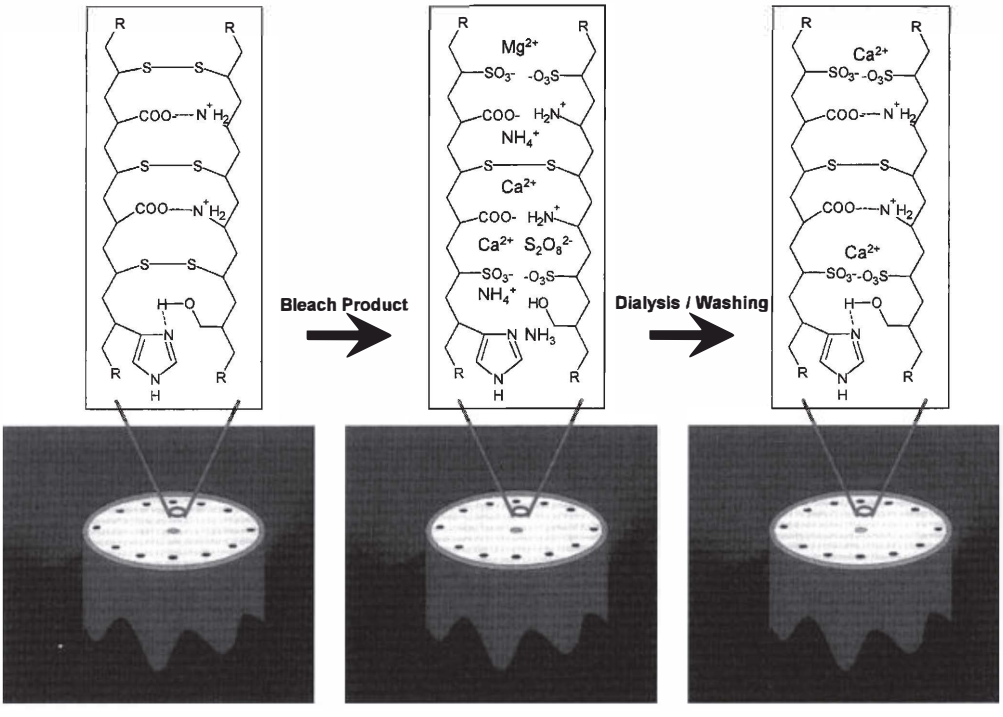

Figure 1. Proposed mechanism: Left is virgin hair fiber: Middle is bleached fiber with compromised covalent cross-linking and residual formulation components. Right is bleached and dialyzed fiber with covalent bonds disrupted but formulation components removed.

CONCLUSION

We have used High Pressure Differential Scanning Calorimetry to measure changes in the denaturation temperature of the crystalline component of the hair cortex from two commercial hair bleach products which have the same lightening and tensile strength but very different HPDSC peak temperatures and enthalpies. Importantly, we have demonstrated that components of these products, such as the alkalizer, can induce large changes in the denaturation temperature that is not due to oxidative covalent bond cleavage. These changes in the denaturation properties are at least partially reversible on dialysis in deionised water.

It is hypothesised that during the bleach process a wide variety of bonds will be broken at different locations in the fibre. Close range electrostatic interactions or salt bridges are readily broken and spontaneously reform as the hair dries. The presence of formulation components such as residual alkalizer and/or salts within the fibre can influence the re-formation of salt bridges and hydrogen bonds. Interference with salt bridges will increase protein flexibility and reduce viscosity in the matrix. This in turn leads to a lowering of the denaturation temperature.

By using multiple cycles of a bleach product it can be demonstrated that it is possible to induce both permanent and reversible effects in the fibre. The permanent, irreversible effects are attributed to the oxidative cleavage of covalent bonds such as cystine while the

reversible effects are attributed to the residual formulation components as described above.

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