Investigation of hair dye deposition, hair color loss, and hair damage during multiple oxidative dyeing and shampooing cycles

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Accepted for publication October 5, 2015.

Synopsis

Color fastness is a major concern for consumers and manufacturers of oxidative hair dye products. Hair dye loss results from multiple wash cycles in which the hair dye is dissolved by water and leaches from the hair shaft. In this study, we carried out a series of measurements to help us better understand the kinetics of the leaching process and pathways associated with its escape from the fiber. Hair dye leaching kinetics was measured by suspending hair in a dissolution apparatus and monitoring the dye concentration in solution (leached dye) with an ultraviolet–visible spectrophotometer. The physical state of dye deposited in hair fibers was evaluated by a reflectance light microscopy technique, based on image stacking, allowing enhanced depth of field imaging. The dye distribution within the fiber was monitored by infrared spectroscopic imaging of hair fiber cross sections. Damage to the ultrafine structure of the hair cuticle (surface, endocuticle, and cell membrane complex) and cortex (cell membrane complex) was determined in hair cross sections and on the hair fiber surface with atomic force microscopy. Using differential scanning calorimetry, we investigated how consecutive coloring and leaching processes affect the internal proteins of hair. Further, to probe the surface properties of hair we utilized contact angle measurements. This study was conducted on both pigmented and nonpigmented hair to gain insight into the influence of melanin on the hair dye deposition and leaching processes. Both types of hair were colored utilizing a commercial oxidative hair dye product based on pyrazole chemistry.

INTRODUCTION

Hair dyeing is a common cosmetic practice in the beauty industry and is carried out in the salon by professional cosmetologists as well as at home by the consumer. It is an especially common procedure for individuals with gray hair, but is also practiced by members of the younger population. Usually, hair is periodically dyed (every 4–6 weeks) due to new hair growth. The most suitable kinds of coloration come from the use of oxidative (or permanent) dyes, which offer a great variety of shades and increased wash fastness. In general, oxidative dyes consist of primary intermediates, color couplers, and oxidizing agents (1,2). During the coloring process, the primary intermediates are activated by hydrogen peroxide and then react with color couplers inside the hair shaft. The newly formed molecules inside

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the hair are colored and large enough so that they do not easily leach out from the hair structure. They also have affinity to hair, presumably due to van der Waals or other noncovalent interactions between the dye and the internal structural components of the hair, allowing them to remain in the hair structure even during shampooing or rinse out (3).

Hair dye deposition and water fastness is influenced by the permeability of primary intermediates into the hair and the health condition of the ultrafine structural components of hair. Typically, oxidative hair dyeing is carried out at high pH (~10), utilizing ammonia or ethanolamine, causing cuticle swelling and facilitating entry of the dye intermediates into the fiber structure as well as to decompose hydrogen peroxide so that the dyes can be activated (4). This high pH damages many of the lipids on the surface of the hair, making it a more hydrophilic substrate with greater capacity to absorb ingredients (5). In addition, bleaching, dyeing, and other harsh chemical treatments damage the ultrafine structure of hair leading to an overall more porous, or open structure, allowing dye molecules to easily diffuse into and out of the hair (6–8). The ease with which a molecule diffuses into or out of the fiber is dependent on a number of factors including its molecular size (9). In fact, kinetic studies of dye removal by Wong *et al.* demonstrated that smaller dyes rinse out much more readily than larger dyes (10).

In addition to surface damage, oxidizing agents in permanent hair dye systems also dissolve melanin and oxidize hair keratin substrate (8). Therefore, both the hair surface barrier of the cuticle and the ultrafine structure of the cortex are greatly changed. To date, there has not been a comprehensive study on the mechanism of hair dye deposition and leaching pathways published in the literature due to the complexity of hair damage caused by the dyeing process. In this study, we investigated hair damage by consecutive dyeingshampooing equivalent cycles and elucidated dye deposition and leaching pathways. This information will be helpful for scientists to develop improved technologies that minimize the amount of damage in the coloring process, prevent color fading from washout, and even create specialized products that restore health and brilliance to colored hair.

MATERIALS AND METHODS

A number of experimental procedures and instruments were used to gain a better understanding of the dye leaching process and the damage associated with the bleaching and dyeing of pigmented and nonpigmented hair. As already mentioned above, instrumental techniques were employed to investigate the morphological characteristics of the ultrafine structure of hair, and consisted of atomic force microscopy (AFM), differential scanning calorimetry (DSC), and dynamic contact angle analysis. In addition, we monitored the deposition of dye within the fiber structure using reflected light microscopy and Fourier transform infrared spectroscopy (FT-IR) spectroscopic imaging. Further, studies of the kinetics of dye leaching were carried out by exposing dyed hair to a number of rinse cycles with water while monitoring the aqueous dye concentration.

HAIR TRESS PREPARATION

The experiments were conducted on both European white and dark brown hair purchased from International Hair Importers (Glendale, NY). Hair tresses were prepared by gluing ~ 2 g of fibers together at the hair root to a Plexiglas tab with Duco cement. The resulting dimensions of the hair tresses were 6.0 inches in length by 1.25 inches in width. Hair

tresses were precleaned with a 3% ammonium lauryl sulfate solution, rinsed thoroughly, and dried prior to use in the experiments. Hair was then subjected to a bleaching regimen for 30 min with Clairol BW2 (Procter & Gamble, Cincinnati, OH) beaching powder and 20-volume hydrogen peroxide (Clairoxide 20; Procter & Gamble).

MULTIPLE HAIR DYEING-LEACHING CYCLES

In this study, hair was subjected to a regimen that consisted of a dyeing step followed by a dye leaching step, which was carried out by immersing freshly dyed hair fibers in water for 30 min at 40°C (called one cycle). Five dyeing-leaching cycles were conducted on both white and dark brown hair. During the dyeing steps, 12 hair tresses were dyed with Textures and Tones 4R (Red Hot Red) hair dye (Procter & Gamble, Cincinnati, OH) for 40 min. Hair tresses were then rinsed for 2 min under hot water (~38°C) and excess water was removed by forming a squeegee with the index and middle fingers and running them along the length of the tress. Hair tresses were then dried with a hair blow dryer (temperature set to medium). During the dye leaching process, each freshly dyed hair tress was suspended in a vessel containing 500 ml of water at 40°C with continuous stirring at 50 rpm. The total dye leaching time is 30 min. Leached dye was measured with an automated dissolution system, consisting of a VK7000 dissolution testing apparatus (Varian Inc., Cary, NC) and an ultraviolet-visible spectrophotometer equipped with seven flow cells and a flow pump (Agilent Technologies, Santa Clara, CA). The dye concentration in solution was determined by measuring the absorbance at 490 nm. The amount of the dye leaching from hair was calculated from the ratio of absorbance to the weight of the hair tress. The continuous dye release from hair fibers within 30 min was measured at different times with a 2-min interval. Six repetitions per sample were measured. Hair dye leaching cycles were performed by rinsing with water. Surfactant was not added as it interacts with hair dye and interferes with dye detection.

FT-IR SPECTROSCOPIC IMAGING

A 1-cm-long hair bundle was cut from the middle of the hair tresses and mounted on the top of a sample holder by embedding in ice. The hair bundle was then microtomed at -30° C into 5-µm-thick cross sections with a Leica CM 1850 cryostat (Leica Microsystems Inc., Bannockburn, IL). Hair cross sections were collected onto CaF₂ windows for IR imaging. This preparation technique avoids any possibility of contamination with embedding or fixing medium. Hair cross sections were imaged with a PerkinElmer Spotlight system (PerkinElmer, Inc., Waltham, MA) that couples a FT-IR spectrometer with an optical microscope. The system consists of a linear array mercury cadmium telluride detector and an automated high precision x-y sample stage. Images were acquired with a 6.25 µm step size, eight scans for each spectrum, and 8 cm⁻¹ spectral resolution. IR spectra were analyzed using ISys 5.0 software (Malvern Instruments, Inc., Malvern, Worcestershire, UK).

ATOMIC FORCE MICROSCOPY

The hair cross sections were prepared by the following procedure. A single hair fiber was hung in the center of a cylinder. Buehlers $\text{Epoxicure}^{\text{TM}}$ Resin (Buehler, Lake Bluff, IL) and Buehlers $\text{Epoxicure}^{\text{TM}}$ Hardener (Buehler) were mixed (weight ratio of 5:1) and slowly poured into a cylinder. To ensure the hair fiber remained vertically positioned in epoxy, one end of the hair fiber was attached to a thin pole, and the other end was tied with an

appropriate weight. The epoxy cures slowly at room temperature. After 24 h of curing, the epoxy containing the embedded hair fiber was taken out of the cylinder and cut into ~2 mm thick sections perpendicular to the longitudinal axis of the hair fiber. A standard metallography polishing technique was to use to polish the epoxy until a clean cross-sectional interface of hair was obtained. AFM images of the hair cross section were acquired using a Multimode Nanoscope IIId supplied by Bruker Corporation (Billerica, MA) at ambient conditions (22°C, 50% humidity) in the contact mode. A sharp nitride lever probe combining a sharp silicon tip with a silicon nitride cantilever was used for the topographic imaging acquisition. The nominal radius of the tip was about 2 nm and the spring constant of the cantilever is 0.06 N/m. A scan rate of 2 Hz was used for all measurements. The data collection was set to both the height and deflection channels.

OPTICAL MICROSCOPY

Images of the hair surface were collected in the reflected light microscopy mode using an Olympus BX50 (Olympus America, Center Valley, PA) optical microscope equipped with $10 \times (UMPlanFL 10 \times 0.30)$, $20 \times (LMPlanFL 20 \times 0.40)$, $50 \times (LMPlanFL 50 \times 0.50)$, and $100 \times (LMPlanFLN 100 \times 0.80)$ objectives. Equipped with a $10 \times$ eyepiece and an additional $2 \times$ objective, magnifications of roughly $200 \times$, $400 \times$, $1000 \times$, and $2000 \times$ were obtained in the final images. The microscope also contains a motorized z-stage allowing z-stacks to be generated. Each final image was obtained by generating an image stack and then using an algorithm to combine in focus details in each image of the stack into one final image.

DYNAMIC CONTACT ANGLE ANALYSIS

An Attension tensiometer was used to determine contact angle (Biolin Scientific, Stockholm, Sweden). A single hair fiber, cut to approximately 1 cm near the root, was immersed approximately 3 mm into deionized water and the advancing contact angle was measured. The required hair diameters were measured using a handheld micrometer. Ten fibers in each hair tress were measured.

DIFFERENTIAL SCANNING CALORIMETRY

A Q2000 differential scanning calorimeter (TA Instruments, New Castle, DE) was employed using pressure resistant, high volume stainless steel pans. Samples consisted of 8–12 mg of cut (2–5 mm) hair fibers, along with roughly 55 (\pm 1.5) mg of deionized water. Each pan was sealed and allowed to sit for at least 6 h at room temperature to ensure equilibrium water content and distribution within the hair fibers. Heating at a rate of 2°C/min was performed from 22° to 190°C in standard mode. Three repetitions per lot were conducted. TA Universal Analysis 2000 (TA Instruments, New Castle, DE) was used in conjunction with Windows 4.7A (Microsoft Corporation, Redmond, WA) to determine the denaturation temperature (T_d) and enthalpy of denaturation (ΔH).

RESULTS AND DISCUSSION

To gain more insight into the dye leaching process, we utilized a dissolution apparatus and monitored the amount of dye leached from hair as a function of rinse time. We studied both pigmented and nonpigmented hair to understand the contribution of melanin dissolution to the dye leaching process. Experiments were carried out using a hair dye based on pyrazole chemistry, which is often employed in hair dye products designed to provide red shades. Of the dyes currently used in the market place, the pyrazole dye in this study provides one of the most intense shades of red, and is especially prone to shampoo or water removal. Therefore, this dye chemistry is a good choice to better understand the factors that control color loss.

Several techniques were employed to provide measurements of the physicochemical state of the hair, focusing on its surface properties (dynamic contact angle analysis) and ultrafine structure (AFM and reflected light microscopy). Overall, we find significant damage in both the surface and internal components of hair due to bleaching and dyeing. Furthermore, dye penetration profiles into the fiber were monitored with FT-IR spectroscopic imaging, providing a two-dimensional map across the cross section of hair. And, finally, we followed the condition of hair proteins in the amorphous matrix by measuring T_d of the α -helical, crystalline phase proteins. All of this information allows us to depict a better picture of how synthetic dyes leach from the hair fiber.

DYE LEACHING KINETICS OF DIFFERENT TYPES OF HAIR

Figure 1 presents typical kinetics curves corresponding to dye leaching that were acquired from dyed dark brown and white hair. Dye leaching occurs once dyed hair fibers come in contact with water. Hair dye loss rates can be determined from the slopes of the curves. By calculation, the dye loss rate within the first \sim 3–5 min is generally 5–8 times faster than in the later stages, and the dye loss in the first 3 min is \sim 35% of the total dye loss (within 30 min). More than likely, the dye loss in the early stages of this process corresponds to dye migration from the cuticle region to the solution phase since this is the shortest diffusion route from within the hair structure. Another factor associated with relatively



Figure 1. Plots of leached dye from pigmented and nonpigmented hair fibers as a function of time. The dye concentration in solution was determined by measuring the absorbance at 490 nm. The amount of the dye leaching from hair was calculated from the ratio of the absorbance to the weight of hair tress.

quick dye loss in the early stages is the high pH in the dyed hair fiber. Dyeing hair results in a large increase in pH to \sim 9. A 2-min rinsing step after the hair dyeing procedure is not sufficient enough to lower the pH of hair to 6.5. Under high pH conditions, the cuticles are swelled and therefore provide open channels and pathways for dye to leach out of the fibers. It should be noted that most contemporary hair dye systems contain a conditioning step to bring the pH down closer to physiological conditions.

To probe dye spatial distribution inside the hair, dye species were imaged across hair sections by FT-IR spectroscopic imaging. Figure 2 presents the relative dye concentration across hair sections. The image was generated by integration ratio of the peak at 1116 cm⁻¹ resulting from the dye species to the amide I peak at 1650 cm⁻¹ due to hair proteins. Such an analysis indicates that the cuticle region has higher dye concentrations than the cortex. Free dyes/residues were also visualized in the cuticle areas of the freshly dyed hair fiber surface (Figure 3) with an optical microscope. Studies have shown that the preferred route of reagents to enter the hair fiber is the scale edge between cuticle cells, either through the cell membrane complex or endocuticle (11).

During the dyeing process, dye molecules aggregate in the cuticle area as reagents permeate into the hair fiber. Therefore, the cuticle area is exposed to and contains a higher amount of dye molecules than other areas of the hair fiber. These results explain our observations from the kinetics measurements, which indicate that dye leaching is much faster in the early stages immediately after dye deposition. Much of the initial dye fading results from dye loss from the cuticle structure, which is dissolved once the dyed hair fiber comes in contact with water. Previous work in this area demonstrated that, in addition to the cortex, reactions between the dyes and developer also occur at the surface of hair and in the cuticle (12). In addition, studies by Chandrashekara and Ranganathaiah revealed that dyes diffuse much more quickly into the cuticle than they do into the cortex (13). Therefore, it should stand to reason that since a significant quantity of dyes are located in the cuticle structure, these are likely to diffuse from the hair structure first.

As already noted, most contemporary hair dye protocols involve rinsing, shampooing, and conditioning steps to bring the pH of hair down closer to physiological conditions



Figure 2. FT-IR image of dye distribution in hair fiber cross sections. The image was generated by taking the ratio of the integration of the peak at 1116 cm^{-1} resulting from the dye species to the amide I peak at 1650 cm^{-1} resulting from hair proteins.

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with the aim to "close" the cuticle and, of course, prevent the leaching of dyes from the interior of the fiber. Shampooing plays a major role in the color fastness of hair. In the experiments conducted in this study only the influence of water on hair dye leaching is considered. It is very likely that results may be different in presence of surfactants.

Dye leaching from dark brown hair is generally faster than from white hair. This trend is more obvious in the later stages of dye leaching kinetics, which reflects that dye molecules are transported from the cortex region through the cuticle, and then to the solution. More than likely, more pores and channels are created in the dark brown hair during the dyeing process when melanin granules are dissolved. This would provide extra pathways for dye molecules to leach out. These results are in agreement with previous studies that demonstrated that hair color fading is greater in pigmented than nonpigmented hair when exposed to solar radiation in combination with shampooing cycles (14).

INVESTIGATION OF HAIR DAMAGE AND HAIR DYE LOSS DURING MULTIPLE OXIDATIVE DYEING–LEACHING CYCLES

The hair surface becomes more hydrophilic during the dyeing process. The dyeing procedure removes most lipids from the hair surface since it is generally performed under alkaline conditions. After the first cycle of the dyeing–leaching process, the contact angle of hair decreases from 104° for virgin hair to 84° for dyed-leached hair. There is no visible damage in the internal structure from AFM observations after the first dyeing–leaching cycle alone. However, if a prebleach process is conducted prior to the hair dyeing process, AFM (Figure 4) reveals that the fiber's internal structure is severely damaged. Cracks (over 100 nm in diameter) and holes are observed in the cuticle (e.g., cell membrane complex, endocuticle) and cortex (e.g., cell membrane complex). Such a result suggests that the damage caused by the bleaching process facilitates dye loss from the internal structure of the hair.

To evaluate hair protein damage during the consecutive dyeing-leaching cycles, protein T_d of both dyed European white and dark brown hair were measured after each dyeing and leaching cycle. The results are displayed in Figure 5. The first three column bars in each chart demonstrate that T_d decreases with consecutive dyeing-leaching cycles, indicating that consecutive dyeing cycles progressively degrade hair proteins. More specifically, the amorphous matrix becomes more viscous (or plastic) due to damage to its proteins. It should be noted that T_d represents the denaturation temperature of crystalline phase of the hair; however, changes to the amorphous matrix (which provides support for the crystalline phase) influence the value of T_d . Plasticization with water, e.g., shifts T_d to lower temperature, while cross-linking hair (making the amorphous matrix more brittle) with reactive ingredients increases T_d .

The last two column bars in each chart in Figure 5 reflect how the leaching process alone affects the hair protein structure. We found that hair dyeing alone affects T_d more than hair dyeing in combination with leaching. Perhaps the leaching process aids in the recovery of hair protein structure. Dyeing the hair results in a large increase in pH to ~9. It requires extensive rinsing to lower the pH to 6.5. It is possible that when conducting DSC measurements at high pH the hair protein is in a highly compromised state. These findings are consistent in both pigmented and nonpigmented hair.



Figure 4. AFM images acquired from hair cross sections in (A) virgin hair and (B) bleached then dyed hair. The images in (A) and (B) on the left side are of both cuticle and cortex; the images in (A) and (B) in the middle are from the cuticle region; and the images in (A) and (B) on the right side are from the cortex region.



Figure 5. DSC measurements of multiple dyed and leached hair fibers for both (A) pigmented and (B) non-pigmented hair.

Interestingly, the dye-leaching rate does not always increase with progressively increasing amounts of fiber damage resulting from consecutive dyeing. Figure 6 presents dyeing– leaching profiles of dyed European dark brown hair after each dyeing–leaching cycle. After the first dyeing–leaching cycle, we observe an increase in the rate of dye loss. However, the third, fourth, and fifth consecutive dyeing–leaching cycles are accompanied by a decrease in the rate of dye loss. This trend is less noticeable in dyed white hair (data not shown). These results suggest that deposition of dyes during the initial dyeing steps occurs in pores and voids formed due to the degradation of melanin and other morphological components of hair. After the second dyeing process, dye molecules from subsequent dyeing steps may granulate with pre-existing dyes already in the fiber and form larger particles. The likelihood that large dye particles dissolve slower could explain why the rate of dye loss decreases on consecutive dyeing–leaching cycles.

CONCLUDING REMARKS

The data generated in this study provide a fundamental understanding of the mechanisms involved in hair dye deposition and dye fastness. Key developments were also made



Figure 6. Dyeing–leaching profiles from dyed dark brown after each cycle of the dyeing–leaching process. The dye concentration in solution was determined by measuring the absorbance at 490 nm. The amount of the dye leaching from hair was calculated from the ratio of the absorbance to the weight of hair tress.

in understanding hair fiber damage resulting from the hair dyeing process as well as progressive damage resulting from multiple dyeing cycles. A proprietary sample preparation technique of hair fiber cross sections, used in combination with atomic force microscopy, allowed us to identify key areas of damage within the fiber including the cell membrane complex (in both the cuticle and cortex) as well as other regions of the fiber where we found small pores and other features consistent with physical damage. More than likely, these destructed regions provide exit points for existing hair dyes to leach from the fiber. Utilizing DSC, we monitored structural changes of keratin protein as a result of hair dyeing. In addition to examining the ultrafine structure of dyed hair, we also generated a significant amount of practical data in regard to the kinetics of the leaching process demonstrating differences of leaching in different types of hair and providing real-time data for this commonly employed consumer procedure. This study confirms the profound effects of rinsing/shampooing after dyeing in governing color fastness. Obviously, mitigating the effects of water and shampoo would help alleviate hair dye loss. As more porous, damaged hair is more susceptible to color loss, new technologies that minimize damage during dyeing could facilitate hair dye systems in which the dyes are less prone to undergo leaching during subsequent washing and rinsing steps. Formulators designing color protection products could use the leaching method to quickly evaluate effects of new ingredients, shampoos, or hair treatments on dye leaching. The current work also demonstrates that multiple dyeing processes do not deteriorate hair color loss in spite of progressively damaging the hair fiber structure. It is speculated that larger granular dye molecules are formed inside the hair structure during repeated multiple dyeing cycles. Therefore, any hair dye technologies that facilitate the granulation of dye molecules inside the hair fiber should benefit hair color retention.

ACKNOWLEDGMENTS

We would like to express our gratitude to Bert Kroon and Linda Foltis of Ashland Specialty Ingredients for constructive discussions about hair color science.

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