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Role of Redox Metals in Color Formation in a Hair Colorant

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Synopsis

The objective of this work was to identify if low levels of redox metals such as copper would accelerate color formation on hair and to understand the consequent impact on initial color formation and color fade. Kinetics of color formation with oxidative dyes in solution in the presence of varying concentrations of copper ions were assessed *via* imaging and color measurements. Color uptake on hair and color fade were measured with a spectrophotometer, and copper levels in hair were measured with inductively coupled plasma atomic spectroscopy after hair digestion. In this work, the role of redox metal ions such as copper and iron on accelerating rates of oxidative dye formation was demonstrated. Kinetics of dye formation were measured in solution for three dye couples—*p*-phenylene diamine (PPD) plus resorcinol, PPD plus 5-amino-2-methylphenol (AHT), and 4,5-diamino-1-(2-hydroxyethyl) pyrazole sulfate (HDAP) plus AHT— in a solution that also contained ammonium hydroxide and hydrogen peroxide at pH 10. Low levels of copper were added at a concentration range from 0.01 μg/g to 0.1 μg/g and the rate of color formation measured over 2 h. All three dye couples showed significant color acceleration that increased with increasing levels of copper. A mechanism where initial oxidation of primary intermediate PPD or HDAP is accelerated is proposed. This mechanism is demonstrated to become important when trace levels of copper are in hair and a hair colorant added. Color formation is accelerated outside versus inside hair, and ultimately, color uptake is reduced after the colorant is rinsed off hair. Noticeable color fade versus the starting hair color is also increased. This work provides evidence for the role of copper ions in color formation in hair and strategies to reduce copper levels in hair using a chelant such as histidine in a shampoo or conditioner before coloring.

INTRODUCTION

Hair colorants constitute a significant category in the cosmetics market—it is estimated that more than 70% of women in the developed world have used hair color, and a large proportion of those do so regularly. Although each woman may have a very individual reason for coloring her hair, covering gray can be considered a universal key motivator. Other desired performance aspects include enhancing the existing color, wanting a different color from the one given to oneself by nature, or achieving a more striking looking appearance. The most widely used products in the colorant category are Permanent Level 3 colorants that consist of a combination of an alkalizer (typically ammonia, but ethanolamine is also

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used), hydrogen peroxide, and a range of dye precursors (1). There are two chemical processes that take place during the permanent dying process, both of which contribute to observed final color. First is oxidation of melanin and previously deposited dyes that lightens the underlying hair color. Second is oxidation of dye precursors that have diffused inside hair to form colored chromophores. The rate of color formation is important: if too fast color will form outside hair or in the bottle before application is completed, and if too slow not enough color is formed during a typical development time of 30 min. Penetration of dyes inside hair is important for color longevity as the larger size and reduced solubility of the dyes formed mean they are trapped inside hair, making them harder to be removed during washing. The basic mechanism of dye formation is an oxidative reaction between hydrogen peroxide and a primary intermediate to form a reactive intermediate called a diimine that then reacts with a coupler to form diphenylamine (leuco dye) which then undergoes a rapid oxidation to form a binuclear indo dye. Depending on the coupler structure, there can be subsequent reactions with additional primary intermediates to form trinuclear and larger dyes (2,3).

The role of redox metals in accelerating this chemistry has been studied. A work by Kojma and coworkers showed *via* isotopically labeled dye precursors, and nano-secondary ion spectrometry that formed chromophores were localized in or close to melanin granules and linked this to the presence of transition metals in melanin. A previously published work has shown that consumer hair contains redox metals such as copper and iron that are adsorbed during washing in tap water (4) and that these exogenous metals are present in the outer layers of the hair cuticle (5). The objective of this work was to determine whether these exogenous redox metals are influencing color formation rates and color uptake.

MATERIALS AND METHODS

HAIR SOURCE

Chemically virgin natural white Caucasian-source hair was purchased from International Hair Importers & Products Inc. (Glendale, NY). Individual tresses (2 gm, 6in with hot wax tab at the top), formed by evenly blending hair from multiple ponytails, were used for all experiments. Colored hair was created by treating virgin hair tresses once with an oxidative permanent commercial colorant (Nice ′N Easy Extra Light Blonde Shade, Clairol, Stamford, CT). The mixed colorant was thoroughly massaged onto hair at a dose of 4 g of product per g of hair and then incubated for 30 min in an oven at 30°C. The product was then completely rinsed from hair. Initial copper levels in hair were <20 μg/g.

DETERMINATION OF METAL CONTENT IN HAIR

The metal content of hair samples was determined by inductively coupled plasma atomic spectroscopy (ICP-OES) with an Optima 5300 DV Optical Emission Spectrometer (Perkin Elmer Life and Analytical Sciences, Shelton, CT). Samples of 100 mg of hair were digested overnight with 2 mL of high-purity concentrated nitric acid. The digestive mixture also contained 150 μL of 100 μg/g yttrium internal standard (Inorganic Ventures,

Christianburg, VA). Following digestion, samples were heated to 70°–80°C for 1h, cooled to room temperature, and diluted to 15 mL with deionized water. Each hair sample was analyzed in triplicate.

DYE SOLUTIONS

A solution of dye precursors was made in deionized water with 3% ammonium hydroxide (30% active) and a pH of 10.5. The molarity of the primary intermediate was 0.031 M (PPD, HDAP) and coupler was 0.032 M (AHT, Res). Solutions were made with 0, 0.2, 1.0, and 2.0 μg/g of copper nitrate. In a 2-mL glass vial, 1720 μL of dye solution was mixed with 180 μL of hydrogen peroxide (35% active) and either 100 μL of DI water or copper nitrate solution. Three vials were made for each dye, and copper level and images taken of the color change over time. The images were taken inside a constant illumination light booth with a USB camera. Each image included a reference color chart. This chart was checked to be in-line with the initial values before each measurement. The images were captured and analyzed with National Instrument's LabView (Austin, TX) application using Vision acquisition software.

COLOR MEASUREMENT

Color measurements were taken using a Konica Minolta CM-700d handheld spectrophotometer (Ramsey, NJ). Settings used were as follows: D65 light, 10° observer, 3 mm aperture, and specular excluded. A total of eight color readings were made on each tress: four on each side.

HAIR TREATMENTS

(i) Influence of copper ions in hair on color uptake

Hair tresses pretreated with one cycle of Nice ′N Easy Extra Light Blonde colorant were washed with water containing copper ions (0.06 μg/g) to create four sets of 12 × 2 g, 6-cm tresses with copper levels of 73 (±7) μg/g, 52 (±7) μg/g, 35 (±7) μg/g, and 21 (±7) μg/g. Each set of 12 tresses was divided into sets of four tresses which were then colored with a permanent colorant. The three colorant tints tested were an intense medium blonde/red (77/44), a medium brown/violet (4/6), and an intensive red (66/46). Each tint was mixed with a 20-volume hydrogen peroxide developer (6%) in a 1:1 ratio and applied at a dose of 4 g of mix to each gram of hair. After 30 min, hair was rinsed for 2 min, dried, and color measured using a Minolta spectrophotometer. The hair was then washed with a shampoo containing 10.5% SLE1S, 1.5% SLS, and 1.0% cocamidopropyl betaine surfactants for a total of 14 cycles with color fade measured using a Minolta spectrophotometer after 2, 6, 10, and 14 cycles. Each wash cycle consisted of applying 0.1 g/g shampoo to the hair switch and lathering for 30 s, followed by a 30-s rinse repeated for a total of two shampoo applications. Hair was then dried in a hotbox at 80°C.

(ii) Influence of histidine chelant on color uptake

A shampoo containing 10.5% SLE1S, 1.5% SLS, and 1.0% cocamidopropyl betaine surfactants was formulated with histidine added at the 0.1% active level. Colored hair was washed for 12 cycles in tap water containing 0.06–0.09 μg/g copper with a shampoo with and without histidine and then analyzed for copper uptake using the ICP-OES method. Each wash cycle consisted of applying 0.1 g/g shampoo to the hair switch and lathering for 30 s, followed by a 30-s rinse repeated for a total of two shampoo applications. Hair was then dried in a hotbox at 80°C.

This hair was then colored with two shades: an intense medium blonde/red (77/44) and a medium brown/violet (4/6). Each tint was mixed with a 20-volume hydrogen peroxide developer (6%) in a 1:1 ratio and applied at a dose of 4 g of mix to each gram of hair. After 30 min, the hair was rinsed for 2 min, dried, and then color measured using a Minolta spectrophotometer. The hair was then washed with a shampoo containing 10.5% SLE1S, 1.5% SLS, and 1.0% cocamidopropyl betaine surfactants with and without 0.1% histidine for a total of 12 cycles with color fade measured using a Minolta spectrophotometer after 2, 5, 8, and 12 cycles. Each wash cycle consisted of applying 0.1 g/g shampoo to the hair switch and lathering for 30 s, followed by a 30-s rinse repeated for a total of two shampoo applications. Hair was then dried in a hotbox at 80°C.

HDAP–AHT DYE SYNTHESIS

0.009M HDAP and AHT were added to a 500-mL flask. Ethanol (20 mL) and ammonium hydroxide (0.083 M) were added to completely dissolve the solid. To another 250-mL Erlenmeyer flask, $K_3Fe(CN)_6$ (0.037 M) and water (100 mL) were added. A sonicator was used to dissolve the ferrate salt and form a homogeneous solution. The ferrate solution was then added to the solution of dye precursors. The mixture was stirred continuously at room temperature for 30 min. The precipitate was filtered and washed with water to give a dark red solid (Z)-5-amino-4-((2-amino-1-(2-hydroxyethyl)-1H-pyrrol-3-yl)imino)-2-methylcyclohexa-2,5-dien-1-one. This crude product was triturated with ethyl acetate and then dried in an oven (50°C) to afford 2.15 g of pure compound (yield 91.5%; >98% purity confirmed by electrospray ionisation mass spectrometery and proton Nuclear Magnetic Resonance).

DYE EXTRACTION

300 mL of methanol was combined with 150 mL of DI water in a 500-mL solvent bottle and allowed to equilibrate to room temperature before use. 100 mg of hair (four replicates for each copper level) were weighed into 4-mL vials, and 4 mL of extraction solvent was added to each vial and allowed to stand for 96 h. At the end of the extraction period, extracts were transferred to fresh vials and diluted 1:5 times and transferred to a disposable cuvette immediately before UV spectrophotometric analysis. A Cary 100 UV-vis spectrophotometer (Agilent, Santa Clara, CA) was used (wavelength range: 400–800 nm, scan rate: 600 nm/min, and data interval: 1.0 nm). Absorbance data were normalized to account for differences in sample weight.

RESULTS AND DISCUSSION

SOLUTION EXPERIMENTS

The role of copper in acceleration of color formation was tested first in solution to simplify the system to just dye precursors and hydrogen peroxide in the presence of varying levels of copper. Low levels of sodium sulfite and ascorbic acid were added as reducing agents with the purpose of stabilizing the solution after making so that there was a reproducible starting solution color. Other precautions were taken to minimize air exposure before the reaction started, and tests were carried out to ensure vials were free of redox-metal contaminants. Color formation as a function of time was measured with different copper levels for three dye couples—PPD–AHT, PPD–Res, and HDAP–AHT—after addition of hydrogen peroxide to the dye solutions with added copper levels. Color was measured from images taken of solution vials under controlled lighting. The three dyes showed different overall rates of color formation, with HDAP–AHT being the fastest and PPD–Res being the slowest, but in all dye systems, copper addition increased the rate of color formation significantly. Initial images were only taken after ~30 s after addition of hydrogen peroxide, and dye formation during this time was not measured. All dye solutions started with an L value of 99. Color formation values for L (lightness) for HDAP–AHT color formation show a dose response for increasing copper levels (Figure 1). Similar dose-response curves were obtained for PPD–Res and PPD–AHT. Change in L versus initial was calculated at 7 min for all dyes at each copper level and confirmed a dose response of color formation acceleration versus copper level added for all three dye couples (Figure 2).

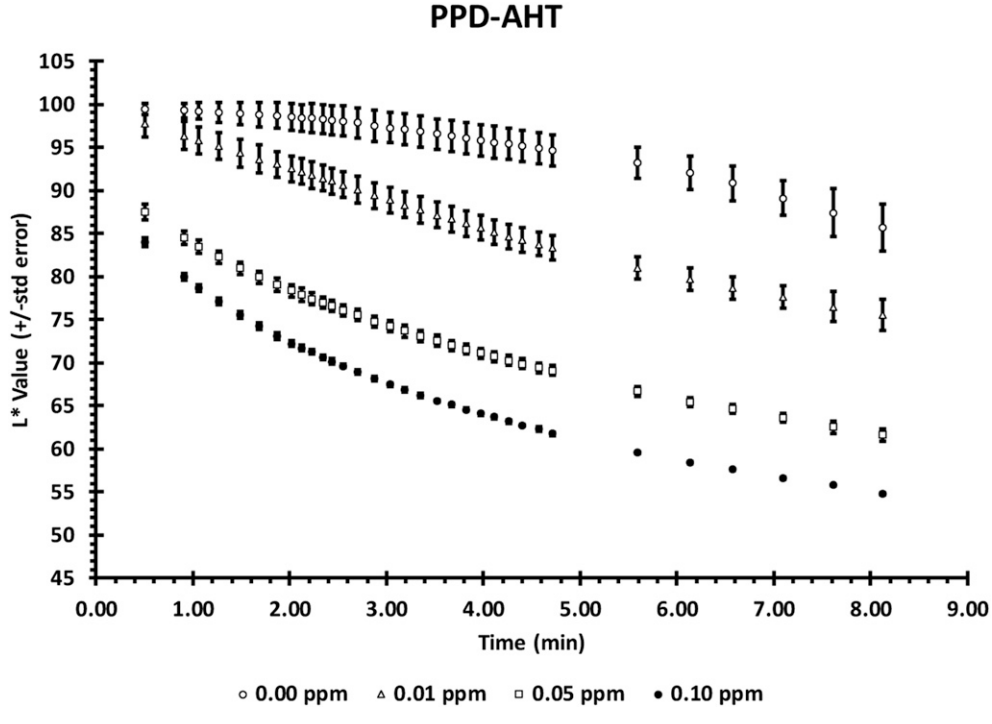

Figure 1. L value change versus time for the HDAP–AHT dye couple.

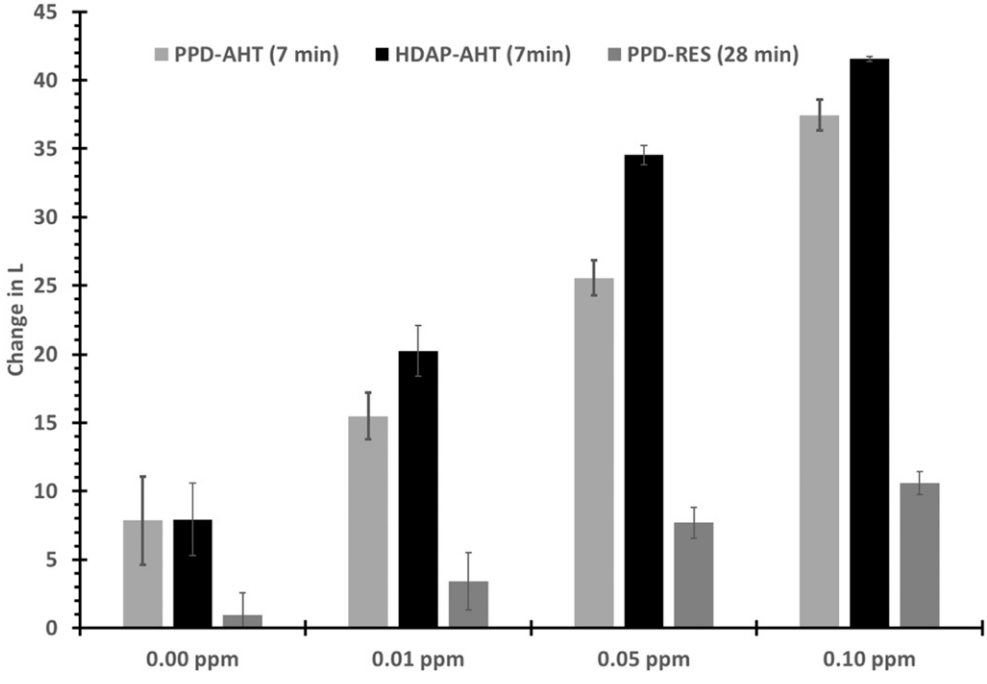

Figure 2. Change in L at 7 min versus initial for PPD–AHT, HDAP–AHT, and PPD–Res dye couples.

The mechanism by which copper accelerates color formation is proposed to be acceleration of the initial primary intermediate to diamine reaction, as shown in Figure 3. This oxidation with hydrogen peroxide in a "clean" solution with no added redox metals is kinetically a slow step that proceeds *via* two one-electron oxidations. Added redox metals accelerate the initial formation of semiquinone radical cation, which then rapidly forms the diimine.

HAIR EXPERIMENTS

Hair experiments were carried out to determine how copper acceleration of dye formation impacts color uptake on hair. Previously colored hair was first rinsed in tap water containing 0.06–0.08 μg/g of copper to create sets of 12 tresses with four different copper levels (Table I). Water hardness was medium (~9 US gpg), and no significant differences in calcium and magnesium were measured between tresses. These copper levels are within the range of levels seen in consumers' hair from previously reported hair harvesting (4).

Each set of tresses was then colored with three different shades {intense medium blonde/red (77/44), medium brown/violet (4/6), and intensive red (66/46)} to asses the impact of copper levels in hair on color formation. The shades were chosen as ones that have the same dye primary intermediates and coupler combinations used in the solution testing. Figure 4A–C shows L*, a*, and b* values for initial color results. In all shades, a significant difference is measured in color readings as a function of copper levels. A higher L* value is seen with higher copper levels, indicating the shade is lighter due to lower dye

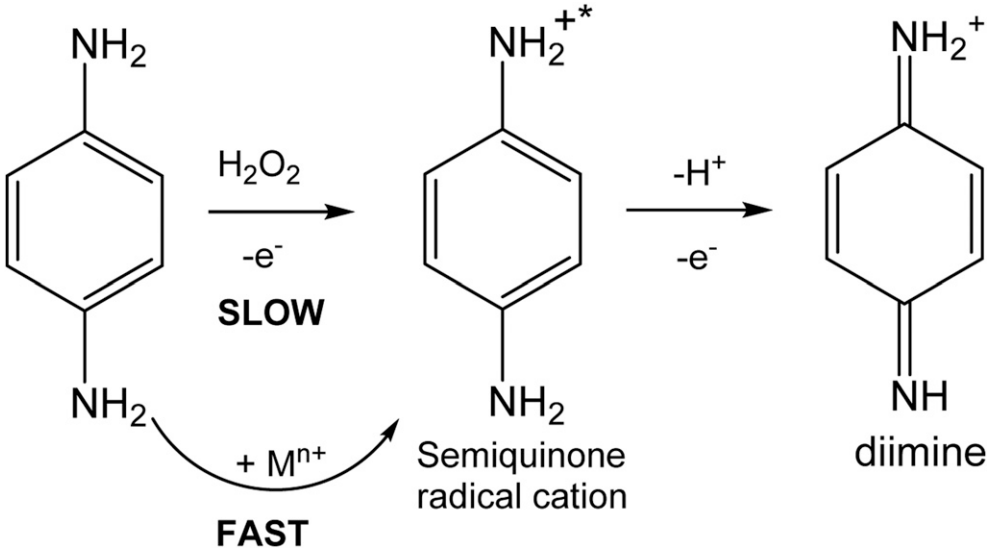

Figure 3. Oxidation of primary intermediate to form diimine.

levels. In addition, a* and b* values are different, indicating the visual color is also a different tone, and this was also observed. In all cases, at lower copper levels in hair the final color was darker and more vibrant, e.g., redder for shade 77/44 and more violet for shade 4/6.

The L*a*b* color space is not linearly correlated with dye intensity, so one shade was used to more accurately correlate copper level in hair before coloring with final dye uptake. Shade 77/44 was chosen as it contains only two dye precursors—HDAP and AHT—and the predominant red color formed has a peak at 477 nm (ε_{EtOH} = 18,900 $M^{-1}.cm^{-1}$). This is the formed dye: (Z)-5-amino-4-((2-amino-1-(2-hydroxyethyl)-1H-pyrrol-3-yl) imino)-2-methylcyclohexa-2,5-dien-1-one (Figure 5). A 2:1 methanol:water extraction for 96 h was used to extract formed dye from hair dyed with four different copper levels, and the absorbance signal correlated with copper levels in hair. The dye (Z)-5-amino-4-((2-amino-1-(2-hydroxyethyl)-1H-pyrrol-3-yl)imino)-2-methylcyclohexa-2,5-dien-1-one was also synthesized and confirmed to have the same 477-nm absorbance. A negative linear correlation of copper level with absorbance at 477 nm was measured with an R^2 of 87% (Figure 6). The data confirmed lower dye formation in hair pretreated with copper and explain the lighter color measured. A previous work has shown that copper taken up by hair from tap water accumulates in cuticle outer layers (6), and thus, it is proposed that more color is formed in the cuticle and outside hair when copper is present,

Table I
Metal Levels of Hair Tresses as Measured by ICP-OES (Standard Error in Parenthesis)

Tress set	Copper levels (μg/g)	Calcium levels (μg/g)	Magnesium levels (μg/g)
1	21 (1.8)	4,339 (55)	515 (7)
2	35 (0.8)	4,526 (36)	517 (3)
3	52 (1.7)	4,426 (78)	534 (8)
4	73 (2.0)	4,713 (54)	535 (7)

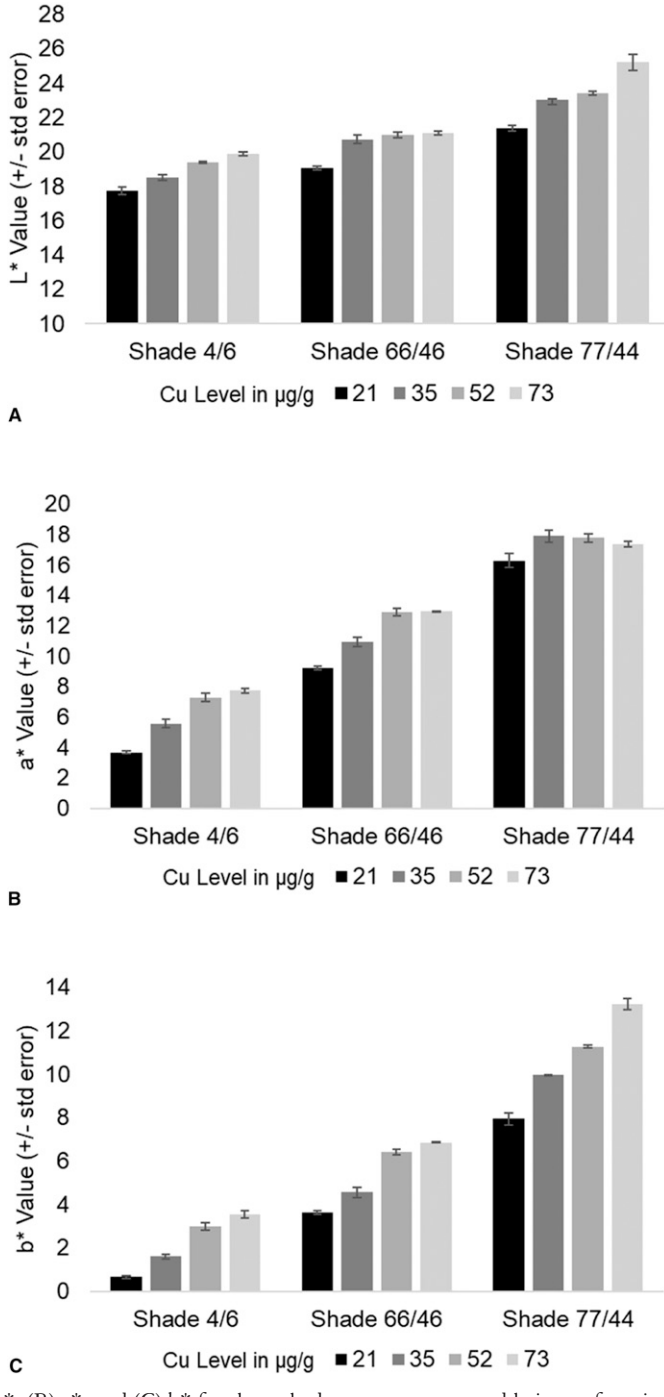

Figure 4. (A) L*, (B) a*, and (C) b* for three shades on copper-treated hair as a function of copper levels.

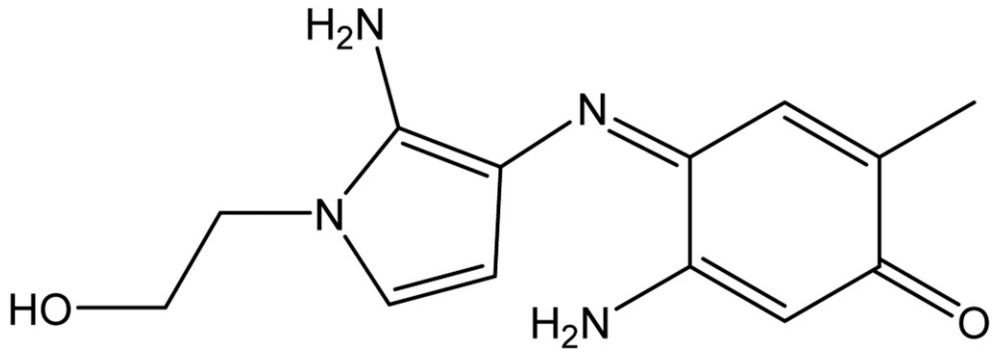

Figure 5. Dye formed in the HDAP–AHT couple.

and this formed color is subsequently washed out during rinsing off the colorant. This leads to a lighter final hair color.

CHELANT EXPERIMENTS

Experiments were performed to demonstrate that chelants added to a shampoo can remove copper from hair and improve color uptake and color intensity over multiple washes. The chelant chosen was L-histidine, an amino acid known to have high binding

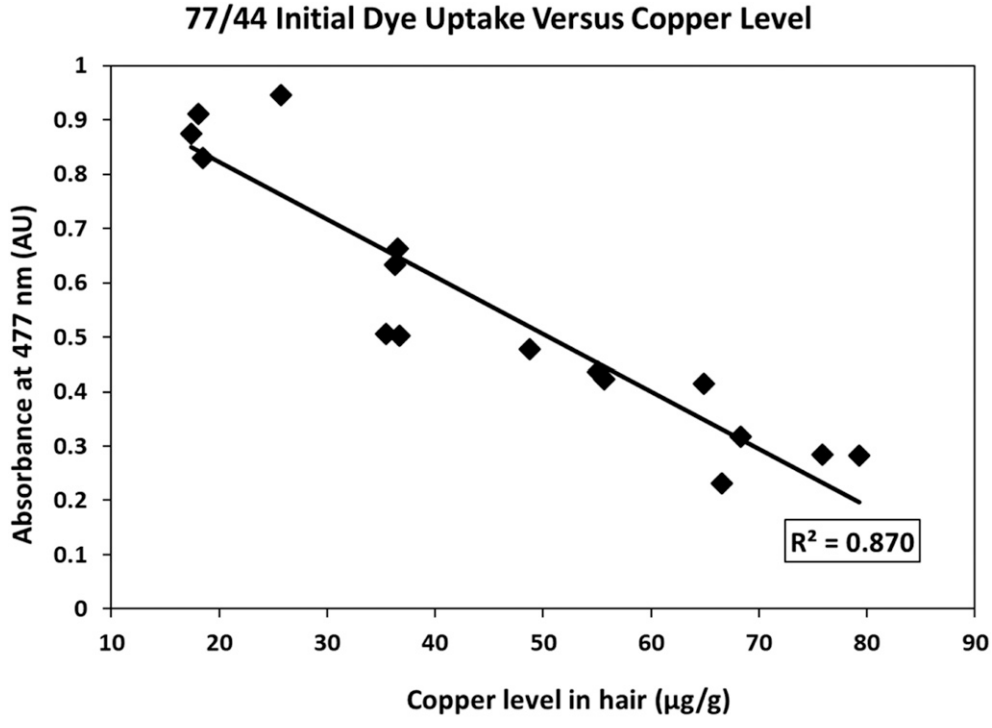

Figure 6. Correlation of UV absorbance of dye extracted versus copper level in hair.

Table II
Copper Levels in Hair before Coloring (Standard Error in Parenthesis)

	Metal levels after 12 wash cycles (μg/g)		
Shampoo details	Copper	Calcium	Magnesium
0% histidine	79.2 (1.7)	5,315 (30)	582 (3)
0.1% histidine	44.2 (1.4)	5,012 (72)	553 (7)

efficiency to copper because of its imidazole group and relatively poor binding to calcium and magnesium (7). In addition, it has been shown to reduce protein damage in hair exposed to UV by reducing copper levels in hair (5). Hair was first washed in water containing copper (0.06–0.09 μg/g) for 12 cycles with a shampoo containing 0.1% histidine and control shampoo containing no histidine. To note, both shampoos contained ethylene diamine tetraacetic acid chelant at 0.13%. Copper levels measured showed lower accumulation in hair washed with 0.1% histidine, a 44% decrease (Table II). A small decrease in calcium (6%) and magnesium (5%) was also measured.

After coloring with shades 77/44 and 4/6, color levels initially were darker (lower L) for hair washed with the histidine-containing shampoo, and this color remained more intense over 12 washes post-coloring (Figure 7). The change in color, delta color change, is also lower than initial color to 12 wash cycles (Table III), indicating improved color fade over time. Color differences were noticeable at all time points between hair pretreated with a 0.1% histidine shampoo versus 0% histidine.

CONCLUSION

The rate of color formation in hair was shown to be accelerated by the addition of low levels of copper, and this chemistry is proposed to be due to one-electron chemistry during the rate-determining initial oxidation step of the primary intermediate dye precursor. This color acceleration was demonstrated across three dye couples (PPD–AHT, HDAP–AHT, and PPD–res) in solution dosed with three different levels of copper. On hair tresses pretreated with different levels of copper, this color acceleration led to a lower level of dye formed inside the hair and consequently a lighter final color. It is proposed

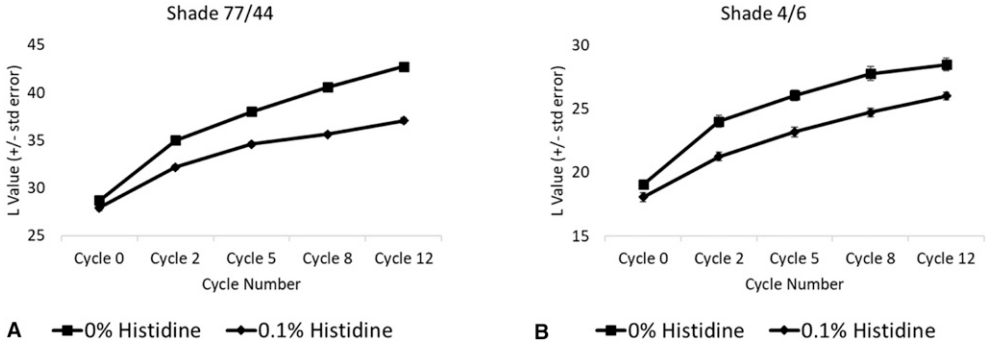

Figure 7. (A) shade 77/44 and (B) shade 4/6—L value versus wash cycle number.

Table III
dE Values for Initial Color versus Wash Cycles (Std Error in Parenthesis)

		dE color change between initial color and Wash cycles			
Shampoo details	Shade	2	5	8	12
0% histidine	77/44	7.59 (0.2)	7.73 (0.3)	6.99 (0.2)	6.70 (0.2)
0.1% histidine	77/44	4.93 (0.3)	5.32 (0.3)	5.71 (0.3)	5.60 (0.3)
0% histidine	4/6	3.42 (0.2)	4.57 (0.2)	5.43 (0.2)	6.12 (0.2)
0.1% histidine	4/6	2.58 (0.3)	3.65 (0.3)	4.68 (0.2)	5.68 (0.2)

that faster dye formation chemistry causes more dye to form outside versus inside hair, and this color is subsequently washed off during the rinsing step. The same effect was seen when prewashing hair in tap water containing copper with a shampoo product containing either no histidine or 0.1% histidine. Addition of low levels of a copper-specific chelant such as histidine in a shampoo prevents copper accumulation in hair as shown in the ICP-OES data and leads to improved color uptake and color over multiple wash cycles.

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