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Comparison of damage to human hair fibers caused by monoethanolamine- and ammonia-based hair colorants

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Synopsis

The number of Level 3 hair color products that substitute 2-aminoethanol [monoethanolamine (MEA)] for ammonia is increasing. There is some anecdotal evidence that higher levels of MEA can be more damaging to hair and more irritating than a corresponding equivalent level of the typical alkalizer, ammonia (in the form of ammonium hydroxide). Our interest was to understand in more quantitative terms the relative hair damage from the two alkalizers, particularly at the upper limits of MEA on-head use. Limiting investigations of oxidative hair damage to increases in cysteic acid content (from cystine oxidation) can underreport the extent of total damage. Hence, we complemented Fourier transform infrared spectroscopy (FTIR) cysteic acid level measurement with scanning electron microscopy (SEM) photomicrographs to visualize cuticle damage, and protein loss to understand not only the oxidative damage but also the damage caused by other damage pathways, e.g., reaction of the more nucleophilic (than ammonia) MEA with hair protein. In fact, all methods show an increase in damage from MEA-based formulations, up to 85% versus ammonia in the most extreme case. Hence, if the odor of ammonia is a concern, a better approach may be to minimize the volatility of ammonia in specific chassis rather than replacing it with high levels of a potentially more damaging alkalizer such as MEA.

INTRODUCTION

Alkalizers have been key components of Level 3 oxidation dyes since these products were developed (1). Typically it was ammonia, which has been the industry standard because of its effectiveness. Alkalizers serve three important functions: swell the hair fiber to allow better penetration of dye precursors, generate the active peroxide species necessary for melanin bleaching and dye formation, and participate in the bleaching of melanin (2).

Because of the characteristic odor of ammonia, other alkalizing agents have been used as replacements, particularly where extreme lightening is not necessary. Although commercial versions of these alternate alkalizers also have characteristic odors, some people find them less objectionable than the odor of ammonia. For example, 2-amino-2-methylpropanol has been used in Level 2 oxidative hair color products. In addition, Level 3 lift was

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achieved for the first time with a high level of MEA in Clairesse® (Proctor & Gamble, Stamford, CT), which was launched by Clairol in 1981. Later, Herbal Essences® Hair Color from Clairol (Proctor & Gamble) used a lower level of monoethanolamine (MEA) to achieve many Level 3 shades, and achieved the higher lift shades by adding a small amount of ammonia. MEA also was used successfully in Level 2 products such as Casting® from L'Oréal (Clichy CEDEX France) and Natural *instincts*® from Clairol, in which less lift is required than for a Level 3 hair colorant.

One of the challenges of using MEA in hair color formulations is that an increased percentage (relative to ammonia) is required to generate the same level of lightening (bleaching, lift) of the hair's melanin (2). This is of particular concern, because Seo *et al.* (3) have observed synergistic causality of dermatitis and hair loss by higher levels of MEA and hydrogen peroxide.

In addition to smell, one of the key concerns with Level 3 colorants is the amount of damage that is done to the hair fiber, either during a single use or repetitive uses as the hair is re-colored. This is particularly true in the salon environment where consumers tend to use the same colorant product for extended periods rather than switching among products. Damage can be masked or repaired with hair treatment agents for both MEA- and ammonia-based products, or mitigated in ammonia-based products with radical scavengers (4) or chelants (5), but starting with ingredients that cause the least damage makes damage mitigation easier.

Among other components, hair is composed of proteins and lipids that are susceptible to a variety of chemical reactions such as oxidation and nucleophilic attack. Of course, the observed rates of these attacks are dependent on a variety of factors such as concentrations, pH, and the individual rate constants. Ammonia is nucleophilic, but less so than MEA. For example, MEA is well known to be nucleophilic enough to be a key reagent in the synthesis of dyes through S_NAr reactions (6) and the removal of ester-protecting groups from air-sensitive coloring agents (7). Whereas excess ammonia quickly leaves the hair because of its volatility, MEA is not volatile under atmospheric conditions, so there is the potential for damage to be exacerbated over time if significant amounts of MEA remain in the hair after rinsing. Our interest was whether there are any differences between ammonia and MEA in the extent of damage to hair fibers when they are used at the concentrations needed to get enough bleaching for Level 3 oxidation dye products.

GENERAL EXPERIMENTAL

MATERIALS

Chemicals used in the bleaching chassis were of the grades commonly used in cosmetic products and were used as received. Cetearyl alcohol was purchased from Cognis Corp. (Monheim, Germany), Crodafos CES® and steareth-200 from Croda Chemicals Europe Ltd. (East Yorkshire, England), xanthan gum from CP Kelco (Atlanta, GA), sodium hydroxide from Brenntag GMBH (Mulheim, Germany), sodium sulfate from Cordenka GmbH (Obernburg, Germany), sodium sulfite from Esseco SRL (Novara, Italy), ascorbic acid from DSM Nutritional (Kaiseraugst, Switzerland), ethylenediaminetetraacetate disodium salt from Akzo Nobel Surface Chemistry Inc. (Amsterdam, Netherlands), propylene

glycol from Lyondell Chemical Co. (Houston, TX), trisodium ethylenediamine disuccinate from Innospec CTD (Littleton, CO), ammonia from EMD (Darmstadt, Germany), and monoethanolamine from Huntsman Corp. (The Woodlands, TX). Deionized water was used to prepare the chassis.

Hair (blended source light brown virgin hair) was purchased from International Hair Importers & Products. Hair treatments were performed with Pantene® Fine Hair Solutions Flat to Volume shampoo and Pantene® Classic Conditioner (Procter & Gamble, Cincinnati, OH). The developer used 20 volume Welloxon® (Procter & Gamble, Cincinnati, OH), which corresponds to 6% by weight hydrogen peroxide.

METHODS

Preparation of Chassis Part 1. To a 10 l vessel was added Crodafos CES® (847.5 g), cetearyl alcohol (45.5 g), and steareth-200 (81 g). The mixture was heated to 85°C. Water (3.63 kg) and sodium hydroxide (34 g) were added during heating and the mixture was homogenized for 1 min at 15 m/s. After the mixture is completely homogenized, agitation was performed for 10 min at 1.1 m/s while heating was continued. To a separate beaker was added xanthan gum (15 g) and propylene glycol (49 g), which was mixed until it was homogeneous. The gum–glycol mixture was then added to the Crodafos CES® mixture, which was >80°C. The combined mixture was then homogenized for 1 min at 20 m/s and then agitated for 10 min at 1.1 m/s. The batch was transferred to large mixing vessel once the temperature reached 85°C. The batch was thoroughly homogenized at 20 m/s for 5 min. Hot water (500 g) was then added to the batch, which then was subjected to cooling under vacuum with stirring at 0.6 m/s. When the temperature decreased to 50°C, cooling was stopped.

Preparation of ammonia and monoethanolamine bleaching chassis. To a dry 2 l Griffin beaker was added Chassis Part 1 (257.5 g). To a separate 450 ml beaker was added sodium sulfate (5.0 g), sodium sulfite (2.0 g), ascorbic acid (1.5 g), ethylenediaminetetraacetate disodium salt (0.5 g), propylene glycol (37.5 g), and water (amount indicated in Table I). The mixture was heated and stirred at 55°C until all salts were dissolved. The solution was then added slowly with stirring (overhead stirrer with 4 paddles; ~270 rpm) to the beaker containing Chassis Part 1. On complete addition, trisodium ethylenediamine disuccinate

Table I
Amounts of Formulation Ingredients Required to Prepare the NH_3 and Monoethanolamine (MEA) Bleaching Chassis. Ingredients That Do Not Vary in Concentration Are Given in the Experimental Procedure

Alkalizer after mixing with H_2O_2 developer	25% Aqueous NH_3 (g)	MEA (g)	Water (g)	pH after mixing 1:1 with H_2O_2
0.27 M NH_3	18.18	0	155.75	10.11 ± 0.02
0.54 M NH_3	36.41	0	139.2	10.35 ± 0.02
0.82 M NH_3	55.76	0	117.9	10.47 ± 0.02
0.27 M MEA	0	16.3	155.75	10.24 ± 0.02
0.54 M MEA	0	32.65	146.6	10.42 ± 0.02
0.82 M MEA	0	50	129.25	10.6 ± 0.02

(16.75 g of a 32 wt% solution in water) was added dropwise with stirring, and the resulting mixture was stirred for 10 min at which time 25% aqueous NH_3 or MEA (amount indicated in Table I) was added. Water was added so that total weight equaled 500 g. The resulting mixture was stirred for 10 min.

Procedure for bleaching tress and rinse/wash cycle. To a plastic weigh boat was added 3 g of dyeless chassis and 3 g of 20 volume Welloxon® developer (6% H_2O_2). The two gels were mixed thoroughly and the pH was measured [Mettler Toledo SevenEasy™ pH meter S20 (Columbus OH)] before applying to the desired 1.5 g hair tress. After uniformly applying the mixture to the tress, the hair was placed in a covered weigh boat and incubated in a 30°C oven for 30 min. After completion of the incubation period, the hair was removed from the oven and rinsed for 2 min (37 ± 2°C water at a flow rate of 1.0 ± 0.2 gal/min), shampoo (0.15 ml per tress) was added and massaged into the hair for 30 s followed by a 30-s rinse (massaging hair once in every 2 s). On completion, the hair was dried using a blow dryer on high heat/high air flow for 3 min (1 min per side + 1 min combing).

Shampoo/conditioner cycles. The tresses were subjected to additional treatments with Pantene® Fine Hair Solutions Flat to Volume shampoo and Pantene® Classic Conditioner. One complete cycle was performed as follows:

- The hair was wetted (37 ± 2°C water at a flow rate of 1.0 ± 0.2 gal/min) for 30 s.
- Shampoo (0.1 ml/g hair) was applied and the hair was massaged thoroughly for 30 s.
- The hair was rinsed (massaging every other second) for 30 s, blotted with a towel, and blown dry (high heat/high air flow) for 3 min (1 min each side with finger pressing; final minute brushing with hair brush).
- The hair was again wetted (37 ± 2°C water at a flow rate of 1.0 ± 0.2 gal/min) for 30 s.
- Shampoo (0.1 ml/g hair) was applied and the hair was massaged thoroughly for 30 s.
- The hair was rinsed (massaging every other second) for 30 s.
- Immediately after the second shampoo rinsing, conditioner (0.1 ml/tress; 1.5 g tresses) was applied for 30 s and rinsed for 30 s (massaging every 2 s).
- The hair was patted dry with a towel before being blown dry (high heat/high air flow) for 3 min (1 min each side with finger pressing; final minute brushing with hair brush).

The above steps were repeated an additional 17 times for a total of 18 cycles after each bleaching treatment.

Cysteic acid determination. Cysteic acid readings were obtained on a Perkin Elmer Spectrum 100 FT-IR (Waltham MA) with universal attenuated total reflectance (UATR) sampling accessory immediately following bleaching as well as after the wash cycles. The hair tress was fastened onto the baseplate of the UATR and was twisted three rotations, then 70 N was applied by means of a piston. The collected absorbance spectrum is normalized by setting the highest peak between 1000 and 2000 cm^{-1} to 1.5 AU. The invariant 1450 cm^{-1} peak is set to zero AU and the second derivative of the normalized spectrum is taken. The second derivative of the 1040 cm^{-1} peak (cysteic acid, S=O stretch) is multiplied by -10^4 to give the cysteic acid value. Four measurements were taken and the arithmetic mean of those measurements was calculated to give the output reading. The process was performed on four locations along the length of the hair tress. Values reported

are the arithmetic mean of all measurements of the four output readings for each bleaching cycle.

Scanning electron microscope (SEM) analysis. SEM images were obtained using a Hitachi S-3000N Scanning Electron Microscope with Oxford detector (Schaumburg, IL), at 500× magnification. The hair tress was separated with a wooden applicator stick so that *ca.* 70 fibers were lifted from the bulk of the tress. The fibers were cut and laid onto a 15 mm × 15 mm SPI aluminum mount that was coated with an adhesive polymer. Loose hair fibers were removed with tweezers and the ends of the fibers were cut and affixed to the disc with a carbon coating. The disc was allowed to dry for 5 min before placing it in the SEM sample chamber for analysis. SEM analysis was performed under low pressure (50 Pa) at 500× magnification examining *ca.* 50 hair fibers for cuticle damage.

Preparation of samples for protein loss analysis. To a plastic weigh boat was added 3 g dyeless tint (0.82 M MEA or NH_3 chassis) and 3 g of 20 volume Welloxon® developer. The two gels were mixed thoroughly before applying to a 1.5 g light brown hair tress. After uniformly applying the mixture to the tress, the hair was placed in a covered weigh boat and incubated in a 30°C oven for 30 min. After completion of the incubation period, the hair was removed from the oven and rinsed for 2 min (37 ± 2°C water at a flow rate of 1.0 ± 0.2 gal/min), shampoo (0.15 ml per tress) was added and massaged into the hair for 30 s followed by a 30 s rinse (massaging the hair once in every 2 s). On completion, the hair was dried using a blow dryer on high heat/high air flow for 3 min (1 min per side + 1 min combing).

Protein loss measurements. Protein loss analysis was performed on hair tresses after a single bleaching cycle using a modified Lowry Assay against a porcine gelatin standard (8), for both the soluble and the insoluble fractions. Hair samples (0.2–0.3 g) were added to scintillation vials. Water was added at a ratio of 10 ml water per gram of hair. Samples were shaken for 1 h at 2500 rpm on a DVX-2500 Multi-2 Vortexer platform (Radnor, PA). For direct measurement of protein, samples were subjected to centrifugation at 14,000 rpm to separate the soluble/insoluble fractions. Pelleted material (insoluble) was solubilized in 3 M urea, 1 M NaOH, 0.06% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate followed by sonication for 30 min in a Branson B300 sonicating water bath (34 kHz) (Danbury, CT). Four replicates were done per sample, and the reported values are the arithmetic mean of the four individual values.

RESULTS AND DISCUSSION

Given that MEA–peroxide is less effective for lightening hair than ammonia–peroxide at equimolar concentrations, we were interested in beginning to quantitate other trade-offs associated with its use. When hair is subjected to oxidative conditions and damage is expected, it is common to measure cysteic acid levels to quantitate that damage. However, this can be somewhat misleading in cases in which other damage pathways exist, and in which cuticle is removed. Because our desire was to have a more complete idea of damage for Level 3 oxidation dye formulations containing MEA versus ammonia, we also used SEM as a damage measure, and for the potentially most extreme examples of high alkalizer concentration, we incorporated protein loss methods that have been successfully used for hair color product damage measurements (9).

Table II
Cysteic Acid Content of Light Brown Hair [Determined by Fourier Transform Infrared (FTIR) Spectroscopy] for Increasing Alkalizer Concentrations and Repetitive Lightening Cycles for Monoethanolamine (MEA) and Ammonia Formulations After 1–5 Bleach Treatments with 18 Shampoo/Conditioner Treatments (as Described Earlier) Between Each Cycle

Alkalizer	Alkalizer (M)	Cysteic Acid–1	Cysteic Acid–2	Cysteic Acid–3	Cysteic Acid–4	Cysteic Acid–5
NH_4OH	0.27	47 ± 3	57 ± 4	68 ± 3	78 ± 5	79 ± 4
MEA	0.27	45 ± 6	61 ± 6	73 ± 5	83 ± 5	88 ± 4
NH_4OH	0.54	45 ± 6	64 ± 2	85 ± 2	90 ± 5	92 ± 3
MEA	0.54	52 ± 7	68 ± 5	86 ± 5	93 ± 6	99 ± 5
NH_4OH	0.82	52 ± 6	75 ± 7	83 ± 6	96 ± 5	104 ± 7
MEA	0.82	55 ± 6	73 ± 4	88 ± 3	101 ± 4	107 ± 3

There are several variables to consider as we determine the effect of a switch from ammonia to MEA as an alkalizer for Level 3 oxidation dye products, important among these is alkalizer concentration. To determine its effect, we prepared formulations without dyes at several molarities of alkalizing agent (0.27 M, 0.54 M, and 0.82 M on-tress concentrations, after mixing with H_2O_2 developer). For each level, batches were prepared using equimolar amounts of NH_3 or MEA (unbuffered), and treatments were done on light brown hair. Protonic equilibria were not considered at this point because they are rapid and we are investigating extent of damage rather than rate of damage, and in the chassis we used, the ambient pH values of the equimolar solutions of MEA and ammonia with H_2O_2 are close (*ca.* ±0.1 units) at each alkalizer concentration. We used five bleaching cycles to simulate approximately 6 months of product usage, and we combined this with 36 shampoo/conditioner cycles between each bleaching to mimic the grooming routine between coloring processes. Cysteic acid levels were measured after each bleach cycle (before washing) and after 36 shampoo/conditioner treatments.

Table II shows that although the values are close within the given sets, there is a trend of increase in cysteic acid content that is dependent on alkalizer concentration and the number

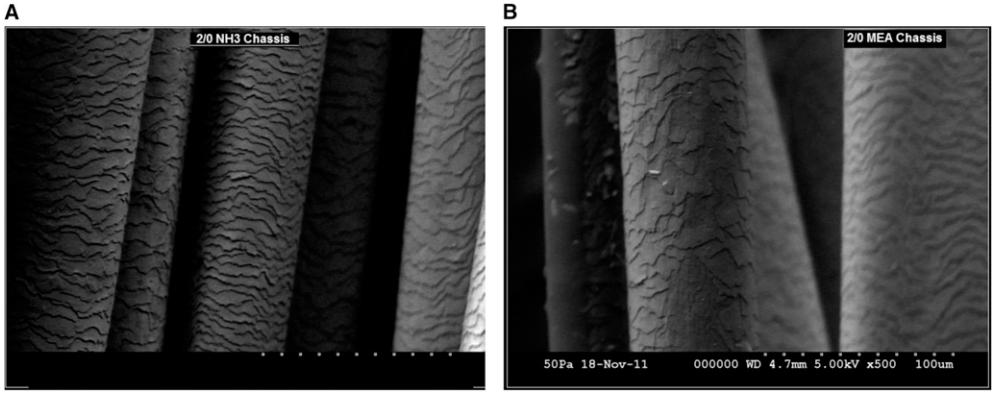

Figure 1. SEM photomicrographs obtained on a Hitachi S-3000N SEM with Oxford detector after the fifth bleaching cycle of light brown hair treated with formulations containing 0.27 M ammonia (A) and MEA (B) for 30 min at 30°C.

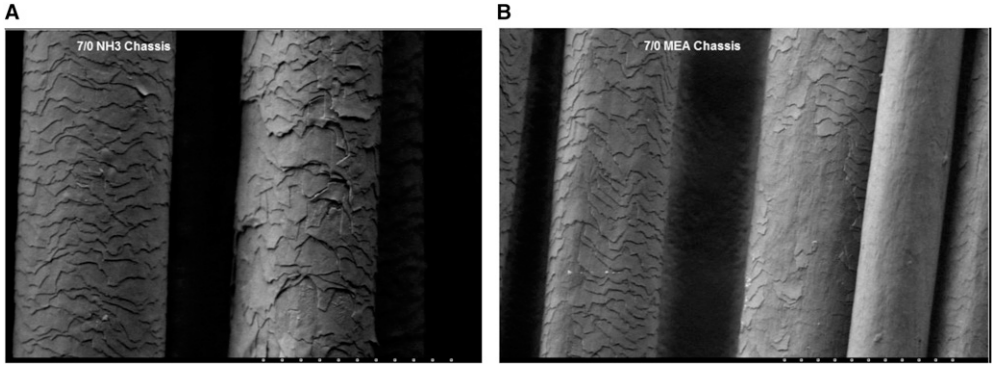

Figure 2. SEM photomicrographs obtained on a Hitachi S-3000N SEM with Oxford detector after the fifth bleaching cycle of light brown hair treated with formulations containing 0.54 M ammonia (A) and MEA (B) for 30 min at 30°C.

of treatments for a particular alkalizer. Cysteic acid content really did not break out large differences between ammonia- and MEA-based chassis, and better measures were needed.

SEM photomicrographs were obtained to complement the cysteic acid values because it is known that cuticle, where the highest percentage of cystine resides, is removed somewhat during chemical treatments and mechanical abrasion. Although the increase in damage as a function of increasing concentrations of alkalizers and increasing numbers of treatments is as we would expect, i.e., more treatments generate more damage, we wanted to ensure that we had a complete damage assessment, because if cuticle is removed, the cysteic acid value may be misleading. Figures 1 to 3 show SEM images for hair treated with 0.27 M, 0.54 M, and 0.82 M alkalizer.

Note that cuticle stripping, and in some cases complete removal of the cuticle, increases as the concentration on MEA is increased. Although there is damage caused by the ammonia formulation, it is less so than for the MEA formulation.

Figures 1 to 3 show that in each case, MEA causes more cuticle lifting and removal than the corresponding ammonia formulation at the same molar concentration.

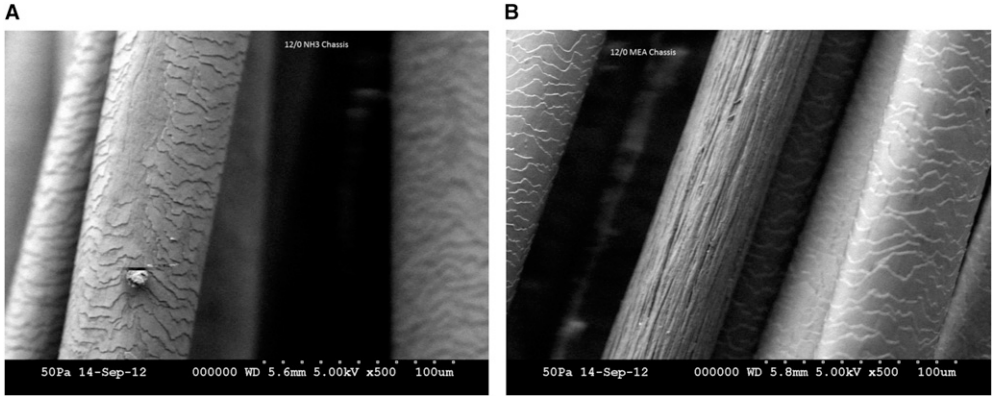

Figure 3. SEM photomicrographs obtained on a Hitachi S-3000N SEM with Oxford detector after the fifth bleaching cycle of light brown hair treated with formulations containing 0.82 M ammonia (A) and MEA (B) for 30 min at 40°C.

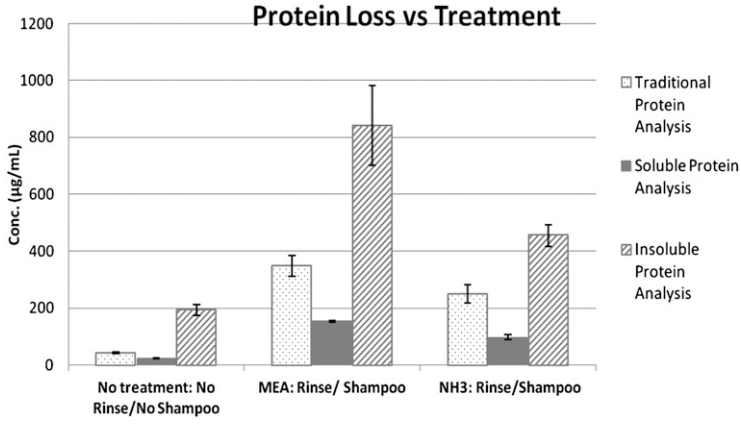

Figure 4. Traditional, soluble, and insoluble protein fractions of hair treated with a 0.82 M MEA/3% H_2O_2 formulation (30°C) or a 0.82 M NH_3/3% H_2O_2 formulation (30°C), measured with the modified Lowry method kit (8).

Given that both Fourier transform infrared spectroscopy (FTIR) cysteic acid analysis and SEM show the most damage for the 0.82 M alkalizer concentration, we performed protein loss analysis on this concentration of both the ammonia and the MEA formulations (Figure 4).

Protein loss analysis complements the cysteic acid method that measures oxidative damage. It enables us to quantify damage that may be due to nucleophilic attack on the keratin fibers by species generated by peroxide (e.g., –OOH), –OH, NH_3, or MEA, as well as the oxidative damage. Naqvi *et al.* (9) have shown that their method to measure protein loss, which quantifies soluble and insoluble protein separately, provides a better understanding of the form in which protein is lost and is a more accurate representation. Traditional protein loss method measurements are included to demonstrate that the observed effect of increased damage by MEA-containing formulations is not a function of the method. Samples of hair were treated once with either a 0.82 M MEA or a 0.82 M ammonia formulation (6% H_2O_2; 30°C). The tresses were rinsed and shampooed once by the procedure described earlier.

By the traditional method (measuring both soluble and insoluble proteins together), we demonstrate that the MEA-based formulation generates 40% more protein loss (damage) than the standard ammonia formulation. Similarly, soluble and insoluble protein losses show 58% and 85% more damage, respectively. This is in keeping with the findings of the original reference, which shows that the traditional protein loss measurement method generally underrepresents the total protein loss.

Clearly, the use of MEA rather than ammonia at 0.82 M (5% by weight), a common concentration of MEA used for higher levels of lift in Level 3 products, results in more protein loss (damage) than an ammonia-based product, even after only one treatment.

CONCLUSIONS

There are trade-offs for less odor in Level 3 hair color: performance and damage. The trade-off in performance (extent of lightening at maximum accepted alkalizer concentration) is

well known. However, more concerning is that if MEA is substituted for ammonia, using cysteic acid level as a measure of damage can be somewhat misleading. However, SEM showing cuticle removal and protein loss, clearly show damage increases for unbuffered equimolar solutions of MEA chassis versus ammonia chassis. Further investigation of other in-use variables and chassis is warranted, as is determination of the specific protein fragments that are lost from the hair. For the time being, though, if the ammonia odor of Level 3 products is a concern, a better approach may be to minimize its volatility rather than replacing it with a potentially more damaging alkalizer.

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