J. Cosmet. Sci., **62,** 383–391 (July/August 2011)

The uptake of water hardness metals by human hair

A. O. EVANS, J. M. MARSH, and R. R. WICKETT,
James L. Winkle College of Pharmacy, University of Cincinnati, 3225 Eden Avenue, Cincinnati, OH 45267-0004 (A.O.E., R.R.W.), and The Procter & Gamble Company, Miami Valley Innovation Center, 11810 East Miami River Road, Cincinnati, OH 45252 (J.M.M.).

Accepted for publication April 19, 2011.

Synopsis

The objective of this work was to examine the variables that influence the interaction between water hardness metals and human hair. Hair extracts various constituents from the tap water used during daily hygiene practices and chemical treatments. Calcium and magnesium metal ions are the most prevalent and give water "hardness." Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was employed to quantify the metal content of hair, which was studied as a function of the following variables: hair condition (oxidative damage), level of water hardness, and water pH. We have demonstrated that these variables impact water hardness metal uptake to varying extents, and the effects are driven primarily by the binding capacity (available anionic sites) of the hair. The condition of the hair, a key representation of the binding capacity, was most influential. Interestingly, water hardness levels had only a small effect on uptake; hair became saturated with notable amounts of water hardness metals even after repeated exposure to soft water. Water pH influenced metal uptake since side chains of hair proteins deprotonate with increasing alkalinity. These insights highlight the importance to the hair care industry of understanding the interaction between water hardness metals and hair.

INTRODUCTION

The uptake of metal cations, specifically redox active metals, by various types of keratin fibers has been investigated in the scientific literature (1–4). However, less attention has been focused on water hardness metals, particularly from a consumer-relevant standpoint. Calcium and magnesium metal ions are most prevalent in tap water and give water "hardness." Acting as an cation-exchange resin, human hair has been reported to extract up to 10,000 ppm of these metal ions from the tap water used during hygiene practices (5), and consumers associate the use of hard (and soft) water with differences in various structural and cosmetic properties of hair. Given that human hair is exposed to water for a significant portion of its lifetime, an understanding of the interaction between the components of water and hair is important.

Past research efforts have established the location of water hardness metals in hair. This work has indicated that calcium is present primarily in the cuticle of hair fibers. Kempson *et al.* and Mérigoux *et al*. used the imaging capabilities of time of flight secondary ion

mass spectrometry (ToF-SIMS) (6) and scanning X-ray fluorescence microscopy (SXRF) (7), respectively, to study calcium in hair. Mérigoux *et al.* found calcium in the cuticle layers and granules in the cortex and within the medulla. Kempson *et al.* showed calcium accumulations at the cuticle edges where carboxyl groups are likely available from the exposed endocuticle, as well as the aforementioned internal structures of this chemical nature. There is also evidence that calcium and magnesium may be present as salts of lipid material (8,9). Using nanoscale secondary ion mass spectrometry (Nano-SIMS), Smart *et al.* (10) confirmed calcium accumulation in the A-layer and exocuticle of bleached hair, areas containing large amounts of sulfur.

Although the uptake of redox metals by human hair has been documented as a function of solution metal concentration, solution pH, and chemical treatment of hair (11,12), only a few studies have examined the uptake of water hardness metals under these conditions. Uzu *et al.* (13) studied calcium uptake by cold-wave permed hair, and observed a positive correlation between uptake, solution pH, and hair treatment. Noble (14) examined the uptake of calcium and magnesium from tap and processed (bottled) water sources from various geographic locations. By measuring the change in the pH and the total hardness of the test waters before and after vortexing the hair in the water, he concluded that both parameters influenced the uptake of hardness metals by hair. As the hair samples were collected from men, women, and children, there was no consideration for the condition of the hair or any parameters that would differ with habits and practices, age, and/or sex.

The highlighted research efforts clearly provide a lead on understanding aspects of the interaction between water hardness metals and hair, but they also indicate the need for well-controlled, consumer-relevant work. We have addressed this opportunity area by systematically studying the uptake of calcium and magnesium as a function of the following key variables: hair condition, water hardness level, and water pH. We hypothesized that these variables influenced the uptake of water hardness metals by hair because they were related to the binding capacity and amount of metal ions available for interaction.

MATERIALS AND METHODS

HAIR SAMPLE PREPARATION AND CHARACTERIZATION

Virgin, dark brown Caucasian hair swatches were the starting substrate for this work (1.5 g/16 cm; International Hair Importers and Products, Glendale, NY). Slightly damaged and highly damaged hair substrates were prepared by treating this hair with a 12% active hydrogen peroxide oxidant crème for one cycle of 15 minutes and three cycles of one hour, respectively. The oxidant crème contained 5% Crodafos® CES (a mix of cetearyl alcohol, dicetylphosphate, and ceteth-10 phosphate; Croda) and 1.2% ammonium hydroxide. The oxidative bleaching treatment was carried out in a 30°C oven to simulate scalp temperature. Immediately following bleaching, the hair swatches were rinsed with deionized water (Milli-Q) for one minute, fan-dried, and equilibrated at room temperature for at least 24 hours before any further treatment. This was considered baseline for the swatches.

The condition of the virgin and bleached hair substrates was characterized by measuring surface cysteic acid content using a previously detailed Fourier transform infrared

spectroscopy (ATR-FTIR) method (5). Four measurements were averaged for each hair swatch. The swatches were then sorted into groups of three, which were balanced for cysteic acid. The lightness value (L*) was assessed to describe the degree of lightening imparted by the bleaching treatment. Eight measurements per hair swatch were made by a Konica Minolta CM3600D spectrophotometer under a D65 illuminant and 10° observer (Konica Minolta Opto, Tokyo, Japan). Table I summarizes the characterization of the hair substrates for this work.

HAIR SAMPLE TREATMENT

To test the effect of water hardness on metal uptake, virgin, slightly damaged, and highly damaged hair was subjected to six wash cycles in 2, 9, or 16 grain per gallon (gpg) water. These water hardness levels represent the categories of soft, moderately hard, and very hard water as identified by the U.S. Geological Survey. The unit originates from the conversion of calcium and magnesium to an equivalent weight of calcium carbonate. A grain is a mass unit that is equal to 64.8 mg of material, and the concentration of water hardness is expressed as this quantity in a gallon (3.78 l) of water. Hence, 1 gpg is equal to 17.11 ppm $CaCO_3$. The properties of the test water are summarized in Table II. Each wash cycle consisted of two thirty-second lathers with a commercial clarifying shampoo, thirty-second rinses before and after these two lathers, and fan-drying. The flow rate and temperature of the rinse water were 1.06 gallons per minute and 37°C, respectively.

To test the effect of water pH on metal uptake, virgin and slightly damaged hair swatches were subjected to ten cycles of treatment with synthetic water hardness solutions containing 1.2 mM calcium sulfate dihydrate (EMD Chemicals), 0.8 mM calcium chloride dihydrate (EMD Chemicals), and 1.2 mM magnesium sulfate (J.T. Baker Chemical) in buffers of pH 7 (5 mM bis-Tris; [bis(2-hydroxyethyl) amino)] tris(hydroxymethyl)methane; Organics Inc.); pH 8 (5 mM Tris; tris(hydroxymethyl) aminomethane; BDH Chemicals); or pH 9 (5 mM ethanolamine; BDH Chemicals). The selected pH values represent a consumer-relevant pH range (14), and the resulting hardness of the solutions was 17 gpg. One treatment cycle consisted of one hour of soaking at a 1:100 hair/liquor ratio

Table I
Characterization of Hair Substrates

Hair condition	Bleaching treatment	L*	FTIR cysteic acid units
Virgin	n/a	22	25
Slightly damaged	15 min	31	56
Highly damaged	1 hour × 3 cycles	49	155

Table II
Characterization of Treatment Water

Water hardness (gpg)	Ca (ppm)	Mg (ppm)	pH	Alkalinity (as ppm HCO_3)
2	11	4	8.4	80
9	40	13	8.4	175
16	71	23	8.5	285

(with stirring) and overnight drying in a fume hood. Hair swatches that were soaked in buffer solutions containing no hardness ions served as the appropriate controls. Metal uptake was calculated by subtracting the average elemental content of the control groups from the respective treated groups.

HAIR AND WATER ANALYSIS

The metal content of hair and water samples was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) with an Optima 5300 DV optical emission spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT). For hair analyses, 200–250 mg of samples were digested overnight with 2 ml of high-purity concentrated nitric acid (70% v/v, Aristar® Plus; BDH Chemicals). This mixture also contained 150 µl of 100 ppm yttrium internal standard (Inorganic Ventures, Christianburg, VA). The samples were then heated to 75°–80°C for one hour, cooled to room temperature, and diluted to 15 ml with deionized water. Three samples from each hair swatch of the treatment groups were analyzed. For water analyses, 10-ml water samples were acidified with 50 µl of trace metal analysis grade 50% v/v nitric acid. Three samples from each water treatment group were analyzed. The alkalinity of the water samples was determined using the Palintest Photometer 8000 and reagent tablets (Palintest Ltd., Erlanger, KY).

STATISTICAL ANALYSIS

The calcium and magnesium content of hair are reported as the mean plus/minus the standard deviation of three hair samples analyzed in triplicate. Treatment (water hardness, damage level, water pH) effects were assessed by univariate analysis of variance (ANOVA) and Tukey-Kramer HSD pairwise comparisons (JMP 7.0.2; SAS Institute Inc., Cary, NC). Multiple linear regression was conducted to further examine the influence of water hardness and hair condition on water hardness metal uptake by hair, and this relationship was characterized by the partial coefficient of determination (r^2) of each independent variable. Statistical significance was established at $p < 0.05$ for all analyses.

RESULTS AND DISCUSSION

The data highlighted the condition of the hair as the key driver for water hardness metal uptake. This was evidenced by the notable differences in metal content between the hair types within each water hardness group ($p < 0.001$) and supports the idea that the binding capacity of the hair is determined by the amount of anionic groups present within the fiber (Figure 1). Upon treatment with alkaline hydrogen peroxide products, peptide and disulfide bonds inside the hair and 18-methyleicosanoic acid (18-MEA) on the hair's surface are cleaved. This exposes anionic carboxylate and sulfonate (of cysteic acid) groups (15–17), which render the hair an ideal cation exchange resin. It should be noted that the calcium and magnesium levels of the highly damaged hair are comparable to the levels found in the hair of consumers who regularly use oxidative colorant products (5).

Selectivity for calcium over magnesium was exhibited by the hair substrates. The treated hair contained seven to nine times more calcium than magnesium, with the virgin hair lying at the lower end of this range and both levels of damaged hair equally lying at the

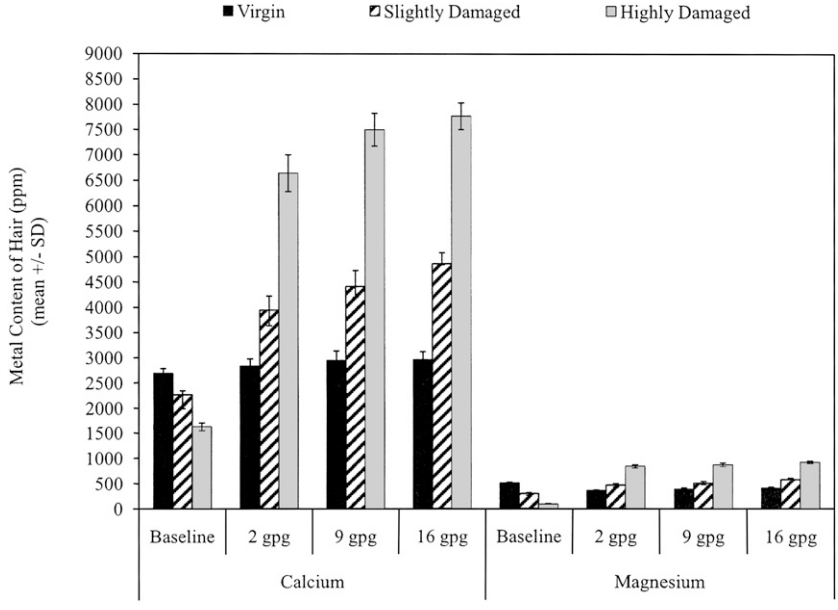

Figure 1. Effect of hair condition on the calcium and magnesium content of virgin and bleached (damaged) hair treated with water of different hardness levels. Calcium content was highly dependent on the condition (binding capacity) of the hair substrate. Tukey-Kramer HSD analysis yielded $p < 0.001$ for all hair condition comparisons within the tested water hardness levels.

upper end. This observation was independent of the water treatment since 3:1 Ca/Mg was maintained for all water hardness levels. However, the selectivity for calcium over magnesium cannot be ascribed solely to the predominance of calcium vs magnesium in the water. This effect has been previously observed in laboratory experiments where bleached hair contained four times more calcium than magnesium following multiple treatments with water containing 1:1 Ca/Mg. In addition to the predominance of calcium vs magnesium in the water, the sizes of the cations likely influenced their keratin-binding affinities. The site-binding interactions that occur between cations in solution and the anionic groups of a polyelectrolyte are governed in part by the hydration characteristics of the cations (18). Smaller cations possess large, tightly held hydration spheres due to high charge density, while the opposite is true of larger cations (19). Since calcium has a larger ionic radius (1.18 Å), and thus a smaller hydrated radius than magnesium (0.82 Å), the former has been reported to adsorb more easily to polyeletrolytes than the latter (19–21).

Baseline calcium and magnesium levels appeared to decrease as oxidative damage increased. However, we believe this trend was due to the diffusion of calcium and magnesium ions out of the swollen hair and into the deionized water used for rinsing the oxidant crème from the hair. This hypothesis has been confirmed by rinsing the oxidant crème from hair using local tap water (8 gpg; 1:1 Ca/Mg ratio). When freshly bleached hair was rinsed with the tap water, the final calcium and magnesium levels were equivalent to the levels in virgin/untreated hair.

The effect of water hardness levels was secondary to the condition of the hair; differences in metal uptake between the test water hardness levels were quite small in virgin hair, but they increased with oxidative hair damage (Figure 2). Interestingly, calcium uptake from hard

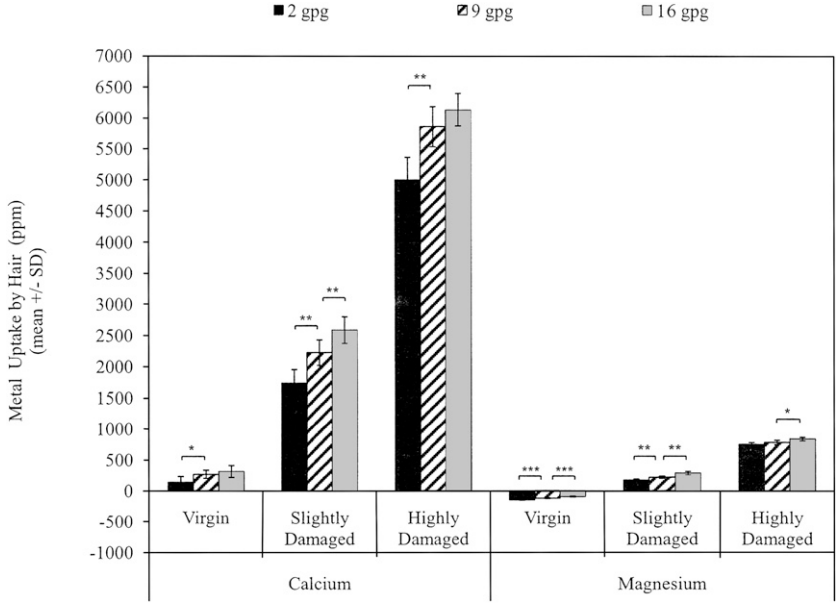

Figure 2. Effect of water hardness levels on calcium and magnesium uptake by virgin and bleached (damaged) hair. n=3. Asterisks (*, **, ***) represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, obtained by Tukey-Kramer HSD analysis. Uptake was calculated by subtracting the average baseline calcium and magnesium content of hair from that of hair treated with water of different hardness levels. Water hardness metal uptake was more driven by the condition or binding capacity of the hair substrate ($r^2 = 0.98$ and 0.99 for calcium and magnesium, respectively, $p < 0.001$) than by the hardness of the water ($r^2 = 0.59$ and 0.72 for calcium and magnesium, respectively, $p < 0.001$). It should be noted that the negative values of magnesium uptake by virgin hair indicate that the hair lost magnesium in an attempt to establish equilibrium between the few binding sites and the low amount of magnesium in the water samples. As the magnesium content in water increased, the hair absorbed more of the ion and approached the baseline levels.

(9 gpg) and very hard (16 gpg) water was not significantly different for virgin and highly damaged hair, and it was different by only 16% for slightly damaged hair ($p = 0.005$). This clearly indicates that the hair has a finite binding capacity, which is determined by the number of available anionic sites. Regardless of the water hardness level, the hair will reach saturation and not associate with any remaining hardness ions in the water. Even upon repeated exposure to soft water (2 gpg), both levels of damaged hair extracted large amounts of water hardness metals, and thus approached their saturation point. Based on a multiple linear regression analysis, it was found that calcium and magnesium uptake was very strongly correlated with hair condition ($r^2 = 0.98$ and 0.99, respectively, $p < 0.001$) and less correlated with water hardness ($r^2 = 0.59$ and 0.72, respectively, $p < 0.001$). This finding is significant because it establishes the fact that water hardness metal uptake is not confined to individuals who live in hard water regions. The popularity of hair treatments that alter the chemical nature of hair, e.g., coloring, bleaching, and relaxing, suggests that even more consumers are susceptible to this uptake if they are exposed to water with any degree of hardness ions.

A positive relationship between water pH and metal uptake was observed (Figure 3). This can be related to the binding capacity of the hair because as pH increases, more groups become available for metal interaction due to the progressive ionization of carboxyl groups

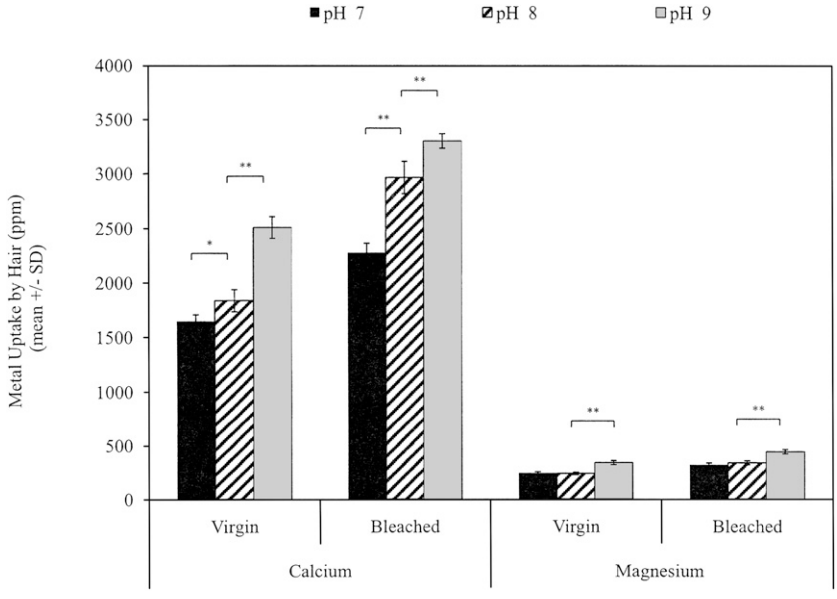

Figure 3. Effect of water pH on the uptake of calcium and magnesium by hair. Uptake was calculated by subtracting the average calcium and magnesium content of hair that was soaked in pure buffer from that of hair treated with buffer solutions containing hardness ions. Water pH influenced calcium and magnesium uptake. Asterisks (*, **) represent $p < 0.05$ and $p < 0.001$, respectively, obtained by Tukey-Kramer HSD analysis.

and amino groups participating in electrostatic interactions with carboxyl groups (4,22). Similar pH-dependent increases in the uptake of cationic moieties by keratin (14,23) and other charged macromolecules (24) have been reported. It is realized that the test pH range exceeds the observed pK_a values of key ionizable protein groups that are capable of binding metals, e.g., sulfonate groups of cysteic acid (pK_a = 1.3), terminal carboxyl groups (pK_a = 3.5-4.3), and carboxyl groups of aspartic and glutamic acids (pK_a = 3.9 and 4.3, respectively) (25). However, since keratin is a polyelectrolyte, the effective pK_a values of the amino acid residues are often different from the intrinsic pK_a values of the isolated amino acid monomers due to electrostatic interactions, hydrogen bonding, solvation effects, conformational changes, and the presence of appreciable levels of counterions (26,27). All of these factors can influence the ionization of protein groups and their ability to bind cations. Creighton (25) reported that the difference in electrostatic environments can cause the pK_a values of one type of amino acid residue to differ by 3–4 pH units within a single protein. It is, therefore, plausible that the pH dependence of metal uptake was influenced by this.

Based on these findings, we can conclude that the hair of consumers who use alkaline water will contain higher levels of water hardness metals than hair from those who use neutral or less alkaline water. This effect will be compounded if the consumer has chemically treated hair.

CONCLUSIONS

Interesting insights about the interaction between water hardness metals and hair have resulted from this work. Our findings suggest that the uptake of water hardness metals is driven primarily by the condition of the hair. Hair that contains more anionic moieties,

the result of chemical treatments such as bleaching and chemical relaxing, has a higher cationic binding capacity and is thus more susceptible to water hardness metal uptake than virgin hair. At a certain level, this binding capacity dominates the effect of water hardness levels, such that the hair will attract significant levels of metal from water that has even a low degree of hardness. This suggests that the effects of water hardness are not just confined to consumers that reside in areas of very hard water, but to a much wider population that encompasses residents of lower water hardness areas. Additionally, the pH of the rinse water can also influence uptake. We have clearly established the significance of water hardness metal uptake by human hair and the conditions under which this occurs. This uptake could potentially impact the structural properties of human hair and the performance of hair care formulations. An investigation of the effect of water hardness on hair properties will be detailed in a future publication.

REFERENCES

(1) P. Kar and M. Misra, Use of keratin fiber for separation of heavy metals from water, *J. Chem. Technol. Biotechnol.,* **79,** 1313–1319 (2004).
(2) S. Kokot, J. Cheng, and N. Gill, Comparative study of metal ion interactions with wool keratin using chemometrics, *Analyst,* **119,** 677–681 (1994).
(3) A. Sheffield and M. Doyle, Uptake of copper(II) by wool, *Textile Res. J.,* **75**(3), 203–207 (2005).
(4) P. Taddei, P. Monti, G. Freddi, T. Arai, and M. Tsukada, Binding of Co(II) and Cu(II) cations to chemically modified wool fibres: An IR investigation, *J. Mol. Struct.,* **650,** 105–113 (2003).
(5) J. M. Marsh, J. Flood, D. Domaschko, and N. Ramji, Hair coloring systems delivering color with reduced fiber damage, *J. Cosmet. Sci.,* **58,** 495–503 (2007).
(6) I. M. Kempson, W. M. Skinner, and P. K. Kirkbride, Calcium distributions in human hair by ToF-SIMS, *Biochim. Biophys. Acta,* **1624,** 1–5 (2003).
(7) C. Mérigoux, F. Briki, F. Sarrot-Reynauld, M. Salomé, B. Fayard, J. Susina, and J. Doucet, Evidence for various calcium sites in human hair shaft revealed by sub-micrometer X-ray fluorescence, *Biochim. Biophys. Acta,* **1619,** 53–58 (2003).
(8) K. M. Attar, M. A. Abdel-Aal, and P. Debayle, Distribution of trace elements in the lipid and nonlipid matter of hair, *Clin. Chem.,* **36**(3): 477–480 (1990).
(9) L. Bertrand, J. Doucet, A. Simionovici, G. Tsoucaris, and P. Walter, Lead-revealed lipid organization in human hair, *Biochim. Biophys. Acta,* **1620,** 218–224 (2003).
(10) K. E. Smart, M. Kilburn, M. Schroeder, B. G. H. Martin, C. Hawes, J. M. Marsh, and C. R. M. Grovenor, Copper and calcium uptake in colored hair, *J. Cosmet. Sci.,* **60,** 337–345 (2009).
(11) G. R. Bhat, E. R. Lukenbach, and R. R. Kennedy, The green hair problem: A preliminary investigation, *J. Soc. Cosmet. Chem.,* **30,** 1–8 (1979).
(12) T. C. Tan, C. K. Chia, and C. K. Teo, Uptake of metal ions by chemically treated human hair, *Water Res.,* **19**(2), 157–162 (1985).
(13) A. Uzu, T. Watanabe, H. Ogino, and H. Hirota, Study on the sorption of calcium ion to human hair, *J. Soc. Cosmet. Chem. Japan,* **22**(2), 88–95 (1988).
(14) R. E. Noble, Uptake of calcium and magnesium by human scalp hair from waters for different geographic locations, *Sci. Total Environ.,* **239,** 189–193 (1999).
(15) M. A. Stranick, Determination of negative binding sites on hair surfaces using XPS and Ba^{2+} labeling, *Surf. Interface Anal.,* **24,** 522–528 (1996).
(16) T. D. Doering, C. Brockmann, A. Wadle, D. Hollenberg, and T. Förster, Super mild oxidation coloring: Preventing hair damage at the molecular level, *IFSCC Mag.,* **10**(4), 323–329 (2007).
(17) W. W. Edman and M. E. Marti, Properties of peroxide-bleached hair, *J. Soc. Cosmet. Chem.,* **12,** 122–145 (1961).
(18) U. P. Strauss and Y. P. Leung, Volume changes as a criterion for site binding of counterions by polyelectrolytes, *J. Am. Chem. Soc.,* **87,** 1476–1480 (1965).
(19) B. Tansel, J. Sager, T. Rector, J. Garland, R. Strayer, L. Levine, M. Roberts, M. Hummerick, and J. Bauer, Significance of hydrated radius and hydration shells on ionic permeability during nanofiltration in dead end and cross flow modes, *Sep. Purif. Technol.,* **51,** 40–47 (2006).

(20) A. E. Martell and M. Calvin, *Chemistry of the Metal Chelate Compounds* (Prentice-Hall, New York, 1952), p. 434.
(21) J. Mattai and J. Twak, Divalent metal ion binding to polyelectrolytes with different polyion structure and functional groups, *Macromolecules,* **19,** 1663–1667 (1986).
(22) T. Hinners, W. Terrill, J. Kent, and A. Colucci, Hair—Metal binding, *Environ. Health Perspect.,* **8,** 191–199 (1974).
(23) I. W. Stapleton, The adsorption of long chain amines and diamines on keratin fibers, *J. Soc. Cosmet. Chem.,* **34,** 285–300 (1983).
(24) W. N. Charman, D. P. Christy, E. P. Geunin, and D. C. Monkhouse, Interaction between calcium, a model divalent cation, and a range of poly(acrylic acid) resins as a function of solution pH, *Drug Dev. Ind. Pharm.,* **17**, 271–280 (1991).
(25) T. E. Creighton, *Proteins: Structures and Molecular Properties, 2nd Ed.* (W.H. Freeman, New York, 1993), p. 272.
(26) J. B. Matthew, Electrostatic effects in proteins, *Ann. Rev. Biophys. Biophys. Chem.,* **14,** 387–417 (1985).
(27) S. Mafé, V. García-Morales, and P. Ramírez, Estimation of pK_a shifts in weak polyacids using a simple molecular model: Effects of strong polybases, hydrogen bonding and divalent counterion binding, *J. Chem. Phys.,* **296,** 29–35 (2004).