Examining cationic polysaccharide deposition onto keratin surfaces through biopolymer fluorescent labeling

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Synopsis

Fluorescein-labeled polyquaternium-10 and guar hydroxypropyltrimonium chloride were employed to study the deposition behavior of these cationic polymers onto hair from a surfactant system. The influence of the covalently attached fluorescein dye on labeled polyquaternium-10 was examined through rheological studies and comparative studies against data previously obtained from radiolabeled polyquaternium-10. A quantitative method for analyzing the amount of cationic polymer that deposits onto hair during a standard shampooing process has been developed using the labeled cationic polymers. The technique requires digestion of the hair and analysis of the resulting solutions against known standardization curves. It has been found that the molecular weight of the cationic polymers plays the most significant role in influencing the deposition of the polymers from surfactant, a far greater role than either cationic charge or washing cycles. The technique also allows for determination of polymer deposition at various tress locations, allowing for the study of the influence of tress age (i.e., damage) on polymer deposition. The use of fluorescein-labeled polyquaternium-10 also provides a unique opportunity to visualize the deposited polymers on individual hair fibers via fluorescent or confocal microscopy.

INTRODUCTION

It is now widely accepted that cationic polymers form deposits onto keratin surfaces treated with polymer/surfactant systems. This was not always the case. It was only through the painstaking application of various analytical methodologies that this fact of great importance to the cosmetic industry was established beyond doubt. Qualitative indication of cationic polymer deposition was gathered, for example, from streaming potential and ESCA analysis of treated hair (1). There are still only a few methods to

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measure the amount of cationic polymer deposited on a keratin surface. The only truly quantitative method was developed by Goddard and coworkers nearly 30 years ago. It requires the use of radiolabeled cationic polymers (2,3). While this technology is quite powerful and accurate, it is rarely used nowadays, as most personal care research laboratories do not have facilities allowing them to use radioactive isotopes. Moreover, it was used mostly to investigate model systems and was only rarely applied to study cationic polymer deposition from surfactant systems (4,5). A titration technique was developed more recently. It requires the use of anionic polymers that form complexes with deposited cationic polymers (6). Such technique does not provide meaningful information if the cationic polymer is deposited in the presence of anionic surfactants, as in the case of body washes or shampoos studies, since the surfactant interferes with the complexation of oppositely charged polymers. Fluorescent and colored dyes have been employed to try and measure cationic polymer deposition (7). However, these dyes are usually added after treatment to the observed keratin surface, where they are expected to interact only with the cationic polymer. Thus, the potential for quantitative error can be significant.

There have been several recent reports on the covalent attachment of a fluorescent dye to a biopolymer, converting a naturally spectroscopically invisible polymer into a spectroscopically visible species. The labeling of proteins (8,9), chitosan (10), dextrans (11), guar (12), and carboxymethylcellulose (13) has been discussed in the literature, and the potential use of these fluorescent polymers in personal care studies has been considered (8,9,12). One of us reported recently the preparation and characterization of a fluorescently labeled cationic hydroxyethyl cellulose using a triazine-modified fluorescein derivative (Scheme 1) (14,15). The labeled cationic polymers were employed in model studies (14). We report here the use of these polymers to study deposition from shampoos. Aspects of this work have been addressed recently (16).

DTAF, 5-(4,6-dichlorotriazinyl)aminofluorescein
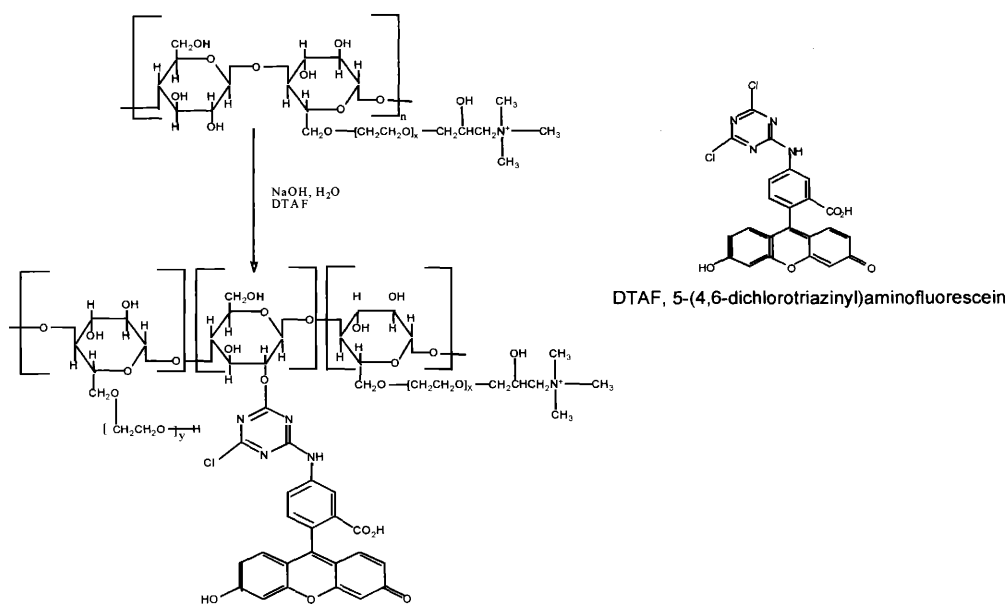
Scheme 1.

EXPERIMENTAL

The preparation, purification, and characterization of fluorescein-modified cationic polysaccharides have been reported elsewhere (14). The same techniques were used to label the cationic polysaccharides used in this study, namely several cationic hydroxyethyl celluloses (polyquaternium-10) and cationic polygalactomannans (guar hydroxypropyltrimonium chloride). Table I shows the cationic polymers examined in this study, their solution viscosities, their cationic charge levels, and the levels of fluorescent dye incorporated. The compositions of the shampoos prepared with the polymers are shown in Table II. Each shampoo was maintained at a pH of 7.0 that provides an optimum pH for fluorescent performance of the dye (13). The polymers were employed at 0.5 wt% in the shampoos for the purposes of this study.

Five-gram swatches of virgin blond hair tresses, from one batch of hair purchased from DeMeo Brothers, were washed once with C11-15 Pareth-9, a non-ionic surfactant (Union Carbide, Danbury, CT), to remove residual oils and surfactants from the hair. Based on previous work, this treatment was considered to be the most practical for bringing all of the hair fibers into a nearly equivalent ionic state (4). One milliliter of the shampoo formulation was spread at three sites along each tress, and the tress was washed for one minute, rinsed in warm water (30°C) for one minute, and dried using a commercial 1500-watt hair dryer. Each data point represents the average value of measurements from five individual tresses washed one time and, in some cases, ten times with each shampoo. In multiple washing studies, the tresses were dried after each shampoo treatment.

One gram of hair was carefully removed from the middle of each tress (unless otherwise specified). It was placed into 99 grams of a 3 wt% NaOH solution. The strongly alkaline solution was kept in the dark at room temperature for 24 hours, whereupon the hair disintegrates and dissolves. The resulting solution was neutralized to pH 7 using aqueous HCl. All types of hair samples disintegrate in this alkaline medium; however, virgin blond hair yields solutions that are nearly void of undissolved residues. Filtration of each solution through a fine fritted glass filter is performed nonetheless. A very small residue was recovered. This residue did not show any fluorescence upon irradiation with a UV light, implying that the amount of polymer trapped, if any at all, is extremely low. The solutions recovered after filtration were then examined by fluorescence spectroscopy, and

Table I
Cationic Polymers

Polymer	INCI name	Viscosity[3]	Dye level[4] (mol g^{-1} polymer)	Charge level[5]
A[1]	Polyquaternium-10	Low	3.2×10^{-5}	High
B[1]	Polyquaternium-10	Low	1.0×10^{-4}	Low
C[1]	Polyquaternium-10	High	5.4×10^{-5}	High
D[2]	Guar hydroxypropyl trimonium chloride	Low	3.7×10^{-5}	Low
E[2]	Guar hydroxypropyl trimonium chloride	High	3.6×10^{-5}	Low

[1] Available from Amerchol Corporation, Edison, NJ.
[2] Available from Rhone-Poulenc, Cranbury, NJ.
[3] Low is <1000 cps at 25°C; high is >1000 cps at 25°C (Brookfield viscosity).
[4] Determined by UV-Vis spectroscopy (14).
[5] Low is <1.2% cationic nitrogen; high is >1.2% cationic nitrogen.

Table II
Shampoo Ingredients

Ingredient[1]	Percent active
Ammonium lauryl sulfate[2]	14.0
Ammonium laureth-3 sulfate[2]	3.9
Cocamidopropyl betaine[2]	3
Cationic polymer	0.5
DMDM hydantoin	0.4
Deionized water	78.2

[1] Nomenclature taken from *International Cosmetics Ingredients Dictionary.*
[2] Available from Henkel Corporation, Hoboken, NJ.

the quantity of deposited polymer was determined by using standardization curves established previously with known quantities of each labeled polymer (17).

Fluorescence images of individual hair fibers were taken using a Zeiss LSM10 laser scanning microscope equipped with Zeiss 20× and 10× objectives. An argon ion laser (488 nm) was used to image green emission. The images were acquired with a digital Sony 3 CCD camera. The hair samples were mounted on clean glass slides using a nonfluorescing aqueous/dry mounting medium (14). Rheological studies were conducted on a Bohlin CS rheometer (Bohlin Instruments, Cranbury, NJ) using a double-gap (DG) 40/50 cup and bob geometry. The rheological studies were run at 30°C, and the measurements were averaged over 40 seconds with a 0.1-second delay between measurements. Polymers employed in the rheological studies were dialyzed against distilled water (Spectra/Por® Membranes, 1000 MWCO, Spectrum Company, Houston, TX) and lyophilized prior to use.

RESULTS AND DISCUSSION

EFFECTS OF THE FLUORESCENT DYE ON POLYMER BEHAVIOR

The fluorescent labeling of cationic hydroxyethyl cellulose was reported earlier (14) (Scheme I). The dye is a modified fluorescein. It is linked to the polysaccharide backbone through an ether linkage between a reactive triazine on the dye and an available hydroxyl group on the polysaccharide. The reaction occurs readily with various polysaccharides. For the present study we prepared labeled cationic hydroxyethyl cellulose and cationic polygalactomannan. Determination of the degree of substitution of the dye, and assessment of the purity of the resulting polymer, i.e., the absence of unreacted dye, were carried out as described earlier (14).

In his seminal studies on the deposition of radiolabeled polyquaternium-10, Goddard reported that in the case of cationic polymers deposited directly from aqueous solution, polymers of lower molecular weight tend to deposit more quickly and more heavily (4,5). Goddard attributed the stronger deposition of the low-molecular-weight polymers to their higher mobility. He took it also as an indication of better penetration of these materials into the intercuticular spaces. Preliminary studies were done to reproduce with the fluorescently labeled polymers the experiments carried out by Goddard and cowork-

ers with radiolabeled polymers. Surprisingly good agreement between the two methods was obtained in the case of polyquaternium-10 deposition on hair (17).

Goddard also studied the deposition of radiolabeled polyquaternium-10 from various simple surfactant platforms (5). The surfactants of most commercial relevance included triethanolamine lauryl sulfate (TEALS) and sodium laureth-3 sulfate (SLES). We reproduced these deposition studies as closely as possible using fluorescently labeled polyquaternium-10 samples of charge and molecular weight characteristics similar to those of the polymers employed by Goddard in his studies. The results of our investigations are shown in Figure 1.

The reproducibility of the deposition measurements for both labeling techniques from the strongly anionic surfactant, TEALS, is good. However, we note that from the milder surfactant system, SLES, deposition of the radiolabeled cationic polymer is greater than that seen for the fluorescently labeled polymer. The reasons for this discrepancy are unknown. One possible explanation is that the dye attached to the polymer somehow affects its deposition on hair when used in a milder surfactant system. Other factors might account for the discrepancy as well. For example, one cannot exclude the possibility of changes in the raw materials used in the original studies twenty-five years ago. Also, Goddard performed his deposition studies on virgin brown hair, while we have conducted ours on virgin blond. We do not believe this change accounts for the differences noted.

To assess whether or not the attached dye affects the behavior of the modified polyquaternium-10 in the presence of strongly anionic surfactants, we investigated the solution rheology of the labeled polymer both alone and in the presence of sodium dodecyl sulfate, SDS, at a concentration high enough to cause gelling of the polymer solution. This latter polymer/surfactant solution behavior was described previously by Goddard (18). The interaction of the cationic polymer and anionic surfactant leading to coacervate phases has been investigated microscopically (19).
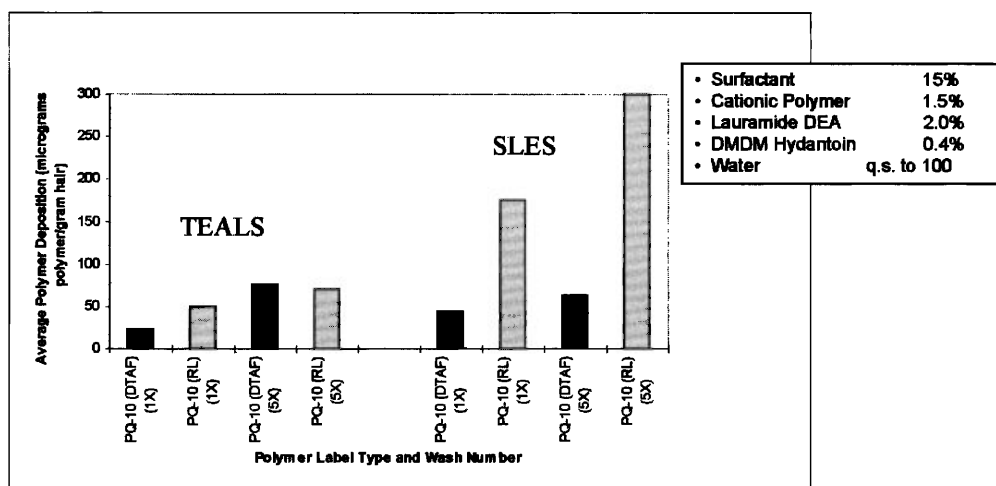

Figure 1. Comparative study between radiolabeled polyquaternium-10 (grey bars) and fluorescently labeled polyquaternium-10 (black bars) from shampoos comprised of the ingredients shown in the small appended table (5). The data on the left was taken from a TEALS-containing shampoo and the data on the right from a shampoo with SLES. Data was generated for one and five wash cycles.

Examination of the solution viscosity of labeled and unlabeled Polymer A shows that the labeled polymer provides a slightly higher viscosity, but its shear-thinning rheology is comparable to that of the unlabeled polymer (Figure 2). The viscosity increase may be attributed to subtle changes of the hydrophobic or electrostatic interactions occurring between polymer chains upon attachment of a few dye residues. The effects intensify in the case of very-high-molecular-weight polymers. It is well known that slight changes in hydrophobicity cause significant changes in the solution behavior of high-molecular-weight polymers.

When we examined the same two polymers in the presence of 0.25 wt% of SDS, we noted that both polymers build significant aqueous solution viscosity, as demonstrated earlier by Goddard (Figure 2). In this case too, labeled and unlabeled polymers produce gels of identical viscosity, although the labeled polymer solutions show some indication of enhanced sensitivity to high shear, as indicated by the disruption of solution viscosity at lower shear rate for this gel. However, we do not consider this change to be significant enough to interfere with the expected deposition mechanism for these polymers.

QUANTITATIVE ANALYSIS OF POLYMER DEPOSITION ON HAIR

It is generally acknowledged that cationic polymers deposited onto hair from shampoos bind to the surface as an associative complex of polymer and surfactant. Therefore, it is not possible to extrapolate deposition data obtained from simple polymer solutions to the behavior of the same cationic polymer in the presence of surfactants. For this reason,
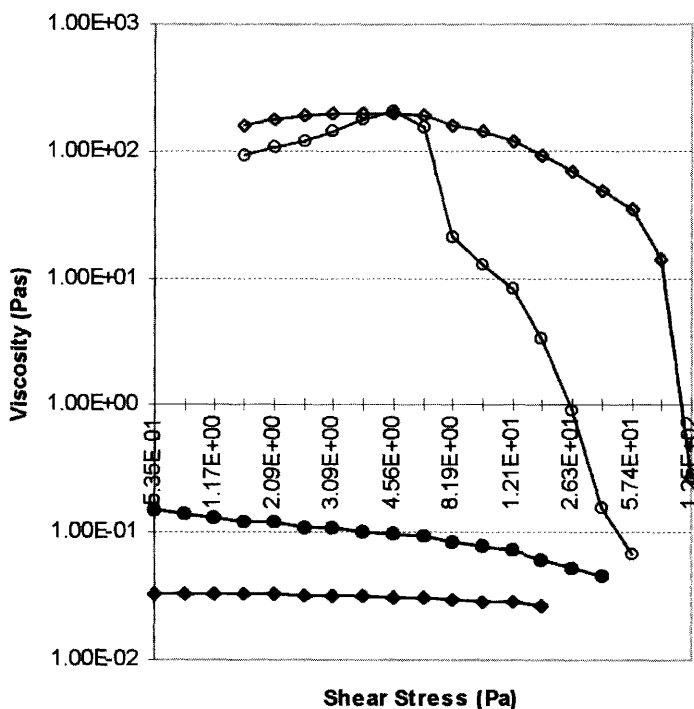

Figure 2. Viscosity versus shear rate response for labeled ($\bigcirc$, $\bullet$) and unlabeled ($\Diamond$, $\blacklozenge$) Polymer A as 1 wt% solutions (closed symbols) and as 1 wt% solutions gelled with 0.25 wt% SDS (open symbols).

we developed the model shampoo system shown in Table II. This shampoo formulation is comprised principally of a strong anionic surfactant, ammonium lauryl sulfate (ALS), a surfactant commonly used in the personal care industry. It closely mimics the TEALS surfactant used in the model studies described previously. It is critical to keep in mind that the data presented below, strictly speaking, apply only to the deposition onto virgin blond hair of the labeled polymers present in a shampoo prepared according to the formulation presented in Table II. Any changes to the shampoo formulation, or changes in hair type, are likely to change the polymer deposition and adsorption behavior.

Influence of cationic polymer viscosity (molecular weight) on deposition. We investigated the effect of polymer viscosity on the cationic polysaccharide's deposition (Figure 3). For this study, we selected Polymers A and C, two polyquaternium-10 samples of similar cationic substitution but different viscosities, and Polymers D and E, two commercial cationic guar derivatives of similar charge levels but different viscosities. As seen in Figure 3, the influence of polymer molecular weight on polymer deposition is significant. For both types of cationic polysaccharides, the deposition of the high-viscosity (high-molecular-weight) polymers is significantly higher than the deposition of the corresponding low-viscosity (low-molecular-weight) polymers. For polyquaternium-10, this trend is the opposite of the sorption behavior reported by Goddard for the polymers when the same polymers were delivered to hair from aqueous solutions, in the absence of surfactant (5)! This result confirms the significant impact of the surfactant on the deposition and adsorption of cationic polymers onto keratin surfaces. This sorption behavior may be related, in part, to the rheology of the polymer/surfactant complexes formed during deposition. Higher-molecular-weight polymers form more viscous associative complexes, hindering the removal of the deposits from the hair during rinsing.

Effect of cationic charge level. To assess the effect of charge on polymer deposition from shampoos, we selected Polymers A and B, which vary in their levels of cationic charge. Polymer A has twice the level of cationic charge, compared to Polymer B, but the two polymers have similar molecular weights. We did not detect a significant difference in the amount of polymer deposited from these two shampoos, at least within the range of
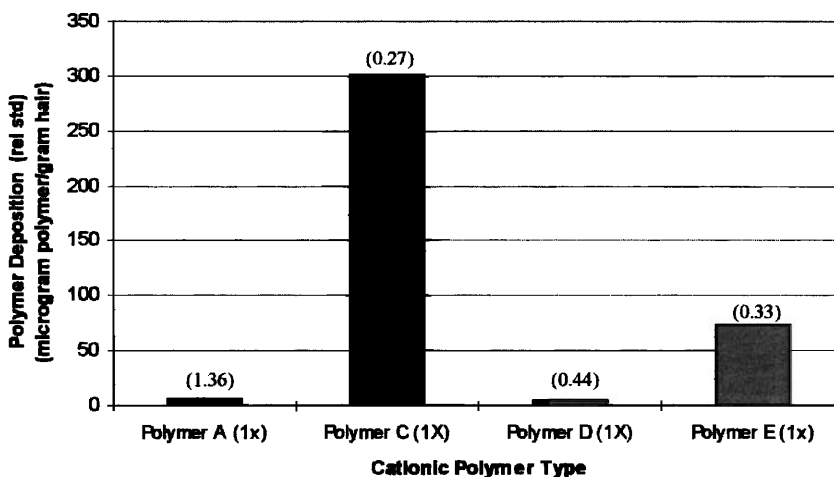

Figure 3. Deposition data (including relative standard deviations) for low-viscosity polymers A and D and high-viscosity polymers C and E after a single wash cycle.

experimental error of the technique (Figure 4). Therefore, while it is important that the polymers carry positive charges for deposition to occur, the amount of charge does not affect deposition to the same extent as molecular weight. It should be pointed out that Goddard demonstrated also by radiolabeling studies that cationic charge was essential for polymer adsorption onto hair and skin (5).

Effect of multiple washings on polymer deposition. The effect of multiple washing cycles on polymer deposition is critical information that the formulator needs to know in order to understand the mechanism of polymer build-up. The perception of build-up of a product, such as a conditioning shampoo, as a result of its repetitive use is detrimental to its acceptability by consumers. The study of the effect of multiple washings on cationic polymer deposition was carried out with a low-viscosity polyquaternium-10 sample, Polymer B. In the case of the strong anionic surfactant platform used here, the sorption of polyquaternium-10 is relatively constant through the course of ten washing cycles (Figure 5). No significant build-up of polyquaternium-10 on the hair tress was detected, within the range of error for the measuring technique. Indeed, polymer build-up depends critically on the hair type and on the nature of the shampoo composition, in particular the choice of surfactants, the polymer concentration, and physical properties.

Polymer deposition at different tress locations. It is well known that as hair ages, damage from various physical, environmental, and chemical factors gradually erodes the protective cuticle layers, eventually exposing the native proteins of the hair cortex. Virgin hair has not been exposed to the ravages of chemical dyeing and perming and is, therefore, relatively damage-free. We examined the amount of polymer deposited in three different locations on the virgin blond hair tresses using a shampoo made with high-viscosity polyquaternium-10, Polymer C. Deposition at the distal end and middle of the tress was similar, but there appeared to be slight reduction in deposition at the proximal end of the tress (Figure 6). This was not unexpected, as the proximal end of the tress is newer hair. We should point out also that during preparation, the tresses were rinsed in such a way that the rinse water flowed from the proximal to the distal end of the hair tress. Thus, it is not unreasonable to consider the possibility that rinsing moves the more weakly bonded polymers down the tress. As this rinsing pattern is typical of standard
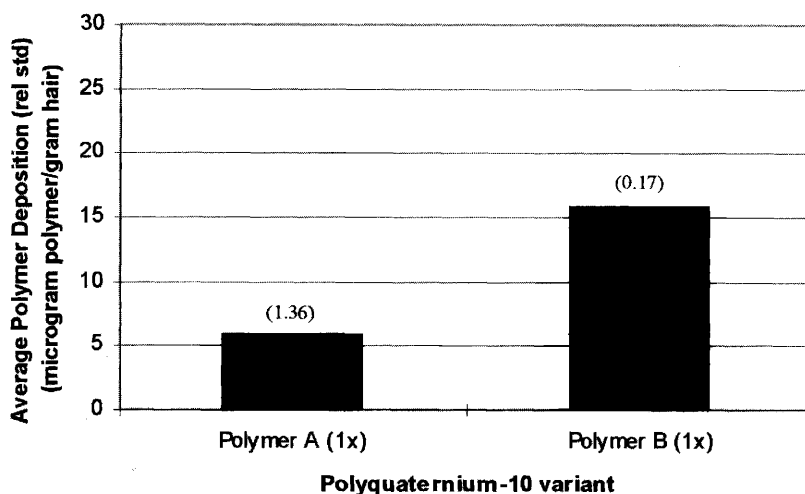

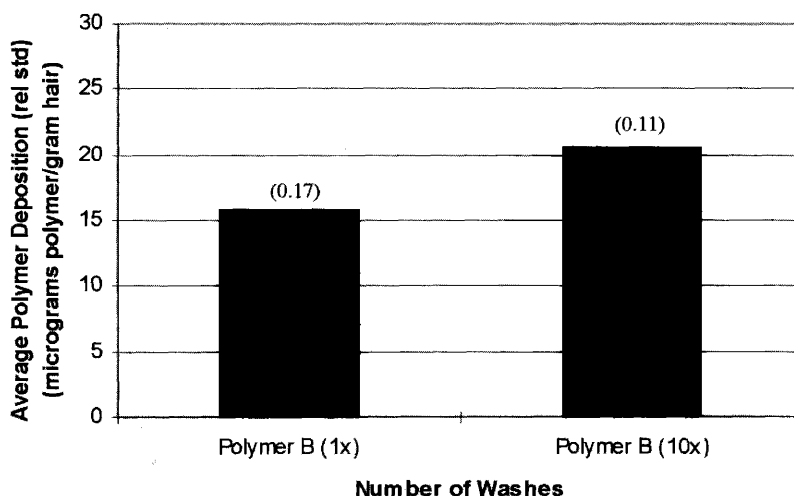Figure 4. Effect of cationic polymer charge level on polymer deposition. Single wash data.

Figure 5. Effect of repeated washings on polymer deposition for shampoos containing Polymer B.
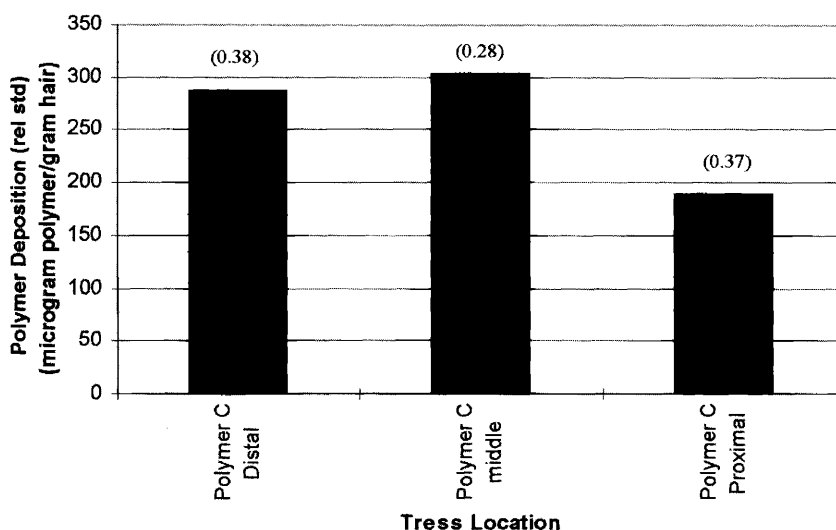

Figure 6. Cationic polymer (Polymer C) deposition as a function of hair tress location. Single wash data.

consumer rinsing practices, the data are relevant to expectations of commercial shampoo performance.

QUALITATIVE EXAMINATION OF FLUORESCENT POLYMER DEPOSITION VIA FLUORESCENT MICROSCOPY

Visualization of fluorescently labeled polymers offers a unique opportunity to study the location and mode of deposition of the polymers onto hair and skin substrates, as discussed in a recent review of the fundamentals of fluorimetry and its applications to consumer product studies (20). Of particular benefit in this regard is the use of confocal fluorescence microscopy to generate visual images of deposited fluorescent materials on

hair and skin. The principles of confocal microscopy and its use in personal care have recently been reviewed (21).

We examined various polymer-treated hair fibers via confocal microscopy to gain further insight to the mode of deposition of the polymers onto the surface of hair fibers. The photomicrograph presented in Figure 7a shows an untreated virgin blond hair fiber under typical fluorescent viewing conditions. Note that hair has a natural fluorescence. Images presented in Figures 7b and 7c display individual fibers taken from tresses treated with shampoos containing Polymer A and Polymer C, respectively. The fluorescently labeled polymer deposited on the hair is easily detected. Qualitatively, the fluorescence intensity of a hair sample treated with a shampoo containing the high-viscosity polymer is much stronger than that of a hair sample treated with the low-viscosity polymer shampoo formulation, in agreement with the quantitative data discussed above.

The appearance of patches of strong fluorescence and areas of weak fluorescence intensity (Figures 7b and 7c) suggests that the polymer is not deposited uniformly on the tress. The highly fluorescent clusters may be regions of stronger deposition of polymer/surfactant complexes, although this cannot be proven by the use of this technique. Interestingly, recent fluorescent images of hair treated with an SLES surfactant system containing a protein labeled with a comparable dye show similar patchiness (9). The authors of the study propose that the images reflect the preferred deposition of the protein on sites of damage on the hair. Our results suggest that this phenomenon may not be limited to proteins, but that cationic cellulose ethers may also show preferential adsorption to specific hair sites. A corollary to this observation is that caution should be placed in attempting to quantify polymer deposition by single-fiber analysis. Measurements using fluorescence imaging alone are prone to significant sources of error and artifact. The bulk hair digestion protocol and the quantitative fluorescence spectroscopic analysis of the digest described in this article circumvent some of these uncertainties. It offers an easy and reproducible means of comparing the adsorption properties of a wide range of synthetic and natural polymers in "real" situations.
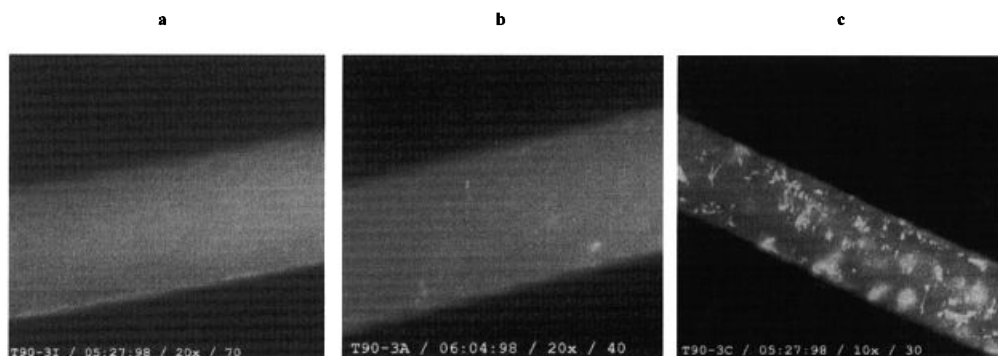
Figure 7. Confocal laser fluorescent photomicrographs of (a) normal virgin blond hair, (b) virgin blond hair treated with a single wash with Polymer A, and (c) virgin blond hair treated with a single wash with Polymer C.

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