

## **Dyestaining and the removal of cationics from keratin: The structure and the influence of the washing anion**

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### **Synopsis**

The Red-80 dye-staining procedure has been modified to help serve as a tool to study the removal of cationic surfactants from keratin surfaces. Results from this method and radiotracer studies demonstrate that monofunctional cationic surfactants are not completely removed from hair by washing with anionic detergents. Shorter chain length anionics are more efficient for removing cationics than longer chain length anionics. When washing hair with sodium lauryl sulfate, significantly more lauryl sulfate is left behind on hair previously treated with cationic detergent than on hair containing no cationic detergent.

Lipid components of conditioning products (including fatty alcohols) adsorb onto hair along with cationic actives. The ratio of lipid/cationic adsorbed increases with the ratio of lipid/cationic in the formulation. Conditioner lipids, to a certain extent, facilitate removal of the cation from the substrate; however, evidence also suggests that they participate in anionic detergent buildup on hair. Fully formulated conditioner products behave analogously to the binary systems containing cationic and lipid. Mechanisms are proposed to provide working models to help explain these results.

### **INTRODUCTION**

In 1969, Scott, Barnhurst, and Robbins (1) described the use of a dye-staining procedure using Direct Fast Rubine W.S. dye to detect cationics on keratin substrates. In 1980, Crawford and Robbins (2) described the use of Red-80 dye to replace Rubine, because the latter dye is no longer being manufactured. This test is now widely used in the cosmetics industry as a rapid qualitative screening tool to detect cationics on keratin surfaces. Normally a cationic conditioner or solution containing a cationic ingredient is applied to either a hair or wool swatch, and after rinsing, dye solution is applied and the keratin is rinsed. If a cationic ingredient with a chain length of more than ten carbon atoms is on the swatch, it stains. If the swatch does not stain, the interpretation is that virtually no cationic is bound to the swatch.

The objective of this work was to determine if the Red-80 staining procedure could be adapted to help study the removal of cationic/conditioning agents by anionic surfactants/shampoos. Thus, we have modified this dye-staining procedure by adding a wash step between application of the cationic and the dye. The new procedure is not intended to replace the former one, but it is intended to be used in conjunction with the former

procedure to complement it. This manuscript describes this new procedure and how varying the structure of the washing detergent influences staining and the interpretation of the results.

## EXPERIMENTAL

### MATERIALS

All sodium alkyl sulfate detergents were purchased from Eastman Kodak Co. and were greater than 96% pure. Ammonium lauryl sulfate (ALS)\* and the alkyl ether sulfates were obtained as 30% aqueous solutions from Henkel Incorporated. Sodium deceth-2 sulfate (SDES) was obtained from Vista Chemical Corporation as a 30% aqueous solution, and the alpha olefin sulfonate (AOS) detergents were obtained from the Witco Chemical Corporation as 30 to 60% aqueous solutions. Stearalkonium chloride (SAC) was used as Ammonyx 4002 from Onyx Chemical, and cetrimonium chloride (CTAC) was obtained as a 25% aqueous solution (Genamin CTAC) from American Hoechst. [ $^{14}\text{C}$ ]SAC and CTAC were synthesized by Andrew Charig along with [ $^{35}\text{S}$ ]ALS and SDES.

### MODIFIED STAINING PROCEDURE

Wet out under running tap water (approximately 100 degrees F) either two wool swatches (3) ( $3 \times 4.5''$  and approximately 1 g each) or one 3-g blonde hair swatch (4) made from 8'' hair. Apply 5 ml of 1 to 2% cationic or fully formulated conditioner and rub into the swatch for 60 seconds. Rinse under running tap water for 60 seconds. Wash the swatches with 5 ml of anionic detergent (5 to 20%) by working into a lather for 60 seconds. Rinse under the tap for 60 seconds. Immerse swatches into a small beaker containing 40 ml of 0.5% Red-80 (5) dye solution for 30 seconds. Rinse the swatches under the running tap for 30 seconds, and dry on a screen rack at room temperature.

### RADIOTRACER EXPERIMENTS

The above-mentioned radiolabeled compounds were diluted with their non-radioactive counterparts to make final solutions of 1.7% CTAC in water, 5% ALS in water, and 1% SAC in 30:70 ethanol water. The final activities of the test solutions were 0.15 microcuries per ml for CTAC, 0.30 microcuries per ml for ALS, 0.30 microcuries per ml for SDES, and 0.075 microcuries per ml for SAC.

Wool swatches were prepared for radiolabeling experiments by treating the swatches, which weighed roughly 1.5 g, with 3 ml of 20% triethanolammonium lauryl sulfate

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\* Acronyms for surfactants used in this work: ALS, ammonium lauryl sulfate; AOS, alpha olefin sulfonate; CTAC, cetrimonium chloride; SAC, stearalkonium chloride; SDES, sodium deceth-2 sulfate; SLS, sodium lauryl sulfate.

(TEALS), rubbing by hand for two minutes, and then rinsing under 100 degrees F running tap water for an additional two minutes.

Following overnight drying, the washed swatches were cut with a pair of pinking shears into 0.14-g segments, having areas of approximately 1.3 square inches, and put aside for use in binding experiments. In general, five swatches were prepared for each treatment. In all binding experiments, stainless steel wire gauzes with rectangular holes cut slightly smaller than the test swatches were placed over beakers. A test swatch was then wet with tap water, excess water squeezed out, and the swatch placed over the hole. Then, 0.3 ml of test solution was applied as evenly as possible to the wool, which was rubbed for one minute between two lengths of rubber tubing mounted on metal rollers.

The wool swatches were rinsed in a beaker of tap water for 45 seconds, followed by a 15-second rinse in a second beaker, after which very little radioactive material could be removed from the wool by further rinsing. A final rinse was performed for one minute under running tap water.

Depending upon the particular experiment, after the above-mentioned treatment, wool swatches could be either hung up to dry or treated again after squeezing out excess water using rubber tubing placed over the ends of crucible tongs. After drying, wool swatches were dissolved by placing each in a counting vial, adding 1 ml of 2M NaOH, and heating in an oven for 1.5 hours. Three reference vials were also prepared, in which a wool swatch was dissolved following addition of a known amount of the radiolabeled compound being tested.

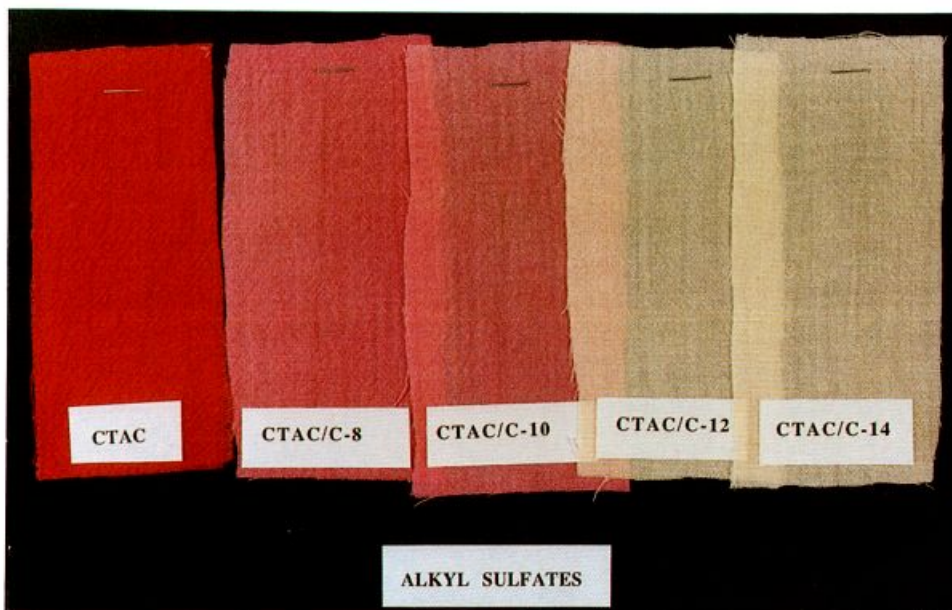
After cooling, approximately 12 ml of Aquasol-2 LSC cocktail was added to each vial, followed by addition of 0.25 ml of concentrated perchloric acid. The vials were then shaken vigorously, resulting in a clear solution ready for counting.

## RESULTS AND DISCUSSION

For initial testing, CTAC and SAC, two commonly used conditioning actives, were selected for study. In one of the first experiments, a series of alkyl sulfates of varying chain length (C8, C10, C12, C14) was selected to simulate the shampooing detergent. These alkyl sulfates were used to wash swatches treated with CTAC and then stained according to the procedure described in the experimental section.

The results of this experiment are depicted in Figure 1, which shows less staining for the swatches washed with C8 and C10 alkyl sulfates compared to the swatch not washed with anionic detergent, and no staining for the swatches washed with C12 and C14 alkyl sulfates. Similar results were obtained for both bleached and light blonde hair tresses, with less overall staining for the unbleached hair. The initial, but erroneous, conclusion was that dodecyl and tetradecyl sulfates are more effective for removing CTAC from the keratin.

The next step was to run a quantitative adsorption experiment to help explain these dye-staining results. In this study we actually compared the removal of labeled SAC from swatches by labeled SDES and ALS. Swatches treated with SAC or CTAC stain similarly after washing with anionic detergents such as SDES and ALS, i.e., washing



**Figure 1.** Wool swatches stained with Red-80 dye after treatment with CTAC and washing with alkyl sulfate detergents.

SAC-treated swatches with either SLS or ALS leaves no stain, while washing with SDES leaves a light orange-red stain (see Figure 2).

The results of this tracer experiment are summarized in Table I. In view of the dye-staining results of Figure 2, the radiotracer results were surprising. Surprisingly, the radiotracer experiment shows more SAC on the lauryl sulfate-washed swatch that did not stain with Red-80 than on the swatch washed with SDES that picked up stain. In addition, the ratio of anion to cation is roughly 1.

Apparently, the stearylalkonium cation interacts with the washing anion and the resultant complex is either washed into the aqueous phase or it deposits on the keratin. When lauryl sulfate is the washing anion, the complex binds so firmly that on subsequent staining with the anionic Red-80 dye, the stearylalkonium ion is not free to interact with the anionic dye and therefore staining does not occur. This lack of staining occurs in spite of the large amount of stearylalkonium species left on the keratin. Not only is there less cationic bound to the swatch after washing with SDES vs. ALS, but some of the anionic dye can displace the deceth sulfate and bind to the stearylalkonium cation and thereby stain the SDES-washed swatch.

In this new modified staining procedure, if the swatch stains, not only is cationic present, but the anionic detergent is either not bound to the cationic conditioner or it does not bind firmly enough to the cation to prevent staining on subsequent treatment with anionic dye. If staining does not occur, then either no cationic conditioner is present, or the deposited cation binds so firmly to the washing anionic detergent that the anionic dye cannot displace the anionic species and staining cannot occur. In our experience, when staining does not occur, this latter condition is the usual situation for most commonly used cationic conditioning agents (6).



**Figure 2.** Wool swatches stained with Red-80 dye after treatment with either CTAC or SAC and washing with C10[SDES] or C12[ALS] detergents.

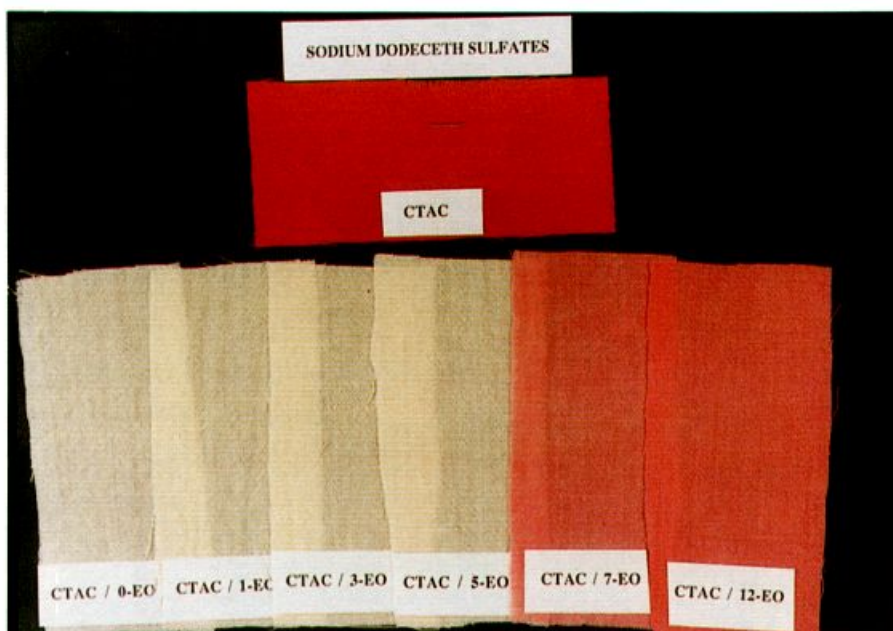
Several other anionic detergents were used in this test scheme to try to shed more light on the mechanism of action in this modified staining procedure. The effect of ethoxylation to SLS was examined for washing CTAC from swatches (see Figure 3). For this series of anionic detergents, those swatches washed with the more water-soluble sodium laureth sulfates with 7 and 12 ethoxy units produced staining, while those treated with the less water-soluble species with up to 5 ethoxy units did not stain.

**Table I**  
Radiotracer Analysis Showing Quantities of Anionic and Cationic Detergents\* Bound to Keratin After Treatment With Pure Detergents

Treatment	mg Ionic moiety/gm Dry keratin		
	Anion	Cation	Total bound
SAC/ALS	4.1*	4.6*	8.7*
SAC/SDES	2.1	2.0	4.1

\* Keratin contained 6.9 mg SAC/gm dry keratin after treatment with SAC, just prior to washing.

\* Significantly different from corresponding value at or beyond the 95% level.



**Figure 3.** Wool swatches stained with Red-80 dye after treatment with CTAC and washing with laureth sulfate detergents.

Thus, the more water-soluble the anionic detergent in this scheme, the more likely for staining to occur. This conclusion is consistent with the results from the initial experiment with the alkyl sulfates that is depicted in Figure 1. Here the cationic-treated swatches when washed with the more water-soluble C8 and C10 alkyl sulfates produced staining, while the swatches washed with the less water-soluble C12 and C14 alkyl sulfates did not stain.

Similar results were obtained in an experiment using a series of AOS detergents (see Figure 4). Here, once again, the cationic-treated swatches washed with the more water-soluble detergents provided staining. However, with the AOS detergents, the cutoff point was between the C12 and the C14 detergents, i.e., the swatches washed with the C10 and the C12 detergents stained, while those washed with the less water-soluble C14 and C16 detergents did not stain.

Cationic conditioners contain lipid materials such as fatty alcohol in addition to the cationic active ingredient. This lipid material adsorbs to the hair along with the cation, and the relative amounts adsorbed depend on the relative concentrations and structures of cationic and lipid (7). The standard Red-80 dye-staining test, involving no washing, often shows a difference in hue of the dye on keratin between swatches treated with cationic vs cationic containing lipid-like cetyl alcohol (see Figure 5). This change in hue of the stain demonstrates that lipids like cetyl alcohol adsorb to the keratin in combination with the cationic. The adsorbed alcohol perturbs the cationic dye interaction product, shifting the light absorption-reflectance character of the dye on the keratin. Thus the change in hue.

Most fully formulated conditioner products or even partially formulated ones containing

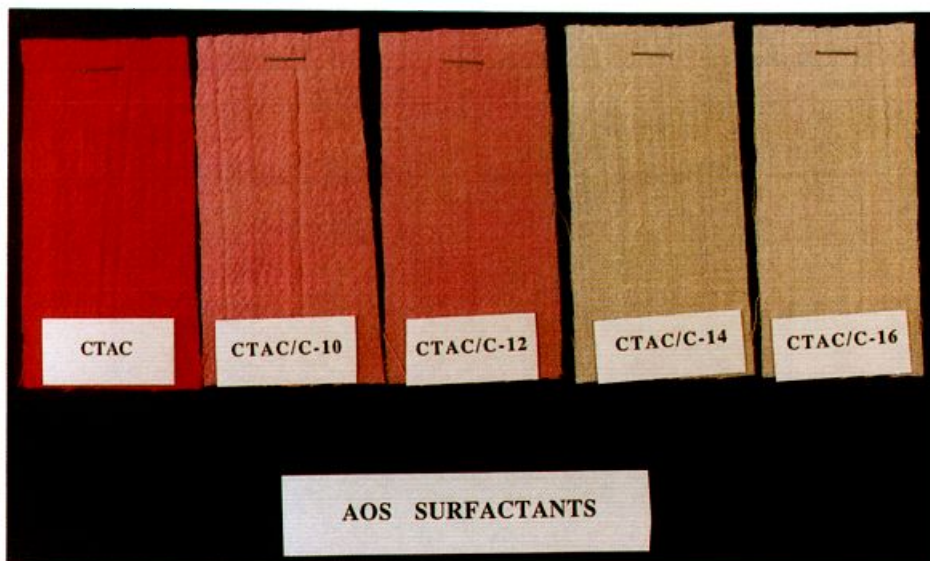


Figure 4. Wool swatches stained with Red-80 dye after treatment with CTAC and washing with AOS detergents.

just cationic and fatty alcohol compare favorably to either pure SAC or CTAC in the modified dye-staining procedure.

Figure 6 compares swatches treated with SAC, a partially formulated conditioner containing SAC and cetyl alcohol, and a commercial conditioner based on SAC and ceteth-2 after washing with both sodium deceth-2 sulfate and SLS. All swatches washed with the deceth-2 sulfate show a light stain, in contrast to those washed with SLS, which do not stain. This suggests that the cationic adsorption, binding, and washing from keratin is similar whether it is used pure in solution or formulated into a conditioner. Nevertheless, we decided to test this hypothesis by radiotracer experiments.

Table II summarizes the results of an experiment in which a formulated conditioner containing radiolabeled SAC and ceteth-2 was washed from wool fabric by solutions of radiolabeled SDES and ALS. The binding of the anionic detergent is similar to the case where pure cationics are washed (compare the results of Tables I and II). Here roughly two times the amount of ALS vs SDES binds to the keratin-containing cationic. Furthermore, in the dye-staining test, the SDES species can be displaced by the anionic dye, but the lauryl sulfate moiety cannot.

In contrast to the case of washing pure cationic from keratin, these results with a fully formulated product show that both of these anionic detergents, ALS and SDES, remove a similar amount of cationic from the swatch, but a large difference exists in the amount of anion that is left behind, with nearly two times the amount of lauryl sulfate vs deceth sulfate being left behind on the keratin.

From all of these staining and radiotracer results, we conclude the following: Swatches of keratin fibers when treated with cationic detergents bind those detergents by electrostatic and Van der Waals attractive forces. When one attempts to remove the cationic detergent from the hair by washing with an anionic detergent, an electrostatic interac-

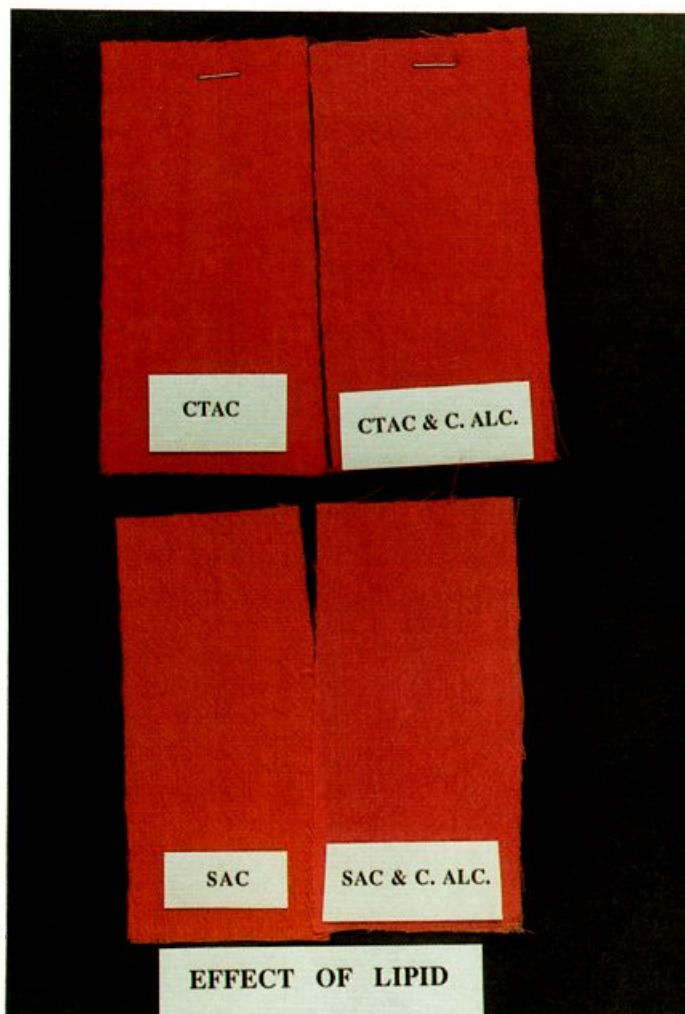


Figure 5. The effect of lipid on the dye-staining properties of cationic surfactants on keratin substrates.

tion occurs between detergents. If the water solubility of this interaction product is in the vicinity of or greater than that of the decyl sulfate-cetrimonium species, much of the cation is removed from the swatch, but staining can still occur because the anionic dye can displace the more water-soluble anionics like octyl or decyl sulfate (see Appendix for a complete list of the surfactants and staining results). However, if the water solubility of this interaction product is less than that of the dodecyl sulfate-cetrimonium species, then staining does not occur because the anionic dye cannot displace the anion from the anionic-cationic complex species.

Buildup on hair occurs because the anionic moiety of the washing detergent binds to the hair with the cationic species, and in the case of fully formulated systems, the ratio of binding anion/cation may be much greater than 1. We confirmed these conclusions by means of light-scattering experiments and subjective comparisons in actual half head tests that demonstrate that shinier, cleaner hair results from washing hair containing cationics with SDES vs. ALS. Therefore, this mechanism forms one basis for the phe-



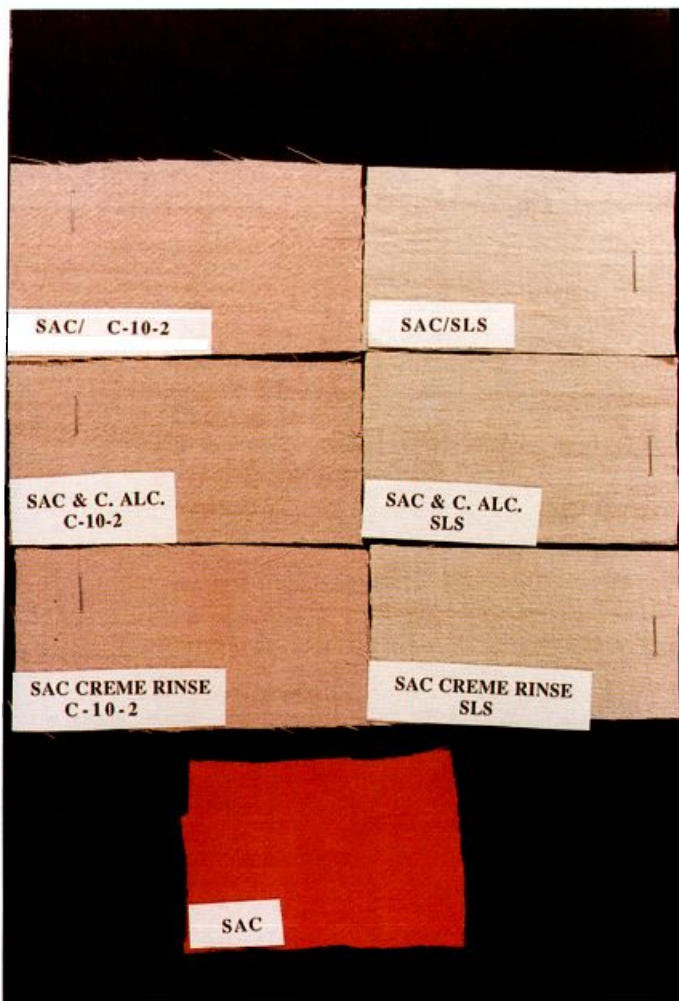


Figure 6. Treatment of wool swatches with a simple cationic vs. formulated conditioners and washing these with deceth vs. lauryl sulfate and staining with Red-80.

**Table II**  
Radiotracer Analysis Showing Quantities of Anionic and Cationic Moieties Bound to Keratin After Treatment With a Conditioner Product\* and Washing With Anionic Detergents

Treatment	mg Ionic moiety/gm Dry keratin		
	Anion	Cation	Total bound
Conditioner/ALS	4.0*	1.7	5.7*
Conditioner/SDES	2.0	1.9	3.9

\* Keratin contained 4.5 mg SAC/gm dry keratin after conditioner treatment, prior to washing.

\* Significantly different from corresponding value at or beyond the 95% level.

nomenon of buildup on hair, where the anionic detergent of shampoo actually builds up on hair with the cationic conditioner by forming association complexes.

Thus, the modified dye-staining procedure is a useful screening tool to help study the removal of cationic conditioners from hair. However, because of the complexity of these reactions, the dye-staining test should be used with other techniques to assist in interpreting the results.

## REFERENCES

- (1) G. V. Scott, C. R. Robbins, and J. D. Barnhurst, Sorption of quaternary ammonium surfactants by human hair, *J. Soc. Cosmet. Chem.* 20, 135–152 (1969).
- (2) R. J. Crawford and C. R. Robbins, A replacement for Rubine dye for detecting cationics on keratin, *J. Soc. Cosmet. Chem.*, 31, 273–278 (1980).
- (3) Purchased from Test Fabric, Inc., Middlesex, N.J.
- (4) Purchased from A. Klugman, New York, N.Y.
- (5) Sirius Red F3BA New, C.I. No. 35780, Mobay Chemical Corp., Rock Hill, S.C. Equivalent products are offered by Sandoz Colors & Chemicals, E. Hanover, N.J., and by American Color & Chemical Corp., Charlotte, N.C.
- (6) C. R. Robbins, C. Reich, and J. Clarke, submitted for publication.
- (7) R. L. Sneath. Presented at the 5th International Hair Science Symposium in Constance, W. Germany, November 1986.

## APPENDIX

Summary of the Staining Reaction Results After Treatment With SAC or CTAC and Washing With Anionic Detergent and Then Staining With Red-80 Dye

Anionic detergent	Staining results
Sodium octyl sulfate	Stains
Sodium decyl sulfate	Stains
Sodium lauryl sulfate	No stain
Ammonium lauryl sulfate	No stain
Sodium tetradecyl sulfate	No stain
Sodium deceth-2 sulfate	Stains
Sodium dodeceth-1 sulfate	No stain
Sodium dodeceth-2 sulfate	No stain
Sodium dodeceth-3 sulfate	No stain
Sodium dodeceth-5 sulfate	No stain
Sodium dodeceth-7 sulfate	Stains
Sodium dodeceth-12 sulfate	Stains
Alpha olefin sulfonate (C10)	Stains
Alpha olefin sulfonate (C12)	Stains
Alpha olefin sulfonate (C14)	No stain
Alpha olefin sulfonate (C16)	No stain