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Selective removal of sebum components from hair by surfactants

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Synopsis

The detergency of three surfactants, sodium laureth 2-sulfate (SLES-2), ammonium lauryl sulfate (ALS), and sodium octeth-1/deceth-1 sulfate (SODS-1), was measured; variables examined were soil/wash cycles plus sebum component vs total sebum removal. After one soil/wash cycle SLES-2 cleans all sebum components from hair equally well (>90%). ALS is not as good, and SODS-1 is poor for all fractions.

With extended use (ten-cycle data), SLES-2 remains superior for all components (>90% removed), but the behavior of ALS and SODS-1 are substantially different from their one-cycle behaviors. Analysis of tresses washed with ALS under test and simulated use conditions suggests a build-up of fatty acid components on hair; this is interpreted in terms of a hard water ion/fatty acid interaction. Extended use data of SODS-1 show increased removal for all components when compared to the one-cycle data, suggesting either a soil release mechanism or inhibition of soiling.

We hypothesize that a technique that provides a rapid assessment of total sebum removed from hair by a detergent can be used to screen surfactants. However, to model extended use behavior, it is useful to monitor the removal of sebum components.

INTRODUCTION

Effective formulation of hair cleaning products begins with an understanding of the substrate. Perhaps of equal or even greater importance is the type of soil found on the substrate and how it is bound to the fibers. Human hair has a chemical composition, physical properties, and histological structure similar to other keratin fibers. However, the cleaning of hair presents a different, and possibly more difficult, problem because of safety restrictions. The use of fairly low temperatures and short cleaning times adds further restrictions. In comparison, products for cleaning textiles do not have to meet such restrictive criteria.

Soils on human hair can be divided roughly into four groups:

(a) Hair lipid, a fatty material composed mainly of sebum (from sebaceous glands) and lipids (from skin surface cells).
(b) Proteinaceous matter from cell debris and sweat.
(c) Extraneous materials from a polluted environment (soot, hydrocarbons).

(d) Hair product soils, e.g., conditioners, hair sprays, mousses, gels, etc.

The perception of dirty or oily hair is probably attributable to hair lipids. These materials may be sticky and can act like a "cement," causing various particulates to stick to the hair surface.

Sebum production is variable (1). This variation is documented to be seasonal, daily, and due to hormonal activity, with changes from preadolescence, through puberty, and into old age. Compositional changes also occur with both subject age (1), and as the sebum ages (1), after distribution on the hair. Furthermore, evidence exists for the classification of hair sebum into two types: external or surface sebum, and internal sebum (2). External sebum contributions combine with the physical properties of the hair fibers (curliness, diameter) to furnish hair with an oily appearance. It is reasonable to assume that the external sebum, which is easily extractable into lipid solvents, can be shampooed off, while the internal lipid is more difficult to remove. In fact, very strong extraction procedures and enzymatic hydrolysis of hair keratin (2) is needed to remove this material. The exact origin of the internal lipid is under debate, but Koch *et al.* (2) have provided evidence that most of the components are those found in external lipid. This suggests at least partial origination of these lipids from the sebaceous glands.

Several published papers detail methods for extracting hair lipid (external and internal) both *in vitro* and *in vivo* (1–4) and for quantifying the data. There are obvious disadvantages in collecting lipid *in vivo* by solvent extraction. Gravimetric analysis, because of the small quantities involved even after *in vitro* extraction, requires sensitive weighing equipment and care.

Several authors have carried out compositional analysis of extracted lipid/sebum. Shaw (5) used gravimetric and spectrophotometric methods to assess total lipid, a fluorimetric technique to determine cholesterol, and thin layer chromatography (tlc) and gas-liquid chromatography (glc) to distinguish between major components of the lipid. Koch (2) determined the total amount and composition of extracted sebum by high pressure liquid chromatography (HPLC). Breuer (6) also reports data for quantifying components of extracted sebum using an HPLC technique. Thompson *et al.* (7) have described a gas chromatography system for the analysis of sebum components extracted into hexane from hair (*in vitro*).

This work arose from our use of a modification of the Thompson *et al.* technique to determine the extent to which a test measuring total sebum removal from wool (by surfactants) was applicable to predicting surfactant performance against sebum, and to investigate certain surfactants of proprietary interest. Thus this work builds upon the published study of Thompson *et al.* We believe that the knowledge acquired in determining surfactant selectivity for removal (cleaning) of sebum components from hair can provide important guidance for formulating shampoos and other hair cleaning products.

MATERIALS AND METHODS

ARTIFICIAL SEBUM

The artificial sebum used in all experiments was prepared according to the Spangler formula (8) shown in Table I.

Table I
Artificial (Spangler) Sebum (8)

Ingredient	%
Linoleic acid	5.0
Squalene	5.0
Oleic acid	10.0
Coconut oil	15.0
Olive oil	20.0
Cholesterol	5.0
Stearic acid	5.0
Palmitic acid	10.0
Paraffin	10.0
Spermaceti wax	15.0

HAIR SUBSTRATE

In all experiments, dark brown, Oriental hair, virgin quality and of 10-inch length was used (DeMeo Brothers, New York). Prior to soiling with sebum, the hair was divided into approximately 3.5-g tresses, washed with 10% TEALS (Standapol T, Henkel) for one minute, rinsed for two minutes under running tap water (105°F), and air dried at room temperature. Tresses were conditioned in a humidity room, 70°F and 60% relative humidity, for 72 hours prior to soiling with sebum. All subsequent weights of hair were made after similar temperature and humidity conditioning.

SURFACTANTS

SLES-2 and ALS were obtained from Henkel Corporation (Standapol ES-2 and Standapol A, respectively), and SODS-1 was obtained from VISTA Chemical Company (Alfonic 8,10–20 ether sulfate). The surfactants were used as provided by the manufacturer, with no further purification. Solutions were prepared with deionized water.

HAIR-SOILING PROCEDURE

Hair tresses were soiled by suspending a preweighed tress in a solution of sebum in hexane (3.5 g hair/250 ml solution), at the required concentration. After 20 minutes in the sebum solution (with constant stirring), the hair was removed and the solvent allowed to evaporate from the tress at room temperature. After conditioning at 60% relative humidity, the tress was weighed to determine the sebum load.

Soiling solutions of 6 and 3 weight percent sebum were used. A 6% solution was used for soiling tresses subsequently washed with 0.01% surfactant (soil/wash condition A) one-cycle experiment. The 3% concentration was used for soiling 1.8-g tresses of the ten-cycle experiment and for soiling tresses washed with 0.1% surfactant (soil/wash condition B). These sebum concentrations produce soiling levels on the tresses of approximately 0.04–0.055 g soil/g hair and 0.03 g soil/g hair, respectively. Hair soiled with the 3% solution is perceived to be "dirty" or "oily" (corresponding to that on heads of consumers who shampoo frequently), whilst tresses soiled in a 6% solution are "very oily," representing perhaps an extreme in hair oiliness for most Western cultures.

The higher soiling level, however, was most often used in this work as it facilitates the subsequent gc analysis of the sebum.

After the soiled tresses were dry, each was split into two swatches of about 1.7 to 1.8 g each. One of each pair was washed with the appropriate surfactant. The other portion remained unwashed and acted as an internal control. This was necessary to compensate for sample-to-sample variation in soiling levels.

TEN-CYCLE SOIL/WASH EXPERIMENT

For the ten-cycle soil/wash experiment, the tresses were split as described above, with one swatch kept as control. The other portion was then washed and dried (described below) and placed in a constant humidity room overnight. The next day these tresses were soiled again with sebum, allowed to dry at room temperature, and placed in the constant humidity room overnight. The following day the tresses were again washed with the appropriate surfactant. This soil/wash cycle was carried out ten times. The order for both soiling and washing procedures was randomized.

HAIR-CLEANING PROCEDURE

Cleaning of the soiled tresses was achieved using a bulk process similar to that described in reference 7. The soiled hair tress was suspended in 100 ml of either 0.1% or 0.01% aqueous surfactant at 110°F and agitated (magnetic stirrer) for five minutes. Tresses were then rinsed under running tap water (105°F) for 20 seconds (total rinse volume 500–600 ml). Heat from a hand-held drier was applied for one minute and the drying completed at room temperature. Conditioning in the humidity room followed.

These surfactant concentrations are very low and for SODS-1 are below the cmc. Since oily soil removal occurs through solubilization via micelles, we would expect poor results with this surfactant. In fact, even at concentrations above the cmc, SODS-1 is a poor detergent for removing oily soil. It is included in this study as a negative control.

EXTRACTION OF SEBUM FROM HAIR

Before the sebum residues were extracted, all tresses were placed in a forced air draft oven at 55–60°C for four hours. This helped to ensure a uniform moisture content throughout the sample set. About 1 g of hair from each tress was weighed into a vial, 20 ml of hexane added, and the sealed vial shaken on a mechanical shaker for 30 minutes. Hexane was used as the extraction solvent based on data presented in reference 7. These data claim chromatographic profiles of the hexane extract of sebum-soiled tresses to be comparable to profiles of standard sebum/hexane solutions.

After shaking, 15 ml of solution was pipetted from each vial into a previously weighed second vial. The sample was evaporated to dryness (at room temperature) by gently blowing filtered nitrogen over the liquid surface. Subsequently the vials were weighed to estimate total extracted sebum, and the residues analyzed by gas chromatography to determine sebum composition. Sample residues were dissolved in hexane containing internal standard, Eicosane, to a concentration of approximately 6 mg/ml. Sample injection amount was 0.4 microliters. The analyses were performed on a Carlo Erba Mega 5360 High Resolution Capillary Gas Chromatograph fitted with a cold on-column

injector and a flame ionization detector. The column is a Supelco 60 meter × 0.75 mm i.d. glass column coated with SPB-1 liquid phase to a film thickness of 1.0 microns. Detector temperature was 325°C. GC oven initial temperature was 220°C, held for eight minutes, ramping up to 310°C at 4°C per minute, and holding for 55 minutes. Figure 1 is a typical gas chromatogram of Spangler sebum; we confirmed peak identifications by mass spectrometry. Note that triglycerides are not detected under the column conditions used; previous data indicate that these materials are easily removed by surfactants (7). We intend to modify our chromatographic system to test this conclusion ourselves.

RESULTS AND DISCUSSION

The objective in this work was to determine if surfactants selectively remove sebum components from hair. Tresses were washed in dilute (0.01 to 0.1%) bulk (100 ml) detergent solution rather than attempting to simulate actual shampooing, because Thompson *et al.* (7) have shown similar results with improved precision by the bulk method.

These low detergent concentrations are used to facilitate analysis of the sebum residues on the hair. If higher concentrations are employed, the recovery and subsequent analysis of the sebaceous residue is not practically feasible because of the very small amount of

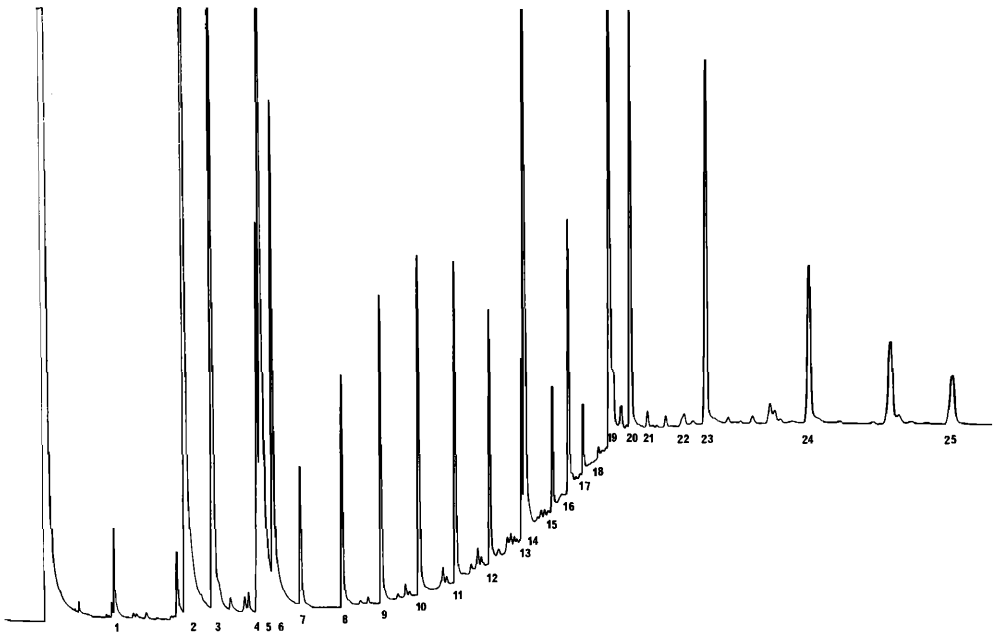

Figure 1. Capillary gas chromatogram of Spangler sebum. 1. Tetradecanoic acid; 2. hexadecanoic acid; 3. n-eicosane (internal standard); 4. 9, 12-octadecadienoic acid; 5. 9-octadecanoic acid; 6. octadecanoic acid; 7. n-docosane; 8. n-tricosane; 9. n-tetracosane; 10. n-pentacosane; 11. n-hexacosane; 12. n-heptacosane; 13. n-octacosane; 14. squalene; 15. n-nonacosane; 16. hexadecyl dodecanoate; 17. n-triacontane; 18. n-hentriacontane; 19. cholesterol; 20. hexadecyl tetradecanoate; 21. n-dotriacontane; 22. n-tritriacontane; 23. hexadecyl hexadecanoate; 24. octadecyl hexadecanoate and hexadecyl octadecanoate; 25. higher molecular weight ester.

residue that is recovered. However, experiments in which soiled hair was handwashed using 10% surfactant, simulating actual use conditions, have shown results similar to those reported here for these test conditions (9). These handwashing tests are discussed later in the text.

Five replicates for each of the following three surfactants have been performed:

- SODS-1—Sodium octeth-1/deceth-1 sulfate
- ALS—Ammonium lauryl sulfate
- SLES-2—Sodium laureth-2 sulfate

The surfactants were chosen on the basis of total sebum removal data obtained using a wool substrate as a model keratin (9). Briefly, sebum removal from wool swatches is measured by monitoring the removal of a lipid-soluble dye (coadsorbed with the sebum) using a reflectance technique. These data (Table II) show that the surfactants may be considered as poor (SODS-1), medium-good (ALS), and good (SLES-2). Data are also shown for a second set of soil/wash conditions (B) (0.03 g soil/g hair; 0.1% surfactant); values are averages of three replicates. As evidenced in Table II, values for the total sebum removed are in good agreement for the three experimental conditions (and two substrates) shown. For both A and B conditions, the amount of soil removed from hair is larger or equal to that for a wool substrate. The order of superiority of surfactants is also maintained (agreement with detergency theory), and the wool and hair values are close in magnitude. The total sebum removed under B conditions is larger than for A: as expected, the combination of lower soil loading and higher detergent concentration promotes better cleaning.

The methods used in this work, soil/wash conditions and component identification, have been adapted from work reported in the literature (7). Three cleaning processes were described (7): bulk bath, finger squeeze, and controlled pressure/sponge; the data show that the bulk bath method produces the most uniform results. Therefore, we have used the bulk method to provide as much precision in our experiments as possible and have drawn conclusions by statistical analysis of the data using a p value of 0.05 as the decision criterion. The conclusions in the Thompson paper (7) are based upon the less reproducible controlled pressure/sponge cleaning process.

Thompson *et al.* (7) evaluated the shampoo detergency of three surfactants commonly used in shampoos: ALS, SLES-2, and AOS (sodium alpha olefin C_{14}-C_{16} sulfonate) against fatty acids, cholesterol, paraffin waxes, wax esters, squalene, and triglycerides. The gc system used in our work did not allow for detection of the triglycerides and all other fractions at the same resolution. The triglycerides have much longer peak reten-

Table II
Data Comparing Total % Sebum Removed for One Soil/Wash Cycle From Hair and Wool Surfaces

Surfactant	% Removed (Cond. A)	% Removed (Cond. B)	% Removed (Wool)
SODS-1	40.7 ± 15	56.7 ± 25	35 ± 4
ALS	72.4 ± 9	97.3 ± 2	79 ± 3
SLES-2	93.7 ± 3	97.6 ± 2	88 ± 2

Condition A: Hair soiled at 0.04–0.055 g soil/g hair and washed with 0.01% surfactant.
Condition B: Hair soiled at 0.03 g soil/g hair and washed with 0.1% surfactant.
Wool: 3-inch × 4.5-inch wool challis swatch soiled with sebum/lipid-soluble dye soil.

tion times, and raising the temperature to speed the elution led to loss of resolution among the other peaks. Since Thompson *et al.* (7) stated that the triglycerides are easily removed by the three surfactants they used, with no increased build-up at 10 or 20 cycles, we elected to concentrate on the other sebum components.

ONE-CYCLE DATA

The sebum component removal data for tresses were analyzed statistically for seven components, i.e., myristic (C_{14}), palmitic (C_{16}), stearic (C_{18}), and unsaturated (oleic and linoleic) acids ($C_{18:21}$), cholesterol (CHOL), paraffin waxes (11 fractions combined) (PW), and esters (from spermaceti wax; five fractions combined) (EST).

The total sebum removal data is shown in Table II. As previously stated, these figures correlate well with data acquired using a wool substrate (9) (Table II).

Figure 2 shows results of component removal after one soil/wash cycle (0.01% detergent). The order of removal for the sebum components is similar: ester and paraffin wax removal is the most difficult, and cholesterol the easiest. The only difference is the magnitude of removal that is determined by the nature of the surfactant, i.e., whether it is a good or poor cleaner of lipid soils.

As mentioned, the data show the relative total sebum removal of SLES-2, ALS, and SODS-1 to be similar from hair and wool surfaces, i.e., SLES-2 > ALS > SODS-1. This order confirms that predicted by surfactant theory for oily soil detergency (10). To determine if there is selective removal of components by a surfactant, one way ANOVA

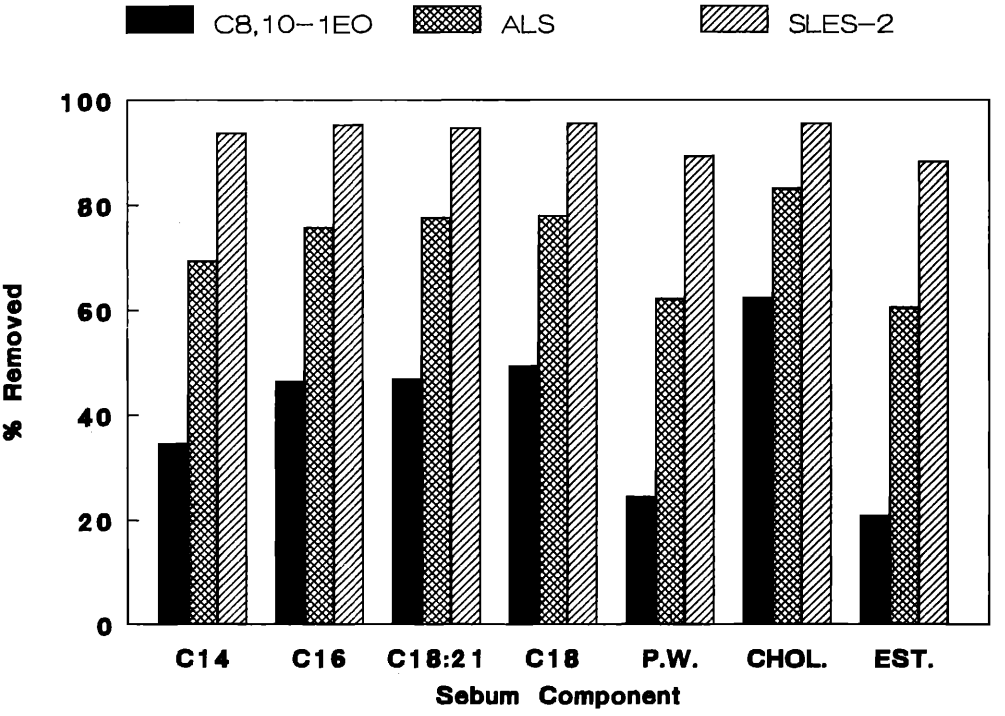

Figure 2. Removal of sebum components by $C_{8,10}$-1EO, ALS, and SLES-2 for one soil/wash cycle.

statistics have been performed on these data and removal of individual component groups compared.

The following summarizes the statistical analyses of the sebum component removal by SODS-1, for one soil/wash cycle (95% confidence level). (Component removals are significantly different when components are not underlined by the same line):

Least removed						*Most removed*
EST	PW	C_{14}	C_{16}	$C_{18:21}$	C_{18}	CHOL

These analyses show that this surfactant is most effective in removing the cholesterol component from hair; it is least effective in cleaning off the esters and paraffin waxes.

For ALS, sebum component removal is as follows:

Least removed						*Most removed*
EST	PW	C_{14}	C_{16}	$C_{18:21}$	C_{18}	CHOL

Here, the order of removal is the same as SODS-1 but the data (Figure 2) show that ALS removes more of each component than SODS-1 (95% confidence level). The esters and paraffin waxes are clearly more difficult for ALS to remove than the other components, with the exception of the C_{14} materials. On the other hand, when soiled hair is washed under similar conditions with SLES-2, there are no significant differences in removal among the sebum components ($p = 0.05$), and SLES-2 removes all components more effectively than either ALS or SODS-1.

Consequently, after one soil/wash cycle (soiling level 0.04–0.055 g/g; 0.01% surfactant), the removal of sebum components by each of the three surfactants tested can effectively be predicted by a value derived for the total sebum removal. SLES-2 is clearly the most effective against all groups of components and SODS-1 the least effective, a confirmation of surfactant theory (10) (Figure 2).

Sebum removal data for hair soiled and washed under a second set of conditions, i.e., 0.03 g sebum/g hair and 0.1% surfactant solution, were also analyzed (9).

The order of component removal for individual surfactants was found to be similar to the order under "A" soil/wash conditions. Additionally, under these conditions of lower soil loading, both ALS and SLES-2 remove all components at >94% levels, approaching the limits of the experiment. Similar to "A" soil/wash conditions, the most difficult fractions to remove are the paraffin waxes and the esters.

Clearly the one-cycle experiments indicate that some sebum components are more difficult to remove, but the same pattern of removal exists for all three surfactants tested. Surfactant theory for oily soil detergency confirms this order (10). Thus, the one-cycle data show that a surfactant with good cleaning power removes all components well, a poor one less well. However, for all surfactants tested, the esters (from spermaceti wax) and the paraffin wax fractions are the most difficult materials to clean from the hair.

TEN-CYCLE DATA AND BUILD-UP OF SOIL

The ten-cycle data (0.01% surfactant) indicate differences relative to the one-cycle data. For the superior lipid soil surfactant SLES-2, there is no change in removal order of the components or in total percent sebum removed (Figure 3). However, there are differences in the ability of SLES-2 to remove different sebum components ($p = 0.001$). Two distinct groupings of components emerge: the esters and waxes are more difficult to remove than the rest (95% confidence level). These data show some selectivity for SLES-2, but it should be noted that removal of all components is high, i.e., >90% There are, however, changes in the removal order for both ALS and SODS-1 compared to their one-cycle behaviors (Figures 4 and 5), and the difference between ALS and the latter surfactant has narrowed. For one-cycle the total sebum removal figures are 72.4% and 40.7%, respectively (significantly different at 95% confidence level); for ten cycles they are 65.2% and 59.2% (not significantly different).

The detergency behavior exhibited by the SODS-1 surfactant is as follows (Figure 4 portrays the one- and ten-cycle data for this material). The dominant feature is the large increase in percent removal of the ester and paraffin wax fractions after ten cycles. In fact, all sebum components show increased removal to some extent; for the aforementioned components this increase is substantial. These results may indicate a soil release mechanism is occurring: SODS-1 may be adsorbing onto the hair during subsequent washes, thus preventing further adsorption of certain sebum components. Regardless, the data show that extended use of this surfactant does not induce build-up, but rather enhances removal.

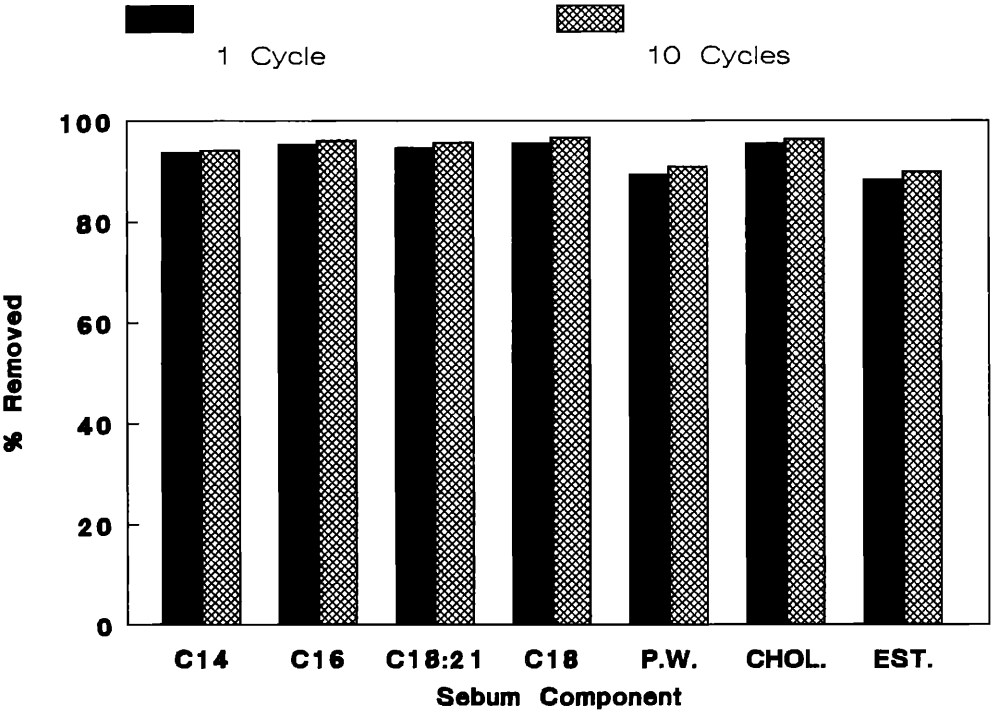

Figure 3. Removal of sebum components by SLES-2 for one and ten soil/wash cycles.

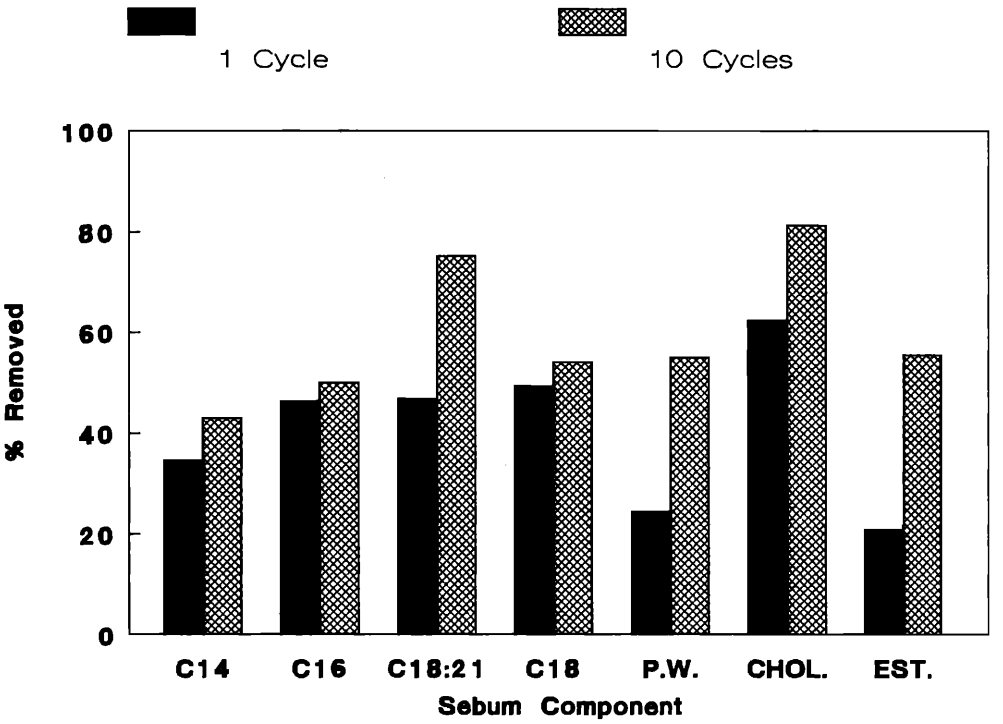

Figure 4. Removal of sebum components by $C_{8,10}$-1EO for one and ten soil/wash cycles.

When data are analyzed (Figure 5) for component residues after ten soil/wash cycles with ALS detergent, there is a decrease in removal for the saturated fatty acid fractions (compared to one-cycle behavior), perhaps indicative of build-up or selective cleaning. The removal of cholesterol, unsaturated acid, ester, and paraffin wax fractions is similar to the one-cycle level.

At the 95% confidence level cholesterol removal is different from the rest (except unsaturated acids); saturated acid fractions remain more readily on the hair. There is distinct evidence of build-up of the saturated fatty acid materials (C_{14}, C_{16}, and C_{18}) on the hair. This build-up is probably due to the interaction between water hardness (Ca^{2+}, Mg^{2+} ions), the fatty acids, and ALS. The tap water used in our experiments is 75–80 ppm (as $CaCO_3$), higher than the 60 ppm reported in reference 7. SLES-2 and SODS-1 do not show this behavior; the ethoxy units apparently aid in preventing this hard water reaction.

In a separate experiment in which 3.5-g hair tresses were successively soiled and hand-washed (ten soil/wash cycles; soil aged overnight between washings), the detergency of 10% ALS and SLES-2 to clean sebum from hair was compared. ESCA data confirmed an increase of calcium ion on the ALS-washed hair as compared to SLES-2 washed tresses. Also, panelists evaluated the ALS-washed tresses to be significantly duller (95% confidence level) than the SLES-2 treated hair. The dulling is presumably a manifestation of the fatty acid residue build-up.

These ALS data affirm that ALS is a good surfactant, although its sebum removal efficacy is less than that for SLES-2. The results are again in accord with surfactant

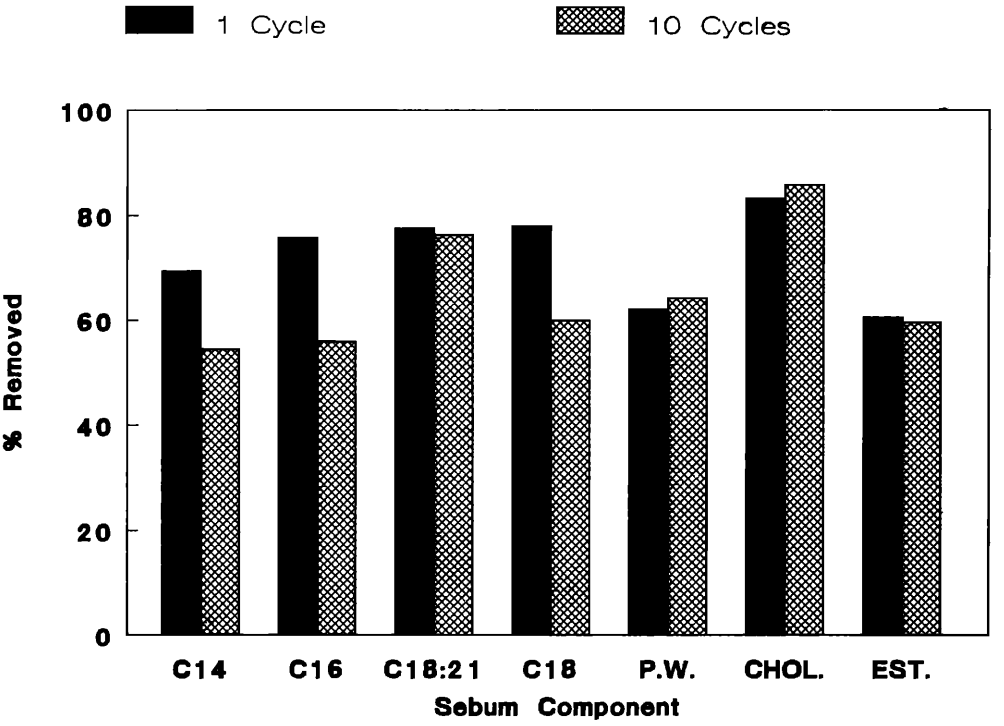

Figure 5. Removal of sebum components by ALS for one and ten soil/wash cycles.

detergency theory (10). Since these results are for a pure surfactant, more data are necessary to draw any conclusions for extrapolating to shampoos, since other ingredients that alter surfactant properties are used in shampoo formulations.

For one soil/wash cycle, Thompson *et al.* conclude that the polar materials are more easily cleaned from hair than the non-polar, and that the degree to which the latter are removed is dependent on the surfactant. Our present data are in general agreement with these conclusions; however, our data show that squalene does not build up after ten soil/wash cycles. We also concur that the paraffin waxes are the most difficult materials to remove (along with the spermaceti esters) and that SLES-2 is superior to ALS for cleaning lipid soils.

The Thompson paper indicates the cholesterol fraction to be difficult to remove for one and 20 cycles. For ten cycles there is a dramatic increase from 65% to 85% removal, a value more consistent with our results showing that cholesterol is easily cleaned from hair.

Squalene is present in the sebum used to soil the hair and appears in calibration chromatograms of the sebum. However, after the extraction procedure it is not found in either control or washed tress extracts. The drying and extraction procedures are those reported (7), so it is not clear why no squalene is detected in practically any chromatogram under either of our soil/wash conditions. Preliminary data does indicate some loss of squalene during the low-level heating to provide a uniform moisture content throughout the sample set. This heating may be enough to remove any squalene not cleaned off by the wash surfactant and thus may explain the absence of squalene in

extract chromatograms. One would expect, though, that if squalene is building up, this effect would be evident in our ten-cycle data even if much is vaporized after one cycle. However, there is no evidence of this, and we are at a loss to explain this anomaly between these and the Thompson data for the squalene component.

In summary, our data show that for one soil/wash cycle, surfactants do selectively clean sebum components from hair at low concentrations. But, as a first approximation, the amount of sebum removed is a function of the detergency of the surfactant, and thus the difference between SLES-2 and ALS is primarily a function of the superior detergency of the former [as predicted by surfactant theory (10); SLES-2 has the lower cmc] rather than differences in selective cleaning. The non-polar sebaceous components (paraffin waxes, esters) are more difficult to remove than the more polar ones, but we contend that the overall surfactant detergency is the determining factor. For ten soil/wash cycles, we find that SLES-2 is superior, and a build-up is found on hair washed with ALS under both (a) the model conditions using 0.01% surfactant and bulk washing and (b) realistic conditions using 10% surfactant and handwashing. This we attribute to hard water/fatty acid interactions. We believe that the data of this paper provide sufficient evidence to warrant extended use testing of potential surfactant systems for oily soil detergency in the manner described.

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