Using time-of-flight secondary ion mass spectroscopy to investigate the reaction of alkylsulfate and alkylethoxysulfate surfactants with keratin

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Abstract

Time-of-flight secondary ion mass spectroscopy (ToF-SIMS) was used to investigate the changes in keratin protein surface chemistry caused by the covalent bonding reactions of commercially available alkylsulfates and alkyl ethoxysulfates surfactants. Due to cystine and cysteine oxidation, plus regular shampooing, the surface chemistry of human hair is different from that of freshly scoured merino wool. Human hair can produce positive ions derived from the reaction of alkylsulfates and alkylethoxysulfates, commonly present in shampoos, with histidine and possibly lysine residues (with little evidence for cysteine thiol reaction). ToF-SIMS analysis of alkylsulfate treated keratin fibers confirmed the reaction of these surfactants with cysteine thiol, tyrosine phenolate, histidine imino, and possibly lysine amino residues. The reaction of alkylsulfates with keratin fiber surface nucleophiles is salutary since similar nucleophiles are present in skin proteins, enzymes, and DNA—which could reasonably be expected to undergo similar modification. In the case of skin, this reaction increases the surface hydrophobicity, which alters the skin biochemistry and microbiome. This results in suitable environmental conditions that could exacerbate existing afflictions such as dandruff, eczema, and mouth ulcers.

INTRODUCTION

Surfactants are long-chain compounds that comprise a hydrophilic (polar) head and a hydrophobic (non-polar) tail, giving them the ability to mix immiscible substances such as water and oil. They are broadly classified as anionic, cationic, amphoteric, or non-ionic according to the nature of the hydrophilic head in aqueous solution (Effendy & Maibach, 1995; Salomon & Giordano, 2022). Anionic alkylsulfate-based surfactants have become the most commonly used surfactants in industrial cleaners, household cleaners, and personal care products due to their relative ability to solubilize oils and fatty substances, lower the surface tension of aqueous solutions, or form microemulsions combined with the additional benefit of being cheap and easy to manufacture.

Popular alkylsulfates include sodium lauryl sulfate (SLS), sodium laureth sulfate (SLES), ammonium lauryl sulfate (ALS), and ammonium laureth sulfate (ALES) as shown in Figure 1. These alkylsulfates possess foaming properties when used with water and have therefore found use in many products such as dish detergents, laundry detergents, body soaps, facial cleansers, shampoos, and toothpastes (Effendy & Maibach, 1995; Presley et al., 2021; Salomon & Giordano, 2022). In the personal care market, shampoos contain relatively large amounts of surfactants in order to produce a wide range of effects (in addition to the expected foaming and cleansing properties) such as controlling rheology, improving “mildness” and serving as depositing agents for ingredients such as anti-dandruff agents (Cornwell, 2018).

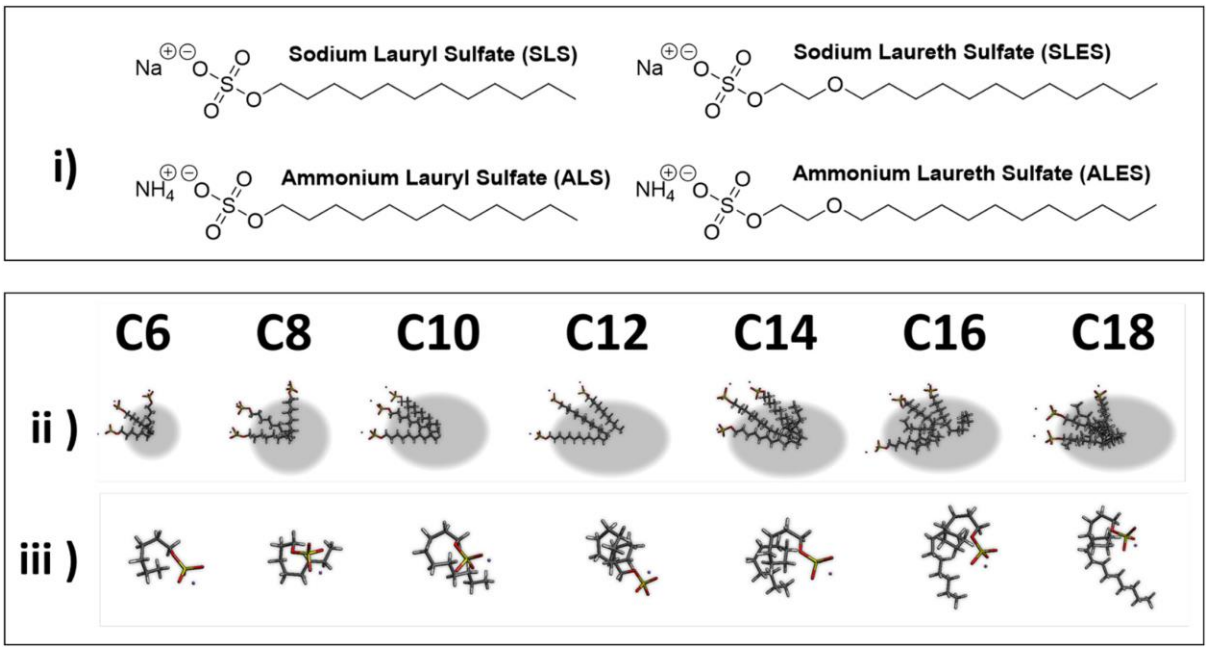

FIGURE 1 (i) Examples of popular alkylsulfates. (ii) sodium alkylsulfates (SAS) alkyl chain length conformational properties above the critical micelle concentration (CMC)—representing a cross section of micelles (Zhao et al., 2023). (iii) SAS alkyl chain length conformational properties below the CMC—coiled to reduce size (Garcia-Dominguez et al., 1977).

Alkylsulfate surfactants used in skin care products are known to reduce the superficial surface tension of proteins and lipids found within the stratum corneum. This serves a role in removing debris (such as excess sebum, oils and dirt) but also presents a risk of damage to the skin barrier function (Salomon & Giordano, 2022; Wilhelm et al., 1994). Many surfactants elicit irritant reactions following application, partially due to their relative ability to solubilize lipid membranes. The swelling effect of topically applied surfactants on corneocyte proteins may result in the removal of natural moisturizing factors — in turn further enhancing the penetration of the surfactant deeper into the epidermis (Paye, 1990). Consequently, this has been associated with skin dryness and irritation. Furthermore, sodium laureth sulfate has been observed inhibiting enzymes that control desquamation, leading to scaling, skin dryness, and impaired barrier function (Schepky et al., 2004). Penetrating the skin further may result in damage to cell membranes and other structural components of keratinocytes, releasing proinflammatory mediators (Seweryn, 2018).

The widespread use of sodium alkylsulfates (SAS) and sodium alkylethoxysulfates (SAES) in hand washes, shampoos, hair dyes, and toothpastes has been questioned due to increasing awareness that alkylsulfates are significant skin irritants. Consumer product ingredient lists describe the surfactant component in the way required by regulatory bodies. For example, sodium lauryl sulfate or sodium laureth sulfate is the official “INCI names” (International Nomenclature of Cosmetic Ingredients) of the compounds. However, there is no reference to the various possible alkylsulfate / alkylethoxysulfate chain lengths (and mixtures thereof) that may be present in the supplied ingredient. It should be noted that such mixtures are well-known and disclosed by the ingredient supplier to the consumer goods manufacturer; however, the ingredients list on consumer personal care products is required to use the relevant INCI name for the ingredient used.

The negative implication of irritancy from certain ingredients such as SLS is never ignored during a product safety assessment; however, it can be reasoned that when contact times are very short and followed by copious water rinsing (i.e. in the case of a shampoo), then it is accepted that residual surfactant is not present. Skin creams and lotions may contain alkylsulfates with much less irritating properties such as sodium cetearyl sulfate (which itself is a mixture of cetyl sulfate and stearyl sulfate) and can conceivably stay on the skin for up to 24 h before water rinsing.

Most SAS and SAES surfactant products are manufactured from palm kernel oil or coconut oil. Subsequently, when these compounds are used in consumer cosmetics, the products applied to the skin / hair will therefore contain numerous fatty chain alkylsulfates and alkylethoxysulfates. Garcia-Dominguez et al. (1977) postulated that in aqueous solutions the lauryl chain surfactant, when below the critical micelle concentration (CMC), possesses a very small (0.2 nm) molecular shape which is readily absorbed through the skin barrier (Figure 1). Longer alkyl chain surfactants are larger in their solution form, and therefore consequently pass less easily through the skin barrier. The latter hypothesis contrasts with the observation that cocoamidobetaine surfactants, having the same alkyl components (but no sulfate residue) are non-irritant, implying that the sulfate moiety may be responsible for irritancy.

A review from the “Cosmetic Ingredient Review Panel” (Fiume et al., 2010) which is supported by the “Personal Care Products Council” (PCPC) concludes that the irritancy potential of alkylsulfate surfactants in cosmetic products is greatly influenced by the alkyl chain length; C_{12} appears to show maximum irritancy whereas C_{16} and C_{18} derivatives are significantly “milder”. Skin irritation can either be via “irritant contact dermatitis” (which is an immediate biological response to irritation from a chemical or abrasion of the skin) or via “allergic contact dermatitis” (which is usually from jewelry, fragrances and preservatives). Mildness can be difficult to define, but generally claims of “mildness” are commonly associated with the “absence of skin irritation symptoms such as redness, swelling and pain” (Cornwell, 2018; Lindberg & Matura, 2011).

A paper by Yanase and Hatta (2018) described the use of x-ray diffraction to follow the disruption of the human stratum corneum lipid structure by sodium dodecyl sulfate and concluded that this surfactant destroyed the intercellular lipid structure. The lipid disruption explanation for irritancy is widely accepted, but there is another factor that seems to have been largely ignored—the possibility of a covalent reaction of the alkylsulfates with nucleophilic sites in proteins and DNA. The sulfate ester group (attached to the alkyl residue) can function as a leaving group in a covalent reaction with nucleophilic sites such as amines, thiols, and hydroxyls. Such reactions with the skin surface proteins would covalently attach hydrophobic alkyl chains, reducing the bound water and subsequently leading to skin dryness. Many publications have assessed the influence of SLS on skin dermatitis and demonstrate that this agent negatively influences basal trans-epidermal water loss (TEWL), probably due to increased hydrophobicity of skin proteins (Basal, 1991). Permanent chemical modification of essential body-function materials such as skin proteins, enzymes, and DNA can also result in a strong immune response. The electrophilic reactive alkylsulfates that are present in skin-contact cosmetic products (i.e. shampoos, soaps and toothpastes) may therefore alter the skin biochemistry and microbiome, which in turn provides suitable environmental conditions that could exacerbate existing afflictions such as dandruff, eczema, and mouth ulcers.

Surfactant manufacturers have modified the sodium alkylsulfates (SAS) structure by incorporating ethoxy residues into the structure to produce SAES in an attempt to reduce irritancy and increase skin hydration. This effect has been confirmed via various studies, such as that published by Loeffler and Happle (2003). This study demonstrated during skin patch testing that, after 7–10 days following patch removal, SLS gave a very pronounced skin reaction whereas SLES

was significantly less aggressive. A study by Wolfenden and Yuan (2007) showed that mono-alkylsulfates covalently react with primary and secondary amine nucleophiles under mild conditions to give the relevant substituted alkylamine, with a bisulfate anion being the leaving group. The latter authors also raised the concern that commercial alkylsulfate detergents may alkylate nucleophiles in biological systems but also suggested that normal (short) exposure minimizes risk. A counterargument would be that frequent use with at least three shampoo applications per week or daily teeth brushing could be regarded as long exposures. Further confirmation of the potential reactivity of lauryl sulfates comes in a review paper by Xiaofei et al. (2014) which describes the alkaline reaction of SLS with dimethylamine to produce dodecyl dimethylamine.

This research paper presents the use of time-of-flight secondary ion mass spectroscopy (ToF-SIMS) to investigate the covalent bonding reactions of alkylsulfates with fibrous keratin protein surfaces. Human hair is regularly shampooed, and hence any permanent chemical modification of the outer surface (1–2 nm) can be easily assessed. As it is impractical to obtain human hair that has never been shampooed, a good substitute is scoured Merino wool — which has similar structural properties and chemistry to hair, albeit being a thinner fiber than hair (~18–23 µm rather than ~80 µm). In this research project, scoured wool fibers were analyzed before and after treatment with a commercially available SAS/SAES surfactant mixture (Tensagex EOC 628BV) to demonstrate the reaction with alkylsulfates that we are hypothesizing (and replicate what would be happening on shampooed hair fibers). ToF-SIMS analysis was used to follow the potential alkylation on the surface of the treated wool fiber.

EXPERIMENTAL

Materials

The human hair tresses used in this study were supplied by Kerling International Haarfabrik GbmH (Backnang, Germany) who are one of the leading suppliers of research hair tresses. It is worth highlighting that the hair tresses are made from real human hair that has been donated by multiple people (or occasionally a single donor). Hair grows on average between 15 and 20 cm per year; therefore, it should be appreciated that a donor giving 10 cm tresses has been growing the hair for quite some time (probably up to 2 years as most donors do not give up hair all the way to the root). Therefore, it can be reasonably assumed that the hair has had a history of being regularly washed in a variety of shampoos and conditioners over several years prior to donation. Scoured wool top (treated with the usual industrial sodium carbonate / non-ionic surfactant washing to remove wool grease) was provided by the Woolmark company (Sydney, Australia).

The simple shampoo used for cleaning the hair tresses in the laboratory was purchased from Morrisons supermarket (Leeds, UK) and was the “Wild Raspberry and Jojoba Extract shampoo”. The INCI ingredients list was as follows: sodium laureth sulfate, cocamidopropylbetaine, perfume, glycerine, citric acid, EDTA, sodium hydroxide, glycol, triethylene glycol, benzyl alcohol, propylene glycol, polysorbate, polyquat-7, benzophenone, sodium benzoate, magnesium nitrate, Rubus Idaeus fruit extract, methylchloroisothiazolinone, Simondsia Chinesis seed oil and hexylene glycol.

To treat the wool fibers, a widely used cosmetic grade of SAS/SAES was used: Tensagex EOC 628BV (28% w/w) solution (Stockmeier Group, Bielefeld, Germany). This product is stated to be a mixture of C_{12}–C_{16} alkylethoxysulfates, having an average of two ethoxy units. This surfactant mixture also contains the antimicrobial preservative Bronopol (2-bromo-2-nitro-1,3-propanediol) when sold in the cosmetic market.

Methods

The normal method for shampooing hair involves a number of steps: (i) Apply the correctamount of shampoo to wet hair; (ii) Massage the shampoo into your scalp and hair; (iii) Lather the shampoo with water; (iv) Work the lather from root to tip; (v) Rinse the hair thoroughly with warm water. The correct amount of shampoo used depends largely on the length and type of hair; for short or fine hair, a teaspoon amount is required, whereas for longer or thicker hair, 2–3 teaspoons may be required (Proctor & Gamble, 2025a, 2025b).

There is generally no fixed amount of time to keep a shampoo product on the hair. The function of a shampoo is to clean the hair by removing grease, oil sebum and dirt, therefore the contact time is dependent on the type and length of hair that the consumer has, and the effectiveness of massaging the lather through the hair. Typically (in real life usage) short hair may require 30–60 s, and longer hair may require up to 3 min of massaging the lather through the hair.

In hair research laboratories, it is quite common to use a “soaking procedure” to test certain actives either from water or a simple shampoo / conditioner formulation. These tests are done to replicate specific contact time experiments. There are a number of published articles that highlight this procedure (although it is normal for hair research laboratories to have their own variation). For example, Patel (1983) reported a soaking procedure where a hair tress was soaked in the test solution at 35°C for 5 min, and Seshadri and Bhushan (2008) disclosed a soaking procedure at room temperature for 5 min.

To mimic the effect of alkylsulfate exposure in multi-use shampooing of human hair, wool samples were dipped into a 28% w/w solution of Tensagex EOC 628BV for 5 min and left overnight at room temperature in a sealed polyethylene bag. Thorough washing with copious amounts of distilled water, followed by drying, completed the procedure. This application procedure was determined to be a suitable method and comparable to the historical shampooing treatment received by the Kerling hair tresses.

ToF-SIMS analyses were performed at Lucideon Ltd. (Stoke-on-Trent, UK). In the case of hair and wool, bismuth ions were used to probe the surfaces. Treated and untreated wool and hair fibers were fixed with adhesive tape in the target area of the ion beam. A sample of Tensagex EOC 628BV (SAS/SAES) was analyzed as a 6% w/w solution that was cast as a film onto an aluminum surface. In all samples, both the negative and positive ions released were investigated and recorded.

Hair and wool fiber surface analysis was conducted using an FEI Quanta 400 environmental SEM (E-SEM) with Oxford IncaSight EDS. Samples were attached to a magnetic lens and examined at 100× magnification. Hair fiber widths were taken from the cross section of different fibers ($N > 40$). The elemental analysis using the EDS system determined the presence of elemental impurities on the fiber surface.

Surface metrology analysis was performed using a Sensofar S Neox 090 instrument with a Nikon DI 50× lens. Hair fiber profiles were analyzed using the ISO4287 method to determine surface roughness profiles to produce the following measurements: total height of the profile (R_t), maximum profile peak height (R_P), maximum profile valley depth (R_V), maximum height of the profile (R_Z), arithmetic mean deviation of assessed profile (R_a), root mean square deviation of assessed profile (R_q), skewness of the assessed profile (R_{SK}) and kurtosis of the assessed profile (R_{ku}). All of these surface profile measurements were averaged from multiple fibers ($N = 5$). Additionally, the peak-to-peak gap (i.e. the distance from peak to peak) was manually recorded – to indicate the varying cuticle length on the hair surface.

All of the relevant fiber surface analysis and ToF-SIMS spectra can be found in the "Supplementary Information File" available online. The ToF-SIMS spectra are shown at a larger scale than those in the manuscript to allow the reader to interpret the spectral intensity data more clearly.

RESULTS AND DISCUSSION

In order to establish comparison between the hair fibers and wool fibers, surface analysis was carried out using scanning electron microscopy with an energy-dispersive X-ray attachment, and surface metrology using white light interferometry profiling. The results of the tests are shown in the supplementary information file. The SEM results confirmed the expectation that the hair fibers were thicker than the wool fibers (an average diameter of 81 µm for the hair compared to 24 µm for the wool fiber). The surface profiling analysis showed that the cuticle cells were of similar overlapping length scales with peak-to-peak gaps of 10 µm for hair compared to 13 µm for the wool fiber. The surface roughness values did not overlap, and most parameters showed a significant difference (t test $p < 0.05$) although both fibers showed equivalent kurtosis (R_{KU}) values of 2.6 nm (hair) compared to 2.4 nm (wool) with t test $p = 0.34$, indicating similar sharpness of the height distributions. Elemental analysis of the hair and wool fibers via SEM-EDS reported similar compositions (>98% being C, N, O, and S) although the hair fibers had more trace elements present than the wool fibers. In summary, the two different types of fibers show some variation in structural composition; however, they present equivalent biological properties for biochemical interactions. These results confirm the well-accepted concept that wool fibers (although thicker) are excellent substitutes for many tests in the hair care industry.

As mentioned previously, it is a reasonable assumption that the purchased hair tresses have had a history of being shampooed (on average twice per week), and as the length of the hair tresses suggests at least 2 years of growth, the estimated number of shampoo treatments can be reasonably assumed to be over 100. Commonly used shampoos often contain a mixture of alkylsulfates and ethoxylated alkylsulfates, derived from naturally available palm kernel oil and/or coconut oil. The synthesis of alkylsulfate surfactants involves trans-esterification of coconut or palm kernel oil with methanol; the ester mix is then hydrogenated to the fatty alcohol mix plus methanol; the latter mixture of compounds is then sulfonated with sulfur trioxide or chlorosulfonic acid, giving a mixture of alkylsulfates. To produce a product with reduced irritation, the intermediate fatty alcohol mixture is reacted with an average of 3 mole of ethylene oxide followed by sulfonation to produce alkylethoxysulfates. The ethoxylation process results in a mixed product that can contain nine or more different ethoxylated alkylsulfates plus numerous non-alkyl poly-ethoxysulfates (Andree et al., 1984; Kölbel & Kurzendörfer, 1969; Kosswig, 2000; Shore & Berger, 1976; Steber et al., 1988).

As previously mentioned, ingredient lists on consumer cosmetic products do not list all possible mixtures of chain lengths (only the INCI names), and therefore the true compositional breakdown of long chain alkylsulfates in shampoos is not disclosed to the consumer. It should be noted that consumer household products do not have the same INCI name requirements as personal care products—in fact, it is normal for such products only to state the percentage of the nonionic and/or anionic surfactant contained within.

The alkyl component analysis of a simple alkylsulfate shampoo was published in the Surfactant Science Series (Vol. 43) book edited by Gloxhuber and Klunstler (1992) and is summarized

in Table 1. The data demonstrate the chemical complexity of a SAS-based shampoo; the major component (50%) is the lauryl (C_{12}) derivative, followed by myristyl (C_{14}) at 16% and then six minor components making up the balance. In the case of Tensagex EOC 628BV, which was selected for this study as a typical commercial ingredient, the C_{12}, C_{14}, and C_{16} derivatives are listed as being present, most likely because coconut oil is the usual source of the original fatty alcohols in similar proportion to that indicated in Table 1.

TABLE 1. The alkyl component distribution in a simple alkylsulfate shampoo.

Alcohol precursor name		Distribution (% w/w)	Partition coefficient[a] (LogP)	CMC[b] (10^{-3} mol/L)	Alkylsulfate	
Traditional	**IUPAC**				**Formula**	**Mol. Wt.**
Capryl alcohol	Octan-1-ol	8	1.64	140	$C_8H_{17}OSO_3^-$ ^+Na	232
Decyl alcohol	Decan-1-ol	8	2.55	33	$C_{10}H_{21}OSO_3^-$ ^+Na	260
Lauryl alcohol	Dodecan-1-ol	50	3.46	8.6	$C_{12}H_{25}OSO_3^-$ ^+Na	288
Myristyl alcohol	1-tetradecanol	16	4.37	2.2	$C_{14}H_{29}OSO_3^-$ ^+Na	316
Palmityl alcohol	Hexadecan-1-ol	8	5.29	0.58	$C_{16}H_{33}OSO_3^-$ ^+Na	344
Stearyl alcohol	Octadecan-1-ol	2	6.20	0.23	$C_{18}H_{37}OSO_3^-$ ^+Na	372
Oleyl alcohol	(Z)-Octadec-9-en-1-ol	5	5.48	<0.05[c]	$C_{18}H_{35}OSO_3^-$ ^+Na	370
Linoleyl alcohol	(9Z,12Z)-Octadeca-9,12-dien-1-ol	2	4.77		$C_{18}H_{33}OSO_3^-$ ^+Na	368

[a] Chemical properties from PerkinElmer Informatics on molecules without sodium counterion.

[b] CMC measurements from Bajpai and Tyagi (2007).

[c] Data from measurements by Ling et al. (2021).

ToF-SIMS of alkylethoxysulfates

Jewett et al. (1999) described the analysis of Dobanol 23PES04, a 62.7% aq. solution of alkylethoxysulfate (Shell Laboratories, Amsterdam) using electrospray mass spectrometry (ESI) and atmospheric pressure chemical ionization. The Dobanol 23PES04 is a similar product to Tensagex EOC 628BV, albeit with a higher solids content (62.7% w/w compared to 28% w/w). Dobanol 23PES04 is supplied as a mixture of C_{12} and C_{13} alkyl chains (indicated by the digits “23”); “PES” stands for (propoxy/ethoxy) sulfate, and the “04” represents 4 ethylene oxide groups, with the

counterion being Na^+. The ESI analysis confirmed that the material was shown to be a complex mixture of C_{12} and C_{13} ethoxysulfates, pure alcohol ethoxylates, and non-sulfated polyethoxylates.

This research paper reports the ToF-SIMs analyses of Tensagex EOC 628BV alkylsulfate surfactant, untreated keratin fibers (merino wool and human hair) and alkylsulfate/alkylethoxysulfate (SAS/SAES) treated wool and shampooed human hair; in the latter cases, any water-soluble, non-bonded surfactant residues were removed by water rinsing.

ToF-SIMS analysis of a SAS/SAES surfactant mixture (Tensagex EOC 628BV)

ToF-SIMS negative and positive ion data were collected from the standard SAS/SAES mixture deposited on aluminum foil. The ion m/z positions of significant species present in Tensagex EOC 628BV, detected in both negative and positive ion modes, are summarized in Table 2.

TABLE 2. Alkylsulfate and alkylethoxysulfate species found in the negative and positive ion time-of-flight secondary ion mass spectroscopy (ToF-SIMS) spectra of standard sodium alkylsulfates (SAS)/sodium alkylethoxysulfates (SAES) (spectral intensities can be seen in the individual spectra).

Negative ion species		**Positive ion species**	
Molecular ion	***m/z***	**Molecular ion**	***m/z***
$C_{12}H_{25}\text{-}OSO_3^-$	265^-	$C_{12}H_{25}OSO_3^-\ Na^+(Na^+)$	311^+
$C_{12}H_{25}\text{-}OC_2H_4\text{-}OSO_3^-$	309^-	$C_{12}H_{25}OC_2H_4OSO_3^-\ Na^+(Na^+)$	355^+
$C_{12}H_{25}\text{-}(OC_2H_4)_2\text{-}OSO_3^-$	353^-	$C_{12}H_{25}(OC_2H_4)_2OSO_3^-\ Na^+(Na^+)$	399^+
$C_{12}H_{25}(OC_2H_4)_3OSO_3^-$	397^-	$C_{12}H_{25}(OC_2H_4)_3OSO_3^-\ Na^+(Na^+)$	443^+
$C_{12}H_{25}(OC_2H_4)_3OSO_3^-$	397^-	$C_{12}H_{25}(OC_2H_4)_3OSO_3^-\ Na^+(Na^+)$	443^+
		$C_{12}H_{25}(OC_2H_4)_4OSO_3^-\ Na^+(Na^+)$	487^+
$C_{12}H_{25}(OC_2H_4)_{10}OSO_3^-$	703		
$C_{12}H_{25}(OC_2H_4)_{12}OSO_3^-$	793		
$C_{14}H_{29}OSO_3^-$	293^-	$C_{14}H_{29}OSO_3^-\ Na^+(Na^+)$	339^+
$C_{14}H_{29}OC_2H_4OSO_3^-$	337^-	$C_{14}H_{29}OC_2H_4OSO_3^-\ Na^+(Na^+)$	383^+
$C_{14}H_{29}(OC_2H_4)_2OSO_3^-$	381^-	$C_{14}H_{29}(OC_2H_4)_2OSO_3^-\ Na^+(Na^+)$	427^+
$C_{14}H_{29}(OC_2H_4)_3OSO_3^-$	425^-	$C_{14}H_{29}(OC_2H_4)_3OSO_3^-\ Na^+(Na^+)$	471^+
		$C_{14}H_{29}(OC_2H_4)_4OSO_3^-\ Na^+(Na^+)$	515^+
$C_{14}H_{29}(OC_2H_4)_7OSO_3^-$	601		
$C_{14}H_{29}(OC_2H_4)_8OSO_3^-$	645		
$C_{14}H_{29}(OC_2H_4)_9OSO_3^-$	689		
$C_{16}H_{33}OSO_3^-$	321^-	$C_{16}H_{33}OSO_3^-\ Na^+(Na^+)$	367^+
$C_{16}H_{33}OC_2H_4OSO_3^-$	365^-	$C_{16}H_{33}OC_2H_4OSO_3^-\ Na^+(Na^+)$	411^+
$C_{16}H_{33}(OC_2H_4)_2OSO_3^-$	409^-	$C_{16}H_{33}(OC_2H_4)_2OSO_3^-\ Na^+(Na^+)$	455^+
$C_{16}H_{33}(OC_2H_4)_3OSO_3^-$	453^-	$C_{16}H_{33}(OC_2H_4)_3OSO_3^-\ Na^+(Na^+)$	499^+
$C_{16}H_{33}(OC_2H_4)_8OSO_3^-$	673		
Bisulfite (HSO_3^-)	80	Na^+	23
Sulfate ($SO_4^=$)	96	$HSO_4^-\ Na^+Na^+$	143
Bisulfate (HSO_4^-)	97	$SO_4^=\ Na^+Na^+Na^+$.	165
Bronopol	200	$HSO_3^-\ Na^+Na^+$	126
		$SO_3^-\ Na^+Na^+Na^+$	149
		Bronopol $Na^+Na^+Na^+Na^+$	292

The negative ion detection analysis indicates that the lauryl (C_{12}) and myristyl (C_{14}) components dominate, with only minor amounts of the palmityl (C_{16}) analogues, somewhat reflecting the composition of the coconut oil/palm kernel oil starting material (Table 1). It is significant that this mass-spec analysis indicated the presence of non-ethoxylated components and components with up to 12° of ethoxylation. Identifiable anionic derivatives are summarized in Table 2.

The positive ion detection analysis again confirms that the commercial SAS/SAES mainly contains lauryl and myristyl residues. The negative ion detection mode showed significant amounts of non-ethoxylated components present along with components having up to 12 ethoxy residues.

ToF-SIMS analysis of scoured wool

Since nature has covalently incorporated alkyl-carbonyl residues as esters and thiol esters in keratin fibers (Anderson & Leeder, 1965; Rivett, 1991) it is firstly necessary to clarify their role in the subsequent analysis of the fatty alkylsulfate covalent bonding interactions with wool and hair. It may be expected that the hair tress has been subject to numerous alkylsulfate treatments in shampooing and regular exposure to light and oxygen. The wool fiber is protected from sunlight and weathering by a layer of wool grease, which is removed prior to processing by scouring; hence, the wool fiber would show more of the surface bonded natural fatty acid esters and amides than human hair. Figure 2 shows the scoured wool ToF-SIMS negative and positive ion analyses, which can be compared to Figure 4 which shows the corresponding analyses after treatment with 28% SAS/SAES.

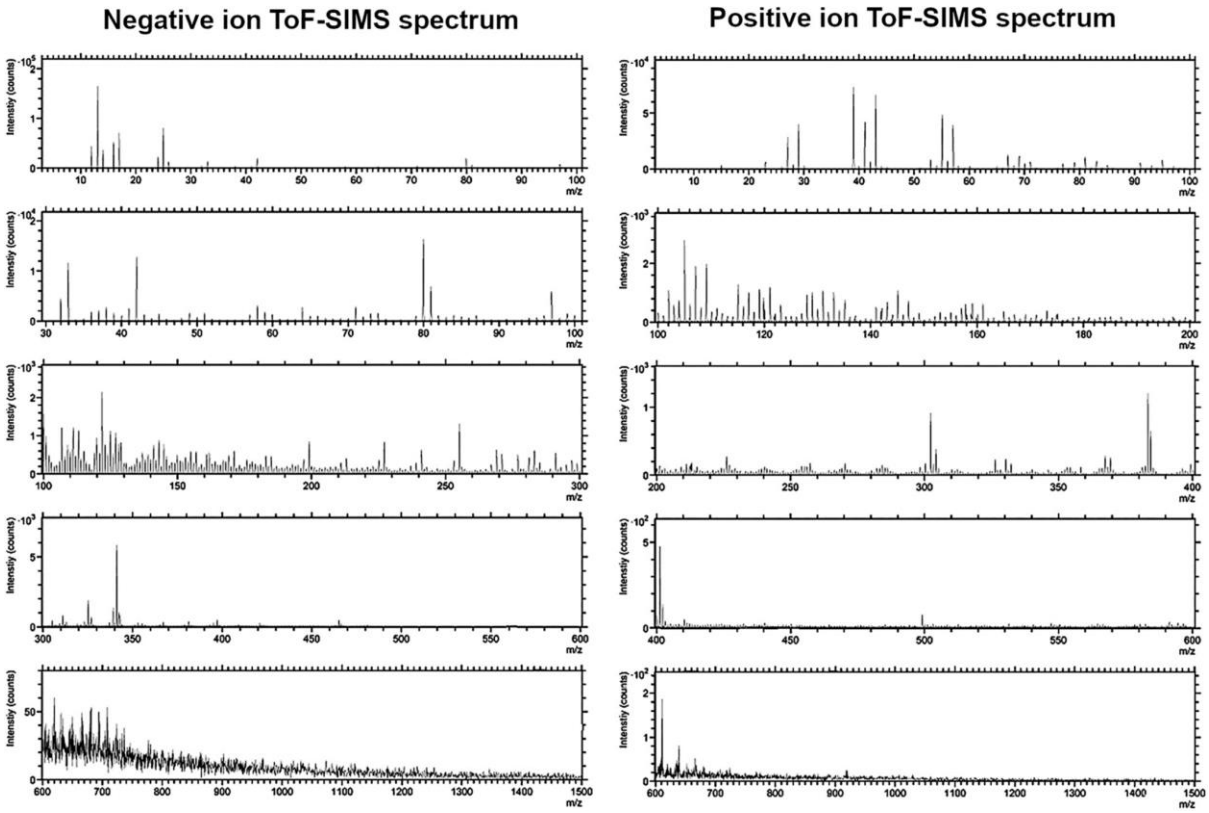

FIGURE 2 Negative (left) and positive (right) time-of-flight secondary ion mass spectroscopy (ToF-SIMS) spectra of scoured wool fibers.

Anderson and Leeder (1965) found 23 different C_{12}–C_{24} saturated and unsaturated acids in wool fabric that had been scoured with a non-ionic detergent. Leeder et al. (1985), when studying the hydrophobic surface of wool epicuticle cells, termed this non-proteinaceous surface the 'F' layer and suggested it was made up of fatty acids covalently bonded to the underlying proteinaceous material. Analysis of extracts obtained using potassium *tert*-butoxide in *tert*-butanol revealed the presence of fatty acids; the major component (about 40%) was found to be 18-methyleicosanoic acid (18-MEA) bonded to the cysteine thiol residue (Anderson & Leeder, (1965). Subsequently, gas chromatography-mass spectra studies on human hair extracts by Wertz and Dowling (1988) confirmed the presence of the 18-MEA species, and these authors proposed that 18-MEA was covalently attached to thiol and hydroxyl residues on the fiber cell surface, supporting the earlier work on wool by Evans et al. (1985). ToF-SIMS was used by Rankin and Carr (2013) to characterize the surface-bound lipids on wool, where about 70% of the acids were found to be bound to the fiber surface as thiol esters and the remainder were bonded as oxygen esters – no evidence of linking as amides through lysine or histidine amino residues was found. The above studies determined that 18-methyleicosanoic acid and other fatty acids are bonded primarily to cysteine thiol as thiol esters; in particular, 18-MEA in mass spec analysis gave a negative ion at *m/z* 341 corresponding to the $C_{21}S^-$ thiol ester derivative (Wolfenden & Yuan, (2007). If 18-methyleicosanoate acyl residues were bonded to tyrosine phenolate or lysine/histidine amino residues, the negative ions would be detected at m/z 325 and/or 326; in the spectrum from scoured wool (Figure 2) a small peak can be discerned in this region to confirm this.

To simplify the subsequent interpretation of the ToF-SIMS spectra of SAS/SAES surfactant treated keratin fibers, it was decided to follow the protocol used by Wertz and Dowling (1989) to evaluate only those fatty acid residues bonded to wool at a level greater than 1% which are: $C_{16:0}$ (17%), $C_{16:1}$ (1.9%), $C_{17:Br}$ (4.6%), $C_{18:0}$ (10.2%), $C_{18:1}$ (5.2%), $C_{19:0}$ (3.8%), $C_{20:0}$, $C_{21:0}$ ante-iso (47.6%).

The proposed m/z values of detectable negative ions from the above fatty acids, which are capable of forming esters, thioesters, and amides (and which can associate with protons and/or sodium cations to produce positive ions) are shown in Table 3.

Examination of the negative ions in Figure 2 reveals a clear m/z peak at 255 that can be attributed to $C_{16}O-$, corresponding to a palmitic acid ester, a small peak at 253 attributed to $C_{16:1O}^-$, a small peak at 269 attributed to $C_{17Br}O^-$, a small peak at 325 attributed to $C_{21Br}O^-$, and a significant peak at 341 attributed to the $C_{21Br}S^-$. Examination of the positive ions in Figure 2 reveals m/z peaks: 302 attributed to $C_{16}O^-\ Na^+Na^+$, a small peak at 327 attributed to $C_{21Br}O^-\ H^+H^+$, and a significant peak at 385 attributed to the $C_{21Br}S^-Na^+Na^+$; the strong peak at 401 could not be readily assigned, but a possible ion could be the sulfoxide derivative $C_{21Br}OS^-Na^+Na^+$.

ToF-SIMS analysis of scoured wool and SAS/SAES treated wool

Organo-sulfates readily undergo covalent bonding reactions with nucleophiles wherein sulfate is the leaving group (Guthrie, 1952; Lewis & Zhao, 2004; Wolfenden & Yuan, 2007; Xiaofei et al., 2014). Sodium alkylsulfate and sodium alkylethoxysulfate, if present in shampoos, would be expected to react with nucleophilic sites in proteins. In keratin fibers, these latter sites are most likely cysteine thiol, lysine ε-amino, histidine imidazoyl imino, and tyrosine phenolate. In terms of nucleophilicity at neutral pH and low temperatures (15–35°C), it might be expected that the cysteine thiol, the amino sites in histidine or lysine, and the phenolic hydroxyl group in tyrosine would most likely be modified. In wool keratin, the cited amino acids are present in the following amounts (Bradbury et al., 1965): cysteine 0.6 mol%; lysine 3.1 mol%; histidine 0.9 mol%; tyrosine 4.0 mol%. Of the amino

acids having sufficient room temperature reactivity with alkylsulfates, tyrosine phenolate is the most abundant, followed by histidine imino and then cysteine thiol. Exemplifying the lauryl derivatives, the proposed general reactions of SAS/SAES with a nucleophilic amino acid side chain in keratin proteins (Keratin–XH) are shown in Figure 3.

TABLE 3. Negative and positive ions from time-of-flight secondary ion mass spectroscopy (ToF-SIMS) spectra of scoured wool.

Negative ion species		**Positive ion species**					
Ion	***m/z***	**Ion**	***m/z***	**Ion**	***m/z***	**Ion**	***m/z***
$C_{16}O^-$	255	$C_{16}O^- H^+H^+$	257	$C_{16}O^- H^+Na^+$	280	$C_{16}O^- Na^+Na^+$	303
$C_{16:1}O^-$	253	$C_{16:1}O^- H^+H^+$	255	$C_{16:1}O^- H^+Na^+$	277	$C_{16:1}O^- Na^+Na^+$	300
$C_{16}S^-$	271	$C_{16}S^- H^+H^+$	273	$C_{16}S^- H^+Na^+$	295	$C_{16}S^- Na^+Na^+$	318
$C_{16:1}S^-$	269	$C_{16:1}S^- H^+H^+$	271	$C_{16:1}S^- H^+Na^+$	293	$C_{16:1}S^- Na^+Na^+$	316
$C_{16}NH^-$	254						
		$C_{16}NH_3^+$	256				
$C_{16:1}NH$	252						
		$C_{16:1}NH_3^+$	254				
		$C_{17}NH_3^+$	270				
$C_{17Br}O^-$	269	$C_{17Br}O^- H^+H^+$	271	$C_{17Br}O^- H^+Na^+$	293	$C_{17Br}O^- Na^+Na^+$	316
$C_{17Br}S^-$	285						
$C_{17Br}NH^-$	268						
		$C_{18}NH_3^+$	284				
$C_{18}O^-$	283	$C_{18}O^- H^+H^+$	285	$C_{18}O^- H^+Na^+$	307	$C_{18}O^- Na^+Na^+$	330
$C_{18}S^-$	299	$C_{18}S^- H^+H^+$	301	$C_{18}S^- H^+Na^+$	323	$C_{18}S^- Na^+Na^+$	344
$C_{18}NH^-$	282						
		$C_{18:1}NH_3^+$	282				
$C_{18:1}O^-$	281	$C_{18:1}O^- H^+H^+$	283	$C_{18:1}O^- H^+Na^+$	305	$C_{18:1}O^- Na^+Na^+$	328
$C_{18:1}S^-$	297	$C_{18:1}S^- H^+H^+$	299	$C_{18:1}S^- H^+Na^+$	321	$C_{18:1}S^- Na^+Na^+$	344
$C_{18:1}NH^-$	280						
$C_{19Br}O^-$	297					$C_{19Br}O^- Na^+Na^+$	344
$C_{19Br}S^-$	313						
$C_{19Br}NH^-$	296						
$C_{21Br}O^-$(or 18-MEA)	325	$C_{21Br}O^- H^+H^+$	327	$C_{21Br}O^- H^+Na^+$	349	$C_{21Br}O^- Na^+Na^+$	372
$C_{21Br}S^-$	341	$C_{21Br}S^- H^+H^+$	343	$C_{21Br}S^- H^+Na^+$	365	$C_{21Br}S^- Na^+Na^+$	388
$C_{21Br}NH^-$	324	$C_{21Br}NH^- H^+H^+$	326				

SLS

SLES

FIGURE 3 Reaction of sodium alkylsulfates (SAS)/sodium alkylethoxysulfates (SAES) with a nucleophilic amino acid side chain, found in keratin proteins.

If X is sulfur, then following a reaction with cysteine thiol, the covalently bonded species would be: $CH_3(CH_2)_{11}S$–Keratin and $CH_3(CH_2)_{11}(OC_2H_4)_nS$–Keratin. In this case, the ToF-SIMS analysis would therefore detect the following negative ions:

- $CH_3(CH_2)_{11}S^-$ peak appearing at 201 m/z
- Mono-ethoxylate $CH_3(CH_2)_{11}(OC_2H_4)S^-$ peak appearing at 245 m/z
- Di-ethoxylate $CH_3(CH_2)_{11}(OC_2H_4)_2S^-$ peak appearing at 289 m/z
- Tri-ethoxylate $CH_3(CH_2)_{11}(OC_2H_4)_3S^-$ peak appearing at 333 m/z
- The alkyl-sulfides that are bonded at the wool surface would be prone to photocatalyzed oxidation in air to produce the mono-oxysulfide, $CH_3(CH_2)_{11}(S=O)^-$ hence the negative ion found at m/z 217.

Positive ions are detected since even apparently negative species will associate with protons and sodium ions. The proton association was not seen when analyzing the Tensagex EOC 628BV SAS/SAES film since there are no protons to extract from the aluminum base. Keratin fibers are rich in protonated species and therefore the association of protons with the negative ions is predictable. Proposed ions that should be detectable in ToF-SIMS following the reaction of SAS/SAES with nucleophilic sites in keratin fibers are listed in Table 4. The negative and positive ion spectra (ToF-SIMS) from wool treated with SAS/SAES are shown in Figure 2.

The following significant negative m/z peaks were detected in the SAS/SAES treated wool negative ion spectrum (Figure 4) but not in the corresponding spectrum of untreated scoured wool (Figure 2): m/z 185 = $C_{12}H_{25}O^-$ (lauryl tyrosine derivative); 200 = $C_{12}H_{25}S^-$ (lauryl cysteine derivative also capryl-monoethoxylate tyrosine derivative); 215 = $C_{13}H_{27}S^-$ (cysteine derivative); 265 = $C_{12}H_{25}OSO_3^-$ (lauryl sulfate); 294 = $C_{14}H_{29}OSO_3^-$ (myristyl sulfate); 309 = $C_{12}H_{25}OC_2H_4OSO3^-$ (lauryl monoethoxylatesulfate); 321 = $C_{16}H_{33}OSO_3^-$ (palmitoyl sulfate); 337 = $C_{14}H_{29}OC_2H_4OSO_3^-$ (myristyl monoethoxylatesulfate); 341 = 18-methyleicosanoic acid (18-MEA) bonded to cysteine thiol (which is a naturally occurring thiol ester).

It may be concluded from the negative ion analysis that in scoured wool, tyrosine–OH is a readily available nucleophilic site, along with cysteine thiol, which can take part in electrophilic substitution reactions with the organo-sulfates.

Figure 4 reveals that SAS/SAES treated wool shows new m/z positive ion peaks that are not present in scoured wool (Figure 2): significant peaks include: m/z 225+ = $C_{12}H_{25}S^-\ H^+Na^+$ (reaction of SLS with cysteine–SH); 231+ = $C_{12}H_{25}OC_2H_4O^-\ H^+H^+$ (reaction of SLES with tyrosine–OH); 231+ = $C_{14}H_{29}OC_2H_4S^-\ H^+H^+$ (reaction of myristylethoxysulfate with cysteine–SH). Peaks of lower significance include: 228+ and 230,+ which are attributed to lauryl ethoxy histidine derivatives, $CH_3(CH_2)_{11}OCH_2CH{=}NH_2$+ and $CH_3(CH_2)_{11}OCH_2CH_2NH_3^+$; 247+ = $C_{12}H_{25}OC_2H_4S^-\ H^+H^+$ (reaction of SLES

with cysteine–SH); 247+ = $C_{12}H_{25}S^-$ Na^+Na^+ (reaction of SLS with cysteine–SH); 275+ = $C_{12}H_{25}OC_2H_4O^-$ Na^+Na^+ (reaction of SLES with tyrosine–OH) and $C_{14}H_{29}OC_2H_4S^-$ H^+H^+; 291+ = $C_{12}H_{25}OC_2H_4S^-$ Na^+Na^+ (reaction of SLES with cysteine–SH); 299+ = $C_{14}H_{29}OC_2H_4S^-$ H^+Na^+ (reaction of SLES with cysteine–SH); 311 = unreacted $CH_3(CH_2)_{11}OSO_3^-$ Na^+Na^+.

TABLE 4. Proposed ions that should be detectable in time-of-flight secondary ion mass spectroscopy (ToF-SIMS) following the reaction of sodium alkylsulfates (SAS)/sodium alkylethoxysulfates (SAES) with nucleophilic sites in keratin fibers.

Cysteinyl species					
Negative ions	***m/z***	**Positive ions**	***m/z***	**Positive ions**	***m/z***
$C_{12}H_{25}S^-$	201	$C_{12}H_{25}S^-$ $H^+(Na^+)$	225	$C_{12}H_{25}S^-$ $Na^+(Na^+)$	247
$C_{12}H_{25}(S{=}O)^-$	217	$C_{12}H_{25}(S{=}O)^-$ $H^+(Na^+)$	241	$C_{12}H_{25}(S{=}O)^-$ $Na^+(Na^+)$	263
$C_{12}H_{25}OC_2H_4S^-$	245	$C_{12}H_{25}OC_2H_4S^-$ $H^+(Na^+)$	269	$C_{12}H_{25}OC_2H_4S^-$ $Na^+(Na^+)$	291
$C_{12}H_{25}(OC_2H_4)_2S^-$	289			$C_{12}H_{25}(OC_2H_4)_2S^-$ $Na^+(Na^+)$	335
$C_{12}H_{25}(OC_2H_4)_3S^-$	333			$C_{12}H_{25}(OC_2H_4)_3S^-$ $Na^+(Na^+)$	379
$C_{12}H_{25}SO^-$	217			$C_{12}H_{25}SO^-$ Na^+Na^+	263
$C_{14}H_{29}S^-$	229			$C_{14}H_{29}S^-$ $Na^+(Na^+)$	275
$C_{14}H_{29}OC_2H_4S^-$	273			$C_{14}H_{29}OC_2H_4S^-$ $Na^+(Na^+)$	319
$C_{14}H_{29}(OC_2H_4)_2S^-$	317			$C_{14}H_{29}(OC_2H_4)_2S^-$ $Na^+(Na^+)$	363
$C_{14}H_{29}(OC_2H_4)_3S^-$	361			$C_{14}H_{29}(OC_2H_4)_3S^-$ $Na^+(Na^+)$	407
Alkoxide species					
$C_{12}H_{25}O^-$	185	$C_{12}H_{25}O^-$ $H^+(Na^+)$	209	$C_{12}H_{25}O^-$ $Na^+(Na^+)$	231
$C_{12}H_{25}OC_2H_4O^-$	229	$C_{12}H_{25}OC_2H_4O^-$ $H^+(Na^+)$	253	$C_{12}H_{25}OC_2H_4O^-$ $Na^+(Na^+)$	275
$C_{12}H_{25}\text{-}(OC_2H_4)_2\text{-}O^-$	273	$C_{12}H_{25}(OC_2H_4)_2O^-$ $H^+(Na^+)$	297	$C_{12}H_{25}(OC_2H_4)_2O^-$ $Na^+(Na^+)$	319
$C_{12}H_{25}(OC_2H_4)_3O^-$	317	$C_{12}H_{25}(OC_2H_4)_3O^-$ $H^+(Na^+)$	341	$C_{12}H_{25}(OC_2H_4)_3O^-$ $Na^+(Na^+)$	363
$C_{14}H_{29}O^-$	213	$C_{14}H_{29}O^-$ $H^+(Na^+)$	237	$C_{14}H_{29}O^-$ $Na^+(Na^+)$	259
$C_{14}H_{29}OC_2H_4O^-$	257	$C_{14}H_{29}OC_2H_4O^-$ $H^+(Na^+)$	281	$C_{14}H_{29}OC_2H_4O^-$ $Na^+(Na^+)$	303
$C_{14}H_{29}(OC_2H_4)_2O^-$	301	$C_{14}H_{29}(OC_2H_4)_2O^-$ $H^+(Na^+)$	325	$C_{14}H_{29}(OC_2H_4)_2O^-$ $Na^+(Na^+)$	347
$C_{14}H_{29}(OC_2H_4)_3O^-$	345	$C_{14}H_{29}(OC_2H_4)_3O^-$ $H^+(Na^+)$	369	$C_{14}H_{29}(OC_2H_4)_3O^-$ $Na^+(Na^+)$	391
$C_{16}H_{33}O^-$	241	$C_{16}H_{33}O^-$ $H^+(Na^+)$	265	$C_{16}H_{33}O^-$ $Na^+(Na^+)$	287
$C_{16}H_{33}OC_2H_4O^-$	285	$C_{16}H_{33}OC_2H_4O^-$ $H^+(Na^+)$	309	$C_{16}H_{33}OC_2H_4O^-$ $Na^+(Na^+)$	331
$C_{16}H_{33}(OC_2H_4)_2O^-$	329			$C_{16}H_{33}(OC_2H_4)_2O^-$ $Na^+(Na^+)$	375
$C_{16}H_{33}(OC_2H_4)_3O^-$	373			$C_{16}H_{33}(OC_2H_4)_3O^-$ $Na^+(Na^+)$	419
Amine species					
$C_{12}H_{25}NH_3^+$	186			$C_{12}H_{25}NH_2$ Na^+	208
$C_{12}H_{25}OC_2H_4NH_3^+$	230			$C_{12}H_{25}OC_2H_4NH_2$ Na^+	252
$C_{12}H_{25}(OC_2H_4)_2NH_3^+$	274			$C_{12}H_{25}(OC_2H_4)_2NH_2$ Na^+	296
$C_{12}H_{25}(OC_2H_4)_3NH_3^+$	318			$C_{12}H_{25}(OC_2H_4)_3NH_2$ Na^+	340
$C_{14}H_{29}NH_3^+$	214			$C_{14}H_{29}NH_2$ Na^+	236
$C_{14}H_{29}OC_2H_4NH_3^+$	258			$C_{14}H_{29}OC_2H_4NH_2$ Na^+	380
$C_{14}H_{29}(OC_2H_4)_2NH_3^+$	302			$C_{14}H_{29}(OC_2H_4)_2NH_2$ Na^+	324
$C_{14}H_{29}(OC_2H_4)_3NH_3^+$	346			$C_{14}H_{29}(OC_2H_4)_3NH_2$ Na^+	368
$C_{16}H_{33}NH_3^+$	242			$C_{16}H_{33}NH_2$ Na^+	264
$C_{16}H_{33}OC_2H_4NH_3^+$	286			$C_{16}H_{33}OC_2H_4NH_2$ Na^+	308
$C_{16}H_{33}(OC_2H_4)_2NH_3^+$	330			$C_{16}H_{33}(OC_2H_4)_2NH_2$ Na^+	352
$C_{16}H_{33}(OC_2H_4)_3NH_3^+$	373			$C_{16}H_{33}(OC_2H_4)_3NH_2$ Na^+	396

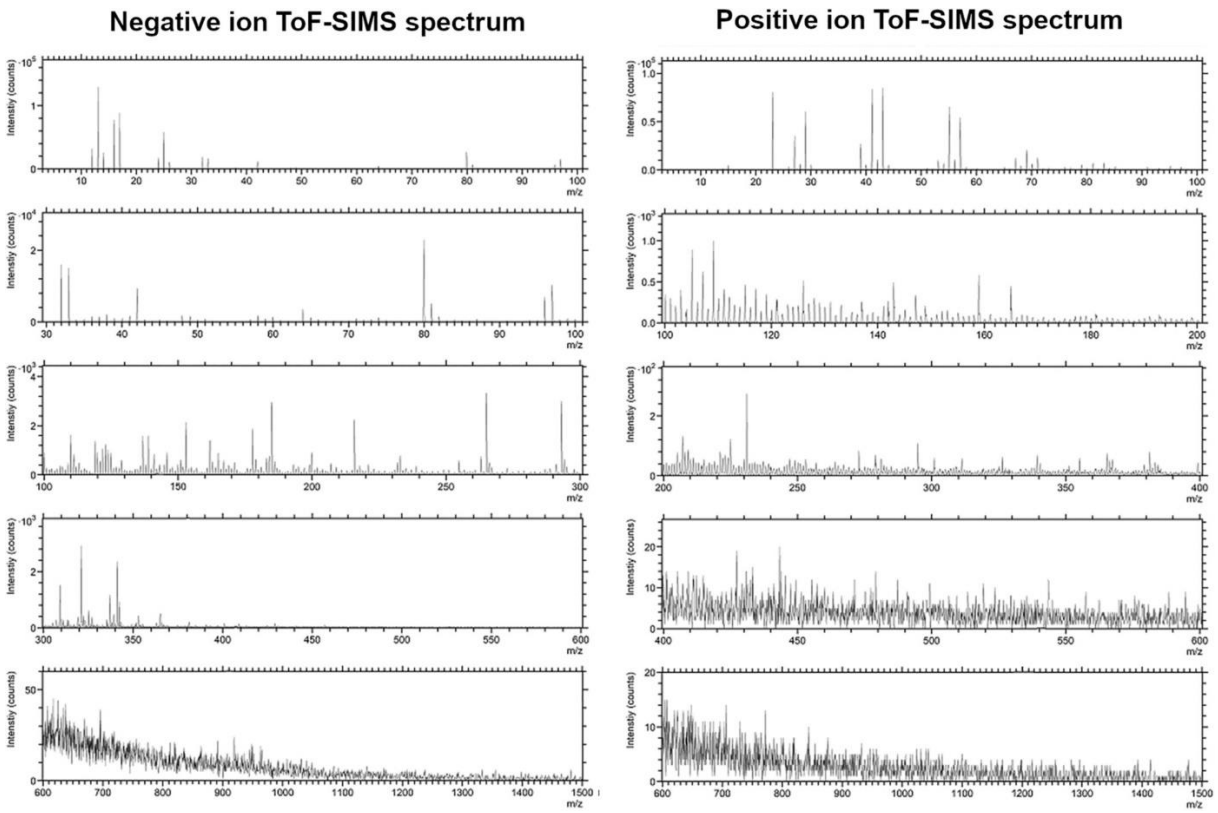

FIGURE 4. Negative ion (left) and positive ion (right) time-of-flight secondary ion mass spectroscopy (ToF-SIMS) spectra of wool fibers treated with sodium alkylsulfates (SAS)/sodium alkylethoxysulfates (SAES) (Tensagex EOC 628BV: 28%).

ToF SIMS analysis of hair fibers

The above positive ion results reveal that tyrosine-OH and cysteine-SH are the nucleophilic sites most available for the surface reaction of wool with alkylsulfate surfactants; in contrast with the hair analysis, the evidence for histidine imino reaction with wool is scant. Highly nucleophilic cysteine thiol is more abundant on the scoured wool surface; in contrast, the human hair surface contains little free cysteine and thus, in the hair case, the main reaction site for the alkylsulfates is the histidine imino residue. The m/z ion TOF-SIMS analysis from the shampooed hair tress (Figures 5 and 6) shows differences to the corresponding SAS/SAES treated wool spectra (Figure 4).

In the case of the hair tress negative ion ToF SIMS spectrum (Figure 5), there are no peaks at m/z 201 or 245 corresponding to a lauryl sulfate / laureth sulfate reaction with cysteine thiol residues (i.e. $C_{12}H_{25}S^-$ and $C_{12}H_{25}OC_2H_4S^-$ ions). This result indicates that since hair surfaces are regularly exposed to air and light, plus frequent shampooing, they possess minimal free cysteine thiols – unlike wool, where the thiol residue is protected from oxidation by wool grease. Figure 5 shows the positive ion ToF-SIMS spectra from shampooed hair.

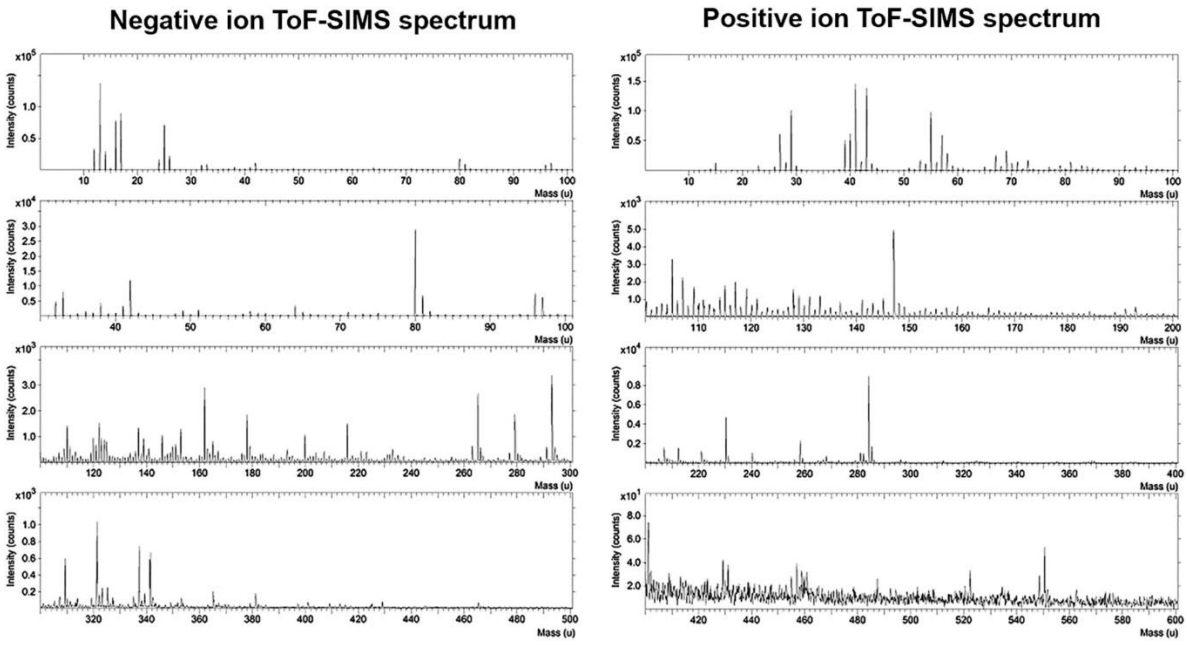

FIGURE 5. Negative ion (left) and positive ion (right) time-of-flight secondary ion mass spectroscopy (ToF-SIMS) spectra of shampooed hair tresses.

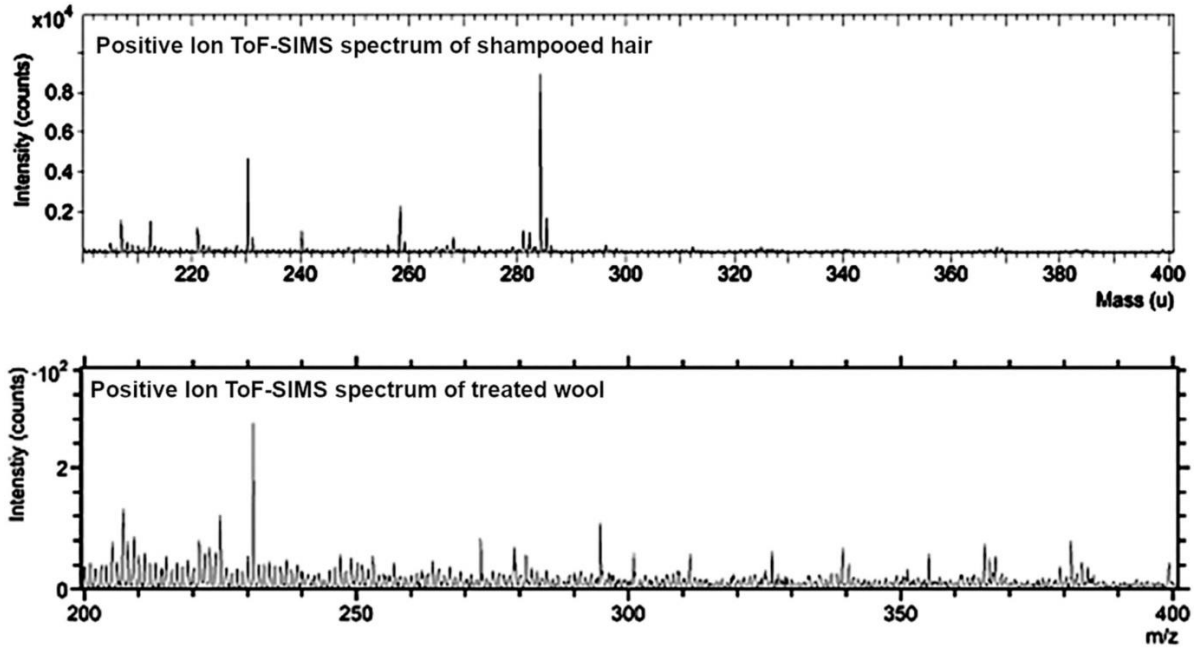

FIGURE 6. Magnified region (200–360 m/z) positive ion time-of-flight secondary ion mass spectroscopy (ToF-SIMS) spectra of freshly shampooed hair tresses (top) and sodium alkylsulfates (SAS)/sodium alkylethoxysulfates (SAES) treated wool (bottom).

To emphasize the differences between hair and SAS/SAES treated wool, the positive ion m/z spectra focused on the region 200–360 are reproduced in Figure 6. If X is the imidazoyl NH residue in histidine or the primary amino in lysine, then the ToF-SIMS analysis would show peaks in the positive ion mode. The reaction of the alkylsulfate with lysine ε-amino residues could also be associated with

the amine derivatives, but under neutral application conditions, the lysine residue is of significantly lower nucleophilicity than the histidine imidazole amine (pK_a of the lysine ε-amino residue is 9.7 whereas the pK_a value of the histidine imidazole amino residue is 6.0).

Figure 7 compares the negative ion and positive ion spectra from shampooed hair and wool in the most significant m/z region (200–400). Both keratin substrates show evidence that the histidine imidazoyl amino residue is a site for alkylation with alkylsulfates; the extent of alkylamine substitution in the hair tress is much more significant than that in scoured wool post-treated with the Tensagex EOC 628BV alkylsulfate mixture.

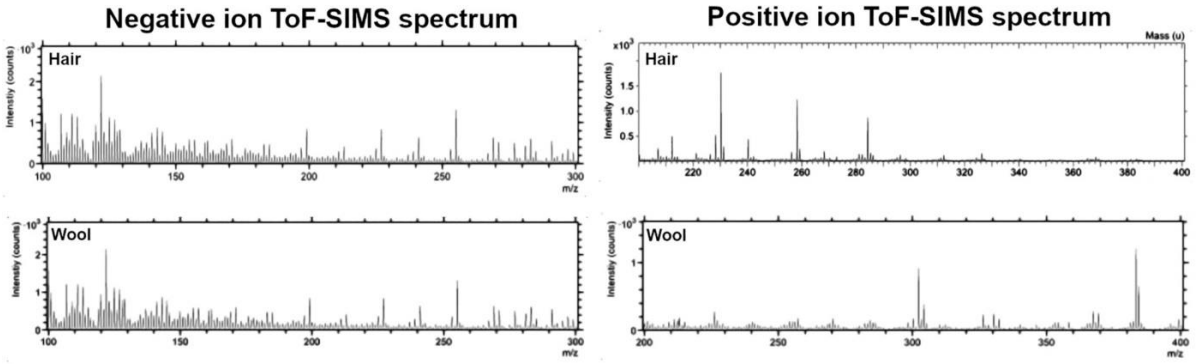

FIGURE 7. Comparison of negative ion (left) and positive ion (right) time-of-flight secondary ion mass spectroscopy (ToF-SIMS) spectra of shampooed hair tresses and wool.

Human hair tresses show the following positive ion mass spec peaks (m/z): 228+ and 230+ attributed to the lauryl ethoxy histidine derivatives $CH_3(CH_2)_{11}OCH_2CH{=}NH_2^+$ and $CH_3(CH_2)_{11}OCH_2CH_2NH_3^+$; 258+ and 259+ attributed to the myristyl ethoxy histidine derivatives $CH_3(CH_2)_{13}OCH_2CH{=}NH_2^+$ and $CH_3(CH_2)_{13}OCH_2CH_2NH_3^+$; 284+ and 285+ attributed to the cetyl ethoxy histidine derivatives $CH_3(CH_2)_{15}OCH_2CH{=}NH_2^+$ and $CH_3(CH_2)_{15}OCH_2CH_2NH_3^+$. Residual conditioner (cetyltrimethylammonium chloride) on the hair tress may also be associated with the m/z 284+ peak.

Scoured wool did not show strong positive m/z peaks at 228, 230, 240, 258, or 284, but there were strong positive ion peaks at 303 and 383; the latter are attributed to naturally bonded fatty alkyl esters bonded to cysteine thiol and tyrosine phenolate. As the latter ions are absent on hair, it is likely that the oxidative degradation at the hair surface has hydrolyzed these naturally bonded residues.

Wool treated with the SAS/SAES surfactant mixture showed the following m/z positive ion peaks: 225+ = $C_{12}H_{25}S^-\ H^+Na^+$ (reaction of SLS with cysteine-SH); 228+ and 230+ = lauryl ethoxy histidine derivatives $CH_3(CH_2)_{11}OCH_2CH{=}NH_2^+$ and $CH_3(CH_2)_{11}OCH_2CH_2NH_3^+$; 231+ = $C_{12}H_{25}OC_2H_4O^-\ H^+H^+$ (reaction of SLES with tyrosine–OH); 231+ = $C_{14}H_{29}OC_2H_4S^-\ H^+H^+$ (reaction of SLES with cysteine–SH); 231+ = $C_{12}H_{25}OC_2H_4O^-\ H^+H^+$ (reaction of SLES with tyrosine–OH); 247+ = $C_{12}H_{25}OC_2H_4S^-\ H^+H^+$ (reaction of SLES with cysteine–SH); 247+ = $C_{12}H_{25}S^-\ Na^+Na^+$ (reaction of SLS with cysteine–SH); 275+ = $C_{12}H_{25}OC_2H_4O^-\ Na^+Na^+$ (reaction of SLES with tyrosine–OH); 291+ = $C_{12}H_{25}OC_2H_4S^-\ Na^+Na^+$ (reaction of SLES with cysteine–SH); 299+ = $C_{14}H_{29}OC_2H_4S^-\ H^+Na^+$ (reaction of SLES with cysteine–H).

The differences in wool and human hair surface chemistry are surprising since both fibers naturally contain very similar keratins. The source of these differences must be that the available human hair is shampooed regularly and daily exposed to light and oxygen (hence hair surface cysteine thiol and cystine disulfide residues are significantly converted to cysteic acid). It is noteworthy that skin and enzyme proteins in the follicle regions can also be chemically modified by alkylsulfates in the same way as keratin fibers.

CONCLUSIONS

ToF-SIMS surface analysis of two similar keratin fibers, merino wool and human hair, has been used to demonstrate surprising differences in surface chemistry. Since human hair by its nature is shampooed regularly and also regularly exposed to light and oxygen, free cysteine thiol and cystine disulfide surface residues are oxidized to cysteic acid and are no longer available to take part in surface reactions with alkylsulfates. The ToF-SIMS results on the hair fibers show that alkylsulfates in shampoos do interact covalently with amino and tyrosine hydroxyl residues at the fiber surfaces. As a comparison, the reaction of scoured wool fibers with the alkylsulfate mixture also indicates a reaction with cysteine thiol and tyrosine phenolate residues.

The reaction of alkylsulfates with keratin fiber surface nucleophiles is salutary since similar nucleophiles are present in skin proteins, enzymes, and DNA – which could reasonably be expected to undergo similar modification. In the case of skin, this reaction increases the surface hydrophobicity, which alters the skin biochemistry and microbiome. This results in suitable environmental conditions that could exacerbate existing afflictions such as dandruff, eczema, and mouth ulcers.

REFERENCES

Anderson CA, Leeder JD. Residual lipid contaminants in processed wool: the composition of the free fatty acids in wool fabric. Text Res J. 1965; 35(5): 416–421.

Andree H, Jakobi G, Schwuger M. CESIO, Proceedings of 1st world surfactant congress, Munich. Vol 4; 1984. p. 5–23.

Bajpai D, Tyagi VK. Laundry detergents: an overview. J Oleo Sci. 2007; 56(7): 327–340.

Basal AT. Transepidermal water loss, skin thickness and skin colour in relation to sodium-lauryl-sulphate-induced-irritation in normal skin. Contact Derm. 1991; 25(2): 108–114.

Bradbury JH, Chapman GV, King NLR. The chemical composition of wool. II. Analysis of the major histological components produced by ultrasonic disintegration. Aust J Biol Sci. 1965; 18: 353–364.

Cornwell P. A review of shampoo surfactant technology: consumer benefits, raw materials and recent developments. Int J Cosmet Sci. 2018; 40(1): 16–30.

Effendy I, Maibach HI. Surfactants and experimental irritant contact dermatitis. Contact Derm. 1995; 33(4): 217–225.

Evans DJ, Leeder JD, Rippon JA, Rivett DE. Separation and analysis of the surface lipids of the wool fibre. Procedings of the 7th international wool textile research conference. Volume I. Tokyo: Society of Fiber Science and Technology; 1985. p. 135–142.

Fiume M, Bergfeld WF, Belsito DV, Klassen CD, Marks JG, Shank RC, et al. Final report on the safety assessment of sodium cetearyl sulfate and related alkyl sulfates as used in cosmetics. Int J Toxicol. 2010; 29(3): 115–132.

Garcia-Dominguez J, Parra JL, Infante MR, Balaguer F, Sastra T. A new approach to the theory of adsorption and permeability of surfactants on keratinic proteins: the specific behaviour of certain hydrophobic chains. J Soc Cosmet Chem. 1977; 28(4): 165–182.

Gloxhuber C, Klunstler K. Anionic surfactants: biochemistry, toxicology, dermatology. Vol 43. 2nd ed. Boca Raton, FL: CRC Press; 1992.

Guthrie JD. Attachment of dyes to cotton by ether link. Am Dyest Rep. 1952; 41(1): 13–30

Jewett BN, Ramaley L, Kwak JCT. Atmospheric pressure ionization mass spectrometry techniques for the analysis of alkyl ethoxysulfate mixtures. J Am Soc Mass Spectrom. 1999; 10: 529–536.

Kölbel H, Kurzendörfer P. Konstitution und Eigenschaften von Tensiden. Angew Chem Int Ed. 1969; 12(2): 252.

Kosswig K. Surfactants. Ullmann's encyclopaedia of industrial chemistry. Wiley; 2000.

Leeder JD, Rippon JA, Rivett DE. Modification of the surface properties of wool by treatment with anhydrous alkali. Proceedings of the 7th International wool textile research conference. Volume 4. Tokyo: Society of Fiber Science and Technology; 1985. p. 312–321.

Lewis DM, Zhao XF. Synthesis of triethanolamine trisulfate and its application to cellulosic fabrics. Color Technol. 2004; 120: 172–179.

Lindberg M, Matura M. Patch testing. In: JD Johansen, PJ Frosch, editors. Contact dermatitis. 5th ed. Heidelberg: Springer; 2011. p. 439–464.

Ling T, Li P, Yang X, Li J, Jiang Z, Ren X, et al. Preparation, adsorption and aggregation behavior of sodium oleyl sulfate with high double bond retention. J Mol Liq. 2021; 342:117506.

Loeffler H, Happle R. Profile of irritant patch testing with detergents: sodium lauryl sulfate, sodium laureth sulfate and alkylglucoside. Contact Derm. 2003; 48(1): 26–32.

Patel CU. Anti-static properties of some cationic polymers used in hair care products. *Int J Cosmet Sci*. 1983; **5**(5): 181–188.

Paye M. Mechanism of skin irritation by surfactants and anti-irritants for surfactant-based products. In: AO Barel, M Paye, HI Maibach, editors. Handbook of cosmetic science and technology. 3rd ed. New York, NY: Informa Healthcare USA Inc.; 1990.

Presley CL, Militello M, Barber C, Ladd R, Laughter M, Ferguson H, et al. The history of surfactants and review of their allergic and irritant properties. Dermatitis. 2021; 32(5): 289–297.

Proctor & Gamble. Head & Shoulders Product Factsheet. How should you really wash your hair? 2025a https://headandshoulders.com/en-us/healthy-hair-and-scalp/hair-care/how-to-wash-your-hair.

Proctor & Gamble. Head & Shoulders product factsheet. How much is too much shampoo? 2025b https://headandshoulders.com/en-us/healthy-hair-and-scalp/hair-care/too-much-shampoo.

Rankin DA, Carr CM. Characterisation of the bound lipids at the wool fibre surface by time-of-flight secondary ion mass spectrometry (ToF-SIMS). J Text Inst. 2013; 104(2): 197–212.

Rivett DE. Structural lipids of the wool fibre. Wool Sci Rev. 1991; 67: 1–25.

Salomon G, Giordano F. Surfactant irritations and allergies. Eur J Dermatol. 2022; 32(6): 677–681.

Schepky AG, Holtzmann U, Siegner R, Zirpins S, Schmucker R, Wenck H, et al. Influence of cleansing on stratum corneum tryptic enzyme in human skin. Int J Cosmet Sci. 2004; 26(5): 245–253.

Seshadri IP, Bhushan B. Effect of ethnicity and treatments on in situ tensile response and morphological changes of human hair characterized by atomic force microscopy. Acta Mater. 2008; 56(14): 3585–3597.

Seweryn A. Interactions between surfactants and the skin—theory and practice. Adv Colloid Interf Sci. 2018; 256: 242–255.

Shore S, Berger DR. Alcohol and alcohol ether sulfates. In: WM Linfield, editor. Surfactant science series, anionic surfactants. Volume 7. New York, NY: Marcel Dekker; 1976. p. 135–217.

Steber J, Gode P, Guhl W. Fatty alcohol sulfates—the ecological evaluation of a group of important detergent surfactants. Fat Sci Technol. 1988; 90(1): 32–38.

Wertz PW, Dowling DT. Integral lipids of human hair. Lipids. 1988; 23(9): 878–881.

Wertz PW, Dowling DT. Integral lipids of mammalian hair. Comp Biochem Physiol. 1989; 92(4): 759–761.

Wilhelm KP, Freitag G, Wolff HH. Surfactant-induced skin irritation and skin repair. J Am Acad Dermatol. 1994; 30(6): 944–949.

Wolfenden R, Yuan Y. Monoalkyl sulfates as alkylating agents in water, alkylsulfatase rate enhancements, and the “energy-rich” nature of sulfate half-esters. Proc Natl Acad Sci. 2007; 104(1): 83–86.

Xiaofei W, Shu L, Tingyun Y, Yang C. Synthesis of tertiary aliphatic amines. Eur Chem Bull. 2014; 3(1): 55–57.

Yanase K, Hatta I. Disruption of human stratum corneum lipid structure by sodium dodecyl sulphate. Int J Cosmet Sci. 2018; 40(1): 44–49.

Zhao H, Yang Y, Shu X, Dong L, Qiao M, Ran Q. Micelle conformation of sodium alkyl sulfate surfactants with different hydrophobic chain length: a molecular dynamics study. Comput Mater Sci. 2023; 229:112452.

ACKNOWLEDGMENTS

Much of this work was carried out in the laboratories of Perachem Ltd. We are grateful for the assistance of Lucideon Ltd. (Stoke-on-Trent, UK) for providing the ToF-SIMS analysis.