# New Anionic Surface-Active Agent Derived from Wool Proteins for Hair Treatment

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**ABSTRACT:** An anionic surface-active agent derived from wool proteins was developed as an alternative to the anionic surfactants extensively used in shampoo formulations. The physicochemical properties of this new surface-active agent prepared form wool proteins and its application for human hair treatments were studied. This new product could be considered a new mild anionic surface-active agent, as evidenced by the results found by the evaluation of its physicochemical properties. The new wool anionic surface-active agent was shown to be very substantive to hair, coating the fiber surface, giving rise to a significant improvement in the mechanical properties of the hair fibers, and providing a certain damage-prevention effect on the hair. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 1461–1467, 2010

**Key words:** fibers; mechanical properties; proteins; strength; surfactants

## INTRODUCTION

Human hair is structured in highly organized strata that are highly resistant to external stimuli. However, morphological changes can occur through daily care routines. Hair damage leads to a change in the physical properties and to modifications in the surface charges of the hair.[1] When hair is damaged, no chemical or physical treatment can bring it back to its original form. This is the reason that cosmetic hair science is focused on preventing, qualifying, and quantifying damage to human hair.[2]

It is well known that shampoos cause hair drying, opacity, and difficulty in handling.[3] Anionic surfactants are extensively used in detergent formulations as shampoos. Hair protein loss has been related to the sodium dodecyl sulfate treatment.[2]

Surfactants are compounds that aggregate at the interface of water–air or water–oil mixtures that give rise to an assortment of properties, such as wetting, emulsifying, solubilizing, and foaming.[4] All surfactants have the same basic structure with a hydrophobic domain and a hydrophilic end. Many amphiphilic compounds are known in nature, with proteins and peptides being two examples.[5] Proteins have some similar features as synthetic surfactants, as they contain both hydrophobic and hydrophilic amino acids that impart some degree of surface activity. A number of natural proteins have very well-known surface-active properties, such as the action of casein in the stabilization of milk-fat emulsions, the foaming properties of egg white, the colloid-protecting effects of gelatin, and the sea foam caused by protein from decomposing marine animals.

There is a growing consumer trend toward the use natural actives in personal care[6]; in fact, the use of natural products in hair care is not a new concept or practice. In this line, protein and protein hydrolysates have been demonstrated to be beneficial to hair, imparting increased moisturization and enhancing softness and flexibility. Hart et al.[7] studied the beneficial effects on hair and skin of hydrolyzed oat protein and oat extracts. Roddick-Lanzilotta et al.[8] also showed the beneficial effects of a natural keratin biopolymer on hair protection.

A commercial trend is well established for environmentally aware companies to move away from traditional synthetic surfactants derived from petrochemicals, which are often harsh and nonbiodegradable, to the use of natural, biologically derived, surface-active agents.[5]

Wool proteins are mild, natural, biodegradable, and sustainable with multiple functionalities and potential for use in the personal care and detergent markets.[9,10] In this study, a new anionic surface-

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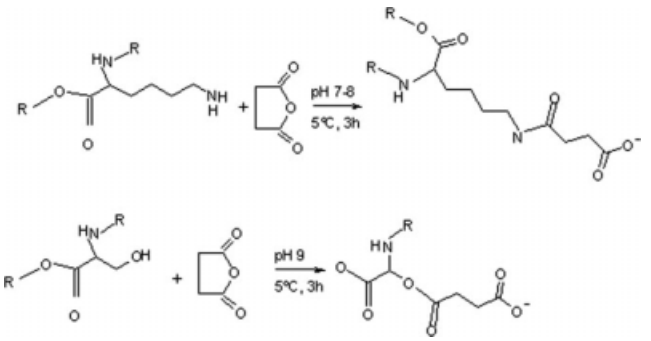


**Figure 1** Succinylation of protein.

active agent derived from a novel keratin protein isolate was developed.[11] Chemical modification provides a useful method for modifying the functional properties of proteins. Many factors affect the surface activity of proteins, with size, charge, structure, and hydrophobicity being important criteria. We can use chemical reactions to influence these factors and thus improve the ability of the protein to act as a surface-active agent. In particular, the chemical modification used in this study was succinylation, which is commonly used in food proteins to improve the solubility, foaming, and emulsifying properties and also taste.[12–14] The succinylation of a protein involves the introduction of negatively charged carboxylic groups, which affect the electrostatic repulsive forces in the molecule (Fig. 1) and cause enhanced electrostatic repulsion between surfaces coated with the protein; this results in greater emulsion stability.

The aim of this study was to determine the physicochemical properties of a new anionic surface-active agent based on succinylated keratin protein (SK-protein) compared with an unmodified protein and its application for human hair treatments. The physical condition of hair was evaluated after repeated washings with the SK-protein compared to an industrial standard surfactant, sodium laureth sulfate (SLES).

## EXPERIMENTAL

### Samples

Materials included chloroform (Merck, Darmstadt, Germany), decane (purum, Fluka, Germany), diethyl ether (Merck), formic acid (85%, Probus S.A., Badalona, Barcelona, Spain), methanol (Merck), natural red hair tresses 20 cm in length (De Meo Brothers, Inc., New York), *n*-hexane (Merck), SLES (Merck), wool keratin surface-active agent (SK-protein, Keratec, Ltd., New Zealand), wool keratin intermediate filament protein (K-protein) solution (Keratec), and wool yarn (Keratec).

Before treatment, each hair tress was washed with a 2% SLES solution (prepared from 70% SLES and diluted to achieve a 2% solution) for 2 min and rinsed thoroughly with warm water ($\sim$ 40°C) for 2 min more. Next, the hair tresses were dried in air.

### Determination of the surface tension in water

The surface tension of the active compounds was measured according to the Wilhelmy plate method[15,16] with a Krüss tensiometer (Processor tensiometer K-12, Hamburg, Germany), which directly determined the real tension values at equilibrium with a series of aqueous solutions at concentrations from 0.10 to 50 g/L of the surface-active agent derived from wool (SK-protein) and the wool unmodified protein (K-protein) at 25°C. The equilibrium time before the surface tension measurements was at least 1 h.

### Wetting properties of the wool-derived proteins

An adaptation of the Draves wetting test[17] was used, with the main difference from the standard method being the use of a fine wool yarn instead of the specified 20 tex greige cotton yarn. The test solution, contained in a 1-L measuring cylinder, was at room temperature (25°C). The yarn was an unscoured Solospun 180 μm wool yarn, with 50/2 tex, 760 tpm singles, and 580 tpm folding. It was wound into 5.0-g hanks 1.00 m in diameter (100 turns), which were tied at two opposite points. The hanks were cut in half at the two points midway between these ties to give two 50 cm long yarn bundles with cut ends, which were then folded at the tie, an arrangement that gave a U-shaped bundle of yarns with the cut ends uppermost; keep in mind that the cutting of the fiber led to an increment of accessible active groups. Air would easily escape from the cut ends of these hanks, and this arrangement gave more reproducible results than the use of hanks without cut ends, where bubbles could collect in the yarn at the top of the hank, prolonging yarn flotation in a nonreproducible way. The yarn tie was attached to a 20-g weight, and the yarn, previously weighed, was carefully dropped into a solution of the wetting agent. At this point, timing was started. Held by the weight on the bottom of the measuring cylinder, the yarn floated in the solution until sufficient air had been displaced for the yarn to begin to sink, at which point the timing was stopped.

### Foaming properties of wool-derived proteins

A foam tester of the Ross–Miles type was used,[17] constructed with the dimensions as specified in an

ASTM standard.[18] The water jacket was maintained at 50°C, and the test method experimental conditions were fully followed.

### Hair treatments

Different washing procedures were performed with the following methodologies. For comparative purposes, tresses of virgin hair were kept as a control [untreated (UT) hair].

#### SLES, SK-protein, and K-protein washing treatments

Hair (1.5 g) was placed daily in a 5% SLES solution, 5% SK-protein solution (pH 7.3), or 5% K-protein aqueous solution (pH 7.8) for 1 h in a rocking table; after this, the hair was rinsed thoroughly with warm water ($\sim$ 40°C) for 2 min and then dried in air. This washing process was performed twice a day for five consecutive days (for 10 washes total).

### Dynamic wetting force measurements of the hair fibers

The contact angles were calculated from the dynamic wetting force measurements carried out in an electrobalance KSV Sigma 70 contact angle meter.[19] A single fiber was mounted, overhanging by 2–3 mm an aluminum support to keep the fiber straight and rigid, prevent the buoyant effect, and obtain a constant fiber perimeter during wetting measurements. The aluminum support was suspended by a hook from the electrobalance. The vessel containing the wetting liquid was raised and lowered with a motorized platform. Both an electrobalance and a motorized platform were connected to a PC for data acquisition and control purposes. Before scanning, the fiber weight was zeroed. The liquid was advanced very slowly until a distinct perturbation in the measured force, corresponding to solid–liquid contact, occurred. The position and time were also zeroed. The fiber was scanned at 1 mm at a velocity of 0.5 mm/min for both the advancing and receding modes. Two hysteresis cycles were evaluated for each fiber. Contact angles were calculated from the dynamic mean wetting force values obtained for the advancing mode on the first hysteresis. All measurements were made at room temperature (20°C) for both scale cuticular directions of immersion: against scale and with scale.

Previous to the wetting measurements, the surface tension of the wetting liquid (water) used in this study was measured by the Wilhelmy plate technique with a platinum plate. Furthermore, the perimeters of the scanned fibers were estimated from the wetting force measured in the total wetting liquid (decane).

### Scanning electron microscopy (SEM)

A SEM study was performed with all of the hair samples to evaluate the possible changes in the surface morphology of the hair fibers due to the different treatment done.

For this, the hair sample was mounted onto 10-mm brass stubs with conductive carbon adhesive tape and sputter-coated from a gold/palladium source. The coating thickness was about 200 Å. The samples were studied with a JEOL JSM 6100 scanning electron microscope. The microscope was operated at 7.0 kV, and samples were viewed at a working distance of 15 mm. Ten fibers from each hair sample were viewed, and representative images were taken.

### Panel testing of luster and smoothness

A panel testing with 14 judges was used to evaluate the luster and smoothness of the differently treated hair fibers. The tests were performed in a conditioned room (23°C and 50% relative humidity) where all hair samples (UT hair, SLES-washed hair, K-protein-washed hair, and SK-protein-washed hair) were evaluated.

The judges scored the hair fibers' luster and smoothness as 0 (none), 1 (little), 2 (moderate), 3 (considerably), 4 (strong), or 5 (very strong).

Analyses of variance were used to determine significant differences between the values obtained from different treatments (significance level accepted as $P < 0.05$) with the Statgraphics program.

### Tensile properties of the hair fibers

#### Stress–strain test

Ten fibers were randomly taken from samples previously conditioned for 48 h in a standard atmosphere (20°C and 65% relative humidity) and centrally attached to a pair of cardboard frames with an internal rectangular cut frame of 50 $\times$ 25 mm$^2$ following the longest direction.

Fiber fineness along the 50 mm subjected to testing was examined by image analysis, and the minimum diameter was taken as fiber fineness because breakage is normally produced at the thinnest (weakest) point.

Samples on the cardboard were attached to an Instron 5500R dynamometer with a gauge length of 50 mm. The two sides of the cardboard were cut before the beginning of the stress–strain test to enable just the fiber under testing to be stressed. The test was performed according to ASTM D 3822 (1980) with some modifications. A gauge length of 50 mm, a rate of strain of 30 mm/min, and the breaking stress (MPa) and strain (%) were recorded. The multiplication of breaking stress and percentage strain gave

**TABLE I**
**Physicochemical Properties of Both Products**

| Product | pH | Surface tension (mN/m) | Wetting time | Foam | |
|---|---|---|---|---|---|
| | | | | Height at zero time (cm) | Collapse time (min) |
| K-protein (10 g/L) | 7.8 | 43.20 | 8.2 min | 10 | 3 |
| SK-protein (10 g/L) | 7.3 | 48.62 | 16 s | 2 | 16.03 |

rise to the evaluation of the breakage work, which evaluated the fiber conditions.

### Stress–relaxation test

Ten fibers were randomly taken with the same procedure of the stress–strain test and were also attached to the Instron 5500R dynamometer to perform the stress–relaxation test. The fibers were strained at 30% at the same rate of the stress–strain test, and the stresses at 0, 2, 5, 10, 15, 30, 45, 60, 120, and 180 s were recorded. With the results obtained and by the application of nonlinear regression, the high-rate, medium-rate, low-rate, and nonrelaxed stresses were estimated.

## RESULTS AND DISCUSSION

### Characterization of the surface activity of the wool-derived protein

Different methods were used to study the physicochemical properties of the new anionic surface-active agent derived from wool proteins (SK-protein). The physicochemical properties of wool unmodified keratin protein (K-protein) were also evaluated for comparison. Table I summarizes the results obtained for the pH, the surface tension evaluation, and the wetting and foam studies for both products.

As is well known, surfactants are molecules with the ability to reduce the surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures.[20] Plots of the surface tension against the logarithm of concentration are specific for surfactants, and a critical micelle concentration can be assigned to the concentration at which the surface tension reaches a constant value. In our case, the two wool-derived proteins were able to slightly reduce the water surface tension; however, no break in the surface tension versus concentration curves were found, which indicated that no critical micelle concentration was found.

The Draves wetting test measures the time required for a yarn to completely sink in a cylinder of surfactant solution. When the surfactant displaces sufficient air from the yarn (by spreading wetting), the yarn suddenly sinks to the bottom of the cylinder. The faster the yarn sinks, the greater the wetting ability of the surfactant solution. Wetting is an important criterion in the selection of surfactants for cleaning, coating, emulsion polymerization, pesticide application, and many other applications. Table I shows the time required to wet the yarn in both of the solutions studied. The wetting time for the surface-active agent derived from wool solution was much shorter than that for the wool protein. This difference resulted in a significant advantage in wetting efficiency.

The foaming property of a surfactant system is one of the most important characteristics for the formulation of cosmetics. Two of the most important factors in foaming a liquid are its initial foam power and its foam stability.[21] The results of the Ross–Miles foam testing (Table I) show that the surface-active agent studied (SK-protein) had a lower initial foam height compared with the unmodified wool protein (K-protein). However, differences were apparent in the foam stability. The wool anionic surface-active agent showed a significant increase in the foam time collapse, which indicated a higher foam stability.

### Contact angles for the UT and treated hair fibers

The dynamic wetting force was measured for all hair samples. The wetting properties of a solid surface can change as a consequence of chemical treatment. There is a relationship between the molecular structure of a surface and the macroscopic properties of this surface, such as wetting, adhesion, biocompatibility, corrosion, and lubrication. Wetting has the advantage of experimental simplicity and applicability to complex systems.[22] Contact angle determination is a simple technique for examining the immediate surface of low-energy solids, such as natural synthetic polymers.[23] Contact angle measurements are attributed to several factors, such as surface chemical heterogeneity, roughness, surface deformability, surface configuration change, surface polarity, and adsorption and desorption.[19,22–25]

As a natural fiber hair has an isoelectric point that characterizes its surface charge, the $\zeta$ potential is not a constant value for fibers but gives information about the nature and dissociation of functional groups, hydrophilicity or hydrophobicity of the fiber surface, and ion or water sorption.[26] The $\zeta$ potential for fibers varies from $-10$ to $-60$ mV. It depends on

**TABLE II**
**Average Values of the Advancing Contact Angle of Different Hair Samples**

| | UT hair | SLES-washed hair | SK-protein-washed hair | K-protein-washed hair |
|---|---|---|---|---|
| Contact angle (°) | 84.06 ± 7.10 | 84.86 ± 9.75 | 76.46 ± 5.47* | 82.48 ± 7.08 |

* $P < 0.05$ (analysis of variance) between the washed hair and UT hair.

factors such as the chemical composition, surface polarity, microstructure, porosity, specific surface, fiber swelling capacity, interaction energy of the fiber and solution, pH of the solution, and electrolyte addition. Different accessible hair fibers' surface-active groups at different pH values are found. Immersed in water (pH 6.5–7.0), hair fibers show negative values of $\zeta$ potential. When the pH is increased, the $\zeta$ potential becomes more negative.[26]

Table II shows the results for the contact angle of the different hair samples studied. Although the UT hair fibers and fibers washed with the SLES surfactant had the same value of contact angle, a decrease in the contact angle was found for the hair fibers that were treated with both keratin products. This change in the contact angle value indicated a modification of the fiber surface due to the keratin treatment and was attributed to both keratin proteins being substantive to hair and coating the fiber surface. This decrease was much more significant for the fibers submitted to the washing protocol with the anionic surface-active agent derived from wool proteins (SK-protein); this indicated an increase in the hair substantivity of the wool anionic surface agent. The keratin coating was detected by a reduction in the contact angle, which indicated an increase in the wetting properties of human hair due to SK-protein treatment.

A significant influence on the sorption properties of hair fibers is the amount of accessible groups.[27] In this study, both keratin treatments, which were shown to be substantive to hair fibers, were performed at neutral pH (ca. pH 7.5). Under these conditions, the hair fibers were slightly anionic, and a significant capacity to absorb anionic surfactant has been demonstrated.[27] This was explained in terms of the treatment environment pH, where the fibers had a slightly anionic character but also had a large number of amino acids with cationic groups (e.g., Arg or His) available for complexation with both keratin samples.[28] Moreover, succinylation is expected to result in a protein with an increased negative charge present and less positive charge, as the positively charged lysine groups are made into negatively charged $COO^-$ groups; this makes the derived anionic surface-active agent have an increase capacity to highly bind the hair fibers.

### Surface changes

The SEM results allowed us to visualize some differences in the conditions of the surface morphology of the hair samples due to the treatments of each sample (Fig. 2).

The damaging effects of the different washing processes applied on the UT hair samples were compared. Both keratin treatments caused very little damage to the cuticle surface; hair samples washed with SLES showed the most substantial damage; this indicated the deleterious effect of the SLES washing process. This damage, specifically cuticle lifting, can occur when solids are washed off of the surface of the hair.

The results also showed that although all of the samples presented some kind of residue fixed on the hair surface, the largest residue was observed on

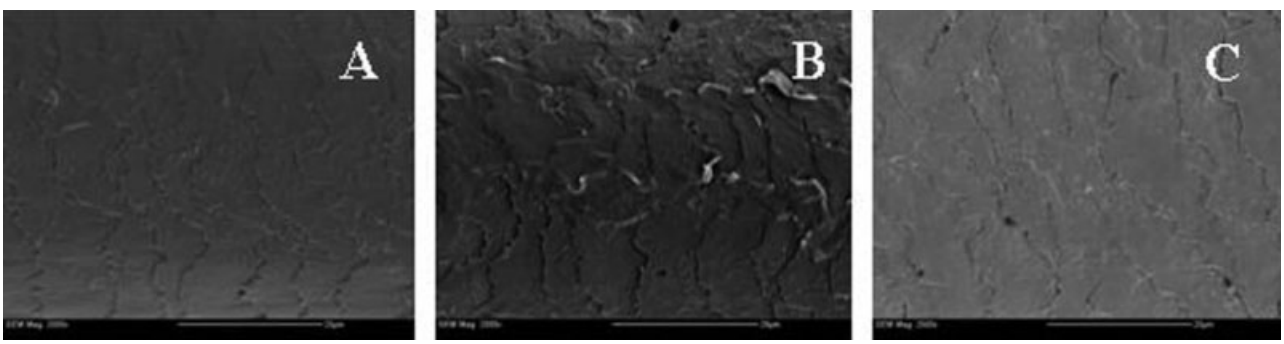


**Figure 2** SEM micrographs of (A) UT hair fibers, (B) hair fibers after being treated with SLES, and (C) hair fibers after being treated with SK-protein.

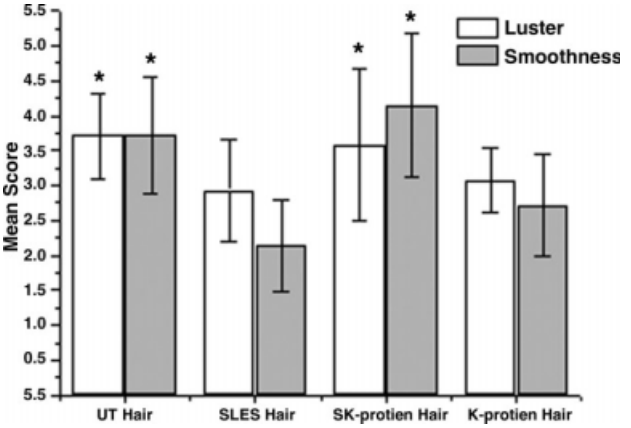


**Figure 3** Mean values of the luster and smoothness scores of all judges for the different hair samples ($P < 0.05$ with respect to the SLES-washed hair).

the hair samples washed with the SK-protein. The cuticle detail was obscured in areas in which fixation occurred; this suggested a relatively persistent layer of surface-active protein protecting the cuticle.

### Panel testing of luster and smoothness

After the SEM results, where important surface differences were found between SLES and SK-protein-washed hair fibers, a subjective assessment of UT-, SLES-, K-protein-, and SK-protein-washed hairs was performed to evaluate possible differences in fiber luster and smoothness. Figure 3 shows the results for the scores of fiber luster and smoothness of the judges on the panel testing. The data demonstrated that there was a clear trend of the judges toward selecting the SLES-washed hair as being the more damaged with a statistically significant decrease in both fiber luster and smoothness. However, the SK-protein-washed fibers had almost the same luster and even an increase in the smoothness compared with the UT hair.

### Tensile properties

Initially, a stress–strain test was performed with all of the hair samples. The mean values of stress and deformation at break for the different hair samples are given in Table III. Breaking stress evaluates the fiber integrity; thus, higher values of this parameter indicate a larger number of bonds present in the fiber structure. The hair fibers washed with SLES had the lowest value of breaking stress when compared with the other hair samples. This result indicates that the fibers with a higher internal deterioration were the ones that were submitted to the SLES washing treatment. As explained before, the breakage work for the different hair samples were calculated, and the results are also shown in Table III. The results for the breakage study, which enabled us to evaluate the fiber conditions, show that worst fiber condition was found for the SLES-treated hairs.

**TABLE III**
**Breaking Stress, Deformation at Break, and Breakage Work of UT Hair and Hair Washed with SLES, SK-Protein, and K-Protein**

| | Breaking stress (MPa) | Deformation at break (%) | Breakage work |
|---|---|---|---|
| UT hair | 304.17 | 47.65 | 14,493.70 |
| SLES-washed hair | 213.01 | 45.39 | 9,668.52 |
| SK-protein-washed hair | 252.83 | 46.16 | 11,670.63 |
| K-protein-washed hair | 271.80 | 45.22 | 12,290.80 |

To obtain additional information on the possible structure modification produced in the hair fibers, a stress–relaxation test was performed. The relaxation was attributed to the breaking of various chemical crosslinks on extension, and their reformation may have occurred with the passage of time. On the basis of a timescale ranking, these bonds were categorized into three groups: (1) weak bonds (relaxation time $< 0.1$ min), including hydrogen bonds, salt linkages, and van der Waals and electrostatic forces; (2) bonds of intermediate strength (relaxation time $= 0.1$–$10$ min) due to a certain disengagement of bonds between the matrix and filament components; and (3) strong bonds (relaxation time $> 10$

**TABLE IV**
**Values of the Nonrelaxed Stress and Relaxed Stress at 5.8 and 79 s for UT Hair and Hair Washed with SLES, SK-Protein, or K-Protein**

| | Nonrelaxed stress (%): Strong bonds | Relaxed stress at 5.8 s (%): Weak bonds | Relaxed stress at 79 s (%): Intermediate bonds |
|---|---|---|---|
| UT hair | 73.62 | 16.71 | 10.32 |
| SLES-washed hair | 71.15 | 18.48 | 9.73 |
| SK-protein-washed hair | 75.11 | 15.11 | 10.09 |
| K-protein-washed hair | 73.29 | 15.37 | 11.46 |

min) consisting of covalent crosslinks, mainly disulfide bonds.[29] Table IV summarizes the percentage of bonds found in each hair sample evaluated so that the nonrelaxed stress corresponded to the strong bonds, the relaxed stresses at 5.8 s corresponded to the weak bonds, and the relaxed stress at 79 s corresponded to the intermediate bonds.

Different bond percentage distributions were found for the different hair samples studied. A diminution in the percentage of intermediate and strong bonds due to the SLES washing process when compared with the UT hair sample was found. This diminution was explained by the damaging effect of the SLES surfactant on the hair fibers. Fibers washed with the anionic surface-active agent showed a small increase in the strong bond percentage. This increase was attributed to an increase in the protein assembly integrity due to the presence of the modified keratin protein in the hair fibers. As explained before, the anionic groups of the surfactant and the cationic groups of the basic amino acids (Arg or His) of the hair fiber, which were positively charged in the pH environment of the keratin treatment, were shown to produce strong bonds between them and result in a coating of the modified keratin protein on the hair fibers.

## CONCLUSIONS

Succinylation of keratin increased important surfactant characteristics; however, micelle formation was not observed. SK-protein may be considered a mild anionic surface-active agent, in particular with regard to its wetting properties and foam stability.

From contact angle measurements and SEM visualization, the SK-protein appeared highly substantive to human hair and gave rise to good luster and a smoothness improvement.

Better behavior was detected in the mechanical properties of hair washed with SK-protein in comparison with hair washed with SLES. It seemed that the protein assembly integrity of human hair was improved after washing with SK-protein. From a tensile property analysis, SK-protein imparted less damage to hair than the conventional surfactant treatment by SLES.

SK-protein shows potential as a mild anionic surface-active agent that might improve hair properties.

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