

## **A role of the anteiso branch of 18-MEA in 18-MEA/SPDA to form a persistent hydrophobicity to alkaline-color-treated weathered hair**

HIROTO TANAMACHI, SHIGETO INOUE,  
SHINICHI TOKUNAGA, HISASHI TSUJIMURA,  
NORIYUKI TANJI, MASASHI OGURI, and TAICHI HABE,  
*Beauty Research Center, Kao Corporation, 2-1-3, Bunka, Sumida-ku,  
Tokyo 131-8501, Japan (H.T., S.T.); Analytical Science Research  
Laboratories, Kao Corporation, 1334 Minato, Wakayama-shi,  
Wakayama, 640-0112, Japan (S.I., N.T, T.H.); and Analytical Science  
Research Laboratories, Kao Corporation, 2606 Akabane, Ichikai, Haga,  
Tochigi 321-3497, Japan (H.T., M.O.).*

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### **Synopsis**

The effect of the anteiso-branch moiety of 18-MEA (18-methyleicosanoic acid) to create a persistent hydrophobicity of alkaline-color-treated weathered hair treated with 18-MEA/SPDA (stearoxypropyldimethylamine) was investigated by comparing a straight-chain fatty acid (n-heneicosanoic acid, n-HEA) and an iso-branch fatty acid (19-methyleicosanoic acid, 19-MEA) with the anteiso-branch fatty acid (18-MEA), using dynamic contact angle measurements, quantification of 18-MEA by LC/MS, and temperature controlled atomic force microscopy (AFM). The dynamic contact angle measurements indicated that the anteiso-branch moiety of 18-MEA is critical for the creation of a persistent hydrophobicity to alkaline-color-treated weathered hair. The temperature-controlled AFM investigations revealed that the anteiso-branch moiety of 18-MEA in the 18-MEA/SPDA system produces a persistent hydrophobicity to alkaline-color-treated weathered hair by providing higher fluidity to the upper region of the 18-MEA/SPDA layer.

### **INTRODUCTION**

Since healthy and beautiful hair is of interest to many females who have had their hair damaged by chemical treatments, heat styling, and environmental factors, various hair care products have been developed for improving the beauty of hair. Detailed knowledge of the surface structure and properties of hair is essential for developing unique hair care technologies. The cuticle is the outer surface of the hair fiber, protecting the cortex. The outermost surface of the cuticle cells has been suggested to be covered by a monolayer of covalently bound fatty acids, a major component of which is 18-MEA (18-methyleicosanoic acid). 18-MEA has been subjected to study for years in hair research science, since the discovery of its presence on keratinous fibers (1–3). 18-MEA is thought to be covalently

bound, probably via a thioester or ester linkage, to the outer surface of the cuticle (4–7) and locates specifically in the cuticle, not the cortex (6). It is also known that 18-MEA makes the surface hydrophobic and acts as a boundary lubricant to decrease friction resistance (8–13).

Although it is expected that a damaged hair surface could be repaired if the 18-MEA layer could be restored, few studies have been made regarding the restoration of 18-MEA on damaged hair surfaces. In our previous study, however, we reported that treatment of alkaline-color-treated weathered hair with 18-MEA, combined with a specific tertiary amine (SPDA), formed a persistent hydrophobicity on alkaline-color-treated weathered hair surfaces, and its hydrophobicity was maintained even after shampooing. The mechanism for the persistent hydrophobicity by 18-MEA/SPDA was analyzed by various analysis techniques, including AFM (atomic force microscopy), ARXPS (angle-resolved X-ray photoelectron spectroscopy), and surface pressure-area isotherms, and it was suggested that the sustainable hydrophobicity could be achieved by forming a durable hydrophobic layer of 18-MEA/SPDA on the hair surface (14,15). In this study, we have investigated whether the anteiso-branch moiety of 18-MEA in 18-MEA/SPDA is essential for providing persistent hydrophobicity to alkaline-color-treated weathered hair treated with 18-MEA/SPDA, by comparing a straight-chain fatty acid (n-heneicosanoic acid, n-HEA) and an iso-branch fatty acid (19-methyleicosanoic acid, 19-MEA) with the anteiso-branch fatty acid (18-MEA). The chemical structures of these fatty acids and SPDA are shown in Figure 1.

## EXPERIMENTAL

### MATERIALS

*Hair samples.* Hair fibers were kindly provided by a Japanese female aged 30. The fibers were cut at a distance of approximately 20 cm from the root end on the back of the head. The hair had never been treated with any chemical agents, such as bleaching, coloring, or permanent waving.

*Preparation of alkaline-color-treated weathered hair.* The hair was exposed to alkaline-coloring treatment four times, coupled with model weathering 360 times, where model weathering

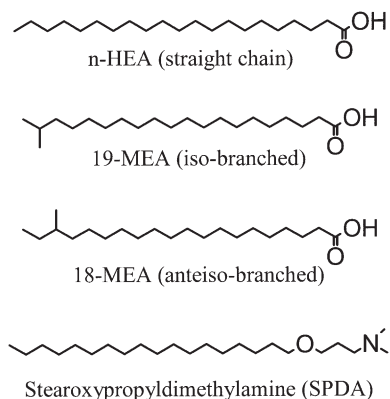


Figure 1. Chemical structures of fatty acids and SPDA.

(90 times) was done between every interval of alkaline coloring. This resulted in a process of alkaline coloring combined with daily weathering, that simulated a one-year period, assuming the hair is alkaline colored every three months. The model weathering treatment consists of a series of daily hair care procedures: shampooing, conditioning, drying with a hot dryer, and brushing. Hair tresses (5 g) were treated with an alkaline-colored lotion (3.5 wt% hydrogen peroxide, 0.76 wt% ammonia, 5.6 wt% ammonium bicarbonate, 2.0 wt% cethyl trimethylammonium chloride, 0.05 wt% EDTA/2Na, and water) for 20 minutes at room temperature at a liquor: fiber ratio of 1:1. The tresses were then rinsed for one minute under running water. One-half milliliter of a plain shampoo (15 wt% of sodium polyoxyethylene lauryl ether sulfate (2.5 E.O.) with 2 wt% N,N-bis(2-hydroxyethyl)-dodecanamide solution adjusted to pH7 with phosphoric acid) was applied to the wet hair tresses, which were massaged by hand for 30 seconds. The hair tresses were then rinsed for 30 seconds under running water. One-half milliliter of a plain conditioner (2 wt% SPDA, 0.5 wt% benzyl alcohol, 3.0 wt% stearyl alcohol, 0.6 wt% lactic acid, and water) was applied to the wet hair tresses and distributed manually for 30 seconds, then left on for one minute. The hair tresses were rinsed for 30 seconds under running water. The hair tresses were then towel dried, dried under a hot dryer for three minutes, and brushed 20 times.

*Mica in conditioner treatment.* A fresh mica sheet was used as a model for the hydrophilic surface of alkaline-color-treated weathered hair. Mica sheets of approximately  $10 \times 10 \times 0.1$ -mm were cleaved on both sides immediately prior to use. They were then immersed in a conditioner solution for one minute at 40°C. The mica sheets were then rinsed under running distilled water for 30 seconds and naturally dried.

*Chemicals.* The investigators obtained 18-methyleicosanic acid (18-MEA), 19-methyleicosanic acid (19-MEA), and stearyloxypropyldimethylamine (SPDA) by chemical synthesis (16,17). Other chemicals were commercially available.

## METHODS

*Measurement of dynamic contact angles of hair.* The wetting forces of hair were measured by the Wilhelmy method, using a K100MK2 tensiometer (Kruss). Single hair fibers were scanned over 3 mm at a velocity of 2 mm/min for the advancing mode. Dynamic contact angles were calculated from

$$F = \pi d \gamma \cos \theta$$

where  $F$  is the wetting force,  $d$  is the diameter of the hair,  $\gamma$  is the surface tension of water, and  $\theta$  is the contact angle of the fiber surface. The hair fiber diameter was measured on the transverse section of each fiber with a rotating fiber diameter measurement system equipped with a laser (Kato Tech Co.) at 20°C and relative humidity (RH) of 65%. The wetting force measurements were also performed at 20°C, 65% RH.

*Quantitative analysis of fatty acids.* The amount of fatty acids absorbed to the hair fiber was measured using liquid chromatography/mass spectrometry (LC-MS, Agilent Technologies, Palo Alto, CA). Hair fibers were immersed in chloroform/methanol (1:1 by volume) for one hour at room temperature. The extracts were dried using a nitrogen stream. The residues were then dissolved in chloroform/methanol (1:9 by volume). In the system, an

1100 binary pump was connected to two mobile phases [M1, methanol/water (8:2 by volume) containing 100 mM ammonium acetate and 50 mM acetic acid; and M2, methanol containing 100 mM ammonium acetate and 50 mM acetic acid] that were eluted at a flow rate of 0.2 ml/minute. The mobile phases were programmed consecutively, as follows: a linear gradient of M1 100–0% (M2 0–100%) between 0 and 20 minutes, an isocratic elution of M1 0% (M2 100%) for 10 minutes, and an isocratic elution of M1 100% (M2 0%) from 30.1 to 40 minutes for column equilibrium (a total run time of 40 minutes). The injection volume was 5  $\mu$ l. The column (L-column ODS 2.1-mm inner diameter  $\times$  150 mm, Chemicals Evaluation and Research Institute, Tokyo, Japan) temperature was maintained at 40°C. ESI measurements in the mass spectrometer were performed with the following settings: polarity, negative; heater temperature of nitrogen gas, 350°C; flow of heated dry nitrogen gas, 11.0l/minute; nebulizer gas pressure, 30 psi; capillary voltage, –4000V; fragmenter voltage, 200V. The selected ion monitoring (SIM) measurement in negative ion ESI was performed using unit mass resolution mode. To detect deprotonated ions for 18-MEA, 19-MEA, or n-HEA,  $m/z = 325.2$  was monitored.

*Measurement of surface properties of mica by atomic force microscopy (AFM).* AFM images of the adsorbed layer on the mica surfaces were obtained using a Nanoscope IIIa multi-mode AFM (Veeco Instruments, Santa Barbara, CA) with an E-scanner. Tapping mode imaging was used to obtain the topographic images of the adsorbed membrane layers. To ensure that imaging the membrane caused no damage, the tapping force was set at the lowest possible level. The nominal spring constants of the cantilevers are reported by the manufacturer to be 20–100 N/m. All images presented in this work were obtained reproducibly over at least three spots on the sample surfaces. The images were acquired with a scan rate of either 0.5 or 1.0 Hz and were flattened with a first-order polynomial prior to analysis.

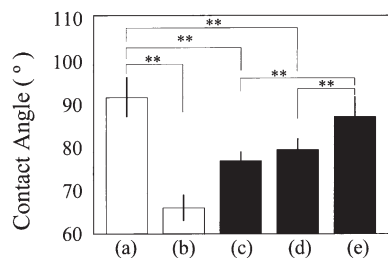
The mechanical properties of the adsorbed membrane were analyzed by the AFM scratching method (18–21). Scratching of the adsorbed membrane was performed in contact mode at a constant force, and a micro-fabricated tip made of silicon nitride (Si-N) and a cantilever, having a spring constant of 0.38 N/m, were used. First, an image (typically, 5  $\mu$ m  $\times$  5  $\mu$ m) of the adsorbed membrane was acquired; then, a smaller area (typically 1  $\mu$ m  $\times$  1  $\mu$ m) was scanned while loading the hard tip onto the surface. Following this, the scanning was repeated over the larger area. This method is hereafter called “scratching.” Thus, if the adsorbed layer was strongly bound to the surface, it was harder to remove.

Measurements of the thicknesses of the adsorbed layers on the mica surfaces were obtained using a NanoScope V multi-mode AFM, equipped with an environment control cell, and the substrate temperature was controlled using a heating stage, which has been modified to be programmable. First, the membrane thickness was measured at room temperature; then, the temperature of the sample stage decreased at a rate of 1°C/minute to a temperature (25°C, 15°C, 5°C, 0°C, –5°C, and –10°C) where it was maintained for at least an hour.

## RESULTS AND DISCUSSION

### DYNAMIC CONTACT ANGLE MEASUREMENTS

The contact angles of normal hair and alkaline-color-treated weathered hair are shown in Figure 2. The contact angle of normal hair was around  $91.4^\circ \pm 4.6^\circ$ , which means



**Figure 2.** Contact angle of hair after shampooing. The bars represent means for  $n = 10$ ; the whiskers represent the standard deviations. The asterisks indicate  $p$ -values obtained from ANOVA analysis. (a) Normal hair. (b) Alkaline-color-treated weathered hair. (c) Alkaline-color-treated weathered hair treated with n-HEA/SPDA. (d) Alkaline-color-treated weathered hair treated with 19-MEA/SPDA. (e) Alkaline-color-treated weathered hair treated with 18-MEA/SPDA.

it is hydrophobic because of the presence of 18-MEA. On the other hand, that of alkaline-color-treated weathered hair was around  $66.3^\circ \pm 3.1^\circ$ , which means it is more hydrophilic. The specific feature of the 18-MEA/SPDA complex was to confer a persistent hydrophobicity and low friction to the alkaline-color-treated weathered hair surface (14). This means that the alkaline-color-treated weathered hair treated with the 18-MEA/SPDA complex could maintain its hydrophobicity and low friction even after one instance of shampooing with a plain shampoo. The contact angles of alkaline-color-treated weathered hair treated with 18-MEA/SPDA, 19-MEA/SPDA, and n-HEA/SPDA after one instance of shampooing with a plain shampoo are shown in Figure 2. The conditioner formulations were as follows: 2 wt% SPDA; 0.5 wt% benzyl alcohol; 3.0 wt% stearyl alcohol; 1 wt% fatty acid; 0.3 wt% lactic acid; and water. The contact angles of alkaline-color-treated weathered hair treated with n-HEA/SPDA and 19-MEA/SPDA conditioners after one instance of shampooing with a plain shampoo were  $76.4^\circ \pm 2.5^\circ$  and  $79.6^\circ \pm 2.6^\circ$ , respectively, which were higher than that of alkaline-color-treated weathered hair but lower than that of normal hair. We demonstrated that there were significant differences in the contact angles between normal hair and alkaline-color-treated weathered hair treated with n-HEA/SPDA or 19-MEA/SPDA by conducting analysis of variance (ANOVA). The contact angle of alkaline-color-treated weathered hair treated with 18-MEA/SPDA conditioner after one instance of shampooing with a plain shampoo was  $88.0^\circ \pm 3.1^\circ$ . This showed that there was no significant difference in the contact angles between normal hair and alkaline-color-treated weathered hair treated with 18-MEA/SPDA. In addition, there were significant differences in the contact angles between alkaline-color-treated weathered hair treated with 18-MEA/SPDA and alkaline-color-treated weathered hair treated with n-HEA/SPDA or 19-MEA/SPDA. The fact that the surface of alkaline-color-treated weathered hair treated with the 18-MEA/SPDA complex could maintain its hydrophobicity even after one instance of shampooing with a plain shampoo, while the hair treated with other complexes could not create a hydrophobic surface, indicated that the anteiso-branch moiety of 18-MEA was vital for providing sustainable hydrophobicity to alkaline-color-treated weathered hair. The question remained as to how the anteiso-branch moiety of 18-MEA in the 18-MEA/SPDA complex provided the persistent hydrophobicity to the alkaline-color-treated weathered hair surface compared to the straight-chain moiety of n-HEA in n-HEA/SPDA or the iso-branch moiety of 19-MEA in 19-MEA/SPDA.

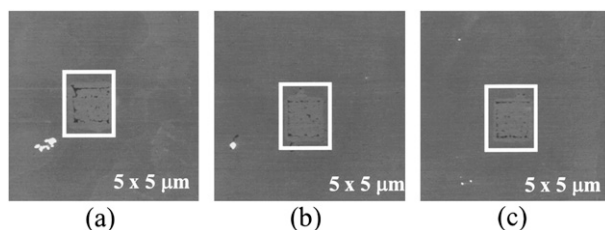
## DEPOSITION OF FATTY ACID

The amount of fatty acid sorption of alkaline-color-treated weathered hair treated with n-HEA/SPDA, 19-MEA/SPDA, and 18-MEA/SPDA conditioners and after one instance of shampooing measured by LC-MS were  $25.4 \pm 6.4$   $\mu\text{g/g-hair}$ ,  $24.2 \pm 3.8$   $\mu\text{g/g-hair}$ , and  $18.7 \pm 6.0$   $\mu\text{g/g-hair}$  (for  $n = 3$ ), respectively. There were no significant differences between these three kinds of fatty acid in the amount of fatty acid sorption by ANOVA. The results indicated that the amount of fatty acid sorption on alkaline-color-treated weathered hair was not just directly related to the branch structure of fatty acid. In our previous paper a surface model of hair treated with 18-MEA/SPDA was proposed, in which 18-MEA/SPDA formed a layer 1 nm in thickness, having a hydrophilic area of molecules binding tightly to the surface and orienting the hydrophobic part to the air interface at an angle of approximately  $25^\circ$  (14). In this model the anteiso-branch moiety of 18-MEA does not interact with the surface directly, and therefore it seems reasonable that there were no significant differences in the deposition of fatty acid among the alkaline-color-treated weathered hair samples treated with n-HEA/SPDA, 19-MEA/SPDA, and 18-MEA/SPDA conditioners.

## AFM INVESTIGATION USING MICA SURFACE

It is very difficult to examine the exact situation of an 18-MEA/SPDA layer adsorbed onto an alkaline-color-treated weathered surface, since the surface of hair is too rough for AFM investigation. In order to investigate the thickness and physical properties of the layer formed by n-HEA/SPDA, 19-MEA/SPDA, or 18-MEA/SPDA, an atomically flat surface is needed for the AFM observation. In this study, mica was also used for AFM investigation as a hydrophilic model surface instead of hair, as in our previous study (14), although it might be open to question whether these surfaces would behave differently due to the different compositions of the surfaces. We believe, however, that the attachment and orientation of these complexes could be similar in human hair.

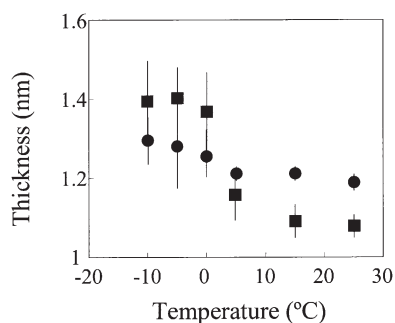
The AFM height images of the adsorbed layers on mica surfaces treated with n-HEA/SPDA (a), 19-MEA/DAPS (b), and 18-MEA/SPDA (c) conditioner solutions (Figure 3) showed that the all conditioner formulations were homogeneously adsorbed on the mica surfaces. The mechanical properties of the adsorbed membranes were analyzed using the AFM scratching method, as shown in Figure 3. A layer that is difficult to remove indicates that the adsorbed layer is strongly bound to the surface. The images were corrected after  $1\text{-}\mu\text{m} \times 1\text{-}\mu\text{m}$  scratching tests by rastering a tip with constant force. The images of the adsorbed films treated with n-HEA/SPDA (a), 19-MEA/SPDA (b), and 18-MEA/SPDA (c) showed small grooves. The results indicated that the n-HEA/SPDA, 19-MEA/SPDA, and 18-MEA/SPDA conditioner solutions adsorbed homogeneously on the mica surfaces and had a high wear resistance. The observation that the adsorbed layer on the mica surfaces treated with the n-HEA/SPDA, 19-MEA/SPDA or 18-MEA/SPDA conditioners had a high wear resistance corresponded to the observation that there were no differences in the amount of fatty acid adsorbed on alkaline-color-treated weathered hair. The fact that the adsorbed layers on the mica surfaces treated with the n-HEA/SPDA, 19-MEA/SPDA, or 18-MEA/SPDA conditioners had a high wear resistance, however, did not correspond with the observation that the anteiso-branch



**Figure 3.** AFM height images of adsorbed layer on mica surface. Dark areas are mica without sorbed compound; bright areas are sorbed chemicals from conditioner. The white squares indicate where  $1\text{-}\mu\text{m} \times 1\text{-}\mu\text{m}$  scratching tests were performed by rastering the tip at constant force. (a) n-HEA/SPDA. (b) 19-MEA/SPDA. (c) 18-MEA/SPDA.

moiety of 18-MEA was responsible for maintaining persistent hydrophobicity to alkaline-color-treated weathered hair.

The question still remains as to why the anteiso-branch structure of 18-MEA is essential for providing a persistent hydrophobicity to alkaline-color-treated weathered hair surfaces. In untreated healthy hair, the precise role of 18-MEA remains unclear, but the large segmental volume of the anteiso-moiety is expected to provide molecular mobility and exhibit liquid-like behavior compared with a straight-chain fatty acid (22,23). It is generally accepted that a liquid crystalline membrane is very soft and fluid. This means that the AFM tip cannot trace the surface of the membrane exactly. It is very difficult, therefore, to analyze the thickness of a liquid crystalline membrane. Investigating the thickness of an adsorbed layer as a function of temperature is a useful technique for judging the situation of the layer, whether the layer is solid-like or liquid-like. Here, analysis of the effect of the anteiso branch in the adsorbed membrane by using temperature-controlled AFM was attempted. Figure 4 plots the thickness of the adsorbed layers on the mica surfaces treated with n-HEA/SPDA and 18-MEA/SPDA conditioners as a function of temperature. The thickness of the layer on the mica surface treated with 18-MEA/SPDA conditioner was 1.08 nm at  $25^{\circ}\text{C}$  and increased to 1.39 nm as the temperature reached  $-10^{\circ}\text{C}$ , while the thickness of the layer on the mica surface treated with n-HEA/SPDA conditioner was 1.19 nm at  $25^{\circ}\text{C}$  and 1.29 nm at  $-10^{\circ}\text{C}$ , showing more stability in the temperature range of  $25^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$ . This change is considered to be chiefly due to the anteiso-branched alkyl chains coagulating upon the transition from the liquid crystalline



**Figure 4.** AFM height images of adsorbed layer on mica surface for 18-MEA/SPDA (■) and n-HEA/SPDA (●). The data represent means for  $n = 5$ ; the whiskers represent the standard deviations.

state to the solid state. The results suggest that molecular mobility at the upper region of the 18-MEA/SPDA layer, generated by the anteiso-branch moiety of 18-MEA, may be the key for the persistent hydrophobicity of the alkaline-colored-treated weathered hair treated with 18-MEA/SPDA.

The dynamic contact angles of alkaline-color-treated weathered hair treated with n-HEA/SPDA and 18-MEA/SPDA after one instance of shampooing were  $76.9^\circ \pm 2.5^\circ$  and  $88.0^\circ \pm 5.1^\circ$  (for  $n = 10$ ), respectively, and the difference was about  $11^\circ$  (Figure 2). Here, we would like to discuss whether the difference of approximately  $11^\circ$  between the n-HEA/SPDA and 18-MEA/SPDA would be reasonable or not. In order to identify the role of the anteiso-branch moiety of fatty acid on the surface properties of natural healthy hair (normal hair), it is very important to examine the hair in which anteiso-branch fatty acids, such as 18-MEA, are completely substituted with straight-chain fatty acids. The absence of 18-MEA from the hair of patients with maple syrup urine disease (MSUD) provides an interesting route to examine the important role of the anteiso-branch moiety of fatty acid. MSUD is an inherited disease involving failure to metabolize branched-chain amino acids in proteins. Hair from a patient with MSUD in which 18-MEA is replaced by straight-chain fatty acids, such as n-eicosanoic acid (23), is the most suitable for this purpose. The dynamic contact angles of normal hair and MSUD hair were already reported by Naito as  $91.5^\circ \pm 0.7^\circ$  and  $82.5^\circ \pm 1.5^\circ$  (for  $n = 5$ ), respectively (24). The observation that the dynamic contact angle values of normal hair were higher than that of MSUD hair by about  $9^\circ$  suggested that the anteiso-branch moiety of fatty acids on the surface of hair makes the surface hydrophobic by providing fluidity at the end of the alkyl chain of fatty acids. The difference in contact angle of approximately  $11^\circ$  between the hair treated with 18-MEA/SPDA and n-HEA/SPDA after one instance of shampooing with a plain shampoo was much closer to the difference in dynamic contact angle of approximately  $9^\circ$  between normal hair and MSUD hair. It was thus concluded that one of the roles of the anteiso-branch moiety of 18-MEA in 18-MEA/SPDA for the persistent hydrophobicity of alkaline-color-treated weathered hair was to give higher fluidity to the 18-MEA/SPDA layer.

Taking into account the results obtained here and in our previous report (14), the following revised model is suggested (Figure 5). 18-MEA/SPDA forms a layer 1.4 nm in thickness, the upper region of which has higher fluidity due to the anteiso-branch moiety of 18-MEA, with both 18-MEA and SPDA bound tightly to the surface by the carbonyl and amide groups, orienting the hydrophobic part to the air interface at an angle of approximately  $35^\circ$ .

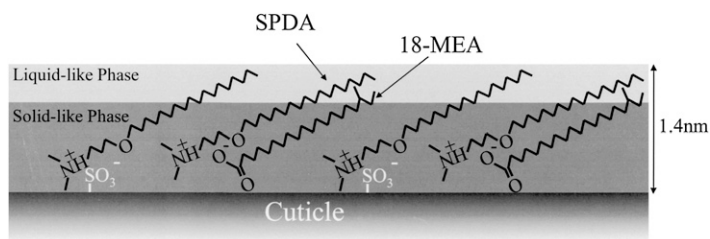


Figure 5. Schematic diagram of the cuticle surface of alkaline-colored-treated weathered hair treated with 18-MEA/SPDA.



## CONCLUSIONS

The following conclusions are based on the findings reported in this paper:

1. The surface of alkaline-color-treated weathered hair treated with 18-MEA/SPDA complex can maintain its hydrophobicity even after one instance of shampooing with a plain shampoo, while the hair treated with n-HEA/SPDA or 19-MEA/SPDA complex cannot create a hydrophobic surface. The results indicate that the anteiso-branch moiety of 18-MEA is vital for providing persistent hydrophobicity to alkaline-color-treated weathered hair.

2. Characterization of adsorbed layers of 18-MEA/SPDA on a mica surface, as a possible hydrophilic surface model, was performed using AFM, although it might be open to question whether these surfaces behave differently due to the different compositions of these surfaces. The results reveal that the mechanism of the sustainable hydrophobicity of the hair surfaces generated by the anteiso-branch moiety of 18-MEA is that the anteiso-branch moiety of 18-MEA in 18-MEA/SPDA can produce higher fluidity to the upper region of the 18-MEA/SPDA layer compared to the straight chain of n-HEA in n-HEA/SPDA or the iso-branch moiety of 19-MEA in 19-MEA/SPDA.

## APPENDIX

### ERRATUM

A previous paper (14) by the present authors [H. Tanamachi *et al.*, Deposition of 18-MEA onto alkaline-color-treated weathered hair to form a persistent hydrophobicity, *J. Cosmet. Sci.*, **60**, 31–44 (2009)] contained an incorrect representation of Table II. The corrected table is reprinted below:

Table II  
Formulation of Conditioners

	1	2	3	4	5	6 (control)
Stearoxypropyldimethylamine	2	—	—	—	—	2
Dimethylaminopropylstearamide	—	2	—	—	—	—
Stearyltrimethylammonium chloride	—	—	2	—	—	—
Docosyldimethylamine	—	—	—	2	—	—
Stearoxyhydroxypropyldimethylamine	—	—	—	—	2	—
Benzyl alcohol	0.5	0.5	0.5	0.5	0.5	0.5
Stearyl alcohol	3	3	3	3	3	3
18-MEA	1	1	1	1	1	—
Lactic acid	0.3	0.3	—	0.3	0.3	0.6
Water				Balance		

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