IFSCC 2025 full paper (IFSCC2025-603)

"A Novel Mechanism For Removing Keratin Plugs Using Selective Cleansing Properties Of Sebum Components"

Satoko ITO[1], Tomonori HIGUCHI[1], Yuji YAMASHITA[2], Wencai JIANG[3], Shiyue DUAN[4], Masayoshi MIYAMOTO[5]

1 Taiyo Kagaku Co., Ltd., 2 Kanagawa University, 3 Shanghai Skin Disease Hospital, 4 Chanel China Beauty Innovation & Performance Center, 5 G.K. Chanel R&I,

1. Introduction

It is known that keratin plugs (KP) not only cause rough skin, but also have a negative impact on the skin's impression, such as skin texture and dullness. KP is formed from the protein and sebum which are difficult to be removed by washing with common surfactant cleansings. Recently we have developed the novel bicontinuous microemulsion (BCME) cleansing water that selectively removes oleic acid through spontaneous emulsification. The addition of Sodium surfactin (SF) to this BCME cleansing water further improved the selective cleansing ability, decreasing the ability to remove oils that are not desired to be removed from the skin, such as squalane, without changing the ability to remove oleic acid[1-3]. We therefore hypothesized that the components of KP include oleic acid derived from sebum, and that selectively removing this oleic acid would loosen the strong structure of the KP, causing them to collapse and be easily removed. In this study, we have discovered that KP can be easily removed by this selectively cleansing tech.

2. Materials and Methods

2.1 Materials

Polyglyceryl-6 caprylate (C8-HG), Polyglyceryl-6 dicaprate (diC10-HG) and Polyglyceryl-4 lauryl ether (C12E-TG) were cosmetic-grade products (without further purification). These surfactants and isononyl isononanoate (I-I, cosmetic grade product) were used to form BCME. Sodium surfactin (SF) is a cyclic lipopeptide-type biosurfactant produced by Bacillus subtilis, a member of Bacillus natto. It has a unique structure in which seven amino acids are connected to form a ring, and is known to exhibit high surface active performance. SF was a cosmetic grade product and used as a selective agent to remove oil. Water was purified by ion filtration.

2.2 Preparation of cleansing water

The cleansing water (BCME-SF) used for the experiments were made from the components shown in Table 1.

Table 1 Components and compositions of BCME-SF

	BCME-SF
SF	0.25
C8-HG	2.40

DiC10-HG	0.60
C12E-TG	0.25
I-I	1.00
Water	to 100 (wt%)

2.3 Preparation of artificial sebum and artificial keratin plug

The artificial sebum used for the experiments was made from the components shown in Table 2. All the components were weighed out, dissolved by heating at 70°C, and allowed to cool. The artificial KP was made from the components shown in Table 3. Keratin powder was dispersed in artificial sebum that had been heated and dissolved at 70°C, and the mixture was allowed to cool.

Table 2 Composition of artificial sebum

	(wt %)
Soybean oil	48
Oleic acid	13
Myristic acid	12
Squalene	12
Paraffin wax	10
Oleic acid monoglyceryl	3
Cholesterol stearate	2

Table 3 Composition of artificial KP

	(wt %)
Artificial sebum	50
Keratin powder	50

2.4 Observation of the behavior on the interface between the artificial KP and the BCME-SF

Microscopic observation of the contact surface between artificial KP and BCME-SF. Warm the artificial keratin plug to 70℃, drop one drop onto a slide glass, and let it cool at room temperature. Using a 0.15 mm thick cover glass as a spacer, inject washing water (0.1 mL) from the side of the glass. The contact behavior between the BCME-SF and artificial KP was observed under the digital microscope (VHX-6000/KEYENCE, Osaka, Japan) at 200x magnification at room temperature.

2.5 Observation of the behavior on the interface between a human KP and the BCME-SF

To collect human KP, a commercially available pore pack was used, and the KP attached to the pore pack were collected with tweezers according to the method described on the package, and then subjected to observation using two different methods.

First, the KP collected from the pore packs was placed on a slide glass and crushed from above with a cover glass. 0.1 mL of BCME-SF was placed next to the cover glass, and the BCME-SF was allowed to penetrate into the cover glass by capillary action. The contact surface between the BCME-SF and the human KP was observed under the digital microscope (VHX-6000/KEYENCE, Osaka, Japan) at 500x magnification at room temperature.

Next, the collected KP was embedded in compound and cut to a thickness of 10 μm using a microtome (CRYOSTAR NX70/Thermo Fisher Scientific, Waltham, MA, USA) to prepare section. The KP section was placed on a slide glass, the compound was removed, and then a cover glass was placed on the slide glass to prepare the observation sample. 0.01 mL of BCME-SF was placed next to the cover glass, and the cleansing water was allowed to penetrate into the cover glass by capillary action. The contact behavior between the BCME-SF and the human KP section was observed under the digital microscope (VHX-8000/KEYENCE, Osaka, Japan) at 500x magnification at room temperature.

2.6 Clinical trials

2.6.1 Preparation of massage gel

To improve the massage properties of BCME-SF, we added Acrylates/C10-30 alkyl acrylate crosspolymer, Sorbitol and so on to prepare a massage gel. All ingredients were cosmetic grade.

2.6.2 Test procedure

Twenty subjects completed the study, and their data was analyzed in the report. The age of the subjects ranged from 18 to 37 years old. The average was 28.65 ± 5.84. All of them were female.

As for study period, in the morning, the subjects cleaned face with water only, and in the evening, cleaned face using the test products. After wetting face with water, subjects took appropriate amount of massaging gel wash (the diameter of the product shape is about 2 cm) in the palm, gently rub with both hands, and then applied on both cheeks, forehead, nose, chin, to slowly spread in a circular way, and then rinse with water. For pores clogged and blackheads more parts, subjects could appropriately increase the amount.

The first application by subjects themselves was instructed by the technician in the lab. During the study period, subjects were asked not to use other cleanser other than massaging gel wash assigned. Other routine skin regimen kept constant.

Candidates would be NOT allowed to apply facial care products, as well as sunscreen and makeups at the screening visit.

After arriving at the investigation center, candidates washed their whole face with tap water, and then sebumeter measurement was taken immediately. After acclimation for 30 minutes under controlled condition with environmental temperature of 18-22℃ and related humidity of 40-60%, the clinical evaluations including the scores of blackheads, whitehead, skin smoothness, skin evenness, pore visibility of global face were performed. If the subject still met the inclusion and exclusion criteria, the skin moisture/sebum content, TEWL and porphyrin distribution by facial image analysis were measured, and the volume change of the KP was measured using reflectance confocal microscopy (RCM) were taken. And then, each subject was instructed to use the test products properly. After 15 minutes (15 Mins), the clinical evaluations, instruments evaluations were taken again. After that, each subject was given verbal and written instructions regarding study requirements/restrictions and a Daily Usage Diary to note use of the assigned test products.

Subjects were required to return to the testing facility at Week 2 (W2), Week 4 (W4), with any remaining test products and completed usage diary. And for these visit days, subjects did not wash face at home and not apply the test products, sunscreen, makeup, or any other skin care products. All clinical and instrument evaluations were performed at BL, 15Min, W2, W4.

2.6.3 Study Instruments and Methods

2.6.3.1 Clinical Evaluation

Clinical evaluation was performed by dermatologist at each visit, and the following items were evaluated: i) The grading the blackhead, ii) The grading the whitehead, iii) The grading the skin smoothness, iv) The grading the skin evenness and v) The grading the pore visibility of global face were performed with 0-9 points scale.

2.6.3.2 Instrument Evaluation

The skin moisture content was measured by Corneometer 825 (Courage & Khazaka, Köln, Germany), the sebum content was measured by Sebumeter SM815 (Courage & Khazaka, Köln, Germany), and the TEWL was measured by Tewameter TM Hex (Courage & Khazaka, Köln, Germany). The distribution of porphyrin, an indicator of KP formation, was analyzed by the facial skin image analysis system of UV-mode (Visia-CR/Canfield Scientific, Parsippany,

USA). The volume change of the KP was measured using reflectance confocal microscopy/RCM (VivaScope 1500/VivaScope GmbH, Munich, Germany). RCM was an equipment that enables the en-face (horizontal plane) visualization of the skin at a depth of 150~200 μm, with a resolution at the cellular level (0.5-1.0 mm in the lateral dimension and 4-5 mm in the axial ones). RCM images were obtained horizontally from the targeted site. Each single image displays a 500 mm×500 mm large field-of-view on the screen. RCM allowed the scanning of the entire area of the targeted area up to 8 mm×8 mm and an automated stepper could generate a mosaic grid of contiguous horizontal images. A targeted area was marked and tracked by a transparent film, and a 5mm×5mm RCM image was captured at an optimal depth, where hair follicles opening was clearly visible.

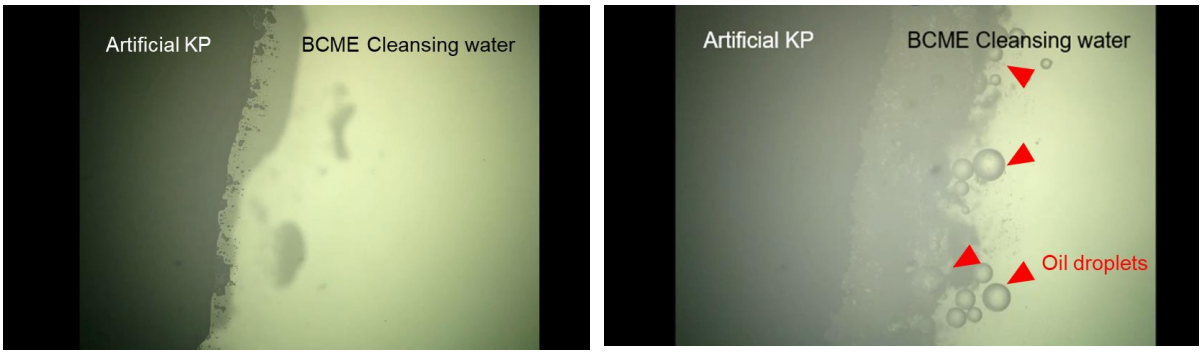
2.6.4 Statistics

All data was analyzed using the SPSS statistical software 22.0 for Windows. For ordinal data, descriptive analysis was performed for each parameter including Sum (Minimum, Median, Maximum). For measurement data, descriptive analysis was for each parameter including mean ± standard deviation (SD). Ordinal data was evaluated by two-related samples Wilcoxon Signed Rank test. Analysis of the normality distribution of the measurement data was evaluated by means of one sample Shapiro-Wilk test. According to data distribution, data sets were analyzed using one of the two following methods: Paired Student t test and Wilcoxon Signed Rank test. All statistical tests were 2-sided at significant level with $P<0.05$.

3. Results

3.1 Observation of the behavior on the interface between an artificial KP and the cleansing water

When the BCME contacted with the artificial KP, it was observed that it immediately penetrated into the inside, causing it to swell and collapse. At the same time, it was observed that an emulsion was spontaneously formed from the edge of the KP. The results are listed in Figure 1.



(**a**) (**b**)

Figure 1. Observation of contact behavior between artificial KP and the cleaning water: (a) Immediately after contact between artificial KP and the cleansing water; (b) 1 minute after contact between artificial KP and the cleansing water.

3.2 Observation of the behavior on the interface between a human KP/ human KP section and the cleansing water

When the BCME contacted with the human KP, it was observed that it immediately penetrated into the inside. After that, oil droplets were formed near the contact surface, and

swelling of the KP was observed. As the formation of oil droplets increased over time, collapse of the KP was observed. The results of typical example are listed in Figure 2.

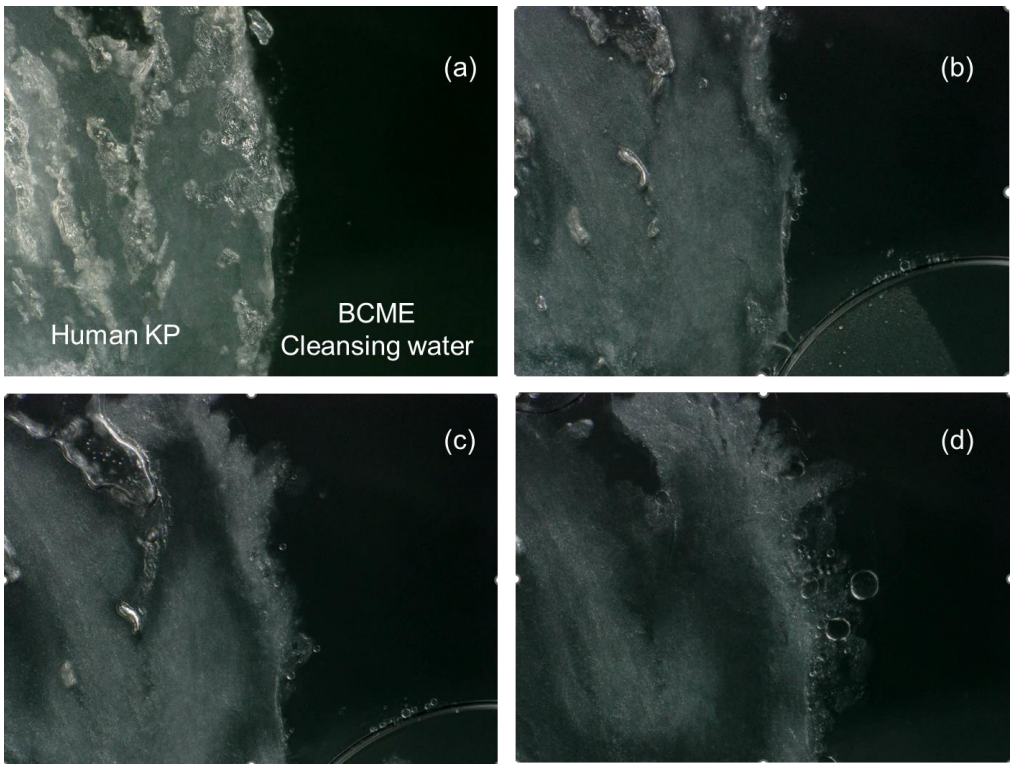
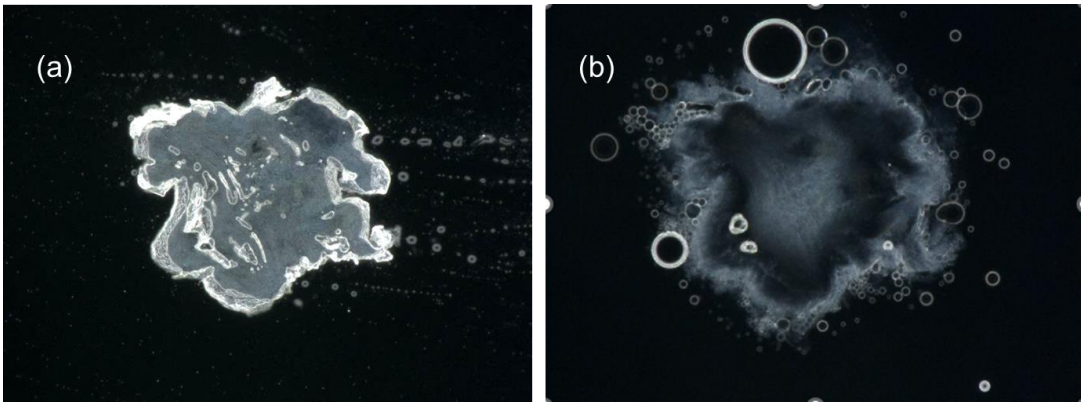


Figure 2. Observation of contact behavior between human KP and the cleaning water: (a) Before contact between human KP and the cleaning water; (b) 15 seconds after contact between human KP and cleaning water; (c)1 minute after contact between human KP and cleaning water; (d)5 minutes after contact between human KP and cleaning water

In the case of human KP sections, the changes were more pronounced than in the case of human KP. The formation of oil droplets was observed immediately after contact. The speed of oil droplet formation was also faster, and swelling and KP collapse were also observed in a shorter time. The results of typical example are listed in Figure 3.



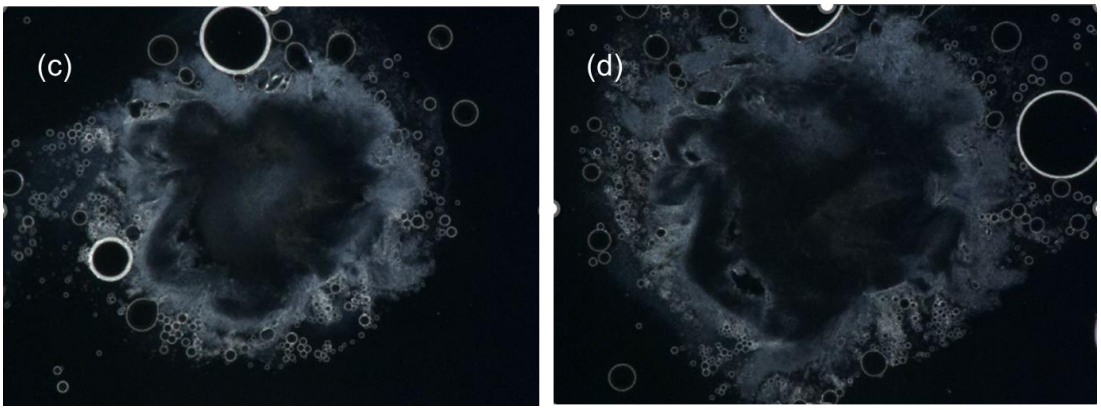


Figure 3. Observation of contact behavior between human KP section and the cleaning water: (a) Before contact between human KP section and the cleaning water; (b) 15 seconds after contact between human KP section and cleaning water; (c)1 minute after contact between human KP section and cleaning water; (d)3 minutes after contact between human KP section and cleaning water

3.3 Clinical evaluation

3.3.1 Clinical evaluation of closed and opened comedones

Compared with BL, the scores of closed and opened comedones decreased significantly at W4 after test product application ($P<0.01$), while they had no significant difference at 15 Mins and W2 after test product application ($P>0.05$). The results are listed in Table 4.

Table 4. The scores of closed and opened comedones at different time points (Sum (Minimum, Median, Maximum))

	BL	15 Mins	W2	W4
Closed comedones	69(1,4,5)	68(1,4,5)	66(1,3,5)	61(1,3,5)**
Opened comedones	67(1,3,6)	67(1,3,6)	63(1,3,6)	55(1,2,5)**

*Notes: * significantly different compared with BL, ** P<0.01, performed by Wilcoxon Signed Rank test. The lower values indicate the better effect.*

3.3.2 Clinical evaluation of other skin attributes

Compared with BL, the scores of skin smoothness decreased significantly at W2 and W4 after test product application ($P<0.05$), the skin evenness and pore visibility decreased significantly at W4 ($P<0.05$). Compared with BL, all had no significant difference at 15 Mins, and the scores of skin evenness and pore visibility had no significant difference at W2 after test product application ($P>0.05$). The results are listed in Table 5.

Table 5 The scores of other skin attributes at different time points (Sum (Minimum, Median, Maximum))

	BL	15 Mins	W2	W4
Skin smoothness	106(3,6,7)	103(2,6,7)	100(3,5,7)*	97(3,5,6)**
Skin evenness	106(3,5,7)	106(3,5,7)	102(3,5,7)	99(3,5,7)*
Pore visibility	58(1,3,5)	58(1,3,5)	55(1,3,5)	46(1,2,4)***

*Notes: * significantly different compared with BL, * P<0.05, ** P<0.01, *** P<0.001, performed by Wilcoxon Signed Rank test or Paired Student t test. The lower values indicate the better effect.*

4.3 Non-invasive instrumentation

4.3.1 TEWL values

Compared with BL, the TEWL values decreased significantly at 15 Mins, W2 and W4 after test product application (P<0.05). The results are listed in Figure 4.

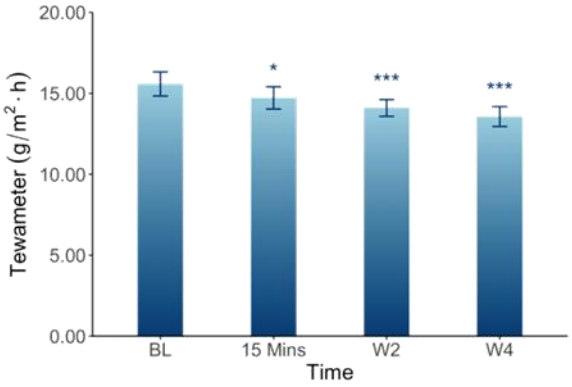


Figure 4. The TEWL values at different time points (Mean ± SD, g/m^2)

4.3.2 Corneometer values

Compared with BL, the Corneometer values increased significantly at W2 and W4 after test product application (P<0.01), while there was no significant difference at 15 Mins after test product application (P>0.05). The results are listed in Figure 5.

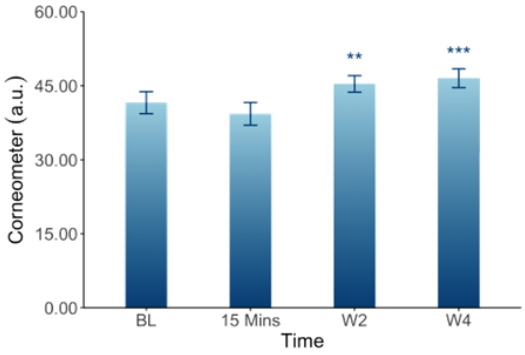


Figure 5 The Corneometer values at different time points (Mean ± SD, a.u.)

4.3.3 Sebum secretionvalues

Compared with BL, the sebum secretion values had no significant difference at W2 and W4 after test product application (P>0.05). The results are listed in Figure 6.

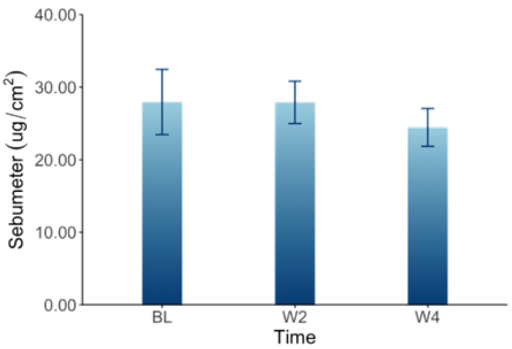


Figure 6 The Sebumeter values at different time points (Mean ± SD, ug/cm^2)

4.3.4 Observation of porphyrin distribution

Compared with BL, the porphyrin distribution decreased at W4 after test product application. The results of typical example are listed in Figure 7.

(**a**) (**b**)

Figure 7. The porphyrin distribution observation: (a) BL; (b) W4

4.3.5 The proportion of in K

Compared to BL, the percentage of KP was significantly decreased in W4 after application of the test product (P<0.01). The mean value was -46%. However, there was no significant difference in the percentage of KP after application of the test product in W2 (P>0.05). The results are shown in Table 6.

Table 6 The proportion of the KP in hair follicles at different time points (Mean ± SD, ug/cm^2)

BL	W2	W4
0.148±0.109	0.152±0.073	0.080±0.030**

*Notes: * significantly different compared with BL, ** P<0.01, performed by Paired Student t test. The lower values indicate the better effect.*

4. Discussion

The BCME cleansing water used in this study is known to have a unique cleaning mechanism: (1) it penetrates quickly when it comes into contact with the KP, forming an emulsion with oleic acid, a component that forms the KP inside; (2) as the emulsion is discharged from the KP, the KP itself swells, loosening its strong structure; and (3) finally, the KP structure collapses and is easily removed. In addition, in a similar observation using artificial sebum/artificial KP without oleic acid, the emulsion formation, KP swelling, and collapse were

significantly slower, making it clear that the selective cleaning mechanism of oleic acid is greatly involved in this KP removal function.

Furthermore, in this study, the use of a massage gel for four weeks showed significant improvements in skin smoothness, skin evenness, and pore visibility. It was thought that this was related to an increase in skin moisture and a decrease in TEWL. However, it did not affect sebum, and did not affect metabolic functions. Using UV-mode measurements from a facial image analysis system, it was observed that the distribution of porphyrin, an indicator of KP, had decreased in all areas, size, and quantity. Detailed changes in the amount of KP measured by RCM showed a 46% decrease after 4 weeks of use, demonstrating that the use of this massage gel significantly removed KP.

5. Conclusion

Due to its structural characteristics, BCME has a high affinity for both the hydrophilic/hydrophobic site with low interfacial tension, allowing it to penetrate into the inside of KP. KP is formed from the protein exfoliated stratum corneum and sebum, and selective removal of its component oleic acid loosened its strong structure, promoting its spontaneous collapse and successfully removing the KP easily. It is also known that an increase in the proportion of oleic acid in sebum with age is related to an increase in TEWL, and it has been suggested that its selective removal improves barrier function. Thus, despite the rinsing-off cosmetic, we have succeeded in developing a novel formula that not only improves concerns with pores and KP, but also improves skin condition.

Acknowledgment

I would like to express my appreciation to Mr. Shunsuke KADOTA and Mr. Yoichi MIZUTANI from Taiyo Kagaku Co., Ltd. Food Analysis Technology Center for filming the video using human KP section.

References.

1. *Ito S., Matsumoto Y., Higuchi T., Yamashita Y., Sakamoto K.,* Less Is More for a Water-based Makeup Cleansing Lotion, IFSCC Magazine, **24** (2021) 47-54.

2. *Yamashita Y.,* Application of “Sponge Phase” – Road to Sustainable Formulation Concept “Less-is-More”, IFSCC Magazine, **25** (2022) 147-150.

3. *Miyamoto M., Ito S., Higuchi T., Kang J., Yamashita Y.,* Selective Cleansing Using a Novel Water-Based Makeup Remover, IFSCC Magazine, **27** (2024) 55-58.