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"Innovative analysis of emulsion behavior on skin"

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1. Introduction

In skincare, the permeability of the skin when cosmetics are applied is the most important factor in understanding the function and characteristics of the formulation. Generally, methods using epidermal models are employed to assess skin permeability. These methods evaluate the amount of specific components that accumulate on or penetrate into epidermal model skin. Previous studies have used epidermal models to evaluate the percutaneous absorption of various ingredients. However, such models tend to have higher transepidermal water loss and lower durability than human skin, making them difficult to use in some evaluation tests. Furthermore, evaluating emulsions is even more complicated, as it is necessary to take into account the inclusion of aqueous components, oily components, surfactants, and other components, as well as differences in the emulsion state.

Given this background, we formulated samples with various emulsion states, applied them to excised human skin, and used imaging to observe how the emulsions underwent structural changes on the skin. We also investigated the rheology using a new method referred to as rheo-impedance measurements (RIMs) [1] to determine what changes occurred in samples with different emulsification states.

2. Materials and Methods

2.1 Preparation of samples with different emulsion states.

Three types of emulsion sample were prepared for evaluation: oil in water (O/W), water in oil (W/O), and water in oil in water (W/O/W). Each sample used the same oil and moisturizer, and the emulsification state was changed by varying the surfactant and processing conditions (Table 1). The viscosity of each emulsion was adjusted to be between 15,000 and 20,000 mPa·s to minimize the effect of viscosity differences on the rheology. The oil-to-water ratio was also adjusted to within the range of 25:75 to 20:80, to ensure that the composition of the emulsions was as consistent as possible.

Table 1. Emulsion composition. For the O/W and W/O, the oil phase and water phase components were all dissolved by heating and then emulsified using a homomixer. For the W/O/W formulation, the W/O phase was prepared first, then added to the external water phase and emulsified using a homomixer.

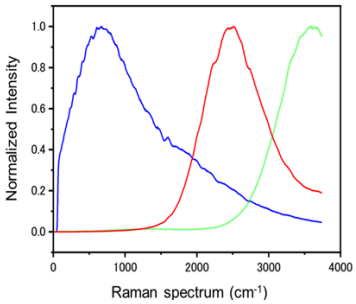
Phase	Ingredient	O/W (%)	W/O (%)	W/O/W (%)
	Water	65.5	-	53.5
	BG	7.0	-	7.0
External water	Thickener	q.s.	-	q.s.
	pH adjuster	q.s.	-	q.s.
	Polysorbate 80	2.5	-	2.5
	Squalane	15.0	15.0	15.0
Oil	Cetyl ethylhexanoate	10.0	10.0	10.0
	Cetyl dimethicone copolyol	-	2.5	1.5
	Water	-	65.0	10.0
Internal water	Inorganic salts	-	0.5	0.5
	BG	-	7.0	-

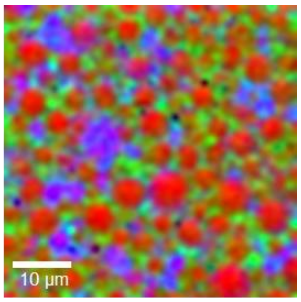
2.2 Rheo-Impedance Measurements (RIMs)

RIMs were performed using a rheometer (MCR 702e Space MultiDrive, Anton Paar). The electrochemical impedance was measured by placing an emulsion sample between the electrodes of the rheometer. The plate diameter was 25 mm, the Gap Control was 0.1 mm, the frequency range was 4 Hz to 8000 kHz, and the shear rates were 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20 and 50 s–1. All measurements were performed at 25 °C.

2.3 Observation by Confocal Raman Microscopy (CRM)

To perform confocal Raman microscopy (Witec alpha300R, Oxford Instruments), frozen excised human skin was first acclimatized to 35 °C and then placed on an observation plate. Next, phosphate buffered saline (PBS, 0.01 mol/L) and penicillin-streptomycin solution (penicillin G, 10,000 units/mL; streptomycin sulfate, 10,000 μg/mL) were mixed in a weight ratio of 99:1 to prepare a PBS(-) solution. The surface of the excised human skin was washed with PBS(-) solution and excess water was then wiped off. The emulsion sample was applied to a cotton swab, the weight of which had been measured in advance, and the swab was used to spread the emulsion evenly over the skin surface. The amount of sample was calculated so that approximately 1 g was applied per 300 cm^2, and was adjusted to match the size of the excised human skin. CRM observations were then performed at 0, 1, 2 and 4 hours after application. The CRM laser wavelength was 532 nm, the laser intensity was 0.1 mW, 50× objective lens, the scan time was 0.02 s/point, and the scan range was 50 μm wide and 50 to 70 μm deep. A fluorescent dye was added to the emulsion to be observed in order to distinguish the water and oil phases. Since W/O/W emulsions have two aqueous phases, a fluorescent dye with a different fluorescent wavelength was added to each phase, and it was confirmed that they could be distinguished by CRM, as shown in Figure 1(1a,1b).





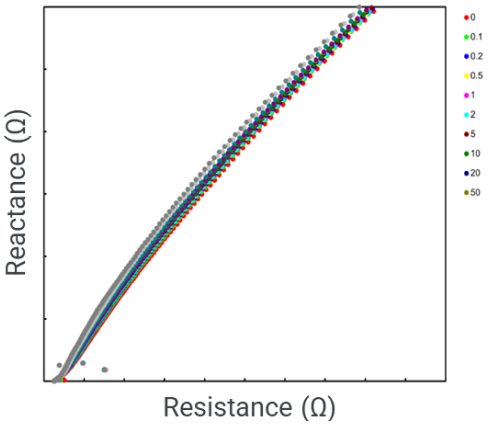
(1a) Peaks of each fluorescent dye **(1b)** Visualization of each W/O/W phase

Figure 1. W/O/W visualization. (1a,1b) Blue identifies the external water phase, red the internal water phase, and green the oil phase.

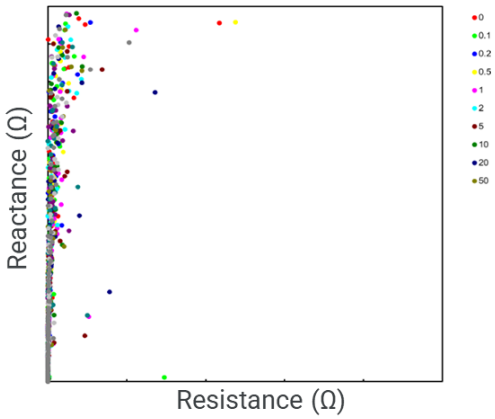
3. Results

3.1 RIMs results

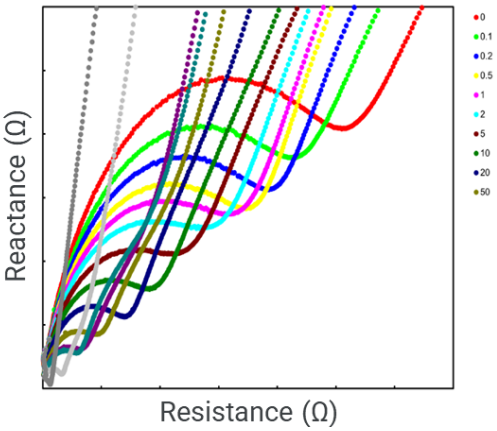
Figure 2 shows the impedance results for each emulsion as determined by RIMs.



(2a) O/W



(2b) W/O



(2c) WO/W

Figure 2. Nyquist plots for each emulsion samples.

The Nyquist plot for the O/W emulsion (2a) indicates a constant impedance, while that for the W/O emulsion (2b) indicates a high resistance and low dielectric constant. On the other hand, the Nyquist plot for the W/O/W emulsion (2c) shows a dependence of the impedance on the

shear rate, suggesting a change in the W/O/W structure due to shear, which is a unique property not found for other emulsions.

3.2 CRM results

To observe changes in the skin over time, cross-sectional CRM images were obtained centered on the sulcus cutis, as shown in Figure 3. Figure 4 shows the results obtained when an O/W emulsion was applied. Observations were performed while slightly shifting the observation point each time to avoid laser-induced fading of the fluorescent dye. It was found that the distribution of the two components varied depending on the observation point.

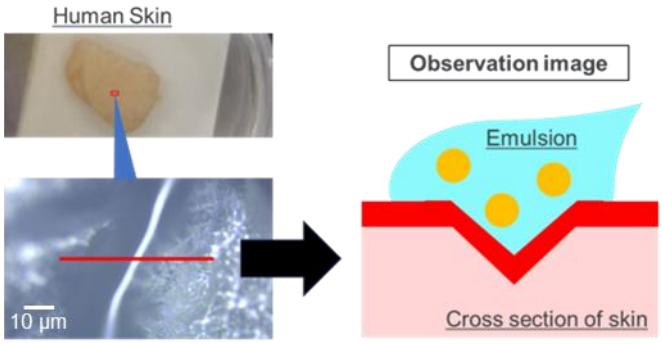
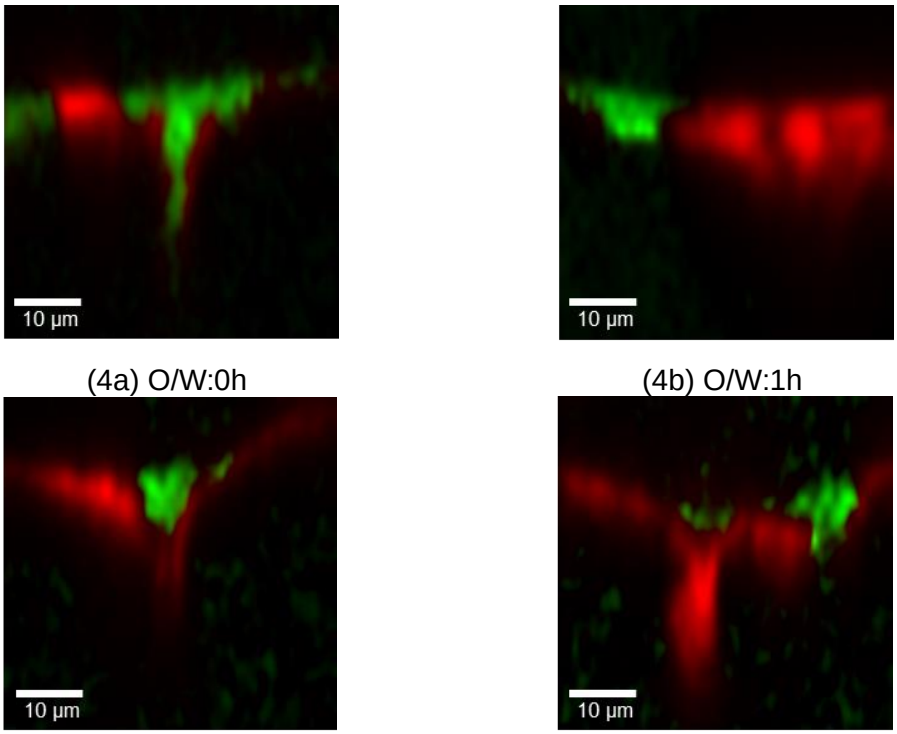


Figure 3. Observation flow.

Cross-sectional data was obtained by focusing the observation on the skin sulcus of the excised human skin.



(4a) O/W:0h (4b) O/W:1h

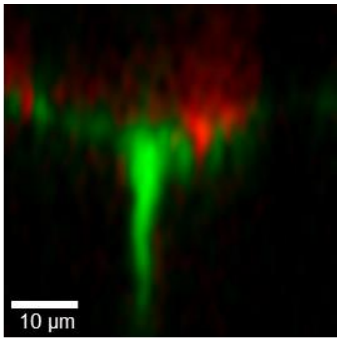
(4c) O/W:2h (4d) O/W:4h

Figure 4. Raman images of O/W emulsion from 0 to 4 hours.

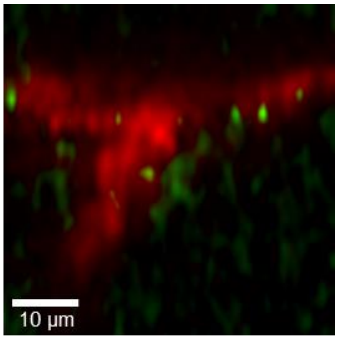
Red : Water phase, Green : Oil phase.

The observation results for the W/O emulsion are shown in Figure 5. In this case, the oil phase spread over time as it blended with the skin, and eventually become difficult to observe. On the other hand, the internal water phase remained in place over time, probably because its movement was suppressed by the oil and the surfactant. However, the Raman peak associated with the aqueous phase is seen to decrease in intensity from 0 to 4 h, suggesting that the agent penetrated into the skin over time.

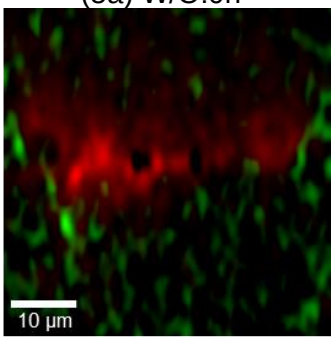
Figure 6 shows the CRM results for the W/O/W emulsion. At 0 h, three separate phases were observed, but as time passed, these phases behaved differently. The intensity of the Raman peak associated with external water rapidly decreased, indicating that percutaneous absorption was occurring. The oil phase spread over the surface of the skin, eventually completely covering it. The behavior of the internal water phase was similar to that for the W/O emulsion, remaining in the formulation for a long time before gradually being absorbed into the skin.



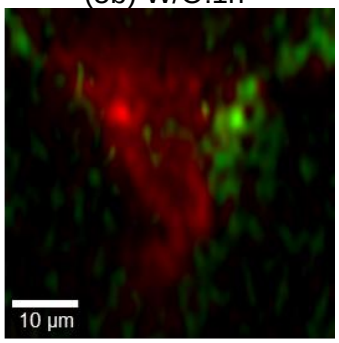
(5a) W/O:0h



(5b) W/O:1h



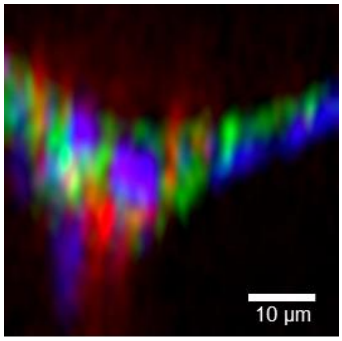
(5c) W/O:2h



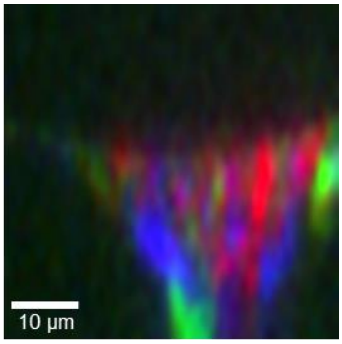
(5d) W/O:4h

Figure 5. Raman image of W/O emulsion. (1-4h)

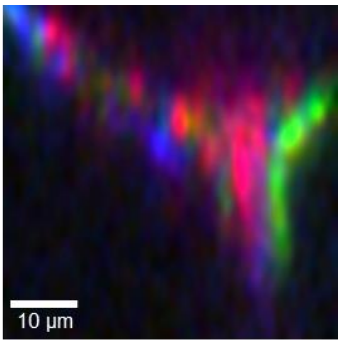
Red : Water phase, Green : Oil phase. After 2 hours, almost no peaks of the fluorescent dye dissolved in the oil phase were observed.



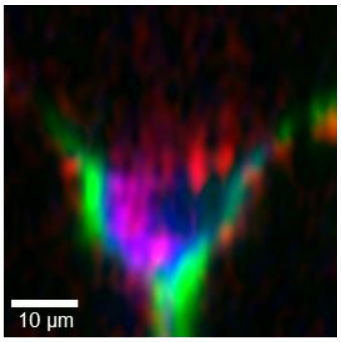
【0h】W/O/W



【1h】W/O/W



【2h】W/O/W



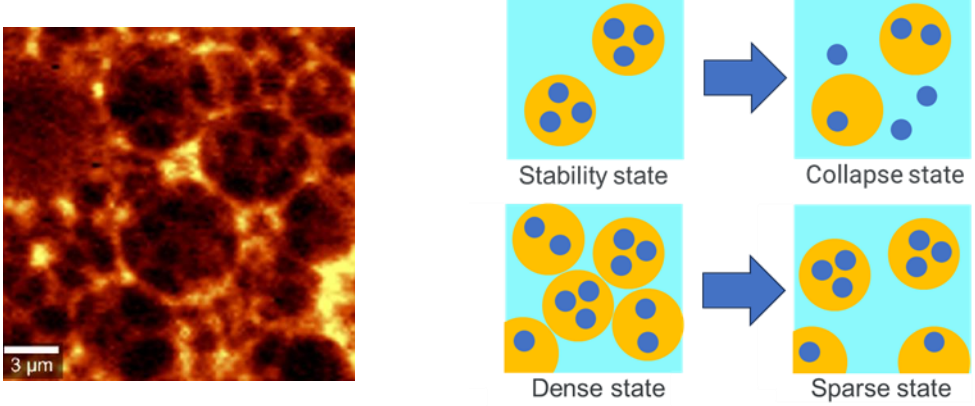
【4h】W/O/W

Figure 6. Raman image of W/O/W emulsion. (1-4h)

Red : Internal water phase, Green : Oil phase, Blue : External water phase.

4. Discussion

Although RIMs have previously been used to determine the dispersion state and dielectric constant of a slurry when shear is applied, the present study shows that they can also be used to investigate the characteristics of specific emulsions. In particular, it was found that a W/O/W emulsion exhibited impedance changes that were clearly different from those for O/W and W/O emulsions, and underwent complex changes in structure in response to shear. One possible reason for this is a simple collapse of the W/O/W structure, but the RIM results also suggested that the dielectric constant may have changed as the W/O particles went from a densely packed state to a dispersed state (Figure 7). Further rheological analysis may reveal the details of these structural changes, thereby allowing a deeper understanding of multiphase emulsions. Because it is difficult to predict the structural changes that occur in multiphase emulsions such as W/O/W emulsions, it is not entirely clear how they will behave when they are applied to the skin, or what the mechanism for percutaneous absorption is.



(7a) W/O/W CRM imaging (7b) Structural changes due to shear

Figure 7. Structural changes predicted from W/O/W composition.

There are several possible reasons for the behavior of W/O/W under shear, such as the collapse or dispersion of the structure.

Although there have been many reports on the use of CRMs [2-5], none of these previous studies have observed any changes that occur between human skin and the emulsion. Observation of emulsions using CRM revealed the dynamics of various formulations. In an O/W emulsion, the water and oil phases were found to be distributed differently depending on the observation point, and there was little uniformity. For the W/O emulsion, the internal water phase was maintained for a long time, while the oil disappeared as it became absorbed by the skin. This may be due to the fact that human skin has a high affinity for hydrophobic components.

Finally, each phase in the W/O/W emulsion had its own unique characteristics. While the internal water phase was retained for a long period of time, the external water phase was observed to be absorbed by the skin over time, thus demonstrating delayed penetration into the skin. Even more interestingly, the oil phase eventually spread over the entire skin surface, so exhibiting protective behavior. This is highly likely to contribute to efficiently suppressing moisture loss from the skin, and future research should focus on investigating the relationship between this effect and skin moisture content.

5. Conclusion

In this study, we used RIM and CRM to investigate the behavior of various types of emulsions. We found that RIM can detect structural changes in W/O/W emulsions that were difficult to identify using conventional rheology. Furthermore, by using CRM, we were able to observe the dynamic changes of emulsions on excised human skin with high resolution.

In particular, the behavior of a W/O/W emulsion could be directly observed for the first time in the world. We believe that the evaluation system developed in this research will advance our understanding of the characteristics of emulsions and contribute to the development of formulations with high functionality and good texture.

6. References

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