Development of emulsifier-free formulations suitable for sun protection for sensitive skin by reducing skin penetration

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Abstract

Sunscreen products play an important role in daily life, as they can prevent the appearance of aging, the darkening of the skin, and lower the risk of developing skin cancer. Previous researches have demonstrated that sunscreen agents can penetrate through the skin, and even trace amounts of sunscreen ingredients have been found in the bloodstream. In this study, we sought to reduce the penetration of sunscreen ingredients by employing a novel emulsification system. Furthermore, we employed *in vivo* confocal Raman spectroscopy to obtain data on penetration with a high degree of accuracy, ensuring that the integrity of the body was not compromised. Finally, samples prepared with the Pickering emulsification system exhibited significantly lower permeation rates than those prepared with traditional emulsifiers. Moreover, after two-week in-vivo test including TEWL, a* value, and *stratum corneum* hydration, Pickering emulsions demonstrated a notable improvement in sensitive skin conditions. The results of our research will provide a basis for the development of sun protection products for sensitive skin and an investigation of the potential application of Pickering emulsion in skin care products.

Keywords: emulsifier-free; sun protection; sensitive skin; skin permeation; Pickering emulsion;

Introduction.

As we know, there is a significant number of individuals with sensitive skin [1]. As the public becomes increasingly aware of the importance of skincare, sunscreen products are being selected by an ever-increasing number of individuals with sensitive skin. These products offer the dual benefit of protecting the skin from the sun's harmful UV rays and slowing down the natural aging process [2]. Nevertheless, some studies have indicated that the sunscreen ingredients in sunscreen products may be able to permeate the epidermal layer and even enter the bloodstream [3,4].

The potential adverse effects induced by UV-filters in experimental animals include reproductive/developmental toxicity and disturbance of hypothalamic–pituitary–thyroid axis (HPT) [5].

Emulsifiers, as necessary ingredients in sunscreen products, are added to almost every sunscreen product. Emulsifiers, such as PEG-based emulsifiers, function to reduce interfacial tension. However, they may weaken the brick-and-mortar structure of the epidermis to some extent, leading to the penetration of sunscreen ingredients [6].

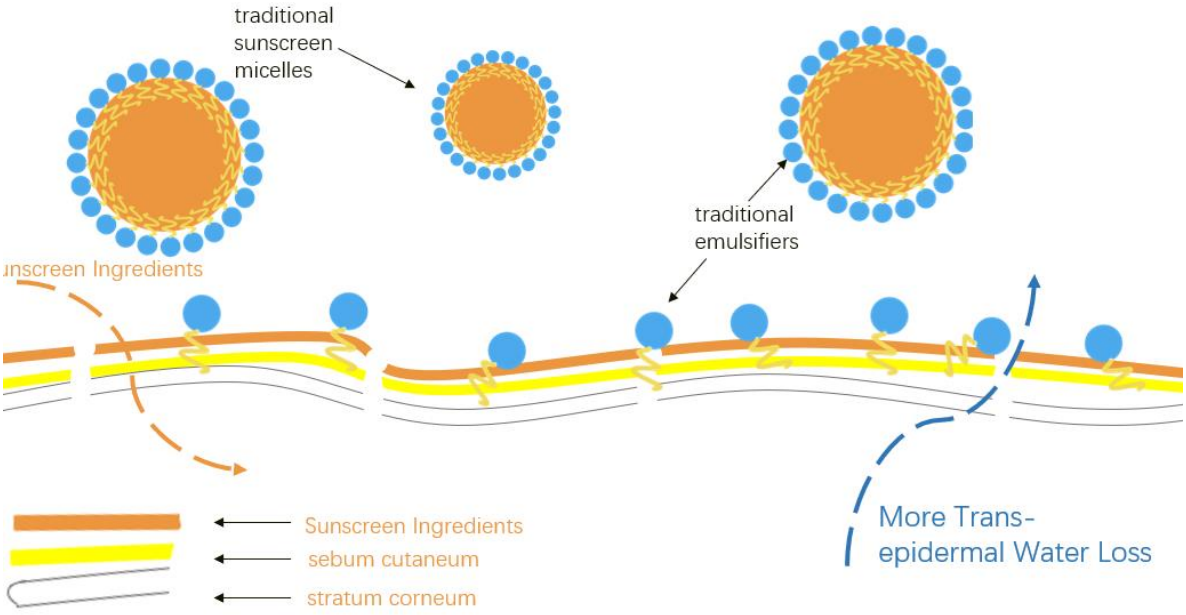

Figure 1. The schematic diagram of traditional emulsifiers causing sunscreen ingredients penetration

Inspired by the pharmaceutical field, we developed the Pickering emulsion system. Because it consists of solid amphiphilic powders, it does not increase skin permeability like conventional non-ionic surfactants. Therefore, it combines strong emulsifying properties with safety [7,8].

Regarding penetration testing, the commonly used method is the Franz diffusion cell. To obtain accurate data using this method, human skin tissue, which is difficult to obtain, is required. Artificial membranes or animal membranes cannot completely replace human skin in researches [9].

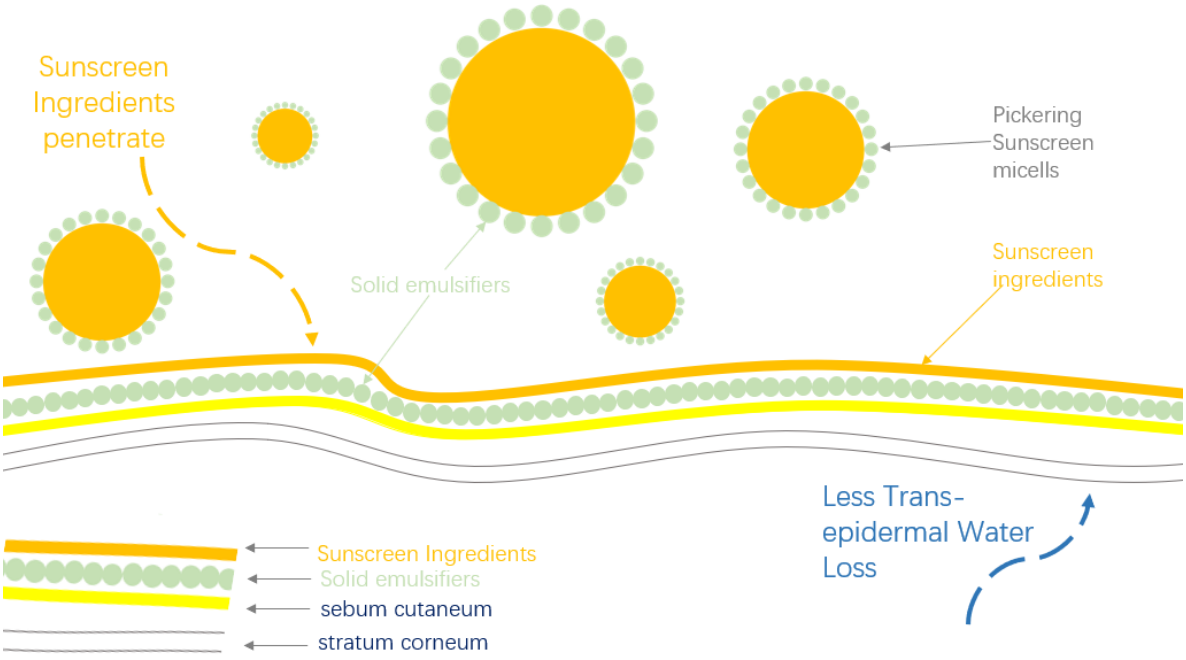

Figure 2. The schematic diagram of Pickering emulsifiers causing less sunscreen ingredients penetration

With the advancement of confocal Raman spectroscopy instruments, Raman devices are widely utilized across various disciplines such as biology, medicine, and food science [9]. In recent years, the development of confocal Raman spectroscopy devices has enabled significant

advances in Raman spectroscopy [10]. Previously, Raman spectroscopy required the combination of scanning Raman and tape stripping for the joint study of organic sunscreen distribution in the skin [11]. However, the advent of confocal Raman has now made it possible to independently utilize this technique to discover that sunscreen products can penetrate the stratum corneum up to 20 micrometers without damaging the skin [12]. Confocal Raman spectroscopy represents a new generation of skin component analyzers, which are user-friendly, thoroughly optimized, and capable of rapidly acquiring high-quality Raman spectra from the skin surface to the epidermal depth.

In summary, confocal Raman technology serves as an effective means to explore the penetration of sunscreen ingredients into the stratum corneum. Consequently, we employed this method for relevant tests.

Materials and Methods

All volunteers participating in the experiment signed informed consent forms before *in vivo* tests, in accordance with Helsinki Declaration.

Materials

Generally, sunscreen is roughly categorized into physical sunscreen and chemical sunscreen. Research indicates that both types of Sunscreen ingredients carry the risk of penetrating the skin. Therefore, this study simultaneously used physical and chemical sunscreens in the formulation design of sunscreen. For the Pickering powder, we selected Lactobacillus/rice ferment and Silica Dimethyl Silylate based on the contact angle and other citations. All other ingredients are cosmetic-grade.

Preparation of Pickering sunscreen

The experiment only varies the type of emulsifier while keeping the types and amounts of sunscreen ingredients, as well as the types and amounts of the aqueous phase.

Phase A consists of commonly used chemical sunscreen filters (Diethylhexyl Butamido Triazone, Octocrylene, Ethylhexyl Salicylate, s-Ethylhexyloxyphenol Methoxyphenyl Triazine, Butyl Methoxydibenzoylmethane) and solvents.

Formulations for followed tests are prepared with Phase A to F as presented in **Table I**. Phase A and phase B were heated to 85 °C in order to facilitate the complete dissolution of solid materials. Subsequently, phase B and C or D were added into phase A, which was then homogenized at 6000 rpm for 5 mins. After cooling the mixture to 45 °C, phase E and F were added with homogenized at 6000 rpm for 3 mins.

Table I. Composition of Sunscreen

Phase	Ingredients	Control	Blank	Picking sunscreen
A	Chemical sunscreen ingredients	15	15	15
	Diisopropyl Sebacate C12-15 Alkyl Benzoate	10	10	10
	Behenyl Alcohol	2	2	2
B	Pure water	To 100	To 100	To 100
	moisturizer	9	9	9
	Silica	1	1	1
	Xanthan Gum Microcrystalline Cellulose	0.08	0.08	0.08

	Titanium Dioxide (CI 77891)	3.5	3.5	3.5
	Cyclopentasiloxane	7.5	7.5	7.5
C	Dimethicone	3.5	3.5	3.5
	Lactobacillus/rice ferment	0	0	0.5
	Silica Dimethyl Silylate	0	0	0.5
D	Glyceryl Stearate	2.5	0	0
	PEG-100 Stearate	0.5	0	0
E	Pure water	5	5	5
	Terephthalylidene Dicamphor Sulfonic Acid	3.5	3.5	3.5
F	Arginine	0.15	0.15	0.15
	preservative	0.3	0.3	0.3

Surface activity measurements by contact angle method

As stated, Pickering powder should be amphiphilic. We judge the hydrophilic properties of the test powder by the contact angle.

At the junction of solid, liquid and gas phases, the angle between the solid-liquid interface passing through the liquid interior to the gas-liquid interface is called the contact angle, also known as the infiltration angle. When a droplet is placed on a smooth and uniform horizontal surface, it may diffuse onto the substrate and the contact angle will approach zero if complete wetting occurs. Conversely, if the wetting is partial, the resulting contact angle equilibrates within the range of the material's surface energy. The smaller the contact angle, the greater the wettability or surface energy of the substrate.

A drop of pure water is placed on the substrate pressed by the powder to be measured. The droplet adheres to the surface of the substrate and casts a shadow. The projection screen micrometer uses optical magnification to project the image onto the screen, determining the contact angle by recording the droplet image and automatically analyzing the droplet shape. In this test, the water drop is purified water.

Pickering emulsions characterization by polarization microscope

Due to the unique emulsification structure of Pickering emulsion, it exhibits distinct features at the microscopic level. Therefore, observation specimens are prepared by diluting the sample with pure water at a ratio of 1:10. Optical microscopy is then used to observe the samples, adjusting bright field and polarization field settings as well as the magnification to identify any Pickering characteristics presented in the sample**.**

***In vivo* Raman spectroscopy tests for penetration of sunscreen ingredients**

Three healthy volunteers aged 24-26 participated in this test, with the inner arm as the testing site. Their Fitzpatrick skin types were categorized as type II and type III. All participants signed informed consent forms before the trial, in accordance with the Helsinki Declaration.

Before testing, the forearms of all participants were cleaned. They were then placed in a constant temperature and humidity environment (temperature: 20±1°C, relative humidity: 50±10%) for 30 minutes to reach stability. Three 3*3 cm^2 areas were identified on the inner arm of each participant, and each area was tested five times in parallel. 18 μL of the product was applied to the inside of the volunteer's arm, and the residue was removed from the skin surface after 1 hour. Handheld Raman probe was used for testing, with an excitation light source wavelength of 660nm, excitation power of 100 mW, and a spectral range of 400-3600 cm^{-1}. The collection range was 0-30 μm, with a step size of 2 μm. The exposure time for each depth is 2 seconds. The Raman spectra were collected and processed using Raman analysis software. All spectra

underwent the same processing steps, with cosmic rays removed prior to processing. Smooth processing was conducted using a Savitzky-Golay filter, applying a 9th-order polynomial to remove fluorescence baseline.

***In vivo* SPF & PFA tests**

i) For SPF

Volunteers underwent a phototherapy experiment on their backs while lying in a prone position. The minimum erythema dose (MED) of the Volunteers' skin to ultraviolet irradiation was predicted 24 hours before the experiment. Based on the prediction results, the dose of ultraviolet irradiation was adjusted for the test samples. On the day of the experiment, a normal skin area on the volunteers' backs, no less than 30 cm^2 in size, was selected. The test samples or standard control samples were evenly applied to the selected area at a dosage of (2.00±0.05) mg/cm^2, and then left for 15 minutes or as instructed on the label. Subsequently, appropriate doses of ultraviolet irradiation were administered in three scenarios:

① no sample applied to the subject's skin;

② standard control sample applied;

③ test sample applied. The experimental results were observed 16-24 hours later, and the MED values for each of the three scenarios were recorded.

The Sun Protection Factor (SPF) is defined as the ratio of the MED required to produce erythema in skin protected by sunscreen cosmetics to the MED required in unprotected skin.

It can be expressed as:

$$SPF = MED\rho / MED\mu$$

Where,

MEDρ: MED of the skin protected by the test product

MEDμ: MED of the unprotected skin

This experimental project is a small-sample SPF value determination, with data from 3 cases. The results of the experiment are for reference only.

ii) For PFA

Volunteers underwent a phototherapy experiment on their backs while lying in a prone position. Four hours before the experiment, the predicted Minimal Persistent Pigmentary Darkening (MPPD) value of the volunteers' skin to ultraviolet irradiation was determined. Based on the prediction results, the dose of ultraviolet irradiation was adjusted for the test samples. On the day of the experiment, a normal skin area on the volunteers' backs, no less than 30 cm^2 in size, was selected. The test samples or standard control samples were evenly applied to the selected area at a dosage of (2.00±0.05) mg/cm^2, and then left for 15 minutes or as instructed on the label. Subsequently, appropriate doses of ultraviolet irradiation were administered in three scenarios:

① no sample applied to the subject's skin;

② standard control sample applied;

③ test sample applied. The experimental results were observed 2-4 hours later, and the MPPD values for each of the three scenarios were recorded.

The Photoprotective Factor (PFA) value of the sample for an individual subject is calculated as follows:

$$PFA = MPPD\rho / MPPD\mu$$

Where,

MPPDρ: MPPD of the skin protected by the test product

MPPDμ: MPPD of the unprotected skin

This experimental project involves a small sample size for PFA value determination, with valid data from 3 cases. The results of the experiment are provided for reference purposes only.

***In vivo* sensitive skin repair tests**

Repair *(n*=10*)*: The Transepidermal Water Loss (TEWL) value reflects the barrier function of the *stratum corneum* and can be used to evaluate the repairing ability of cosmetics on skin barrier function. Under test conditions, a higher TEWL value indicates a greater loss of epidermal moisture per unit time and unit cross-sectional area, while a lower TEWL value indicates less loss of epidermal moisture per unit time and unit cross-sectional area.

Soothing: The skin erythema index a* value is measured, with a higher a* value indicating a higher content of skin hemoglobin, which suggests more obvious skin redness.

Moisturizing: The skin moisture content of the *stratum corneum* is determined using the capacitance method. Different skin capacitance values are obtained according to the moisture content of the *stratum corneum*, and these values represent the skin's moisture content.

Before the test: the volunteer rested for 15 minutes in a room with constant temperature and humidity after cleaning theirs face.

1. Before using the products, the erythema index (a* value) was measured using the Skin-Colorimeter; The Transepidermal Water Loss was measured using the Tewlmeter on the facial area; and the moisture content of the *stratum corneum* was measured using the skin surface hydrometer Corneometer.
2. Following the product usage instructions, apply the product every morning and evening.
3. Repeat step 1 after 1 week and 2 weeks of product use.

Results.

Based on the preparation principle of the Pickering emulsion, we used pure water as the object of investigation. From **Figure 3**, we can clearly see that the contact angles formed by the plane pressed from Lactobacillus/rice ferment powder with water droplets is 38.9°.

This indicates water droplets can spread well on the plane, demonstrating that Lactobacillus/rice ferment powder is relatively hydrophilic, making it suitable as the main

emulsifier for O/W Pickering emulsion. Due to the relatively high oil phase content and variety of types in sunscreen products, we have added silica dimethyl silylate, which has excellent lipophilic properties, as a Pickering co-emulsifier.

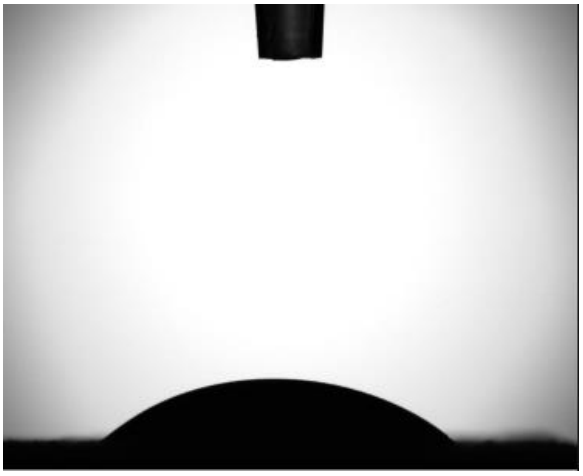

Figure 3. The contact angle of the substrate compressed from Lactobacillus/rice ferment with water

In accordance with **Table I**, we have prepared the blank and Pickering sunscreen samples. The powders mentioned above are the only difference between these two samples. From **Figure 4 (a)** we can see that the blank sample, without the addition of the two powders, showed oil-water separation. The Pickering sunscreen sample was homogeneous and stable. It should be noted that the TiO_2 in the formula is not only a physical sunscreen, but also a color powder. Consequently, it can cause the final image to appear unsatisfactory. In order to address this issue, an alternative sample was prepared without TiO_2 shown in **Figure 4 (b)**. So, we can see that the two powders in our example are the key to emulsification. They are the Pickering emulsifiers.

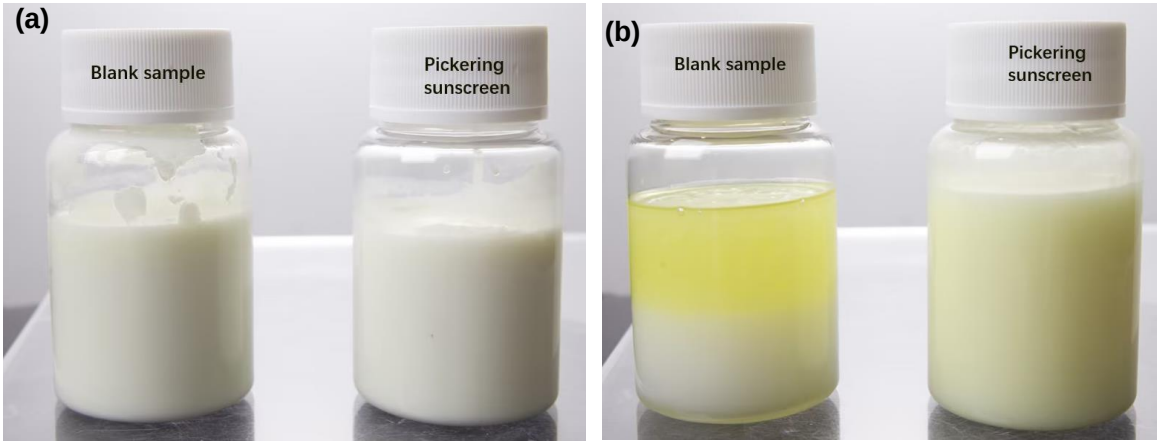

Figure 4 Sample of Blank and Pickering sunscreen (a); Sample of Blank and Pickering sunscreen without Tio_2 (b)

We used a polarized microscope to observe the diluted sunscreen samples from different fields. In the bright field **Figure 5 (a)**, We could clearly see that the surface of the droplets was enriched with numerous small particles. In the polarized field **Figure 5 (b)**, we observed that the particles dispersed in the phase were relatively orderly clustered on the surface of the emulsion droplets, rather than inside the droplets.

In conclusion, we can confirm that our sunscreen product emulsified by Pickering emulsification technology.

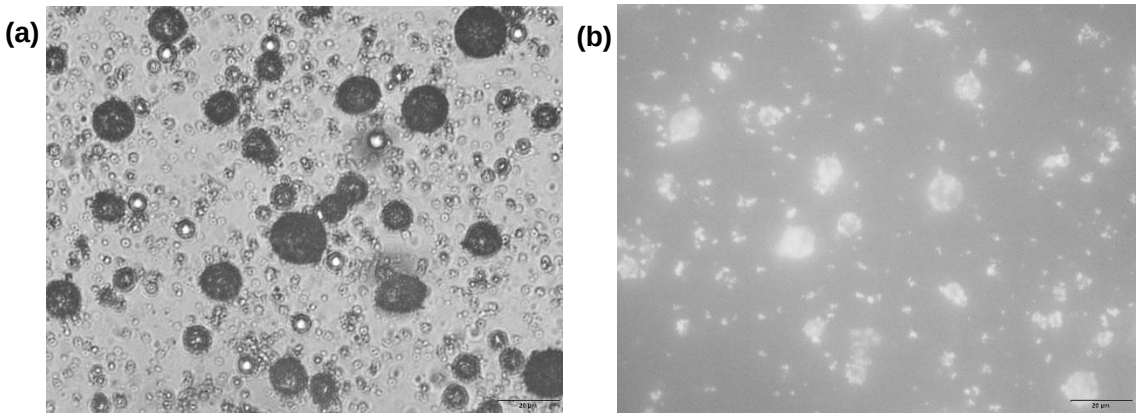

Figure 5. Pickering Sunscreen under bright field (a) and polarized field (b)

The processed data is presented in the form of a heat map as shown in **Figure 6**, illustrating the extent of sunscreen penetration into the skin. The purple portion in the graph represents the sunscreen ingredients, while the black bars themselves represent the depth of the skin. It is evident that the depth and quantity of penetration in the control group are significantly higher than the control group. The only variable we controlled was the type of emulsifier, with the control group using the traditional emulsifier and the experimental group using Pickering powders.

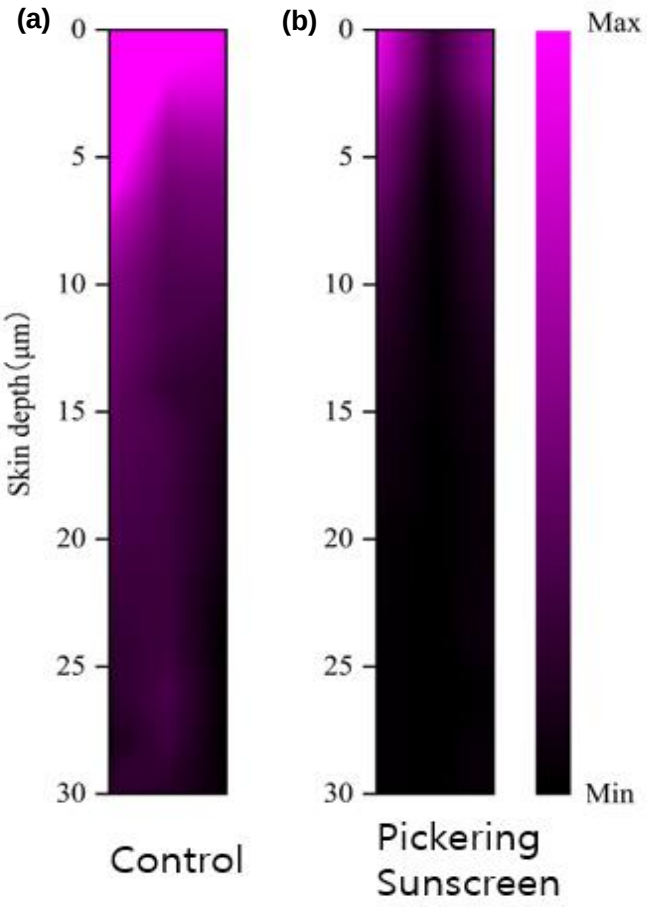

Figure 6. Epidermal penetration simulated diagram of Pickering sunscreen and control

Based on a small-scale population test conducted in accordance with the current Chinese guidelines for SPF and PFA testing of sunscreen products as shown in **Figure 7**, it can be

roughly estimated that the sunscreen made with Pickering emulsifier has an SPF greater than 50 and a PFA value greater than 15.

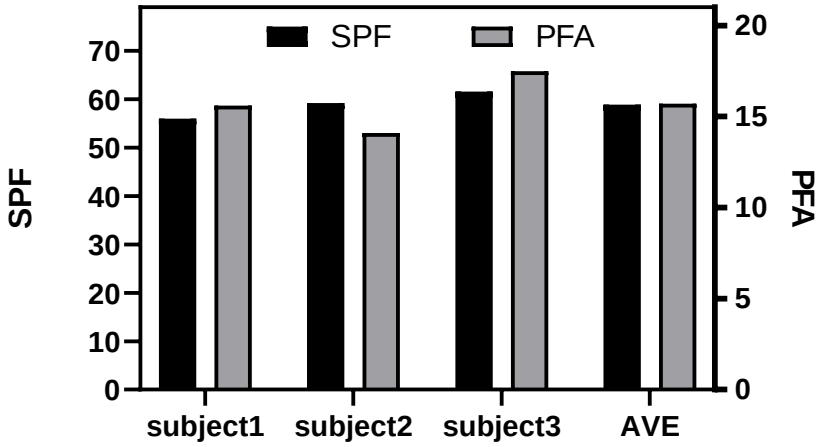

Figure 7. SPF & PFA *in vivo* tests of Pickering sunscreen

From the data in **Figure 8 (a)**, a decrease in TEWL after 2 weeks indicates an improvement in the skin's barrier function.

From the data on *stratum corneum* hydration in **Figure 8 (b)**, there is a significant increase in skin hydration after two weeks. Water, as a crucial component of cellular structure, plays a vital role in various physiological activities of the skin. The level of skin hydration also reflects the overall health of the skin.

The skin color was analyzed using the L*a*b* color space system as specified by the International Commission on Illumination (CIE). The a* value primarily represents the shift in skin color from green to red, with a lower a* value indicating less redness in the skin. When the a* value decreases, it can be said to have an improvement effect on skin redness.

Clearly, as shown in **Figure 8 (c)**, there is a decrease in the a* value after two weeks, indicating an improvement in skin redness.

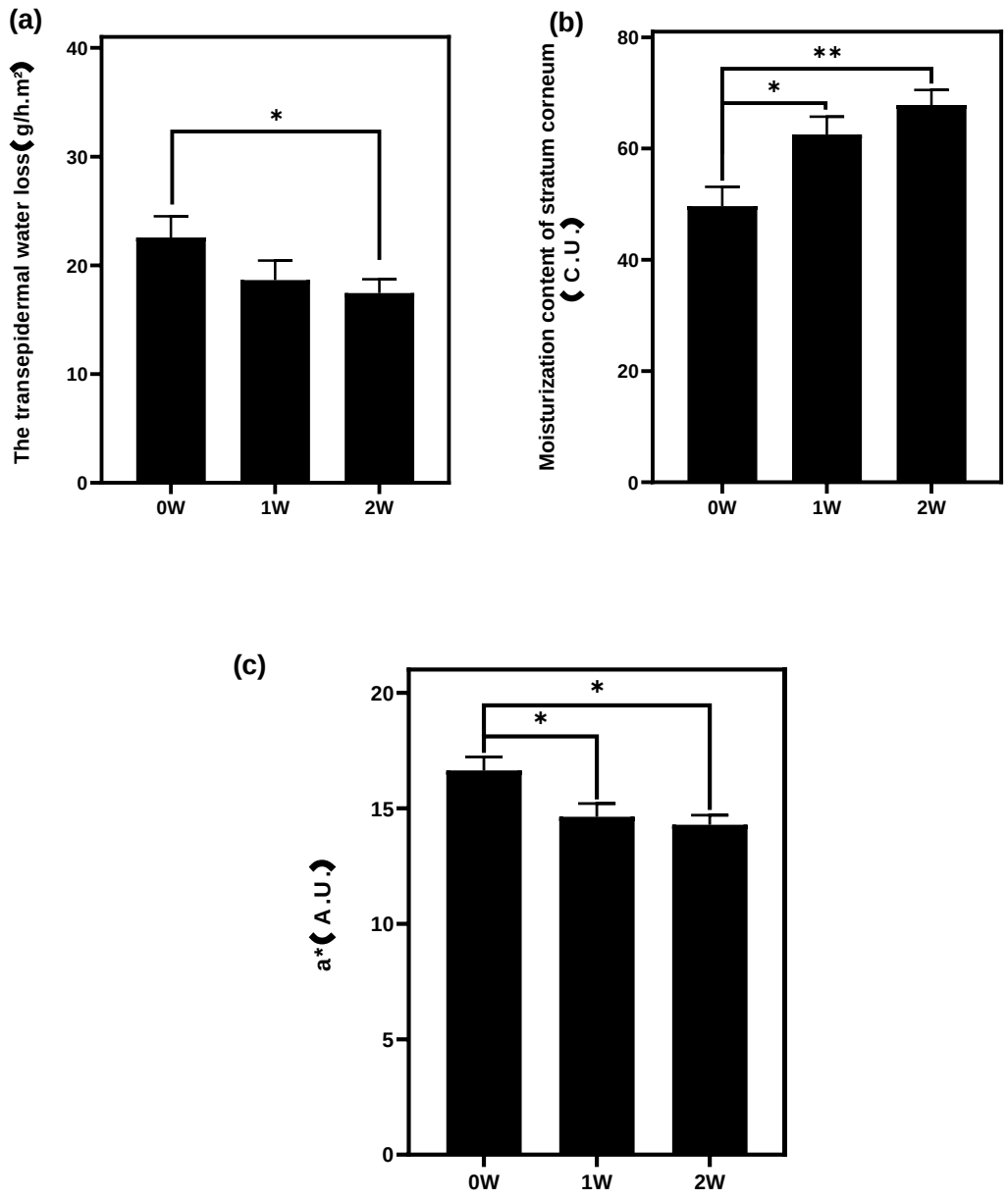

Figure 8. TEWL *in vivo* test of Pickering sunscreen (a); *stratum corneum* hydration *in vivo* test of Pickering sunscreen (b); a* value *in vivo* test of Pickering sunscreen (c)

Discussion.

Confocal Raman is a non-invasive approach for in-depth analyses about the penetration of skincare productions. Base on confocal Raman equipment and Raman spectroscopy, we studied the currently popular organic and inorganic sunscreens. We found that they both exhibit a

significant peak at about 1590-1610 cm^{-1}, which we believe may be related to the structure of the sunscreens[12]. Comparing with the spectrum of the skin, the peak at 1590-1610 cm^{-1} can be easily regarded as a specific peak of the sunscreen. Studying the variation of the 1590-1610 cm^{-1} peak with skin depth can thus be considered as tracking the distribution of the sunscreen with skin depth. From the result of confocal Raman, it shows that the penetration of Pickering sunscreen is weaker than control. This result also shows that our sunscreen stays on the surface of the *stratum corneum* rather than deep inside the skin. It helps sunscreen ingredients to absorb the UV rays [13].

We believe this is a result of emulsifiers. The solubilization ability and association structure of various emulsifiers are different. These differences in solubilization capacity may lead to changes in the thermodynamic activity of the penetrant in the formulation. Examples include liquid crystal emulsions and liposome, where the arrangement of the emulsifier in the water phase helps to increase its permeability [14]. In addition, HLB, particle size, ionic properties of emulsifiers, etc., can affect the permeability of the emulsion [15,16]. Especially the amphiphilic emulsifiers, they tend to enter the keratin interstitial and bind to the skin lipids. It has been reported that these emulsifiers have the potential to be absorbed by the lipid bilayer, thereby increasing the TEWL of normal skin. The higher the TEWL, not only the more water lost through the epidermis, but also the increased permeability of the skin to a certain extent [6]. Therefore, the decrease in TEWL in this experiment not only shows that our Pickering sunscreen has a good repair effect, but also shows that we do not increase the permeability of the skin.

For the repair effect, we did three experiments. The results of human trials have demonstrated that the product exhibits an SPF greater than 50 and a PFA greater than 12. In terms of sensitivity improvement, a reduction in TEWL of 22.62% was observed following a 2-week period. TEWL is an index that is used to evaluate the water-holding capacity of the *stratum corneum*. A reduction in TEWL indicates an improvement in cuticle function. This result can be corroborated by the water content of the *stratum corneum*. After two weeks of use, the average water content

of the subjects' skin increased by 36.6%. Skin redness is also an important characteristic of sensitive muscles. Skin color is analyzed using the colorimetric system prescribed by the International Commission on Lighting (L*a*b* colorimetric system), where the a* value indicates the change in color from green to red. Skin color is analyzed using the colorimetric system prescribed by the International Commission on Lighting (L*a*b* colorimetric system. The a* value mainly measures the change in color from green to red, and the lower the a* value, the less red the skin appears. Two weeks of testing showed a 14.14% reduction in the volunteers' a value.

Conclusion.

The phenomenon of solid particles gathering at the interface of an emulsion was observed by an optical microscope under different field of view. It was observed that the interface of the emulsion drop was covered in powder, which was distributed evenly across the interface.

Moreover, a Pickering sunscreen with high sunscreen efficacy and improved functions for sensitive skin has been successfully developed. Four *in vivo* tests have demonstrated that Pickering sunscreen not only provides effective protection from ultraviolet radiation but also has a beneficial repairing effect. Confocal Raman was used to verify the penetration of sunscreen ingredients. By comparing the Raman spectra of different types of sunscreen lotion, it was shown that Pickering lotion indeed reduce the risk of penetration. The usual cuticle thickness is about 15 μm. According to the Raman results we can see that the depth of the Pickering sunscreen is 12 μm, while the control group is significantly deeper than 15 μm. Therefore, it can be concluded that for the same sunscreen composition and content, Pickering emulsifier has a lower penetration depth than traditional emulsifiers (PEG type) and Pickering emulsifier has higher safety.

We have developed a sunscreen system that does not use traditional emulsifiers, has low skin permeability and is suitable for people with sensitive skin. This offers a new way of developing sunscreens for people with sensitive skin.

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NONE

Conflict of Interest Statement.

NONE

References.

1. Farage, M.A. (2019) The Prevalence of Sensitive Skin. *Front. Med.*, **6**, 98.
2. Hughes, M.C.B., Williams, G.M., Baker, P., and Green, A.C. (2013) Sunscreen and Prevention of Skin Aging. *Ann. Intern. Med.*, **158** (11), 781–790.
3. Adler, B.L., and DeLeo, V.A. (2020) Sunscreen Safety: a Review of Recent Studies on Humans and the Environment. *Curr. Dermatol. Rep.*, **9** (1), 1–9.
4. Matta, M.K., Zusterzeel, R., Pilli, N.R., Patel, V., Volpe, D.A., Florian, J., Oh, L., Bashaw, E., Zineh, I., Sanabria, C., Kemp, S., Godfrey, A., Adah, S., Coelho, S., Wang, J., Furlong, L.-A., Ganley, C., Michele, T., and Strauss, D.G. (2019) Effect of Sunscreen Application Under Maximal Use Conditions on Plasma Concentration of Sunscreen Active Ingredients: A Randomized Clinical Trial. *JAMA*, **321** (21), 2082–2091.
5. Krause, M., Klit, A., Blomberg Jensen, M., Søeborg, T., Frederiksen, H., Schlumpf, M., Lichtensteiger, W., Skakkebaek, N.E., and Drzewiecki, K.T. (2012) Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV‐filters. *Int. J. Androl.*, **35** (3), 424–436.
6. Bárány, E., Lindberg, M., and Lodén, M. (2000) Unexpected skin barrier influence from nonionic emulsifiers. *Int. J. Pharm.*, **195** (1–2), 189–195.
7. San Miguel, A., Scrimgeour, J., Curtis, J.E., and Behrens, S.H. (2010) Smart colloidosomes with a dissolution trigger. *Soft Matter*, **6** (14), 3163–3166.

8. Wu, F., Deng, J., Hu, L., Zhang, Z., Jiang, H., Li, Y., Yi, Z., and Ngai, T. (2020) Investigation of the stability in Pickering emulsions preparation with commercial cosmetic ingredients. *Colloids Surf. Physicochem. Eng. Asp.*, **602**, 125082.
9. Zsikó, S., Csányi, E., Kovács, A., Budai-Szűcs, M., Gácsi, A., and Berkó, S. (2019) Methods to Evaluate Skin Penetration In Vitro. *Sci. Pharm.*, **87** (3).
10. Baena, J.R., and Lendl, B. (2004) Raman spectroscopy in chemical bioanalysis. *Curr. Opin. Chem. Biol.*, **8** (5), 534–539.
11. Adlhart, C., and Baschong, W. (2011) Surface distribution and depths profiling of particulate organic UV absorbers by Raman imaging and tape stripping. *Int. J. Cosmet. Sci.*, **33** (6), 527–534.
12. Tippavajhala, V.K., de Oliveira Mendes, T., and Martin, A.A. (2018) In Vivo Human Skin Penetration Study of Sunscreens by Confocal Raman Spectroscopy. *AAPS PharmSciTech*, **19** (2), 753–760.
13. Frelichowska, J., Bolzinger, M.-A., Pelletier, J., Valour, J.-P., and Chevalier, Y. (2009) Topical delivery of lipophilic drugs from o/w Pickering emulsions. *Int. J. Pharm.*, **371** (1–2), 56–63.
14. Otto, A., Wiechers, J.W., Kelly, C.L., Dederen, J.C., Hadgraft, J., and du Plessis, J. (2010) Effect of Emulsifiers and Their Liquid Crystalline Structures in Emulsions on Dermal and Transdermal Delivery of Hydroquinone, Salicylic Acid and Octadecenedioic Acid. *Skin Pharmacol. Physiol.*, **23** (5), 273–282.
15. Montenegro, L., Carbone, C., Paolino, D., Drago, R., Stancampiano, A.H., and Puglisi, G. (2008) In vitro skin permeation of sunscreen agents from O/W emulsions. *Int. J. Cosmet. Sci.*, **30** (1), 57–65.
16. Otto, A., and du Plessis, J. (2015) The Effects of Emulsifiers and Emulsion Formulation Types on Dermal and Transdermal Drug Delivery, in *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Drug Manipulation Strategies and Vehicle*

Effects (eds.Dragicevic, N., and Maibach, H.I.), Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 223–241.