Application Note

Strat-M® Membranes and Sunscreen

Substituting Strat-M® membrane for human skin in evaluating effect of encapsulation on diffusion of sunscreen formulations

Introduction

Transdermal delivery is commonly used for delivering active skin care compounds in the cosmetic and cosmeceutical industries. Development of effective sunscreens is a large focus of skin care research, given that proper use of sunscreens can prevent occurrence of multiple skin ailments, such as melanomas, skin cancers, wrinkles and sunburns.

For sunscreens to be useful in protecting against damaging ultraviolet (UV) rays, the active compound in the formulation should stay on the skin surface instead of penetrating the skin. Once a compound penetrates the skin, the compound can no longer exert its UV protection effect.

Avobenzone and octocrylene are two commonly used sunscreen actives. Avobenzone offers protection against the entire UVA spectrum, whereas octocrylene is mainly effective against UVB rays; therefore, these two actives are frequently combined in sunscreen formulations. Because there are always concerns about skin absorption of these compounds in their formulated forms, sunscreen developers typically perform *in vitro* transdermal diffusion studies using human skin (from cadavers or cosmetic surgery) as a model. In particular, octocrylene is known to penetrate into skin, wherein it acts as a photosensitizer, leading to DNA damage and malignant melanomas.

There are several barriers to using human skin for *in vitro* diffusion studies. First, human skin is plagued with high lot-to-lot variability. Second, human skin samples are expensive and not easy to obtain.

Finally, use of human tissue also poses additional experimental challenges, such as poor stability, sensitivity to storage conditions, biohazard concerns and disposal costs.

Recently, we introduced Strat-M® membrane, a synthetic membrane-based model with diffusion characteristics well-correlated to human skin (Figure 1). Because Strat-M® membrane is a synthetic product, its performance is highly consistent, it is easy to procure and shelf-stable, and it also provides data that are highly correlated to data obtained from human skin studies.

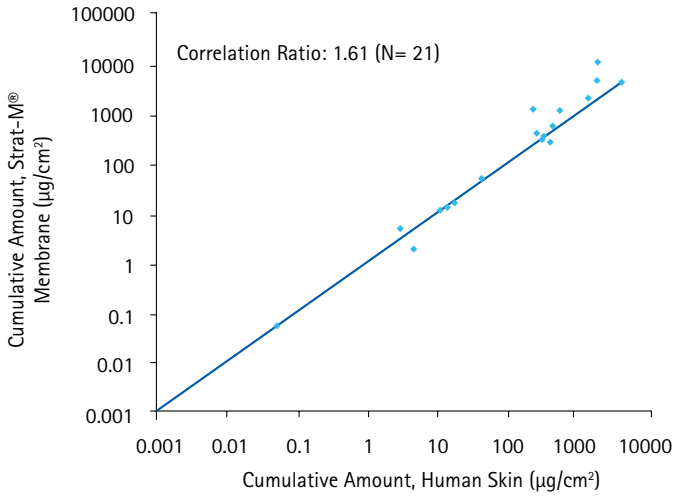


Figure 1.
21 compounds, covering a wide range of molecular weight and lipophilicity, were tested for diffusion through human skin and Strat-M® membrane. The average cumulative flow (amount diffused per unit surface area over 8 hours) of each compound through human skin and through Strat-M® membrane were nearly equivalent, with a mean ratio of 1.61.

In this report, we provide data showing the utility of Strat-M® membrane in comparing transdermal diffusion of two sunscreen actives, avobenzone and octocrylene, in unencapsulated (Formulation A) and encapsulated (Formulations B and C) formulations. By encapsulation of the actives, diffusion of both the actives were substantially reduced. These results show that Strat-M® membrane is a reliable and practical model for screening diffusion properties of sunscreen formulations. Also, the data show that Strat-M® membrane is able to distinguish between encapsulated and unencapsulated forms of active compounds in the formulation, thereby providing a useful model for formulation optimization studies.

Materials and Methods

Preparation of formulations

Oil-in-water emulsions were prepared using encapsulated and unencapsulated avobenzone and octocrylene. Amount of actives in the formulations are described in Table 1.

Formulation A consisted of unencapsulated avobenzone and octocrylene, whereas formulations B and C consisted of encapsulated avobenzone and octocrylene. Formulations A and B contained the same percentage of the active, the only difference being encapsulated vs unencapsulated version of the active. The rest of the formulation mainly consisted of water (65 – 75%) and isohexadecane (10%) along with emulsion stabilizers.

Chromatographic analysis of actives.

High performance liquid chromatography coupled with a diode array detector (HPLC-UV) was used for analysis and quantitation of avobenzone and octocrylene.

Table 2 provides detailed information about HPLC conditions used for both compounds.

Table 1.
Compositions of Formulations A, B and C for two sunscreen actives.

	Formulation A	Formulation B	Formulation C
	Unencapsulated	Encapsulated	Encapsulated
Avobenzone	1%	1.05%	2.1%
Octocrylene	3.5%	3.5%	6.99%

Table 2.
Separation parameters used for HPLC-UV analysis of sunscreen actives.

	Mobile Phase A	Mobile Phase B	Column Used	Injection Volume, µL	Detection Wavelength
Avobenzone	Acetate Buffer, pH 3.5 in water (10%)	Methanol (90%)	Alltech® Hyperprep C18, 4.6 X 250 mm, 8 µm	50	270 nm
Octocrylene	Acetate Buffer, pH 3.5 in water (10%)	Methanol (90%)	Alltech® Hyperprep C18, 4.6 X 250 mm, 8 µm	50	270 nm

Human skin samples

Cadaver human skin samples were purchased from a skin bank. Skin donors were Caucasian males (age 30 – 60), and skin was obtained from the posterior torso.

All experiments were conducted with three lots of human skin from three different donors. Individual experiments were conducted in triplicate and data were averaged for human skin from all the three skin lots as well as the membrane lot.

Upon receipt, human skin samples were partially thawed and cut into small pieces, which were individually wrapped in gauze and stored at –20 °C. At this point, skin was used within 6 months of storing at –20 °C. Prior to use, skin samples were fully thawed and hydrated (45 – 60 min) to room temperature in presence of receptor solution and further cut to fit the Franz cell setup.

Vertical Franz cell diffusion studies

A 6-cell semiautomatic Franz cell setup (Logan Instruments, Cat. No. FDC 6T) was used for all diffusion experiments. For initial setup, circulating water baths were turned on and set to 37 °C ± 0.5 °C. The water bath required approximately 30 minutes for equilibration. Strat-M® membrane (25 mm discs) was placed between the donor and receptor compartments of the Franz cells. Fully hydrated cadaver skin was placed between donor and receptor compartments. The available open area between donor and receptor compartments measured 0.635 cm^2. Compartments were clamped together, ensuring that the shiny side of the membrane was facing the donor compartment. The receptor compartment was filled with phosphate-buffered saline (PBS, pH 7.4, Sigma-Aldrich) containing 0.005% bovine serum albumin (BSA), which was permitted to overflow through the side tubes of the Franz cell to ensure that no air bubbles formed. After 20-30 min, when the receptor solution reached 37 °C, 500 µL of sunscreen formulation was added to the donor compartment. The point at which the formulation was added was recorded as Time T=0. An aliquot of each sample (500 µL) was removed from the receptor compartment at times T=0, 1, 2, 3, 4, 5, 6, 8, 10, 24 and 26 hours. Prior to removal of each 500 µL sample, a 1 mL sample from the Franz cell was discarded to ensure representative sampling. At each time point, the amount withdrawn was replaced with fresh PBS so that constant contact was maintained between the donor and receptor compartment.

Results and Discussion

As expected, very low amounts of avobenzone and octocrylene were observed in the receptor fluid when formulation A was tested using the vertical Franz cell arrangement. Avobenzone and octocrylene showed very similar diffusion properties when compared between Strat-M® membrane and human cadaver skin. In the case of avobenzone, the cumulative amount diffused was between 20 and 70 ng/cm^2, while in the case of octocrylene, the cumulative amount was between 5 and 20 µg/cm^2. For both these compounds, the diffusion profile between human skin and Strat-M® membrane was very similar, indicating the utility of Strat-M® membrane as a screening tool for transdermal diffusion studies in the development of cosmetic products.

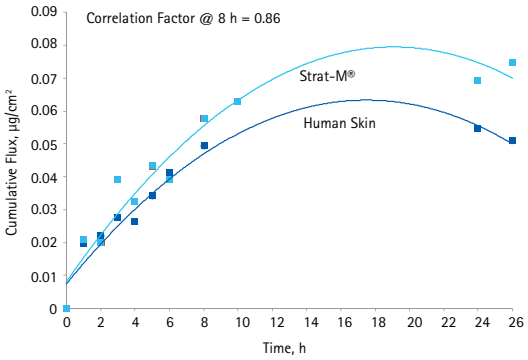


Figure 2.
Diffusion of unencapsulated avobenzone through human skin and Strat-M® membrane (Formulation A).

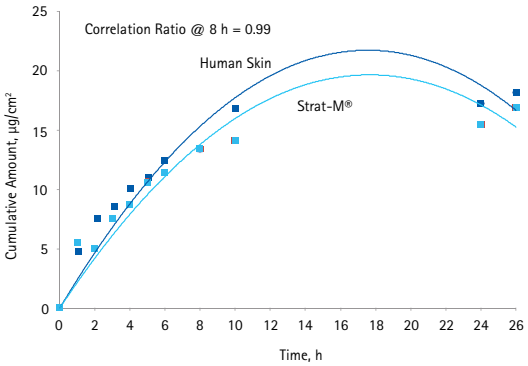


Figure 3.
Diffusion of octocrylene through human skin and Strat-M® membrane (Formulation A).

In the case of formulation A, the active ingredients were unencapsulated and in their native form. When the same actives were encapsulated and added to the sunscreen formulation, the amounts of both these actives diffusing through the Strat-M® membrane was significantly reduced. As can be clearly seen in Figure 4, diffusion of encapsulated avobenzone was approximately 10 times less than unencapsulated avobenzone. In fact, Formulation C, which contained twice as much avobenzone as in Formulation A, still showed a lower amount of diffused avobenzone compared to that observed with unencapsulated avobenzone (Formulation A).

We also measured the diffusion of encapsulated octocrylene (Formulations B and C) through Strat-M® membrane. As can be seen in Figure 5, encapsulation significantly reduced diffusion of octocrylene to levels that are almost undetectable by downstream HPLC-UV analysis. Diffusion of octocrylene decreased from a few µg/cm^2 to a few ng/cm^2. The difference in diffusion between formulations B and C was not statistically significant, as both these formulations exhibited octocrylene diffusion levels that were close to limit of detection of HPLC-UV downstream analysis.

Even though formulation C contained double the amount of active as formulation B (therefore potentially acting as a more effective sunscreen), encapsulation provided a significant barrier to octocrylene diffusion through Strat-M® membrane, indicating that the encapsulation would likely be sufficient to prevent octocrylene diffusion into human skin.

Figure 4.
Diffusion through Strat-M® membrane of avobenzone in unencapsulated (Formulation A) and encapsulated (Formulations B and C) formulations.

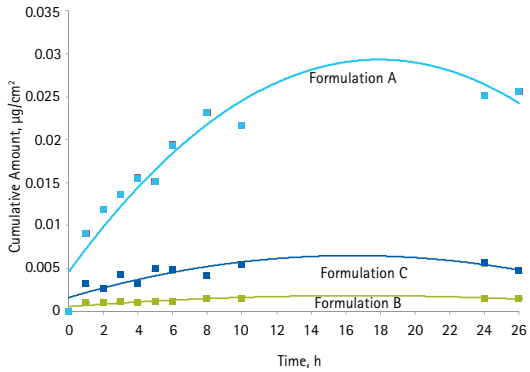
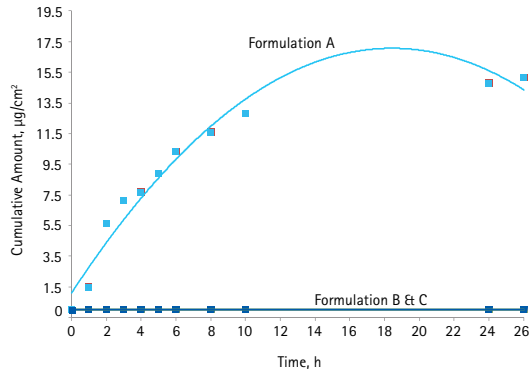


Figure 5.
Diffusion through Strat-M® membrane of octocrylene unencapsulated (Formulation A) and encapsulated (Formulations B and C) formulations.



Conclusions

Encapsulation of an active ingredient in a formulation can be crucial for modulating delivery of the active. Usually, encapsulation is used for extended delivery of actives or reducing toxic side effects. In the case of sunscreen formulations, where the desired sunscreen activity depends on the active remaining on the surface of skin, encapsulation can effectively reduce the diffusion of sunscreen actives through skin, thereby increasing their activity as well as reducing any potential toxic effects of diffusion.

For *in vitro* diffusion studies using transdermal formulations, human skin has been traditionally used as a model and gold standard, but human skin suffers from high variability, high cost and limited availability. Strat-M® membrane provides a synthetic membrane alternative to human skin for conducting *in vitro* diffusion studies, providing data that are consistent and correlated to human skin. Furthermore, the membrane is easily available and can be stored at room temperature.

Strat-M® membrane showed very close correlation to human skin when comparing diffusion of unencapsulated active. As a previous study using pig skin has shown, encapsulation can decrease the transdermal diffusion of a sunscreen active by over three-fold[1]. Consistent with these published observations, we determined that encapsulation of both avobenzone and octocrylene greatly decreased diffusion through Strat-M® membrane.

Comparing the diffusion of avobenzone and octocrylene in formulations A, B and C through Strat-M® membrane, we have shown that encapsulation effectively reduces diffusion of sunscreen actives through the membrane. For avobenzone, the reduction in diffusion by encapsulation was ten-fold, whereas for octocrylene, diffusion was reduced by almost 1000-fold. Since diffusion of sunscreen actives can potentially cause adverse reactions, encapsulation appears to be a strategy to significantly reduce diffusion-related side effects of sunscreens.

We conclude that, for screening formulations for effectiveness of encapsulation on reducing transdermal diffusion of skin care actives, Strat-M® membrane is a reliable, inexpensive, easy-to-handle alternative to human or animal skin.

References

1. Jimenez, MM et al. Influence of encapsulation on the *in vitro* percutaneous absorption of octyl methoxycinnamate. International Journal of Pharmaceutics. 2004; 272: 45–55.

Ordering Information

Description	Cat. No.
Strat-M® Membrane, 25 mm, 60/pk	SKBM02560
Strat-M® Membrane, 47 mm, 60/pk	SKBM04760

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