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COMMUNICATION

Release of Benzimidazole and Benzylidene Camphor from Topical Sunscreen Formulations

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ABSTRACT

Absorption of two ultraviolet (UV) filters was evaluated through a lipophilic synthetic membrane (Folioxane®) and excised hairless rat skin using a flow-through diffusion cell. Folioxane membrane is an artificial skin used in the treatment of third-degree burns. Diffusion tests were performed with aqueous solutions and galenic formulations (one water-in-oil [W/O] emulsion and two oily gels). Analyses were achieved with high-performance liquid chromatography (HPLC) with UV detection at 295 nm. Diffusion kinetics of 17 β estradiol, a reference compound, through rat skin, human skin, and Folioxane membrane were performed to validate the in vitro model. Phenylbenzimidazole and methylbenzylidene camphor in aqueous solutions were diffused at a regular rate through the Folioxane film. The release of phenylbenzimidazole was very slow, whereas the release of benzylidene camphor was more pronounced: a decrease of the quantity was observed in the donor compartment (30 % at 6 hr and 93% after 72 hr). A significant flow of benzylidene camphor was also measured through excised skin of rat in the first 3 hr. The skin absorption was 38% over 72 hr. The W/O emulsion had low penetration of UV filter: 20% of the initial amount for Folioxane membrane and 0.4% for rat skin. In contrast, the penetration of two oily gels was identical: 28% on Folioxane membrane and 0.6% on rat skin. This study demonstrates the transcutaneous diffusion of two

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important classes of sunscreens through a lipophilic Folioxane membrane and through excised hairless rat skin. From the results, Folioxane membrane appears to be an alternative model for studying diffusion of topical molecules and as a tool for guiding formulation choices.

Key Words: *Benzimidazole; Benzylidene camphor; Cosmetic formulations; Percutaneous absorption; Sunscreen filters.*

INTRODUCTION

Sunscreen formulations are conceived to protect the skin and are designed to absorb ultraviolet (UV) radiation selectively. According to the hydrolipidic balance, sunscreen filters are added to the aqueous or the oily phase of emulsions or to both phases. Each filter is characterized by its maximum absorption wavelength in the UV spectrum. The UV-B absorbers will protect the skin against wavelengths in the UV-B range (290–320nm), which are partially responsible for erythema and inflammation; the UV-A absorbers protect against the wavelengths in the UV-A range (315–380 nm), which promote skin aging, chronic photopathy, and denaturation of cellular protein structures.

Sunscreen preparations are usually applied to large skin areas. They must remain on the skin to be efficient. Therefore, *effectiveness* implies that sunscreen filters adhere to skin like a protective film. They should have a high affinity for the stratum corneum or should diffuse into intercorneocyte ceramide spaces or through the phospholipidic structures of corneocyte membranes. All these modalities should prevent the rapid withdrawal of the sunscreen agents during bathing or sweating and should reduce diffusion toward the deepest cellular layers of the corium or epidermis.

Sunscreen filters are now incorporated in most cosmetics. The effectiveness of sunscreen preparations against UV radiation has been extensively investigated, but studies relative to the penetration of these chemicals through skin are rather scarce. In fact, these molecules are not supposed to penetrate skin, but now the matter is worth interest. Benzophenones are the second most common UV filter chemical used around the world (1). They contain either ternary or quaternary amines, and their substantivity on hairless rat skin has been investigated in vitro and ex vivo (2,3). Benzophenones with a quaternary ammonium structure revealed the highest substantivity for keratin, whereas those with ternary amines groups showed low substantivity, but an appreciable skin penetration rate. Moreover, sunscreen filters have structures comparable to various drugs with already known percutaneous absorption (4–6). Percutaneous penetration of other sunscreens should then be hypothesized, although very few studies have been performed.

The aim of this study was to compare the diffusion of two important sunscreen filters, phenylbenzimidazole and methylbenzylidene camphor, and to find an alternative method for the penetration studies using human skin. Penetration tests were performed through Folioxane®, a multilayer silicone membrane employed as artificial skin for ''third-degree burns,'' and through excised hairless rat skin. The study relates the release and diffusion of the UV absorbers from an aqueous solution and from galenic formulations: water-in-oil (W/O) emulsion and oily gels.

MATERIALS AND METHOD

A hydrosoluble filter, 2-phenylbenzimidazole-5-sulfonic acid (Eusolex® 232), and a liposoluble one, 4-methylbenzylidene camphor (Eusolex® 6300), were kindly supplied by Merck (Darmstadt, Germany). Each filter was dissolved at the concentration of 0.02% in a solution of PEG 300 (Prolabo, Marseille, France) and sodium chloride 0.9% (1:1 v/v). Polyethylene glycol allows better interaction between the sunscreen and the lipophilic membranes. E6300 was also incorporated at a concentration of 6% into a W/O emulsion and into two oily gels, aerosil 200/parrafine and labrafil/parrafine.

In vitro diffusion cells were made of Pyrex glass and were composed of a donor and a receiver compartment with a running 37°C thermostated water jacket around the receiver compartment (Fig. 1). The receiver compartment (10 ml) was separated from the donor compartment by a synthetic Folioxane membrane or by excised rat skin (area 5.3 cm^2). Five ml (0.2 mg/L) of each UV filter solution or 2 mg/cm^2 of each galenic formulation were deposited on the upper face of the barrier. The dermis side of membrane or skin was in contact with the receptor compartment fluid containing 9 g/L NaCl, 15 g/L bovine serum albumin, and 5 g/L of Volpo 20 (polyoxyethylene oleic ether, a surfactant used for increasing the lipophilic affinity) (7). A peristaltic pump (Ismatec®, MSA Reglo,

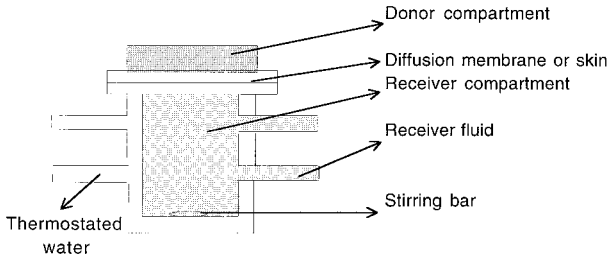

Figure 1. Diffusion cell.

Bioblode, Illkirch, France) was adjusted to a flow rate of 2 ml/hr and allowed the circulation of the receptor liquid and the thermostating water. Stirring bars were used to mix the content of the receptor compartment. Folioxane D4 membrane film (0.25 mm deep) was kindly supplied by Axion (Aubagne, France).

Ex vivo experiments were achieved on hairless male rat skin, OFA-hr SSC (IFFA Credo), 5 to 10 weeks old.

Table 1

*Linearity and Reproducibility (*n = *10)*

Concentration (μg/ml)	Benzimidazole Area × 10^{-3}	Benzylidene Camphor Area × 10^{-3}
0.2	11.6 + 0.01	1 + 0.02
0.4	12.4 + 0.019	2 + 0.018
1	24.6 + 0.011	4 + 0.022
2	29.8 + 0.021	8.7 + 0.036
4	47.0 + 0.027	18.4 + 0.028
Linear coefficient of regression	$r = 0.995$	$r = 0.999$
Equation	$Y = 9.37X + 10.32$	$Y = 4.58X - 0.138$
rsd	14.6	7

Table 2

17 β Estradiol Diffusion

	17 β Estradiol Cumulated Quantities (μg)		
Time (hr)	Folioxane	Rat Skin	Human Skin
1	0.216	1.75	0.961
4	11.03	14.8	12.05
6	6.75	18	15
24	47.53	187.8	69.3

Patterns of abdominal skin were taken off and used immediately or after storage at −80°C. Preliminary studies (data not shown) did not reveal any difference between freshly scraped skin and defrosted skin in regard to the penetration profile of the sunscreens being tested.

Studies of the 72-hr kinetics were performed on the Folioxane membrane and on rat skin. Samples from donor and receptor compartments were collected at T0, T1 hr, T3 hr, T6 hr, T9 hr, T24 hr, T48 hr, and T72 hr. The linearity of the method was determined with the two filters in ethanol solutions from 0.2 μg/ml to 4 μg/ml. The reproducibility was measured by 10 injections of 10 μl (Table 1).

Samples of the sunscreen filters were collected in the donor and the receiver compartments. High-performance liquid chromatography (HPLC) was performed using Lichrosorb RP 18 as the stationary phase (25 cm × 4.1 cm, 10 μm). The eluent was acetonitrile-water (85:15) at a flow rate of 1 ml/min. Detection was achieved at 295 nm with a photodiode-array detector.

RESULTS AND DISCUSSION

Because of regulatory limitations, availability, and sample fluctuations related to the use of excised human

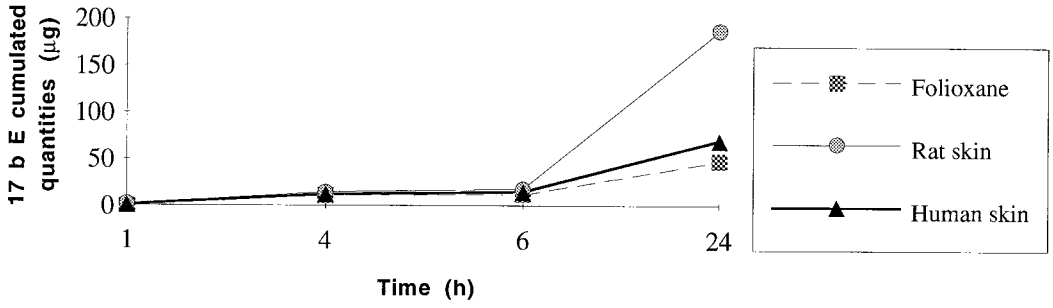

Figure 2. Diffusion of 17 β estradiol through Folioxane, rat skin, and human skin ($n = 6$; rsd % = 5).

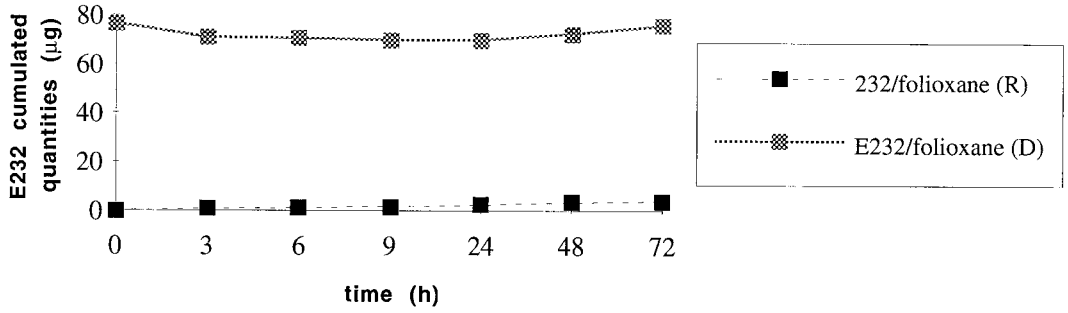

Figure 3. Diffusion of phenylbenzimidazole (E232) in solution through Folioxane (n = 6; rsd % = 14.6).

skin, it appeared judicious to compare the diffusion of UV sunscreens through a synthetic membrane, Folioxane used as artificial skin, and through hairless rat skin. Diffusion kinetics of 17 β estradiol (a reference compound) through rat skin, human skin, and Folioxane membrane were performed to validate the in vitro model (Fig. 2; Table 2). The three kinetics are superimposed for 6 hr after application. At the end of the assay (T24 hr), the kinetics through Folioxane membrane and through human skin were still similar, while the kinetics through rat skin appeared more accentuated. According to these results, Folioxane membrane seems to be a good alternative model for modeling studies of the diffusion of topical molecules.

Release and Diffusion of Ultraviolet Absorbers from Polyethylene Glycol/ Sodium Chloride Solutions

Diffusion Through Synthetic Folioxane Membrane

The kinetic data of phenylbenzimidazole in aqueous solution shows that the UV filter quantity was not significantly modified in the donor compartment, whereas the cumulative amount permeated through the membrane slowly and regularly increased over 72 hr in the receptor compartment (Fig. 3; Table 3).

The kinetic data of methylbenzylidene camphor in solution decreased steadily in the donor compartment during the first 24 hr and over 72 hr: The decrease was 30% at 6 hr and 93% after 72 hr. In parallel, the permeated cumulative amount increased regularly in the receptor compartment; 0.6% of the sunscreen filter had diffused through the membrane after 72 hr (Figs. 3 and 4).

Diffusion Through Hairless Rat Skin

Following precedent results through Folioxane membrane, the diffusion of methylbenzylidene camphor through excised hairless rat skin seemed to be most interesting. Effectively, the diffusion was greatly significant from the third hour and increased up to the end of the kinetic measurement (T24 hr); the decrease in the donor compartment was 38%. This decrease correlated with the increase of the permeated quantity in the receptor compartment (+26%) (Fig. 4; Table 4).

Release and Diffusion of Ultraviolet Absorbers from Galenic Formulations

The assay from galenic formulations was conducted under the same conditions as above, but sunscreen solutions were replaced by an emulsion and by gels containing 6% methyl-4-benzylidene camphor. The same amount of methylbenzylidene camphor was applied on the Folioxane membrane or on rat skin surface. Determinations were made in the receptor compartment T4 hr, T8 hr, T24 hr, and T54 hr after application (Figs. 5 and 6; Tables 5 and 6).

The results showed the lowest permeated quantities in the receptor fluid following application of O/W emulsion; the increase was 20% of the initial amount for Fo-

Table 3

E232 Diffusion Through Folioxane

	E232 Cumulated Quantities (µg)	
Time (hr)	E232/Folioxane (R)	E232/Folioxane (D)
0	0	76.7
3	0.91	70.8
6	1.35	70.5
9	1.71	69.7
24	2.38	69.7
48	3.42	72.3
72	3.99	75.9

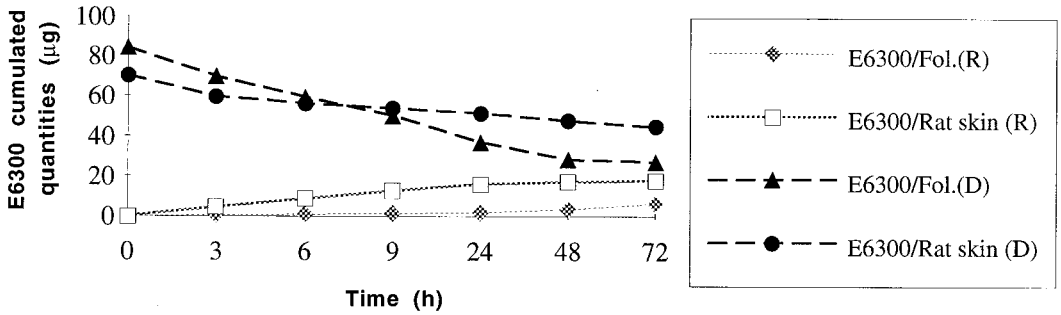

Figure 4. Diffusion of methylbenzylidene camphor (E6300) in solution through folioxane and rat skin (n = 6; rsd % = 7).

Table 4

E6300 Diffusion in Solution Through Folioxane and Rat Skin

Time (hr)	E6300/Folioxane (R)	E6300/Rat Skin (R)	E6300/Folioxane (D)	E6300/Rat Skin (D)
0	0	0	83.9	69.8
3	0.5	4.4	69.7	59.6
6	0.88	8.6	59.2	56.1
9	1.19	12.5	50.1	53.5
24	1.86	15.85	36.9	51.2
48	3.41	16.78	28.3	47.3
72	6.32	18.02	26.9	44.4

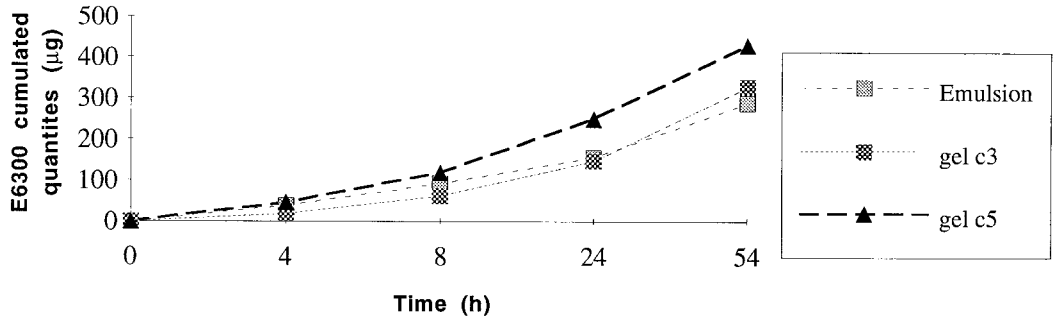

Figure 5. Diffusion of methylbenzylidene camphor in galenic formulations through Folioxane (n = 6; rsd % = 7.6).

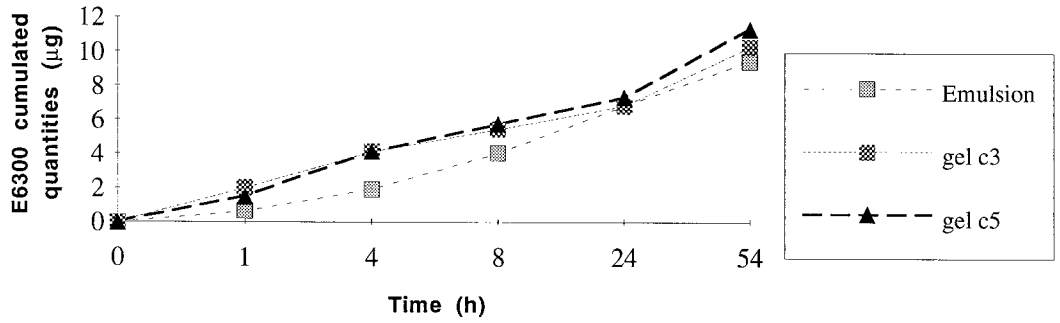

Figure 6. Diffusion of methylbenzylidene camphor in galenic formulations through rat skin (n = 6; rsd % = 7.2).

Table 5

E6300 Diffusion in Galenic Formulations Through Folioxane

	E6300 Cumulated Quantities (μg)		
Time (hr)	Emulsion	Gel c3	Gel c5
0	0	0	0
4	39.2	18.98	44.5
8	92.5	61.37	117.66
24	156.35	146.5	249.11
54	286.7	326.71	428.24

Table 6

E6300 Diffusion in Galenic Formulations Through Rat Skin

	E6300 Cumulated Quantities (μg)		
Time (hr)	Emulsion	Gel c3	Gel c5
0	0	0	0
1	0.636	2	1.5
4	1.9	4.1	4.1
8	4.03	5.4	5.7
24	6.78	6.8	7.3
54	9.44	10.28	11.3

lioxane membrane and +0.4% for rat skin. In contrast, penetration profiles for the two gels were identical: 28% on Folioxane membrane and 0.6% on rat skin.

CONCLUSION

To be effective, a sunscreen UV filter should interpose between the skin and solar radiation for a long time. Any removal of the sunscreen filter exposes the skin to erythema and biochemical impairments of cellular constituents by UV radiation. Thus, withdrawal of the sunscreen filters from the skin should be explained partly by some cutaneous penetration of the molecules. In fact, from the standpoint of chemical structure, many substances used as sunscreen filters are analogous to some topical drug molecules. Molecular weight, steric environment, and hydrolipidic balance of these sunscreen filters are of the same magnitude as skin-diffusible molecules already investigated (8–11).

This paper showed the diffusion of two major sunscreen filters, a hydrophilic (phenylbenzimidazole) and a lipophilic one (methyl-4-benzylidene camphor) through Folioxane, a synthetic lipophilic membrane considered a model of skin. Our results are in accordance with studies carried out on other sunscreen molecules on rat skin. The diffusion pattern was validated. Folioxane membrane may thus be used as a valuable model for studying skin permeation of drugs. A transcutaneous diffusion of the sunscreen filters studied here can also be stated.

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