Department of Drug Sciences, University of Catania, Italy

Evaluation of sunscreen safety by *in vitro* skin permeation studies: effects of vehicle composition

L. Montenegro, G. Puglisi

Received June 6, 2012, accepted June 16, 2012

Dr. Lucia Montenegro, Department of Drug Sciences, University of Catania, V.le A.Doria 6, 95125 Catania, Italy
lmontene@unict.it

Pharmazie 68: 34–40 (2013) *doi: 10.1691/ph.2013.2117*

For sunscreens to be safe and effective, the lowest possible UV-filter percutaneous absorption should be achieved. In this paper, we evaluated *in vitro* release and permeation through human skin of two UV-filters, octyl methoxycinnammate (OMC) and butyl methoxydibenzoyl methane (BMBM) from six commercial O/W emulsions and we estimated their margin of safety (MoS). OMC and BMBM *in vitro* release and skin permeation were investigated in Franz-type diffusion cells and permeation data were used to calculate MoS. OMC *in vitro* skin permeation depended on both its concentration and vehicle composition while BMBM skin permeation depended on its release from the vehicle. MoS values were well beyond the lowest limit accepted for safe products. Although sunscreen skin permeation may depend on many factors, the commercial products investigated are safe under normal “in use” conditions.

1. Introduction

Sun protecting agents are known to prevent many harmful effects of solar radiations such as erythema, DNA pyrimidine dimer formation, sunburn cell formation, basal and squamous cell cancers, photoaging and immune suppression (Peguet-Navarro et al. 2004; Afaq et al. 2005). For decades, the UVB portion of sunlight (290–320 nm) has been regarded as the main cause for such acute and chronic skin damages (Heck et al. 2004; Matsumura and Ananthaswamy 2004). However, recent studies have shown that UVA (320–400 nm) can cause long-term damages such as photoaging, photodermatoses, immunological suppression and carcinogenesis (Afaq and Mukhtar 2001; Wendel 2003).

To reduce the harmful effects of sunlight, sunscreen use is widely recommended. A successful UV-filter should posses several requisites and it should protect the skin without penetrating it so as to avoid systemic effects (Nohynek and Schaefer 2001).

Sunscreen products available on the market show different values of sun protection factor (SPF) obtained using different cosmetic vehicles containing variable concentrations of UV-filters (Ferguson et al. 1996). Vehicle effects on active ingredient skin permeation have been widely investigated to optimize active ingredient delivery and formulation characteristics such as stability, efficacy, safety and aesthetic acceptability (Forster and von Rybinski 1998). However, to date these effects are not fully understood, particularly for more complex formulations such as emulsions. These vehicles are widely used in the cosmetic and pharmaceutical field because of their ability to solubilize lipophilic and hydrophilic active molecules and their good acceptability by the consumers. However, their complexity makes it difficult to predict the active ingredient/vehicle/skin interactions that may occur. Many studies have pointed out that emulsion type and structure, type of emulsifiers, presence of penetration modifiers and/or emollients are factors that may strongly influence active ingredient dermal and transdermal delivery (Otto et al. 2009; Ghafourian et al. 2010).

The development of a cosmetic formulation requires a precise assessment of its safety and efficacy and current legislation imposes increasing measures of safety. For solar formulations to be safe and effective, the lowest possible sunscreen percutaneous absorption should be achieved (Watkinson et al. 1992; Nohynek and Schaefer 2001; Nash 2006). Since these products are often applied on large skin areas, even small differences in skin permeation could cause significantly different amounts of UV-filters to enter the body, causing concerns for human health. To overcome real or perceived human health concerns due to the use of sunscreen products, their margin of safety (MoS) can be easily estimated by assessing UV-filters skin permeation through human skin from currently marketed sunscreens.

Therefore, in this paper we evaluated *in vitro* release and skin permeation through human skin of two commonly used UV-filters, octyl methoxycinnamate (OMC) and butyl methoxydibenzoylmethane (BMBM) from six commercial O/W emulsions designed for face or body application with different composition and SPF. OMC and BMBM permeation data were used to estimate the safety of these solar formulations by calculating their MoS with respect to UV-filter ability to enter the body.

2. Investigations, results and discussion

Although the use of finite dosing more closely resembles “in use” situations and it is recommended by OECD guidelines 428 (OECD 2006), we evaluated OMC and BMBM *in vitro* release and skin permeation using the infinite dose technique, i.e. applying a large amount of formulation (20 mg/cm^2) on the skin surface. The choice of using a larger amount of product than that recommended was based on the following considerations: a) preliminary experiments performed using a finite dosing (2 mg/cm^2) did not allow us to detect any skin permeation of both sunscreens because of the poor sensitivity of a common HPLC

Table 1: Release rate (flux ± S.D.), lag time, cumulative amount released after 24 h (Q_{24} ± S.D.), and percentage of dose released after 24 h (% D) of OMC and BMBM from emulsion 1-6

Emulsion		Flux ± S.D. (μg/cm²/h)	Lag Time (h)	Q_{24} ± S.D. (μg/cm²)	% D
1	OMC	4.33 ± 0.20	3.51	59.86 ± 3.07	5.44
	BMBM	—	—	1.26 ± 0.07	3.15
2	OMC	3.23 ± 0.15	2.39	46.95 ± 2.14	3.61
	BMBM	—	—	1.17 ± 0.06	0.43
3	OMC	22.65 ± 1.26	4.46	299.02 ± 16.56	14.95
	BMBM	—	—	1.72 ± 0.09	0.86
4	OMC	5.00 ± 0.28	3.91	67.78 ± 3.90	3.39
	BMBM	—	—	1.40 ± 0.07	0.13
5	OMC	14.16 ± 0.67	4.47	212.73 ± 11.55	10.63
	BMBM	—	—	1.60 ± 0.08	0.80
6	OMC	3.59 ± 0.19	0.43	58.48 ± 3.19	4.87
	BMBM	—	—	1.42 ± 0.10	0.89

analytical method that was not able to detect the low amount of UV-filter permeated; b) active compound depletion from the donor compartment was avoided, thus ensuring a constant driving force for the permeation process during the experiment and allowing the detection of both sunscreen skin permeation; c) *in vitro* skin permeation studies on octyl salycilate from sunscreen formulations showed that no significant difference of the percentage of the applied dose of UV-filter permeated from the same emulsions was observed using a finite or an infinite dose technique (Walters et al. 1997).

As reported in the literature (Wester and Maibach 1983), the ability of an active compound to exert its action, after topical application, depends basically on two consecutive steps. First, the active compound must diffuse from the vehicle to the skin surface and second, it must be able to penetrate the skin barrier. Both these processes can influence the percutaneous absorption rate, the slowest step being rate-limiting. Therefore, to evaluate which of these two processes was the rate-limiting step in OMC and BMBM skin permeation, we determined UV-filter release parameters from emulsions 1-6 and release data are reported in Table 1. BMBM release rate was not determined since this UV-filter could not be detected in the receiving compartment until 6 h from the beginning of the experiment and no release rate could be calculated under these conditions. For BMBM, only the cumulative amount released after 24 h (Q_{24}) and the percentage of dose released after 24 h (%D) were determined. While BMBM Q_{24} values were similar from all the products tested, the percentage of dose released decreased in the order $1 > 6 \cong 3 \cong 5 > 2 > 4$ and a 24-fold decrease was observed comparing emulsion 1 with emulsion 4. As no relationship between the percentage of BMBM contained in the product and that released from the vehicle was observed, it could be hypothesized that vehicle composition affected BMBM release. However, a trend different from that of OMC was observed, thus indicating that vehicle effects may depend on sunscreens' physico-chemical properties. As OMC and BMBM show different lipophilicity (BMBM Log P = 4.8; OMC Log P 5.6; calculated using Advanced Chemistry Development software Solaris V 4.67), a different release from the vehicle could be expected. However, the different percentages of OMC and BMBM used to prepare emulsions 1-6 could not account for the lower release of BMBM compared to OMC since sink conditions were maintained during all the experiments for both UV-filters.

Figure 1 shows that a pseudo-first order release rate for OMC from emulsions 1, 2, 4 and 6 was obtained, while formulations 3 and 5 showed an initial slow release of this sunscreens followed by a faster release about 4 h after applying the formulation on the membrane. Therefore, OMC release from products 3 and 5 showed the highest lag time values (4.46 h and 4.47 h, respectively). OMC release rate from emulsions 1-6 decreased in the order: $3 > 5 > 4 > 1 > 6 > 2$ (Table 1). Although emulsions 3, 4 and 5 contained the same proportion of OMC (9.9% w/w), the amount of UV-filter released from these formulations was significantly different, suggesting that vehicle composition affected OMC release.

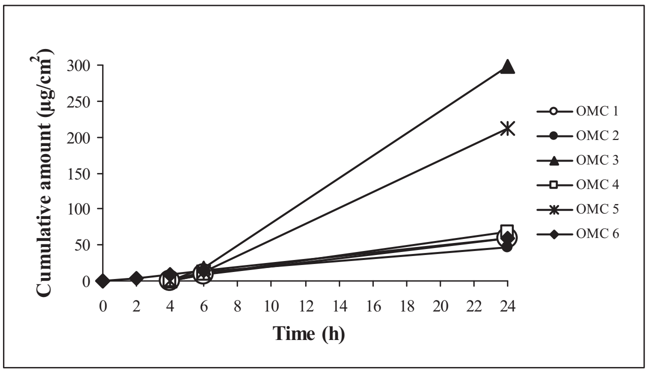

Fig. 1: Cumulative amount of OMC released from products 1-6 vs time. Standard error bars were omitted for clarity

Calculating OMC permeability coefficient (K_p), apparent diffusion coefficient (D_m) and membrane/vehicle partition coefficient (K_m) from emulsions 1-6, the highest diffusion coefficient was observed for emulsion 6 while the best OMC partitioning was observed for emulsions 3 and 5 (see Table 2). In an attempt to explain these results, OMC and BMBM release from different oil mixtures with the same HLB values of emulsions 1-6 (Table 3) was evaluated. Although the percentages of ingredients used to prepare emulsions 1-6 were not disclosed, some information

Table 2: OMC permeability coefficient (K_p), apparent diffusion coefficient (D_m) and membrane/vehicle partition coefficient (K_m) through cellulose membrane from emulsions 1-6

Emulsion	$K_p \times 10^{-4}$ (cm/h)	$D_m \times 10^{-13}$ (cm²/h)	$K_m \times 10^2$
1	0.78	2.97	6.56
2	0.49	4.35	2.81
3	2.26	2.33	24.3
4	0.49	2.66	4.58
5	1.46	2.33	15.62
6	0.59	24.22	0.62

Table 3: Composition (% w/w) of oil vehicles A-D and required HLB values (HLB_r) of oil ingredients

Oil	HLB_r	Vehicle			
		A	B	C	D
Mineral oil	4,5	86	90	92	95
Olive oil	7	14	—	—	—
Octyl palmitate	8	—	10	—	—
C12-15 alkyl benzoate	9	—	—	8	—
IPM	11	—	—	—	5

All vehicles contained 0.2% w/w BMBM and 5.5% w/w OMC. Oil percentages were calculated so as to obtain vehicles with the same HLB_r value (4.85)

can be deduced taking into account their normal percentages of use. All the emulsions under investigation contained the same emulsifiers, sorbitan stearate and PEG-30 dipolyhidroxystearate whose HLB values, as reported in their technical data sheet, are 4.7 and 5.5, respectively. According to their normal percentage of use to obtain stable emulsions, the resulting HLB value of emulsions 1-6 could be estimated to be about 4.85. Therefore, this HLB value was used to prepare mixtures of oils with different physico-chemical properties so as to assess the influence of lipid composition on UV-filters release. As according to the European laws cosmetic products have to be labelled reporting their ingredients in a decreasing order of concentration, from the label of emulsions 1-6 it could be deduced that the main lipid, contained in all the emulsions under investigation, was mineral oil (paraffinum liquidum). Therefore, the lipid mixtures A-D were prepared using this oil as basic component. The cosmetic lipids to be added to mineral oil were chosen on the basis of a significant increase of their polarity, even if they were not contained in the emulsions under investigation. As shown in Table 4, the amount of OMC and BMBM released after 24 h was not significantly different comparing oil vehicles A-D and was similar to that released from emulsion 1, which contained the same amount of UV-filters of vehicles A-D. In Table 5, only OMC release parameters are reported because BMBM release could not be detected until 6 h from the beginning of the experiment and, as mentioned above, no release parameters could be calculated under these conditions. As shown in Table 4, OMC D_m values from oil vehicles decreased in the order C > A > D > B while K_m values showed the opposite trend. These results suggest that although the different polarity of the oils used did not affect OMC and BMBM release rate from these vehicles, the release mechanisms were influenced by the different lipophilicity of the oil ingredients. However, no relationship between oil polarity and OMC ability of partitioning or diffusing out of the vehicle was observed. Therefore, OMC release data obtained from vehicles A-D pointed out that the oil lipophilicity alone could not account for the results obtained from emulsions 1-6 and could not be regarded as a predictive parameter to evaluate OMC release from these vehicles, suggesting that other factors, such as UV-filter interactions with emulsions ingredients and vehicle viscosity could affect UV-filter release. For instance, the presence in emulsions 1-6 of hydrophilic ingredients with different polarity such as butylene glycol (Log P −0.29, emulsions 1,2,6), propylene glycol (Log P −0.92, emulsions 1,2,4,6) and dipropylene glycol (Log P −1.49, emulsions 3,5) could also affect OMC release since the highest OMC release rates were obtained from emulsions 3 and 5 that contained, among the other ingredients, the least polar glycol derivative.

Furthermore, it is interesting to note that calculating OMC release parameters from emulsions 1-6, the highest OMC D_m

Table 4: Cumulative amount released after 24 h (Q_{24} ± S.D.), release rate (flux ± S.D.), lag time, permeability coefficient (K_p), apparent diffusion coefficient (D_m) and membrane/vehicle partition coefficient (K_m) of OMC and BMBM from vehicles A-D

Vehicle	UV-filter	Q_{24} ± S.D. (μg/cm²)	Flux ± S.D. (μg/cm²/h)	Lag time (h)	$K_p \times 10^{-4}$ (cm/h)	$D_m \times 10^{-13}$ (cm²/h)	$K_m \times 10^2$
A	OMC	55.47 ± 4.32	3.85 ± 0.45	2.24	0.70	4.65	3.76
	BMBM	1.31 ± 0.09	—	—	—	—	—
B	OMC	58.92 ± 3.79	4.01 ± 0.56	3.27	0.73	3.19	5.72
	BMBM	1.44 ± 0.15	—	—	—	—	—
C	OMC	54.77 ± 5.03	3.39 ± 0.58	1.68	0.62	6.20	2.50
	BMBM	1.54 ± 0.12	—	—	—	—	—
D	OMC	59.06 ± 2.58	3.87 ± 0.51	2.69	0.70	3.87	4.52
	BMBM	1.12 ± 0.20	—	—	—	—	—

Table 5: Permeation rate (flux ± S.D.), lag time, cumulative amount permeated after 24 h (Q_{24} ± S.D.), percentage of dose permeated after 24 h (% D), systemic exposure dose (SED), and margin of safety (MoS) of OMC and BMBM from products 1-6

Product		Flux ± S.D. (μg/cm²/h)	Lag Time (h)	Q_{24} ± S.D. (μg/cm²)	%D	SED (mg/kg/day)	MoS
1	OMC	1.43 ± 0.07	4.70	18.60 ± 0.91	1.69	3.98	113
	BMBM	—	—	1.26 ± 0.06	3.15	0.27	1666
2	OMC	1.95 ± 0.09	2.27	29.99 ± 1.49	2.31	6.43	70
	BMBM	—	—	1.29 ± 0.07	0.8	0.27	1666
3	OMC	5.92 ± 0.28	4.21	79.24 ± 4.05	3.96	16.80	27
	BMBM	—	—	1.32 ± 0.07	0.66	2.83	159
4	OMC	5.32 ± 0.27	2.46	70.41 ± 3.28	3.52	14.93	30
	BMBM	—	—	1.27 ± 0.09	0.42	0.27	1666
5	OMC	6.64 ± 0.33	3.43	92.63 ± 4.97	4.63	0.48	937
	BMBM	—	—	1.33 ± 0.08	0.65	0.007	64285
6	OMC	2.45 ± 0.13	2.60	36.09 ± 1.83	3.00	0.19	2368
	BMBM	—	—	1.31 ± 0.06	0.82	0.007	64285

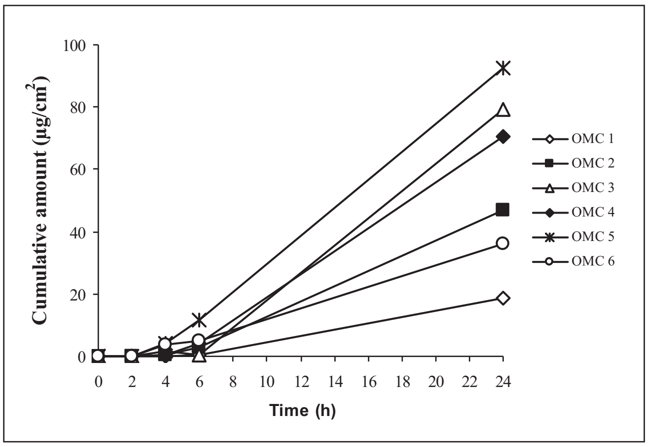

Fig. 2: Cumulative amount of OMC permeated through excised human skin from products 1-6 vs time. Standard error bars were omitted for clarity

Table 6: OMC permeability coefficient (K_p), apparent diffusion coefficient (D_m) and membrane/vehicle partition coefficient (K_m) through excised human skin from emulsions 1-6

Emulsion	$K_p \times 10^{-4}$ (cm/h)	$D_m \times 10^{-6}$ (cm^2/h)	K_m
1	0.26	0.10	0.44
2	0.30	0.21	0.24
3	0.59	0.11	0.90
4	0.54	0.19	0.48
5	0.67	0.14	0.80
6	0.40	0.18	0.37

value was observed from emulsion 6 that had the lowest viscosity. However, although OMC D_m value from emulsion 6 was about one order of magnitude higher that that obtained from the other emulsions, its release rate from emulsion 6 was similar to that obtained from emulsions 1, 2 and 4, indicating a lack of relationship between OMC release and emulsion viscosity. In a previous work (Bonina and Montenegro 1994), studying *in vitro* release and skin permeation of heparin sodium salt from different gel vehicles, we observed a similar lack of relationship between active ingredient release and viscosity of the vehicle, that was attributed to different active compound interactions with the gel network.

As shown in Fig. 2, a pseudo-first order skin permeation rate for OMC was obtained from all the products under investigation. OMC *in vitro* skin permeation from formulations 1-6 (expressed as flux through the skin and as cumulative amount permeated after 24 h) decreased in the order $5 > 3 > 4 > 6 > 2 > 1$ and a 4.5-fold increase was observed comparing emulsion 1 with emulsion 5 (Student's t-test; $p < 0.05$). On the contrary, the cumulative amount of BMBM permeated through human skin after 24 h was not significantly different from all the emulsions tested ($p > 0.05$) and was lower than that of OMC (Table 5). As sink conditions were maintained during all the experiments for both sunscreens, the different percentages of OMC and BMBM used to prepare emulsions 1-6 could not account for the lower skin permeation of BMBM. Comparing the cumulative amount of BMBM released after 24 h with that permeated highlighted that BMBM skin permeation was limited by its release from the vehicle, therefore its lower skin permeation with respect to OMC could be due to a poorer release from the vehicle.

An almost liner relationship ($r^2 = 0.90$) between the percentage of OMC contained in the vehicle and the cumulative amount permeated though the skin was observed. However, OMC skin permeation from emulsion 5 was higher than that obtained from emulsions 3 and 4, even though all these products contained the same percentage of OMC (0.9 % w/w). As for emulsion 4, OMC flux through the skin was similar to its release rate from the vehicle, thus indicating that, in this case, the rate-limiting step was OMC release from the formulation. Since different interactions between sunscreen/vehicle, vehicle/skin and sunscreen/skin may affect OMC skin permeation, to assess the mechanisms of OMC permeation through the skin from emulsion 1-6, we calculated OMC skin permeation parameters (permeability coefficient, apparent diffusion coefficient and partition coefficient). As shown in Table 6, OMC D_m values from emulsions 1-6 decreased in the order $2 > 4 > 6 > 5 > 3 > 1$ while K_m values decreased in the order $3 > 5 > 4 > 1 > 6 > 2$. These results suggest that both OMC partitioning and diffusion through the skin contributed in the determining OMC ability to permeate the skin as the emulsion that provided the highest OMC K_m value (emulsion 3) did not produce the highest OMC flux through the skin. OMC skin permeation data from oil vehicles A-D (Table 7) showed that OMC flux from oil mixtures containing olive oil (vehicle A) or IPM (vehicle D) was significantly higher than that obtained from vehicle B and C. These data pointed out that there was no correlation between oil ingredient lipophilicity and OMC skin permeation since the highest OMC fluxes through the skin were obtained from the vehicles that contained the least or the most polar lipid (olive oil and IPM, respectively). As reported in the literature (Barry 1991), IPM is regarded as a skin permeation promoter and olive oil, due to its content of oleic acid, could be regarded a promoter as well. Olive oil was an ingredient of emulsion 6 but OMC skin permeation from this formulation was significantly lower than that observed from emulsions 3, 4 and 5. These last formulations contained, among the other ingredients, phospholipids that are known to be able to improve skin permeability (Williams and Barry 2004). However, other ingredients such as sodium hyaluronate and dipropylene glycol could contribute to promote OMC ability to permeate the skin. In accordance with the literature (Otto et al. 2009), our results suggest that the complexity of emulsion systems, which involves different interactions between various emulsion components and between emulsion components and the skin, makes it difficult to predict the effects of these vehicles on active compound skin permeation and/or penetration.

As regards emulsion viscosity effects on OMC skin permeation, no relationship was found between emulsion viscosity and UV-filter skin permeation. Other authors (Hadgraft 1989) reported that drug skin permeation could depend on the viscosity of the vehicle but this generally holds true for viscosity changes of at least one order of magnitude. Cross et al. (2001) reported that oxybenzone *in vitro* skin permeation from different emulsions was affected by the viscosity of the vehicle depending on the use of finite or infinite dosing. However, the authors (Cross et al. 2001) performed their studies on formulations containing the same emulsifying systems to which a thickening agent was added to increase their viscosity. In our experiments, the different viscosities of the emulsions tested depended on the use of different vehicle ingredients and their effects on OMC *in vitro* skin permeation seem to be related to alterations of sunscreen/skin/vehicle interactions rather than to viscosity changes of the vehicle.

According to the 6th revision of the SCCP's notes of guidance for the testing of cosmetic ingredients and their safety evaluation (European Ccommission 2006), a MoS of at least 100 is generally accepted to declare a product safe for use. As shown in Table 3, MoS values calculated for BMBM from emulsions 1-6 were higher than the lowest accepted limit for safe products. As for OMC, MoS values for emulsions 2,3,4 were less than 100, thus suggesting that these products could not be safe. However,

Table 7: Cumulative amount permeated after 24 h (Q_{24} ± S.D.), permeation rate (flux ± S.D.), lag time, permeability coefficient (K_p), apparent diffusion coefficient (D_m) and membrane/vehicle partition coefficient (K_m) through excised human skin of OMC and BMBM from vehicles A-D

Vehicle	UV-filter	Q_{24} ± S.D. (μg/cm^2)	Flux ± S.D. (μg/cm^2/h)	Lag time (h)	$K_p \times 10^{-4}$ (cm/h)	$D_m \times 10^{-6}$ (cm^2/h)	K_m
A	OMC	25.72 ± 1.38	1.71 ± 0.23	2.02	0.31	0.23	0.23
	BMBM	1.20 ± 0.11	—	—	—	—	—
B	OMC	15.43 ± 1.99	1.13 ± 0.21	3.58	0.24	0.13	0.31
	BMBM	1.36 ± 0.19	—	—	—	—	—
C	OMC	17.66 ± 1.87	1.25 ± 0.26	2.37	0.23	0.20	0.19
	BMBM	1.14 ± 0.13	—	—	—	—	—
D	OMC	26.88 ± 1.76	1.79 ± 0.20	2.51	0.33	0.19	0.29
	BMBM	1.48 ± 0.16	—	—	—	—	—

it should be kept in mind that we performed our experiments applying on the skin surface an amount of sunscreen products 10-fold that recommended by OECD guidelines as representative of normal "in use" conditions. Since we observed a linear relationship between the cumulative amount of OMC permeated and its concentration in the vehicle, it could be reasonably assumed that applying an amount of sunscreen product 10-folds lower, MoS values should be one order of magnitude higher and, therefore, well beyond the limit accepted for safe products.

3. Experimental

3.1. *Materials*

All tested products were O/W cosmetic emulsions for sun protection and they were a kind gift of Terme di Tabiano (Italy). Two types of products, Thermactive body emulsions and Thermactive face emulsions were tested, and their UV-filter concentrations, SPF, and viscosity values are reported in Table 8. All the formulations contained the following basic ingredients: Water, cyclopentasiloxane, polydecene, paraffinum liquidum, PEG-30 dipolyhidroxy stearate, sorbitan stearate, glycerin, alumina, cera alba, cera microcristallina, sodium magnesium silicate, magnesium sulfate, methylparaben, propylparaben, DMDM hydantoin, imidazolidinyl urea, parfum, butylphenyl methylpropional, citronellol, coumarin, geraniol, limonene, linalol, ethylhexyl methoxycinnamate (OMC), butyl methoxydibenzoylmethane (BMBM) and titanium dioxide. Furthermore, each formulation was characterized by different ingredients as reported below. Formulations 1, 2 and 6 contained also: propylene glycol, *Aloe barbadensis*, bisabolol, butylene glycol, acetyl tyrosine, hydrolyzed vegetable protein, adenosine triphosphate, riboflavin and retinyl palmitate. Formulation 1 contained *Zea mays* and Coco - caprylate caprate, in addition to the other ingredients listed above while formulation 2 contained Coco-caprylate caprate, *Zea mays*, *Daucus carota*, and formulation 6 contained caprylic capric triglyceride, Olea europaea and *Daucus carota*. Additional ingredients for formulations 3, 4 and 5 were: *Helichrysum italicum*, phospholipids, escin, beta-sitosterol and tocopheryl acetate. Formulations 3 and 5 contained also dipropylene glycol, dicaprylyl ether, sodium hyaluronate, and *Simmondsia chinensis*. Hydrogenated castor oil was contained in formulations 1, 4 and 5. Additional ingredients for formulation 4 were: propylene glycol, C 12–15 alkyl benzoate and *Persea gratissima*.

Octyl methoxycinnamate (OMC) and butyl methoxydibenzoylmethane (BMBM), used for HPLC calibration curves, were a kind gift of BASF (Ludwigshafen, Germany). Mineral oil (paraffinum liquidum), olive oil, isopropyl myristate (IPM) and octyl palmitate were bought from Galeno (Prato, Italy). C12–12 alkyl benzoate (Finsolv TN) was purchased from Degussa (Cremona, Italy). Oil vehicle A-D composition and oil hydrophilic lipohilic balance (HLB) values are shown in Table 3. Oil vehicles A-D were prepared simply by mixing the weighed amount required of each oil at room temperature. Regenerated cellulose membranes (Spectra/Por CE; Mol. Wet. Cut off 3,000) were supplied by Spectrum (Los Angeles, CA). Acetonitrile and water used in the HPLC procedures were of LC grade and were bought from Merck (Milan, Italy). All other reagents were of analytical grade.

Table 8: Sun protection factor (SPF), percentage (% w/w) of OMC and BMBM and viscosity of formulations 1-6

Formulation	Use	SPF	OMC (% w/w)	BMBM (% w/w)	Viscosity (mPas)
1	body	6	5.5	0.2	5000
2	body	12	6.5	0.8	5200
3	body	25	9.9	1.0	5700
4	body	50	9.9	1.5	8700
5	face	25	9.9	1.0	6200
6	face	12	6.0	0.8	2700

3.2. *Viscosity measurements*

A Mettler Rheomat RM 260 viscosimeter was used to measure the viscosity (mPas) of the emulsions under investigations using a MSDIN 125 spindle at shear rate 3 for 10 s. Samples of the emulsions were left to settle over 30 min at room temperature before measurements were taken.

3.3. *In vitro release experiments*

OMC and BMBM release rates from emulsions 1-6 and oil vehicles A-D were measured through cellulose membranes by means of Franz-type diffusion cells (LGA, Berkeley, CA). This technique has been reported in the literature as a suitable method for evaluating drug release from topical formulations (Shah et al. 1989).

The cellulose membranes were moistened by immersion in distilled water for 1 h at room temperature before being mounted in Franz-type diffusion cells. The surface area was 0.75 cm^2 and the receiving chamber volume was 4.5 ml. The receiving solution consisted of water/ethanol (50/50 v/v) for ensuring pseudo-sink conditions by increasing active compound solubility in the receiving phase (Touitou and Fabin 1988). The receptor fluid was constantly stirred and thermostated at 35 °C so as to maintain the membrane surface at 32 °C. Each formulation (20 mg/cm^2) was applied into the donor compartment and the experiments were run for 24 h. At intervals (0, 2, 4, 6, 24 h), samples of the receptor phase (200 μl) were withdrawn and replaced with an equal volume of receiving solution equilibrated to 35 °C. Samples of the receptor phase were analyzed by the HPLC method described below to determine their active compound content. Each experiment was performed in triplicate.

3.4. *In vitro skin permeation experiments*

In vitro skin permeation experiments were performed as reported in OECD Guidelines 428, with minor changes (OECD 2006). Stratum corneum and epidermis (SCE) membranes were prepared as described by Kligman and Christophers (1963). This kind of membrane is regarded a suitable model to assess *in vitro* skin permeation of UV-filters (Huong et al. 2009). Briefly, subcutaneous fat was carefully trimmed from samples of adult human skin (obtained from abdominal plastic surgery; mean age 39 ± 7 years) and the skin was immersed in distilled water at 60 ± 1 °C for 2 min, after which stratum corneum and epidermis were removed from the dermis using a scalpel blade. SCE membranes were dried in a desiccator at approximately 25% RH. Samples of dried SCE were rehydrated by immersion in distilled water for 1 h prior to the experiment. SCE samples were mounted in the same Franz-type diffusion cells described above. The receiving compartment was filled with water/ethanol (50/50 v/v) that was constantly stirred and thermostated at 35 °C. The use of such a receiving solution has been already described in the literature to ensure solubility of poor water-soluble compounds in *in vitro* percutaneous absorption studies (Baert et al. 2010). As requested by OECD guidelines (OECD 2006), we checked that no alteration of skin permeability occurred due to the receptor fluid we used. Therefore, we carried out preliminary experiments to assess SCE samples for barrier integrity by measuring *in vitro* permeability of [^{3}H] water under the same experimental conditions used for *in vitro* permeation experiments on OMC and BMBM. The value of permeability coefficient for tritiated water was $1.7 \pm 0.3 \times 10^{-3}$ which was in good agreement with that reported by other authors for samples with normal skin permeability (Bronaugh et al. 1996; Sctt et al. 1986). Each formulation (20 mg/cm^2) was applied to the skin surface and the experiments were run for 24 h. At intervals (0, 2, 4, 6, 24 h), 200 (l of the receptor phase were withdrawn and replaced with an equal volume of receiving solution equilibrated to 35 °C. Samples of the receptor phase were analyzed by HPLC to determine their of UV-filter content. Each formulation was tested in triplicate on three different skin specimens.

3.5. *HPLC analyses*

The HPLC system consisted of a Varian ProStar model 230 (Varian, Milan, Italy) with an auto-sampler Varian model 410 and a Galaxie software for data elaboration. The chromatographic analyses were performed using a Waters Simmetry, 4.6×25 cm reverse phase column (C_{18}) at room temperature and a mobile phase consisting of acetonitrile/water (80:20 v/v) under isocratic conditions for both UV-filters. All the analyses were carried out at room temperature at a flow rate of 1.0 ml/min. 20 μl of each sample were injected and the column effluent was monitored continuously at 310 nm and at 360 nm to detect OMC and BMBM, respectively. The amounts of each compound were calculated by reporting the peak area of the sample on a standard calibration curve that was built up by relating known concentrations of UV-filter with the respective peak areas. No interference of the other formulation components was observed. The sensitivity of the method was 0.1 μg/ml for all the compounds tested.

3.6. *Data analysis*

Active compound flux (μg/cm^2/h) through the cellulose membrane or through the skin was calculated by plotting the cumulative amount of compound released or permeated against time and dividing the slope of the steady-state portion of the graphs by the area through which diffusion took place. The lag time was determined from the x-intercept values of the regression lines.

Results were expressed as mean values ± standard deviation (SD) and Student's t-test was used to evaluate the significance of the difference between mean values. Values of $p < 0.05$ were considered statistically significant.

UV-filters apparent diffusion coefficients (D_{app}) through cellulose membranes or through the skin were calculated according to the relation:

$$D_{app} = \frac{h^2}{6t_L}$$

where h is the thickness of the membrane and t_L is the lag time. The thickness of the membrane was 25 nm when cellulose membranes were used (Montenegro et al. 2006) while it was assumed to be 16.8 μm when skin samples were used (Bronaugh et al. 1982).

The permeability coefficient K_p was calculated using the following equation:

$$K_p = J/C_0$$

where J is the flux at steady-state (μg/cm^2/h), and C_0 is the initial UV-filter concentration (μg/cm^3). UV-filter membrane/vehicle partition coefficient K_m was calculated from the relationship:

$$K_p = \frac{K_m D_m}{h}$$

Systemic exposure dosage (SED) was estimated as reported by Søeborg et al. (2007) using the following equation:

$$SED = C_{cream} \times C_{applied} \times n_{application} \times area_{exp} \times Perm(\%)/weight$$

where C_{cream} is the concentration of active compound in the cream, $C_{applied}$ is the amount of cream applied to the skin, $n_{application}$ is the number of application per day, $area_{exp}$ is the exposed area of the skin, Perm (%) is the percentage of a topically applied dose of active compound that permeated the skin, and weight is the weight of a standard human. According to Søeborg et al. (2007), an area of 15,000 cm^2 corresponding to treatment of 83% of the skin and a weight of 70 kg of a standard person were used for body formulations while an area of 370 cm^2 was used to calculate SED from face products. A single application of cream per day was used for all calculations. To estimate the margin of safety (MoS), the SED was compared to the NOAEL:

$$MoS = NOAEL/SED$$

where NOAEL is the no observed adverse effect level. NOAEL for OMC and BMBM was obtained from the literature (Nash et al. 2006) and its value was 450 mg/kg/day for both active compounds.

Acknowledgment: We thank Terme di Tabiano for supplying the sunscreen products tested in this work.

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