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Influence of cyclodextrins on in vitro human skin absorption of the sunscreen, butyl-methoxydibenzoylmethane

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Abstract

The effects of hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutylether-β-CD (SBE7-β-CD) on in vitro human skin penetration and retention of the sunscreen agent butyl-methoxydibenzoylmethane (BM-DBM) were investigated. The interaction between the UV filter and the cyclodextrins was studied in water by phase-solubility analysis. Solid complexes were prepared by the co-evaporation method and characterized by ^{1}H NMR spectroscopy, thermal analysis and powder X-ray diffraction. Solutions containing BM-DBM free or complexed with cyclodextrins were applied to excised human skin in Franz diffusion cells and the amount of sunscreen permeated after 6 h into the stratum corneum, viable epidermis, dermis and receptor fluid was assessed by HPLC. As much as 14.10–16.78% of the applied dose of BM-DBM penetrated within the skin tissue. No sunscreen was detected in the dermis and in the receiver phase. The greater proportion (84.6–95.5%) of the absorbed UV filter was localized in the stratum corneum with no significant differences between uncomplexed or complexed BM-DBM. Notable levels (2.29% of the applied dose) of the sunscreen agent accumulated in the epidermis from the preparation containing free BM-DBM. The epidermal concentration of the UV filter was markedly reduced (0.66% of the applied dose) by complexation with SBE7-β-CD, whereas HP-β-CD had no effect. The decreased BM-DBM retention in the epidermal region achieved by SBE7-β-CD limits direct contact of the sunscreen and of its reactive photolytic products with the skin viable tissues.

Keywords: Sunscreen agent; Butyl-methoxydibenzoylmethane; Cyclodextrin; Complexation; Human skin; Percutaneous penetration

1. Introduction

The expanding knowledge on the harmful effects of sunlight UV radiation (290–400 nm), including erythema, cutaneous photoageing, immune suppression and skin cancer (National Institute of Health, 1989; Ziegler et al., 1994; Serre et al., 1997), has led to the widespread use of topical sunscreening preparations (National Institute of Health, 1989; Schauder and Ippen, 1997; Gasparro et al., 1998; Green et al., 1999). The most common active constituents in these products are organic chemicals which attenuate the transmission of the solar energy to the skin by absorbing UV radiation (Jiang et al., 1996; Gasparro et al., 1998; Chatelain and Gabard, 2001). An essential characteristic for the effectiveness of UV filters is a high photostability, since the light-induced decomposition of the sunscreen agent not only decreases its screening capacity but can also generate toxic degradation products (Schauder and Ippen, 1997; Tarras-Wahlberg

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et al., 1999; Maier et al., 2001). Moreover, since the sunscreen agents exert their effect on the skin surface (Treffel and Gabard, 1996; Hayden et al., 1998), a high degree of retention of the UV filters in the outermost cutaneous layers is required to achieve the highest protective effect and minimize the toxicological risk derived from their percutaneous penetration (Hayden et al., 1997; Maier et al., 2001). Published in vitro and in vivo studies have shown that certain sunscreen agents are absorbed through human skin following topical application (Hagedorn-Leweke and Lippold, 1995; Treffel and Gabard, 1996; Hayden et al., 1997; Jiang et al., 1999; Potard et al., 2000). Therefore, there is a need for the development of new systems which achieve reduced penetration of sunscreen chemicals into the skin.

Cyclodextrins are cyclic toroidal-shaped oligosaccharides with a hydrophilic external surface and a hydrophobic cavity interior. They are capable of incorporating appropriately sized non-polar compounds or some lipophilic moiety of a molecule into their apolar cavities, forming non-covalent inclusion complexes (Rajewski and Stella, 1996; Loftsson and Brewster, 1996). This type of molecular encapsulation can lead to changes in some of the physical and chemical properties of the included substance, such as the enhancement of stability to air and light and apparent aqueous solubility (Rajewski and Stella, 1996; Loftsson and Brewster, 1996; Uekama et al., 1998). Moreover, cyclodextrin complexation can affect the availability of topically applied drugs, either increasing or decreasing their permeability into and through the skin (Rajewski and Stella, 1996; Matsuda and Arima, 1999; Loftsson, 2000; Loftsson and Masson, 2001).

The present study reports on the influence of cyclodextrins on the percutaneous absorption of sunscreen agents. In particular, the investigation focuses on butyl-methoxydibenzoylmethane (BM-DBM; Fig. 1), since it is a very efficient, widely used UV filter, approved by the regulatory agencies of Europe, Australia, USA and Japan (Schwack and Rudolph, 1995; Hayden et al., 1998; Tarras-Wahlberg et al., 1999; Chatelain and Gabard, 2001). BM-DBM provides excellent protection against UV-A radiation (320–400 nm) which plays a significant role in sunlight-induced skin damage (National Institute of Health, 1989; Tarras-Wahlberg et al., 1999; Chatelain and Gabard, 2001; Maier et al., 2001). However,

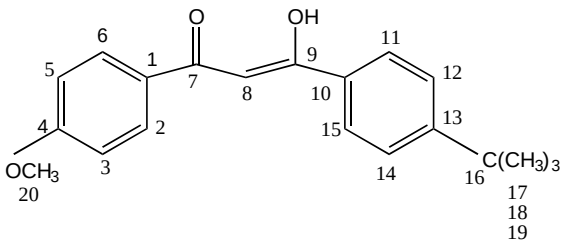

Fig. 1. Chemical structure of BM-DBM.

BM-DBM experiences marked photodegradation (Schwack and Rudolph, 1995; Scalia et al., 1998; Tarras-Wahlberg et al., 1999; Chatelain and Gabard, 2001). Moreover, recent studies (Damiani et al., 1999, 2000; Scalia et al., 2002) have demonstrated that this sunscreen agent produces free radicals under solar UV irradiation and, as a consequence, inflicts in vitro damage to DNA and bovine serum albumin. Hence, the most critical effect of the photoinstability of BM-DBM is the formation of highly reactive photolytic products which can come in contact with the living tissues of the skin (epidermis and dermis) following sunscreen percutaneous penetration.

In earlier investigations (Scalia et al., 1998, 2002) we demonstrated that the degree of decomposition and free radical formation observed upon exposure of BM-DBM to simulated sunlight were reduced by complexation of the sunscreen agent with hydroxypropyl-β-cyclodextrin (HP-β-CD). The current study was undertaken to assess in vitro the extent of penetration and distribution of BM-DBM into human skin and to evaluate the influence of HP-β-CD and the ionic sulfobutylether-β-CD (SBE7-β-CD) on the cutaneous delivery of the UV filter.

2. Materials and methods

2.1. Materials

Butyl-methoxydibenzoylmethane was supplied by Hoffmann-La Roche Ltd (Geneva, Switzerland). The cyclodextrins used in this study included: HP-β-CD (average molar substitution, 0.6) by Aldrich (Sydney, Australia) and SBE7-β-CD (total degree of substitution, 6.6) as a gift from CyDex (KS, USA). Bovine serum albumin (BSA, fraction V) was obtained from

Sigma (St. Louis, MO, USA). Methanol and acetonitrile were high-performance liquid chromatography (HPLC)-grade from Mallinckrodt (Paris, TX, USA). Water was purified by Milli-Q ultrapure water system (Millipore, Billerica, MA, USA). All other chemicals were of analytical-reagent grade (BDH Chemicals, Kilsyth, Australia).

2.2. High-performance liquid chromatography

The apparatus consisted of a modular chromatographic system (Model LC 1120 pump and Model LC 1200 UV–vis detector; GBS, Australia) linked to an injection valve with a 20 μl sample loop (Model 7725, Rheodyne, Cotati, Ca, USA). The detector was set at 355 nm. Data acquisition and processing were accomplished with a Hewlett–Packard HP 3396A integrator. Sample injections were effected with a Model 750 syringe (Hamilton, Reno, NE, USA). Separations were performed on a 5 μm Symmetry C_{18} column (3.9 mm × 150 mm, Waters Corporation, MA, USA) operated at ambient temperature and eluted with acetonitrile-water (85:15, v/v) at a flow-rate of 1.8 ml/min. The identity of the sunscreen peak was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of the peak areas using the external standardization method.

2.3. Phase-solubility studies

Solubility analyses were carried out according to Higuchi and Connors (1965). An excess amount of BM-DBM was added to aqueous solutions (5 ml) containing various concentrations (0–60 mM) of SBE7-β-CD or HP-β-CD. The samples were stirred in 10 ml screw-capped vials at 25 ± 1 °C and shielded from light. After equilibrium was attained (3 days), the content of each vial was filtered through 0.45-μm membrane filters (Whatman, Clifton, NJ, USA) and assayed for BM-DBM by HPLC as outlined above. Data were determined from the mean of at least three tests.

2.4. Preparation of the inclusion complex

The inclusion complexes were prepared at a 1:2 molar ratio of BM-DBM to HP-β-CD or SBE7-β-CD. BM-DBM (155.2 mg, 0.5 mmol) was dissolved in methanol (5.0 ml) and added to 3 ml of purified water containing a two-fold molar quantity of cyclodextrin. The suspension was maintained under stirring for 24 h at room temperature and shielded from light. The solvent was then evaporated under vacuum at 40 °C with a rotary evaporator and the residue was kept in a desiccator until used. The content of BM-DBM in each complex was determined by HPLC after proper dilution.

2.5. X-ray diffractometry

The powder X-ray diffraction patterns were recorded on a D 5000 powder diffractometer (Siemens, Munich, Germany) using a voltage of 45 kV and a current of 25 mA for the generator, with Cu anode material. The wavelength of the graphite-monocromated radiation was 1.5406 Å. The diffractograms were recorded from 3° (2θ) to 50° (2θ) at an angular speed of 1° (2θ) per minute using 1-1-1-0.15° slits.

2.6. NMR spectroscopy

^{1}H NMR spectra were recorded on a Varian Mercury Plus (400 MHz). Samples were solubilized in DMSO-d_6 or C_2D_5OD-$CD_3CD(OD)CD_2OD$-D_2O (40:30:30, v/v). Chemical shifts are reported in ppm (δ) relative to TMS. Typical parameters for the ^{1}H-NMR spectra were: 0.35 Hz/pt resolution, 18 s relaxation delay, 90° pulse.

2.7. Thermal analysis

Differential thermal analysis (DTA) was carried out on a Netzsch STA 409 simultaneous thermal analyzer (Netzsch Italiana, Verona, Italy). The samples (6–7 mg) were accurately wheighed in platinum pans (Netzsch) and heated from 30 to 130 °C, at a scanning rate of 10 °C/min.

2.8. In vitro permeation studies

Ethical approval for using human skin was granted by Curtin University Human Research Ethics Committee. Skin tissues from the breast and abdomen regions of female subjects aged between 29 and 40, were stored at −20 °C before separation. After thawing,

the subcutaneous tissue was removed by dissection and the resultant full-thickness skin cleaned with distilled water, air-dried, visually selected, cut in small pieces, placed onto aluminium foil and stored at 4 °C for 24 h before use. The skin was mounted between the donor and receptor chamber of Franz-type vertical glass diffusion cells. The area available for diffusion was 1.13–1.23 cm^2 and the receptor chamber volume varied from 3.0 to 3.6 ml. The receptor chamber was filled with a known volume of phosphate-buffered saline (pH 7.4) with 4% (w/v) BSA to ensure sink conditions (Jiang et al., 1996). The fluid was maintained at 37 ± 0.1 °C and stirred with a magnetic bar throughout the experiment. Diffusion cells were equilibrated for 1 h prior to vehicle application. Aliquots (1ml) of filtrated BM–DBM solutions in ethanol–propylene glycol–H_2O (20:30:50, v/v), with or without cyclodextrins, were introduced into the donor chamber which was sealed with parafilm to prevent sample evaporation. The concentration of the UV filter solutions was around 15 μg/ml. The diffusion studies were carried out in a blacked out water bath to protect the sunscreen agent from light. Aliquots (200 μl) of the receptor phase were withdrawn immediately following vehicle application (to check the skin barrier integrity) and after 6 h, and replaced with an equal volume of fresh fluid pre-warmed to 37 °C. Each series of experiments was repeated at least 10 times. Samples from the receptor phase were processed by protein precipitation with 2 vol. of MeCN–MeOH (95:5, v/v) and then kept in a refrigerator for 15 min. After centrifugation (5000g for 15 min), the supernatant was analyzed by HPLC as outlined above.

At the end of the experiment, the remaining solution in the donor chamber was removed with a micropipette and the skin surface rinsed with distilled water three times. The treated area was then wiped gently with cotton swabs. Water aliquots and all cotton swabs were added to the formulation residue to determine the unabsorbed sunscreen. The assay of BM-DBM present in the stratum corneum was performed using the stripping technique (Potard et al., 2000). Scotch tapes (2 mm × 3.5 mm) were applied with a constant pressure for 5 s and then removed. In order to strip the stratum corneum uniformly and to minimize the damage induced to the dermoepidermal junction, the tapes were applied in four different directions. The stratum corneum was sequentially stripped up to eight times and the eight tapes were pooled, extracted with 2 ml × 2 ml of MeOH–MeCN (90:10, v/v) and, after filtration, the obtained solution was analysed for BM-DBM by HPLC.

After the stripping technique, the epidermis was separated from the dermis by heat treatment. Each skin compartment was cut into small pieces and extracted with 2 ml × 2 ml of MeCN–MeOH (95:5, v/v) (Jiang et al., 1999). The resulting sample was filtered and the BM-DBM concentration was quantified by HPLC.

Data were analyzed for significance by using the Student's unpaired *t*-test (Instat, Graphpad Software, San Diego, CA). *P*-values <0.05 were considered significant.

3. Results and discussion

3.1. Complex characterization

In a previous investigation (Scalia et al., 1998) on the complexation of BM-DBM with unsubstituted α-, β- or γ-cyclodextrin and their hydroxypropylated derivatives, we showed that HP-β-CD interacts more strongly with the sunscreen agent than the other available cyclodextrins. Moreover, the decomposition of BM-DBM upon illumination with simulated sunlight was significantly reduced by complexation with HP-β-CD. To study the effect of this system on the penetration of the UV filter into human skin, the HP-β-CD/BM-DBM complex was prepared by the co-evaporation method and characterized by powder X-ray diffraction and DTA as described earlier (Scalia et al., 1998). The absence of both the BM-DBM crystalline peaks in the X-ray diffractogram and of the melting peak in the DTA thermogram (data not shown) was taken as proof of the inclusion of the sunscreen agent into the HP-β-CD cavity.

At this point of the experimental work, a sample of SBE7-β-CD became available and hence it seemed interesting to examine also the influence of this charged cyclodextrin derivative on the percutaneous permeation of BM-DBM. Solubility experiments were used for the initial study of the interaction of BM-DBM with SBE7-β-CD, which has not been described before. Fig. 2 illustrates the influence of the cyclodextrin on the aqueous solubility of the sunscreen agent, which is almost insoluble in water. The

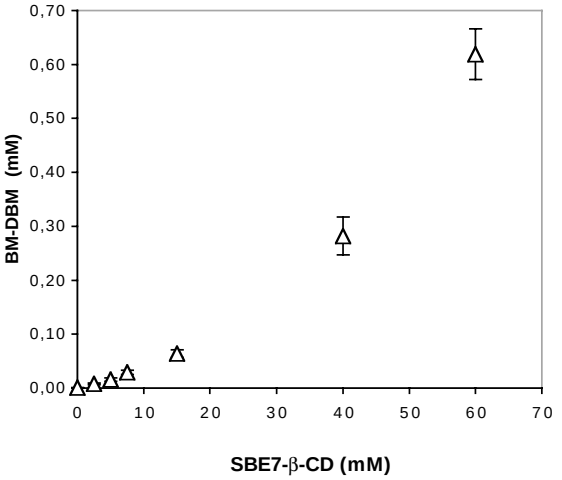

Fig. 2. Phase-solubility diagram for BM-DBM in the presence of SBE7-β-CD in purified water at 25 °C. Each point represents the mean ± S.D. of at least three experiments.

obtained diagram indicated that the apparent solubility of BM-DBM increased non-linearly with increasing SBE7-β-CD concentration, showing an Ap-type profile (Higuchi and Connors, 1965). This plot with a positive deviation from linearity (Fig. 2) indicated that the observed solubility enhancement could be attributed to the formation of inclusion complexes with different stoichiometric ratios, in accordance with the results obtained for the BM-DBM/HP-β-CD system (Scalia et al., 1998). The analysis of the solubility data by the method of Higuchi and Connors (1965) suggested that BM-DBM preferentially forms the 1:1 and 1:2 (guest:host) complexes with SBE7-β-CD. The stability constants were then calculated (Higuchi and Khristiansen, 1970) and their values were found to be 2166.6 M^{-1} for $K_{1:1}$ and 11.9 M^{-1} for $K_{1:2}$ Under the same experimental conditions, HP-β-CD exhibited a solubilizing activity similar to that observed for SBE7-β-CD (data not shown), thus indicating that the efficiency of complexation is comparable for the two examined cyclodextrins.

The interaction between BM-DBM and SBE7-β-CD was investigated in solution (DMSO) also by ^{1}H NMR spectroscopy. The major changes in the chemical shift values of BM-DBM protons (see Fig. 1 for BM-DBM structure and atom labels) induced by the presence of SBE7-β-CD are listed in Table 1. The largest variations were detected for the methyl protons on the *tert*-butyl substituent and the aromatic protons *ortho* to it, suggesting a stronger interaction of this moiety with the cyclodextrin. The H-3, H-5 and OH protons signals were shifted upfield (negative $\Delta\delta$ values) which is indicative of the insertion of these portions of the sunscreen molecule into the macrocycle cavity (Chan et al., 2000).

Table 1
^{1}H NMR chemical shifts (DMSO-d_6) for BM-DBM in absence and presence of SBE7-β-CD

Protons	δ_{free}	$\delta_{complex}$	$\Delta\delta^a$
H-17, H-18, H-19	1.320	1.327	0.007
H-20	3.866	3.870	0.004
H-3, H-5	7.229	7.223	−0.006
H-12, H-14	7.562	7.571	0.009
OH	17.386	17.381	−0.005

[a] $\Delta\delta = \delta_{\text{with cyclodextrin}} - \delta_{\text{BM-DBM alone}}$.

The solid-state characterization of the BM-DBM/SBE7-β-CD complex was performed by powder X-ray diffractometry. The solid complex was prepared in a molar ratio of 1:2 (guest:host) by the co-evaporation method, exactly as described for the BM-DBM/HP-β-CD system (Scalia et al., 1998). As illustrated in Fig. 3, the BM-DBM crystalline peaks present in the X-ray diffraction pattern of the physical mixture (Fig. 3A) were absent in the diffractogram of the SBE7-β-CD complex (Fig. 3B). These results demonstrated the amorphous nature of the co-evaporated system, providing further evidence of the inclusion of the sunscreen agent into the cyclodextrin cavity.

3.2. In vitro skin penetration studies

In order to investigate the effect of HP-β-CD and SBE7-β-CD on the in vitro percutaneous permeation of BM-DBM, excised human skin mounted in Franz diffusion cells was used. A finite dose of the sunscreen agent alone or complexed with cyclodextrins was applied to the skin surface in ethanol–propylene glycol–water solutions and the amount of BM-DBM diffused, over a period of 6 h, within the different skin layers (stratum corneum, viable epidermis and dermis) and in the receiver phase was assayed by HPLC. The recovery values obtained as sum of the unabsorbed UV filter and the BM-DBM permeated into each skin compartment were between 85.3 and

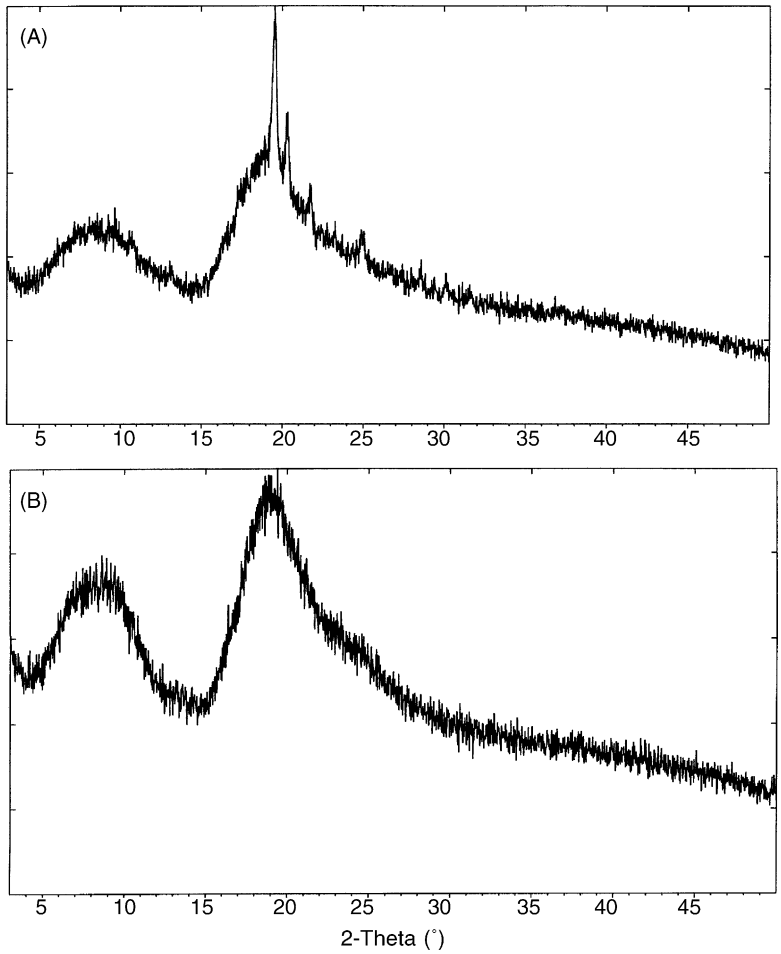

Fig. 3. Powder X-ray diffraction patterns of BM-DBM/SBE7-β-CD (1:2) physical mixture (A) and BM-DBM/SBE7-β-CD (1:2) co-evaporated complex (B).

93.4% of the applied dose. For all tested preparations, the majority of the applied sunscreen dose (70.3–77.2%) remained on the skin surface at the end of the experiment, whereas no BM-DBM was detected in the dermis and in the receptor fluid. However, a substantial amount of the UV filter (14.10–16.78% of the applied dose) penetrated into the skin, the higher proportion (84.6–95.5%) being found in the stratum corneum (see Table 2). These results are in accordance with previous studies on human skin absorption of BM-DBM (Jiang et al., 1999; Weigmann et al., 2001) and of other lipophilic sunscreen agents (Kenney et al., 1995; Treffel and Gabard, 1996). There was no statistically significant difference ($P > 0.05$) in the level of BM-DBM accumulated within the horny layer between the preparations containing free or cyclodextrin-complexed sunscreen agent (Table 2). The UV filter was also detected, though in lower quantities, in deeper skin tissues that remained after removal of the stratum corneum by stripping. Appreciable levels (2.29% of the applied dose) of the sunscreen agent diffused into the epidermis from the preparation containing uncomplexed BM-DBM (Table 2). Even if the fraction of the UV filter penetrated is moderate, it must be kept in mind that commercial sunscreen preparations usually contain high concentrations of sunscreen agents and are repeatedly applied to a large area of the skin for extended periods of time (Watkinson et al., 1992; Hagedorn-Leweke and Lippold, 1995; Jiang et al., 1999). Employing

Table 2
In vitro distribution of BM-DBM in human skin after 6 h from topical application of free or complexed sunscreen agent in ethanol–propylene glycol–H_2O (20:30:50, v/v) solutions

Sample	Percent of applied dose permeated[a]		
	Stratum corneum	Epidermis	Dermis
Free BM-DBM	14.49 ± 5.69	2.29 ± 1.10	0
HP-β-CD complex	13.01 ± 3.34	2.37 ± 1.23	0
SBE7-β-CD complex	14.03 ± 2.63	0.66 ± 0.52[b]	0
SBE7-β-CD complex + 1% SBE7-β-CD	13.14 ± 3.82	0.96 ± 0.63[b]	0

[a] Each value is the mean ± S.D. of 10 determinations.
[b] Indicates $P < 0.05$ compared to free BM-DBM.

optical and UV spectroscopy, Weigmann et al. (2001) have reported that BM-DBM was localized in the upper part of the horny layer after topical application of an emulsion formulation. This apparent discrepancy with the results presented in Table 2, can probably be traced to differences in the vehicle and in the analytical methodologies used for the evaluation of the sunscreen penetration profiles. However, the epidermal concentration of BM-DBM measured in the present study is consistent with those obtained in a previous investigation (Treffel and Gabard, 1996) on the in vitro absorption from petroleum jelly of the lipophilic UV filters, oxybenzone, ethylhexyl methoxycinnamate, ethylhexyl salicylate. The amount of BM-DBM permeated into the epidermal region was not influenced by complexation of the sunscreen agent with HP-β-CD (Table 2). Conversely, a marked decrease of the degree of epidermal penetration to 0.66% of the applied dose was observed upon application of the sunscreen agent complexed with SBE7-β-CD (Table 2). The pronounced difference in the epidermal distribution of BM-DBM generated by the two examined cyclodextrins could be ascribed to electrostatic repulsion between the anionic SBE7-β-CD and the negative charge present in the upper skin layers (Martin, 1993), which may play a major role in the skin permeation of the sunscreen. Because of the presence of cosolvents (ethanol, propylene glycol) in the donor vehicle, their influence on the stability of the BM-DBM/SBE7-β-CD complex was examined. In fact, cosolvents have been shown to destabilize the inclusion complexes (Pitha and Hoshino, 1992; Viernstein et al., 2003), although other studies have reported a synergistic effect of cosolvency on complexation (Li et al., 1999). The interaction between BM-DBM and SBE7-β-CD in the water/cosolvent mixture was examined by ^{1}H NMR analysis which provides the most conclusive evidence of complex formation (Hedges, 1998). As NMR is less sensitive than HPLC, the concentration of ethanol in the donor phase had to be increased to 40% (v/v) to achieve a suitable solubilization of the free sunscreen agent for the NMR experiments. The changes in the chemical shift values of BM-DBM induced by SBE7-β-CD (H-17, H-18, H-19 $\Delta\delta = +0.051$; H-20 $\Delta\delta = +0.016$; H-3, H-5 $\Delta\delta = -0.033$; H-12, H-14 $\Delta\delta = +0.007$) in ethanol–propylene glycol–H_2O (40:30:30, v/v) were of higher magnitude than those measured in DMSO (Table 1), with the exception of H-12, H-14. These results demonstrate the occurrence of interactions between BM-DBM and SBE7-β-CD in the water/cosolvent system. Moreover, these interactions are stronger than in 100% DMSO, in accordance with previous studies (Pitha and Hoshino, 1992; Viernstein et al., 2003) showing that the complex stability constants decrease with increasing cosolvent concentrations. It is reasonable to assume that complex formation should be even more efficient in the donor fluid (ethanol–propylene glycol–H_2O, 20:30:50, v/v) owing to the lower percentage of cosolvent present. A number of studies have demonstrated that the presence of excess cyclodextrin results in decreased drug penetration through the skin (Loftsson and Masson, 2001 and references therein). Accordingly, the effect of the addition of an excess (1%, w/v) of SBE7-β-CD to the preparation used for the in vitro diffusion studies was examined. However, no further reduction in the amount of BM-DBM retained within the epidermis was observed (Table 2).

4. Conclusions

The results described in this study demonstrated that complexation of BM-DBM with SBE7-β-CD, while attaining high sunscreen levels at the skin surface where its action is most desirable (Treffel and Gabard, 1996; Hayden et al., 1998), leads to its reduced retention in the epidermis compartment. The latter aspect is of utmost significance, since BM-DBM generates free radicals when exposed to simulated sunlight (Damiani et al., 1999; Scalia et al., 2002) and hence limited contact of the UV filter with the viable tissue of the skin should minimize potential photo-induced toxic effects to biosubstrates.

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