Journal of Inclusion Phenomena and Macrocyclic Chemistry (2006) 54:275–282
DOI 10.1007/s10847-005-9002-0

Influence of Cyclodextrin Complexation on the *in vitro* Human Skin Penetration and Retention of the Sunscreen Agent, Oxybenzone

SILVIA SIMEONI[1], SANTO SCALIA[1,*], ROSANNA TURSILLI[1] and HEATHER BENSON[2]
[1]*Department of Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara 17, 4100 Ferrara, Italy;*
[2]*Western Australian Biomedical Research Institute, School of Pharmacy, Curtin University, Perth, Australia*

(Received: 30 March 2005; in final form: 17 June 2005)

Key words: cyclodextrins, human skin, oxybenzone, percutaneous penetration, sunscreen agent

Abstract

The objective of this study was to investigate the influence of cyclodextrins on the cutaneous availability of the sunscreen oxybenzone. The interaction between oxybenzone and hydrophilic α-, β- and γ-cyclodextrin derivatives was studied in water by phase-solubility analysis. Among the available cyclodextrins, hydroxypropyl-β-cyclodextrin (HP-β-CD) and especially sulfobutylether-β-cyclodextrin (SBE-β-CD) had the greatest solubilizing activity. Ethanol–water solutions containing oxybenzone free or complexed with HP-β-CD or SBE-β-CD were applied to human skin in Franz diffusion cells and the amount of sunscreen permeated into the different cutaneous compartments was determined by HPLC. As much as 20.5% of the oxybenzone applied dose diffused within the skin tissue after 6 h application. Between 39.4% and 54.9% of the penetrated UV filter was localized in the stratum corneum, with no significant difference between uncomplexed oxybenzone or its complex with HP-β-CD. Conversely, the amount retained in the stratum corneum was markedly decreased (ca. 50%) by complexation with SBE-β-CD. Considerable quantities of oxybenzone accumulated into the viable epidermis (5.7% of the applied dose) and dermis (6.2% of the applied dose) from the preparation containing the free UV filter. The sunscreen penetration to the deeper living layers of the skin was remarkably lower (1.0% and 2.0% of applied dose for epidermis and dermis, respectively) upon application of the sunscreen complexed with SBE-β-CD, whereas HP-β-CD had no effect. In addition, photostability experiments demonstrated that SBE-β-CD complexation did not alter the sunscreen photochemical properties.

Introduction

Protection of human skin against damage by sunlight UV radiation (290–400 nm) has become a major concern due to accumulating evidence of the harmful effects of sun exposure including erythema, cutaneous photoageing, immune suppression and skin cancer, the latter being the most common type of human cancer [1–5]. A widespread strategy advocated by the health care authorities to prevent sunlight-induced damage is based on topical application of sunscreening preparations [1, 4, 6]. The active ingredients in these formulations lessen the transmission of the solar energy to the skin by absorbing, reflecting or scattering the UV radiation [5–7].

Sunscreen photostability represents an essential requirement for the evaluation of its efficacy and safety, since the possible decomposition of the UV absorber during sunlight exposure not only reduces the expected photoprotective power but can also lead to the accumulation on the skin of harmful degradation products [6, 8–10]. Another important characteristic that sunscreens have to fulfil is minimal absorption, since their permeation will leave the skin unprotected and increase the risk of phototoxic and photoallergic reactions [9, 13]. Human skin is a stratified organ composed of distinct layers which are, from the outside of the skin inward, the devitalized outer epidermis (called stratum corneum), the living epidermis and the dermis. Hence, the topically applied UV filters should be localized in the outermost part of the stratum corneum without permeation to deeper viable tissues [5, 11, 12]. However, published studies have shown both *in vitro* and *in vivo* that a number of sunscreen agents penetrate into human skin [11, 14–16]. In particular, 2-hydroxy-4-methoxybenzophenone (oxybenzone), one of the most commonly used UV filters [13, 14, 17] is known to be systemically absorbed after topical application to human volunteers and subsequently excreted in the urine and breast milk [17, 18]. Several strategies have

* Author for Correspondence. E-mail: sls@unife.it

been investigated to reduce the skin penetration of the sunscreen including evaluation of different types of vehicles [5, 11, 12, 16], increase in the formulation viscosity [19], incorporation of the UV filter in nanoparticles [13, 20] or complexation with cyclodextrins [7, 21]. The latter are torus-shaped cyclic oligosaccharides with a hydrophilic external surface and a non-polar core. Cyclodextrins can entrap appropriately sized hydrophobic compounds into their cavities, forming non-covalent inclusion complexes [22, 23]. This complexation process can increase the apparent aqueous solubility and stability to air and light of the included substance [22–24]. Moreover, the inclusion of the guest molecule by cyclodextrins can modify the delivery of topically applied drugs, either enhancing (through an increase in the apparent solubility) or decreasing (by the formation of highly stable inclusion complexes) their percutaneous absorption [23, 25–27].

Previous investigations of the influence of cyclodextrin complexation on the permeability of oxybenzone have been carried out using hydroxypropyl-β-cyclodextrin (HP-β-CD) and a synthetic membrane [7] or mouse skin [21] as cutaneous models. The relevance of these studies for evaluation of the sunscreen transdermal absorption is limited by the poor correlation in permeation with human skin [11]. Moreover, another inherent disadvantage is the lack of information on the skin distribution of oxybenzone, since it is important to ascertain whether the sunscreen accumulate preferentially in the superficial part of the horny layer without reaching substantial concentrations in the more vulnerable living regions of the epidermis and dermis.

The present study reports on the influence of cyclodextrins on the penetration of oxybenzone into and across human skin. In particular, we compared *in vitro* the percutaneous absorption and distribution in the different skin compartments of the UV filter after its application in the free form or as a complex with HP-β-CD or the anionic sulfobutylether-β-CD (SBE-β-CD).

Experimental

Materials

The cyclodextrins used in this study included: HP-β-CD, hydroxypropyl-α-cyclodextrin (HP-α-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD) by Aldrich and SBE-β-CD as a gift from CyDex (KS, USA). 2-Hydroxy-4-methoxybenzophenone (oxybenzone; Figure 1) was supplied by Aldrich (Milwaukee, 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 (Australia). Ethyl alcohol was purchased from CSR distillers (Australia). Water was purified by Milli-Q ultrapure water system (Millipore, USA). All other chemicals were of analytical-reagent grade (BDH Chemicals, Australia).

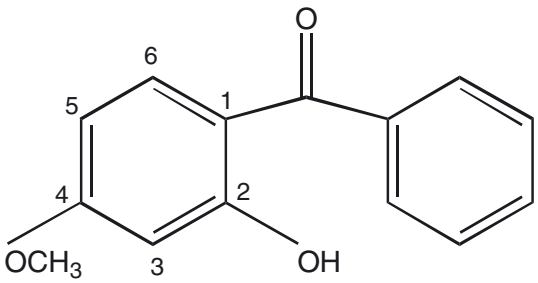

Figure 1. Chemical structure of oxybenzone.

High-performance liquid chromatography

The HPLC apparatus comprised a Model LC 1120 pump (GBS, Australia), a Model 7725 injection valve with a 20 μl sample loop (Rheodyne, Cotati, CA, USA) and a Model LC 1200 UV-Vis detector (GBS) set at 290 nm. Data acquisition and processing were accomplished with a Hewlett-Packard HP 3396A integrator. Sample injections were effected with a Model 750 syringe (Hamilton, Co.Reno, NE, USA). Separations were performed on a 5 μm Symmetry C_{18} column (3.9 mm $\times$ 150 mm; Waters Corporation, MA, USA) operated at ambient temperature and eluted with acetonitrile/water (70:30, v/v) at a flow-rate of 1.5 ml/min. The identity of the sunscreen peak was assigned by co-chromatography with the authentic standard. Sample concentration was calculated from peak areas by the external standard method.

Solubility studies

Solubility analyses were carried out according to Higuchi and Connors [28]. An excess amount of oxybenzone was added to solutions (5 ml) containing increasing concentrations (0–60 mM) of the examined cyclodextrins in water or ethanol-water (30:70, v/v). The samples were stirred in screw-capped vials at 25 $\pm$ 1 °C and shielded from light. After equilibrium was reached (3 days), the content of each vial was filtered through a 0.45-μm membrane filter (Whatman, Clifton, NJ, USA) and assayed for oxybenzone by HPLC as outlined above. Data were determined from the mean of at least three tests. Solubility diagrams were constructed by plotting the molar concentration of the sunscreen in solution against the molar concentration of cyclodextrin. The stability constant values were calculated with the following equation:

$$K = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

where S_0 represents the solubility of oxybenzone in the absence of cyclodextrins and slope is the slope of the obtained phase solubility diagram.

Preparation of the inclusion complex

The inclusion complexes were prepared at 1:1 and 1:2 molar ratios of oxybenzone to HP-β-CD or SBE-β-CD. Oxybenzone (114.8 mg, 0.5 mmol) was dissolved in methanol (3.0 ml) and added to 6 ml of purified water containing an equimolar or a 2-fold molar quantity of the corresponding cyclodextrin. The mixture 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 oxybenzone in each complex was determined by HPLC after proper dilution.

X-ray diffractometry

The powder X-ray diffraction patterns were recorded using a D 5000 powder diffractometer (Siemens, Munich, Germany) operated at a voltage of 45 kV and a current of 25 mA for the generator, with Cu as 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.

NMR spectroscopy

^{1}H-NMR spectra were recorded on a Varian Mercury Plus (400 MHz). Samples were solubilized in C_2D_5OD-D_2O (60:40, 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.

UV spectrophotometry

UV spectra were recorded in ethanol:water (60:40, v/v) on a UV/VIS/NIR Spectrometer (Lambda 19; Perkin Elmer, Norwalk, USA).

In vitro percutaneous penetration

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 until needed. 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. Human epidermal tissue was obtained by heat separation (immersion of the skin in water at 60 °C for 3 min) and blunt dissection of full-thickness skin. Permeation studies were performed using Franz-type vertical glass diffusion cells with a 1.13–1.23 cm^2 cross sectional surface area. The skin was mounted between the donor and receptor chamber of the diffusion cells. The receptor compartment (approx. 3.5 ml) was filled with a known volume of phosphate-buffered saline (pH 7.4) with 4% BSA to ensure sink conditions [12]. The receiver medium was maintained at 37 ± 0.1 °C and stirred with a magnetic bar throughout the experiment. Following equilibration (1 h) of the skin and receptor phase, aliquots (1 ml) of filtrated ethanol–water (30:70, v/v) solutions containing free or cyclodextrin complexed oxybenzone, were spread uniformly over the epidermal surface in the donor chamber which was sealed with parafilm to prevent sample evaporation. The concentration of the UV filter solutions was around 150 μg/ml. The diffusion studies were carried out in a light-proof water bath to shield samples from light. Aliquots (200 μl) from the receptor phase were withdrawn immediately following vehicle application (to check the skin barrier integrity) and after 6 h. Each fraction was replaced with an equal volume of fresh fluid pre-warmed at 37 °C. At least eight replicates were used for each series of experiments. Samples from the receptor phase were processed by protein precipitation with two volumes of acetonitrile-methanol (95:5, v/v) and then kept in a refrigerator for 15 min. After centrifugation (5000 × g for 15 min), the supernatant was analysed by HPLC as outlined above.

In vitro human skin distribution

At the end of the permeation experiment, the residual formulation in the donor compartment was removed with a micropipette and the skin surface quickly washed with distilled water three times. The treated area was then dried gently with cotton swabs. Water aliquots and all cotton swabs were added to the remaining solution in the donor for the assay of the non-penetrated sunscreen. To measure the oxybenzone concentration in the stratum corneum the stripping technique was employed [15]. The skin samples were removed from the Franz cells and Scotch tape strips (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 corresponding tapes were pooled, extracted with 2 × 2 ml of methanol-acetonitrile (90:10, v/v) and, after filtration, the resulting solution was analysed for oxybenzone by HPLC.

Following the stripping of the horny layer, the epidermis was separated from the dermis by heat treatment. The two skin sections were separately cut into small pieces and extracted with 2 × 2 ml of acetonitrile-methanol (95:5, v/v). The resulting samples were filtered for subsequent HPLC determination of the sunscreen content.

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

Photostability studies

Photolysis experiments were conducted in solution (30% ethanol in water). A portion (0.5 ml) of the test preparation containing oxybenzone (150 μg/ml) alone or complexed with SBE-β-CD, was placed into a quartz cuvette (2 mm path-length), closed with screw caps and inserted in the chamber of the solar simulator (Suntest CPS+; Atlas, Linsengericht, Germany) which was equipped with a Xenon lamp, an optical filter to cut off wavelengths shorter than 290 nm and an IR-block filter to avoid thermal effects. The solar simulator emission was maintained at 500 W/m^2. After the selected irradiation time (2 h), the samples were quantitatively transferred into 2-ml calibrated flasks with methanol, diluted to volume and the remaining sunscreen level was quantified by HPLC, as outlined above. All samples were protected from light both before and after irradiation. The degree of photodegradation was evaluated by comparing the peak areas of oxybenzone from the irradiated samples, with those obtained by analysis of an equivalent amount of the unirradiated preparations. The results are the average of 10 experiments.

Results and discussion

Interaction between oxybenzone and cyclodextrins

Previous studies of cyclodextrin complexation with oxybenzone have been restricted to β-CD and HP-β-CD [7, 21]. Conversely, a systematic evaluation of the interaction of various cyclodextrins with the sunscreen agent was carried out in the present work. The highly hydrophilic hydroxypropylated- (i.e., HP-α-CD, HP-β-CD, HP-γ-CD) and sulfobutylated-(SBE-β-CD) derivatives were selected, because of the limited aqueous solubility of native cyclodextrins [22]. Moreover, a previous report has demonstrated the absence of valuable interactions between β-CD and the sunscreen agent [7]. The solubility method was used initially for studying inclusion complexation of oxybenzone with the examined cyclodextrins. Figure 2a shows the influence of HP-α-CD, HP-β-CD, HP-γ-CD and SBE-β-CD on the water solubility of the UV filter. The diagrams obtained pointed to remarkable variations in the activity of the different cyclodextrins, the major solubility enhancements being produced by HP-β-CD (540-fold increase)

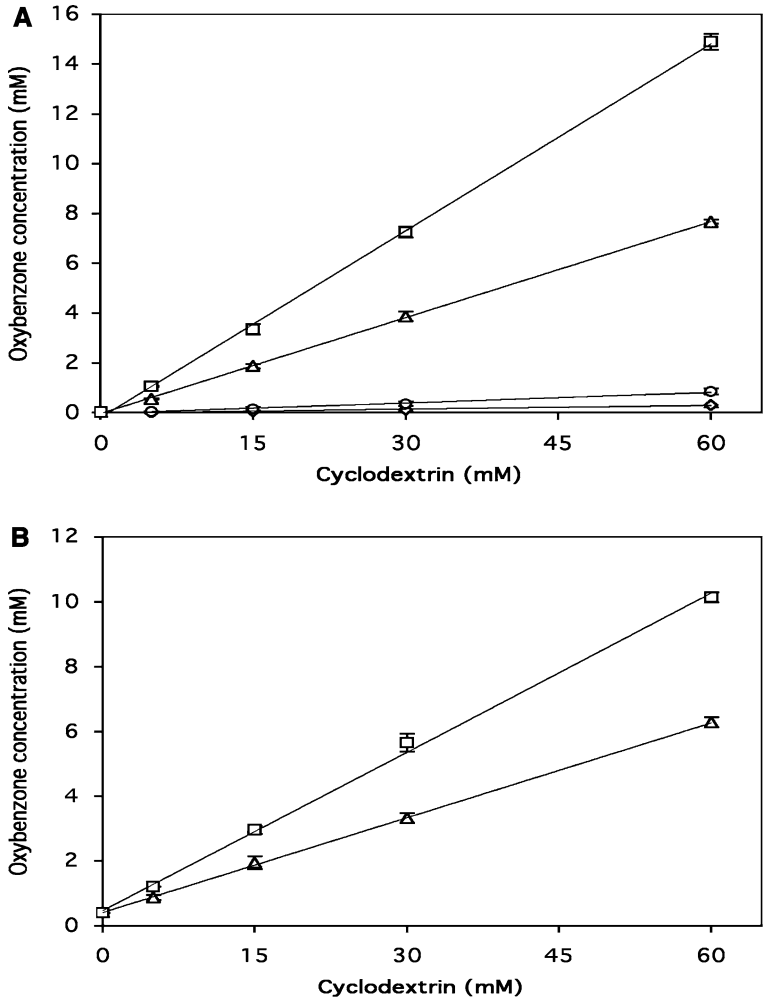

Figure 2. Phase-solubility diagrams for oxybenzone with different cyclodextrins at 25 °C in purified water (a) and in 30% ethanol in water (b). Key: (◇) HP-α-CD; (○) HP-γ-CD; (□) SBE-β-CD; (Δ) HP-β-CD. Each point represents the mean ± S.D. of at least three experiments. The lines are the regression lines obtained using least squares linear regression analysis.

and especially by SBE-β-CD (1049-fold increase). In particular, the graphs illustrated in Figure 2a demonstrated that SBE-β-CD exhibited stronger interactions with the sunscreen agent than HP-α-CD, HP-γ-CD or the previously adopted HP-β-CD [7, 21]. The superior solubilizing effect of SBE-β-CD to the poorly water-soluble oxybenzone suggests that the hydrophobic butyl group, close to the cyclodextrin rim, plays an important role in the inclusion process rather than the ionic sulfonate group protruding from the cavity. The apparent solubility of the UV filter increased linearly ($r^2 > 0.99$) as a function of HP-β-CD or SBE-β-CD concentration (Figure 2a) showing A_L-type profiles and suggesting the formation of complexes of stoichiometry 1:1 [28]. The stability constants ($K_{1:1}$) calculated according to the method of Higuchi and Connors [28] were $9482 \pm 119\ M^{-1}$ and $21353 \pm 531\ M^{-1}$ for the HP-β-CD/oxybenzone and SBE-β-CD/oxybenzone complex, respectively.

The interaction between oxybenzone and SBE-β-CD, which has not been described before, was ascertained in solution also by ^{1}H-NMR spectroscopy. In order to acquire evidence of complex formation in the same vehicle used for the *in vitro* skin penetration studies, ^{1}H-NMR analyses were performed in water–ethanol mixtures. As NMR is less sensitive than HPLC, the ethanol concentration in the donor fluid had to be increased from 30% to 60% (v/v) to achieve a sufficient solubility of the free UV filter for the recording of NMR spectra. Table 1 lists the major changes in the chemical shift values of selected oxybenzone protons (see Figure 1 for oxybenzone structure and atom labels) induced by the presence of SBE-β-CD, other signals present in the spectra being overlapped between them. Pronounced chemical shift variations were observed for all detected oxybenzone peaks (Table 1). Moreover, the H-6 signal was shifted upfield (negative $\Delta\delta$ value) which gave an indication of the inclusion of this portion of the sunscreen molecule into the cyclodextrin cavity [29]. These data demonstrated the occurrence of interactions between oxybenzone and SBE-β-CD in the 60% (v/v) ethanol in water solvent system. It is reasonable to assume that the complex should be even more stable in the donor vehicle (ethanol–water, 30:70, v/v) owing to the lower percentage of ethanol present. In fact, in aqueous solutions the complexation efficiency decreases with increasing cosolvent concentration [22, 30].

Table 1. ^{1}H-NMR chemical shifts for oxybenzone in absence and presence of SBE-β-CD

Protons	δ_{free}	$\delta_{complex}$	$\Delta\delta^a$
H-6	7.553	7.504	−0.049
H-3	6.609	6.651	0.042
H-5	6.570	6.611	0.041
OCH_3	3.900	3.927	0.027

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

Powder X-ray diffractometric studies were carried out to examine the interaction of oxybenzone with SBE-β-CD on solid-state. The complex was prepared in a molar ratio of 1:1 and 1:2 (guest:host) by the co-evaporation method. As shown in Figure 3, crystalline peaks corresponding to oxybenzone were present in the diffractogram of the physical mixture (Figure 3a), whereas the diffraction pattern of the complex with a 1:2 molar ratio did not show the sunscreen signals (Figure 3b). This finding demonstrated the amorphous nature of this co-evaporate, providing evidence of the inclusion of the sunscreen agent into the SBE-β-CD cavity. In the X-ray diffraction pattern of the 1:1 (oxybenzone:SBE-β-CD) complex the main peak (26°) of the UV filter appeared, though with a low intensity (diffractogram not shown). This indicates that complexation of oxybenzone is more efficient when a 2-fold molar quantity of the host is used. Accordingly, the complexes in a molar ratio of 1:2 were selected for the subsequent studies.

In vitro percutaneous penetration

The influence of HP-β-CD or SBE-β-CD complexation on the penetration of oxybenzone through excised human skin was investigated *in vitro* using Franz diffusion cells. A finite dose of free or cyclodextrin complexed (1:2, guest:host) sunscreen agent in ethanol–water (30:70, v/v) solutions was applied to the skin surface and the quantity of oxybenzone permeated over a period of 6 h into the different skin layers (stratum corneum, viable epidermis and dermis) and in the receptor fluid was measured by HPLC, according to previously reported and validated procedures [14], with minor modifications (see Experimental). The recovery values obtained as sum of the unabsorbed UV filter and the oxybenzone diffused into each skin compartment were in the 94.2–98.5% range. For all the examined formulations, the majority (78.1–89.7%) of the applied sunscreen dose remained on the skin surface at the end of the experiment. However, a substantial amount of oxybenzone (19.8% of the applied dose) penetrated into and through human skin from the preparation containing the uncomplexed UV filter, 39.4% of the permeated sunscreen being localized in the stratum corneum (see Table 2). These results are in agreement with those reported in earlier studies on human skin absorption of oxybenzone [13–15, 31]. Moreover, the data presented in Table 2 indicate that oxybenzone readily diffuses across the stratum corneum barrier of human skin. Appreciable levels of sunscreen agent (12.0% of the applied dose) permeated into the viable epidermis and the dermis upon application of the oxybenzone solution without cyclodextrin (Table 2). The concentration of UV filter measured in the viable cutaneous tissue is consistent with the values obtained in previous investigations of *in vitro* human skin absorption of oxybenzone from different vehicles [11, 14, 16]. The skin penetration of the sunscreen agent was not significantly affected by complexation with HP-β-CD

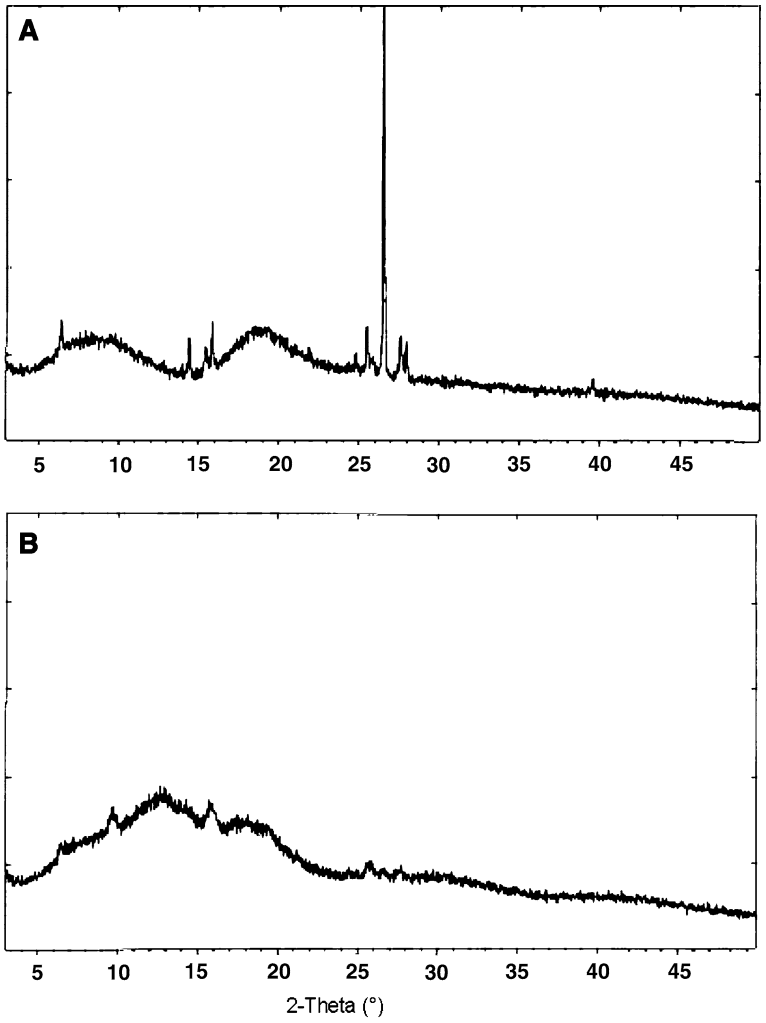

Figure 3. Powder X-ray diffraction patterns of oxybenzone/SBE-β-CD (1:2) physical mixture (A), oxybenzone/SBE-β-CD (1:2) co-evaporated complex (B).

(Table 2). Using mouse skin, Felton *et al.* [21] demonstrated that HP-β-CD significantly increased the cutaneous retention of oxybenzone, although no information regarding the sunscreen localization within the skin was provided. The discrepancy with the results reported here for the oxybenzone/HP-β-CD complex (Table 2) may be ascribed to the difference in permeability between animal and human skin. At variance with the data obtained for HP-β-CD, a marked reduction (by up to 82.3%) in the quantities of UV filter retained in the horny layer and diffused into the epidermal and dermal regions was observed for the formulation containing the SBE-β-CD complex (Table 2). The pronounced difference in the degree of oxybenzone accumulation in the different skin layers produced by the two examined cyclodextrins, can be explained in part on the basis of complexation strength [23, 32] given the higher stability constant value measured from the solubility curves (Figure 2b) in the donor vehicle (30% ethanol in water) for the oxybenzone/SBE-β-CD complex ($K_{1:1}$, 478 ± 27 M^{-1}) as compared to the oxybenzone/HP-β-CD system ($K_{1:1}$, 241 ± 18 M^{-1}).

Table 2. In vitro distribution of oxybenzone in human skin 6 h after topical application of free or complexed sunscreen agent in ethanol–H_2O (30:70, v/v) solutions

Sample	Percent of applied dose permeated[a]		
	Stratum corneum	Epidermis	Dermis
Free oxybenzone	7.81 ± 2.99	5.75 ± 2.19	6.24 ± 0.94
HP-β-CD complex (1:2, guest:host)	10.07 ± 1.88[b]	5.19 ± 3.16[b]	5.22 ± 3.99[b]
SBE-β-CD complex (1:2, guest:host)	3.70 ± 1.04[c]	1.02 ± 0.40[c]	2.01 ± 0.31[c]

[a]Each value is the mean ± S.D. of at least eight determinations.
[b]Indicates $p > 0.05$ compared to free oxybenzone.
[c]Indicates $p < 0.002$ compared to free oxybenzone.

Due to the presence of ethanol acting as competing guest molecule [30], the values for the stability constants obtained in the donor vehicle are lower than those measured in 100% water. However, the difference between the equilibrium complexation constants is not large enough to account for the remarkable decrease in the cutaneous availability of the oxybenzone attained by complexation with SBE-β-CD and hence some other factors must be involved. In particular, the electrostatic repulsion between the negative charge present in the upper skin layers [33] and the polyanionic SBE-β-CD may play a major role in the uptake of the sunscreen by the skin, as reported for the percutaneous penetration of butyl-methoxydibenzoylmethane complexed with SBE-β-CD [34]. In addition, non-inclusion based processes may be involved [35]. A number of studies have demonstrated that addition of an excess amount of cyclodextrin to the vehicle can lead to decreased drug absorption through skin [27 and references therein]. However, the use of high cyclodextrins concentrations has limited practical applicability to finished suncare products due to the influence on the formulation bulk.

After the 6 h study period, no oxybenzone was detected in the receptor fluid, this result being supported by earlier *in vitro* investigations on the transdermal absorption of the sunscreen agent [15, 16]. Using excised human epidermis in Franz diffusion cells, Jiang *et al.* [12, 14] have demonstrated that oxybenzone permeated to the receptor chamber from various solvents and commercial products. Additional experiments performed on isolated human epidermis, under the conditions described in the present study, showed that, in this case, the UV filter did diffuse to the receiver medium. The percentage of applied dose passed through the epidermal tissue after 3 and 6 h (Figure 4) was notably decreased by complexation with SBE-β-CD, whereas HP-β-CD did not have any significant effect, in line with the results obtained with full-thickness skin (Table 2).

Photostability studies

Work from our group [10] and from other researchers [24, 36] has shown that cyclodextrin complexation can also influence the photochemistry of the included substance, either enhancing or decreasing its photolability. Since it is known that oxybenzone is susceptible to some degradation under sunlight [8, 37], it seemed relevant to examine the effect of SBE-β-CD also on the stability of the UV filter during irradiation. The photolysis experiments were carried out on the same vehicle used for the *in vitro* penetration studies. Free or complexed oxybenzone was exposed to the solar simulator and the extent of photodegradation was measured by HPLC. Following irradiation of the solution containing the uncomplexed sunscreen agent, $2.3 \pm 2.2\%$ ($n = 10$) of the oxybenzone content was lost, in good agreement with previous investigations [8]. The photo-induced decomposition of the UV filter was not significantly altered ($p > 0.4$) by complexation with SBE-β-CD, the extent of degradation being $2.9 \pm 2.7\%$ ($n = 10$). Moreover, UV spectrophotometric analysis of oxybenzone and its complex with SBE-β-CD showed that the corresponding spectra were almost superimposable (spectra not shown) and hence complexation doesn't modify significantly the UV absorption characteristics of the sunscreen agent.

Conclusions

The results described in this study demonstrated that, while maintaining the photochemical properties of the UV filter, complexation of oxybenzone with SBE-β-CD markedly reduces its percutaneous penetration thereby minimizing the sunscreen contact with the living area of the skin and the potentially associated toxicological risks. This aspect is particularly important since commercial sunscreen preparations usually contain high

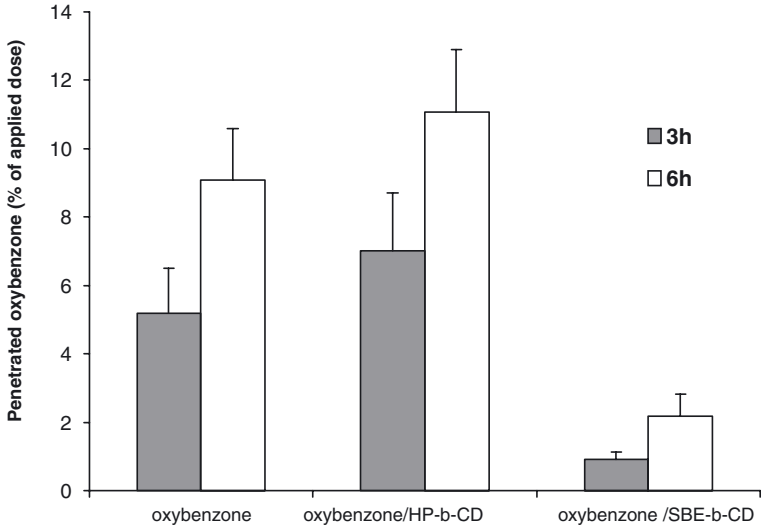

Figure 4. Penetration of oxybenzone across human epidermis to the receptor phase from ethanol–water (30:70,v/v) solutions containing free or complexed sunscreen agent. Data represent mean ± S.D. ($n = 6$–8).

concentrations of the UV filter and are repeatedly applied to large areas of the skin for extended periods of time [14, 18]. In addition, by inclusion into the SBE-β-CD cavity, a more localized distribution of oxybenzone on the skin surface is attained which should enhance the sunscreen photoprotective power [16].

Acknowledgements

The authors are grateful to CyDex for the gift of SBE-β-CD and to MIUR for financial support.

References

1. National Institute of Health, NIH Consens Statement Online. Sunlight, Ultraviolet radiation, and the Skin 7, 1 (1989).
2. A. Ziegler, A.S. Jonason, D.J. Leffell, J.A. Simon, H.W. Sharma, J. Kimmelman, L. Remington, T. Jacks, and D.E. Brash: *Nature* **372**, 773 (1994).
3. I. Serre, J.P. Cano, M.C. Picot, J. Meynadier, and L. Meunier: *J. Am. Acad. Dermatol.* **37**, 187 (1997).
4. A. Green, G. Williams, R. Neale, V. Hart, D. Leslie, P. Parsons, G.C. Marks, P. Gaffney, D. Battistutta, C. Frost, C. Lang, and A. Russell: *Lancet* **354**, 723 (1999).
5. D.A. Godwin, N.H. Kim, and L.A. Felton: *Eur. J. Pharm. Biopharm.* **53**, 23 (2002).
6. F.P. Gasparro, M. Mitchnick, and J.F. Nash: *Photochem. Photobiol.* **68**, 243 (1998).
7. V. Sarveiya, J.F. Templeton, and H.A.E. Benson: *J. Incl. Phenom.* **49**, 275 (2004).
8. N. Tarras-Wahlberg, G. Stenhagen, O. Larkö, A. Rosén, A.M. Wennberg, and O. Wennerström: *J. Invest. Dermatol.* **113**, 547 (1999).
9. H. Maier, G. Schauberger, K Brunnhofer, and H. Hönigsmann: *J. Invest. Dermatol.* **117**, 256 (2001).
10. S. Scalia, A. Molinari, A. Casolari, and A. Maldotti: *Eur. J. Pharm. Sci.* **22**, 241 (2004).
11. P. Treffel and B. Gabard: *Pharm. Res.* **13**, 770 (1996).
12. R. Jiang, H.A.E. Benson, S.E. Cross, and M.S. Roberts: *Pharm. Res.* **15**, 1863 (1998).
13. S.A. Wissing and R.H. Müller: *J. Control. Release* **81**, 225 (2002).
14. R. Jiang, M.S. Roberts, D.M. Collins, and H.A.E. Benson: *Br. J. Clin. Pharmacol.* **48**, 635 (1999).
15. G. Potard, C. Laugel, H. Schaefer, and J.P. Marty: *Skin Pharmacol. Appl. Skin Physiol.* **13**, 336 (2000).
16. E. Chatelain, B. Gabard, and C. Surber: *Skin. Pharmacol. Appl. Skin Physiol.* **16**, 28 (2003).
17. G.H. Gustavsson, A. Farbrot, and O. Larko: *Clin. Experim. Dermatol.* **27**, 691 (2002).
18. C.G. Hayden, M.S. Roberts, and H.A.E. Benson: *Lancet* **350**, 863 (1997).
19. S.E. Cross, R. Jiang, H.A.E. Benson, and M.S. Roberts: *J. Invest. Dermatol.* **117**, 147 (2001).
20. B. Luppi, T. Cerchiara, F Bigucci, R. Basile, and V. Zecchi: *J. Pharm. Pharmacol.* **56**, 407 (2004).
21. L.A. Felton, C.J. Wiley, and D.A. Godwin: *Drug. Devel. Ind. Pharm.* **28**, 1117 (2002).
22. T. Loftsson and M.E. Brewster: *J. Pharm. Sci.* **85**, 1017 (1996).
23. R.A. Rajewski and V.J. Stella: *J. Pharm. Sci.* **85**, 1142 (1996).
24. K. Uekama, F. Hirayama, and T. Irie: *Chem. Rev.* **98**, 2045 (1998).
25. H. Matsuda and H. Arima: *Adv. Drug. Deliv. Rev.* **36**, 81 (1999).
26. T. Loftsson: *Cosmet. Toil.* **115**, 59 (2000).
27. T. Loftsson and M. Masson: *Int. J. Pharm.* **225**, 15 (2001).
28. T. Higuchi and K.A. Connors: *Adv. Anal. Chem. Instrum.* **4**, 117 (1965).
29. L.W. Chan, T.R.R. Kurup, A. Muthaiah, and J.C. Thenmozhiyal: *Int. J. Pharm.* **195**, 71 (2000).
30. H. Viernstein, P. Weiss-Greiler, and P. Wolschann: *Int. J. Pharm.* **256**, 85 (2003).
31. C. Fernandez, F. Nielloud, R. Fortuné, L. Vian, and G. Marti-Mestres: *J. Pharm. Biomed. Anal.* **28**, 57 (2002).
32. M. Motwani and J.L. Zatz: *Cosmet. Toil.* **112**, 39 (1997).
33. A. Martin: *Physical Pharmacy*, 4th ed.,, Lea & Febiger, Malvern, PA (1993), pp. 544.
34. S. Simeoni, S. Scalia, and H.A.E. Benson: *Int. J. Pharm.* **280**, 163 (2004).
35. T. Loftsson, M. Masson, and M.E. Brewster: *J. Pharm. Sci.* **93**, 1091 (2004).
36. B.D. Glass, M.E. Brown, S. Daya, M.S. Worthington, P. Drummond, E. Antunes, M. Lebete, S. Anoopkumar, and D. Maharaj: *Int. J. Photoenergy* **3**, 205 (2001).
37. N. Serpone, A. Salinaro, A.V. Emeline, S. Horikoshi, H. Hidaka, and J. Zhao: *Photochem. Photobiol. Sci.* **1**, 970 (2002).