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Comparative studies of the influence of cyclodextrins on the stability of the sunscreen agent, 2-ethylhexyl-*p*-methoxycinnamate

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Abstract

The effects of β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) on the base-catalyzed degradation and light-induced decomposition of the sunscreen agent, *trans*-2-ethylhexyl-*p*-methoxycinnamate (*trans*-EHMC) were investigated. Reversed-phase liquid chromatography was used to study the interaction between natural and modified cyclodextrins, added to the mobile phase, and the sunscreen. Among the available cyclodextrins (β-CD, HP-β-CD, hydroxypropyl-α-cyclodextrin and hydroxypropyl-γ-cyclodextrin), only HP-β-CD and β-CD produced a significant decrease in the chromatographic retention of *trans*-EHMC. The complexation of the sunscreen agent with HP-β-CD and β-CD was confirmed by thermal analysis and nuclear magnetic resonance spectroscopy. β-CD depressed the decomposition of *trans*-EHMC in alkaline solutions more effectively than HP-β-CD. Moreover, the irradiation-induced degradation of the sunscreen agent in emulsion vehicles was reduced by complexation with β-CD (the extent of degradation was 26.1% for the complex compared to 35.8% for free *trans*-EHMC) whereas HP-β-CD had no significant effect. Therefore, the complex of β-CD with *trans*-EHMC enhances the chemical- and photo-stability of the sunscreen agent. Moreover, it limits adverse interactions of the UV filter with other formulation ingredients.

Keywords: Sunscreen agent; *trans*-2-Ethylhexyl-*p*-methoxycinnamate; Cyclodextrin; Complexation; Hydrolysis; Photodegradation

1. Introduction

There is overwhelming evidence indicating that human skin is damaged in different ways by exposure to sunlight [1]. Within the solar radiation reaching the earth's surface, the UV component (290–400 nm) is the major factor leading to skin

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pathologies including inflammatory responses (i.e. erythema, oedema) and long-term effects such as cutaneous photoageing, immunosuppression, non-melanoma and melanoma skin cancers [1–4]. The expanding knowledge of the deleterious effects of sunlight has promoted the widespread use of topical sunscreening preparations [1,5,6]. Organic sunscreen agents are compounds which decrease the dose of UV light reaching human skin by absorbing the radiation via electron promotion from a lower-energy molecular orbital to a higher-energy one. The activated sunscreen molecule dissipates the excess energy in the form of heat, by fluorescence, phosphorescence, interaction with neighboring molecules or by undergoing photochemical modifications [7,8]. The latter mechanism not only reduces the screening efficiency [8–10] during usage but can also leads to accumulation on the skin of harmful degradation products which have been associated with irritation and photoallergy to sunscreen preparations [11,12]. Therefore, special attention should be paid to the photostability characteristics of sunscreen agents.

The photochemical behavior of *trans*-2-ethylhexyl-*p*-methoxycinnamate (*trans*-EHMC; Fig. 1) is of fundamental interest since it represents the most widely used sunscreen compound [5,7]. It is approved by the regulatory authorities of Europe [13], USA [14], Japan and Australia [5]. *trans*-EHMC is classified as an UV-B filter because it absorbs most effectively the shorter wavelength band (290–320 nm) of the solar UV radiation, which is regarded as the most deleterious [1,4]. The photo-induced degradation of *trans*-EHMC has been demonstrated by several researchers both in solution [7,9,10] and in emulsion formulations [11]. Moreover, photoallergic contact reactions to *trans*-EHMC have been reported in the literature [15,16]. Consequently, in order to ensure adequate efficacy and safety for this sunscreen agent, new systems exhibiting enhanced *trans*-EHMC photostability are required.

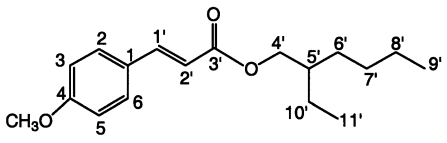

Fig. 1. Chemical structure of *trans*-EHMC.

Cyclodextrins are cyclic oligosaccharides which can entrap appropriately sized molecules into their hydrophobic cavities [17,18]. This non-covalent inclusion complexation can increase the apparent aqueous solubility and dissolution rate of poorly water-soluble drugs and improve the stability to air and light of labile compounds [17]. In previous papers, we demonstrated that the photodegradation of the UV-A (320–400 nm) filter, butylmethoxydibenzoylmethane (BM-DBM) [19] and of the UV-B sunscreen agent, 2-ethylhexyl-*p*-dimethylaminobenzoate [20] was reduced by inclusion in hydroxypropyl-β-cyclodextrin (HP-β-CD), although this effect was more significant in solution than in emulsion formulations. In the present work, the preparation and characterization of the complex between *trans*-EHMC and β-cyclodextrin (β-CD) or HP-β-CD are reported. In addition, the influence of the complexation on the chemical and photochemical stability of this sunscreen agent are also presented.

2. Materials and methods

2.1. Materials

trans-EHMC and BM-DBM were supplied by Hoffmann-La Roche Ltd. (Basel, Switzerland). The cyclodextrins used in this study included: β-CD, HP-β-CD; (average molar substitution 0.6), hydroxypropyl-α-cyclodextrin (HP-α-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD). They were purchased from Aldrich Chimica (Milan, Italy). Methanol, acetonitrile and water were high-performance liquid chromatography (HPLC)-grade from Merck (Darmstadt, Germany). All other chemicals were of analytical-reagent grade (Sigma, Milan, Italy).

2.2. High-performance liquid chromatography

The HPLC apparatus comprised a Model Lab-Flow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 20 μl

sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable wavelength UV–Vis detector set at 307 nm (JASCO, Tokyo, Japan). Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developpements, Le Fontanil, France). Sample injections were effected with a Model 80365 syringe (10 μl; Hamilton, Bonaduz, Switzerland). Separations were performed on a 5-μm Zorbax SB-CN column (250 × 4.6 mm i.d.) fitted with a guard column (5-μm particles, 4 × 2 mm i.d.) and eluted isocratically, at a flow-rate of 0.8 ml/min, with methanol–acetonitrile–water (55:20:25, v/v/v). The identity of *trans*-EHMC 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.

For the HPLC study of the interaction between *trans*-EHMC and cyclodextrins, a Hypersil BDS Phenyl column (150 × 3.0 mm i.d.) was used in conjunction with mobile phases consisting of methanol–water (50:50, v/v) mixtures containing cyclodextrins at various concentrations (0–4 mM). Chromatographic experiments were carried out at 25 ± 1 °C, under isocratic conditions and at a flow-rate of 0.4 ml/min. The retention factor, k' was calculated from the equation: $k' = (t_r - t_0)/t_0$ where t_r is retention time and t_0 the column dead time measured by injecting sodium nitrate as the non-retained sample.

2.3. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) was performed with a GC 8060 gas chromatograph (CE Instruments, Milan, Italy) coupled with a MD 800 mass spectrometer (TermoQuest Italia, Milan, Italy) operating in the electron impact mode (70 eV) with transfer line and ion source temperatures maintained at 250 °C. A SE-54 fused silica capillary column (25 m × 0.25 mm i.d.; CE Instruments) was used. The GC operating conditions were: injector temperature, 280 °C; column temperature, 100 °C for 2 min, then programmed at 10 °C/min to 270 °C; carrier gas (helium) inlet pressure, 70 kPa. The samples (1 μl) were introduced using split injection (split ratio 20:1). The GC–MS was controlled by the Mass Lab 1.12 software (TermoQuest Italia).

2.4. Phase solubility studies

Solubility measurements were carried out according to Higuchi and Connors [21]. An excess amount of *trans*-EHMC was added to aqueous solutions (5 ml) containing various concentrations (0–15 mM) of the different cyclodextrins examined. 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 analyzed for *trans*-EHMC by HPLC as outlined above. Data were determined from the mean of at least five tests.

2.5. Preparation of the complexes

The complex with β-CD was prepared by adding an equimolar concentration of *trans*-EHMC (58 mg, 0.2 mmol) to a solution of β-CD (227 mg, 0.2 mmol) in purified water (20 ml). The mixture was stirred for 24 h at room temperature and shielded from light. A precipitate was obtained which was collected by filtration and stored under vacuum in a dessicator until used. The preparation procedure of the *trans*-EHMC–HP-β-CD complex was as follows: *trans*-EHMC (72.6 mg, 0.25 mmol) was dissolved in methanol (8 ml) and added to 3 ml of an aqueous solution containing an equimolar quantity of HP-β-CD (345 mg, 0.25 mmol). The obtained opalescent 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 by rotary evaporation and the residue was kept in a desiccator until used. The content of *trans*-EHMC in each complex was determined by HPLC after proper dilution.

2.6. Thermal analysis

Differential thermal analysis (DTA) and thermal gravimetric analysis (TGA) were carried out on a Netzsch STA 409 simultaneous thermal analyzer (Netzsch Italiana, Verona, Italy). The

samples (4–15 mg) were accurately weighed in platinum pans (Netzsch) and heated at a scanning rate of 10 °C/min.

2.7. NMR spectroscopy

^{1}H-NMR spectra were recorded on a Bruker AC spectrometer (300 MHz). Samples were solubilized at a concentration of ca. 10 mM in DMSO-d_6. Chemical shifts are reported in ppm (δ) relative to TMS. Typical parameters for the ^{1}H-NMR spectra were: 0.4 Hz/pt resolution, 16 scans, 18 s relaxation delay, 90° pulse.

2.8. Base-catalyzed decomposition studies

The degradation studies were performed by adding *trans*-EHMC (0.18%, w/w) or its equivalent amount of cyclodextrin complex to methanolic solutions containing KOH (0.04 M). The samples were maintained at 25±1 °C in tightly stoppered containers, under continuous shaking and shielded from light. At appropriate time intervals, 50-μl aliquots of the medium were withdrawn and replaced with an equal volume of fresh medium. The test sample was neutralized by addition of HCl (0.04 M) and analyzed directly by HPLC for the assay of the undecomposed *trans*-EHMC and of its degradation product. The degradation profiles were obtained by plotting the logarithm of *trans*-EHMC concentration versus time. The rate constants were determined from the slopes of the linear semilogarithmic plots.

2.9. Photodegradation studies

Photochemical measurements were carried out in lotion preparations (oil-in-water emulsion) containing *trans*-EHMC (0.2%, w/w) alone or complexed with cyclodextrins. In some irradiation experiments the UV-A filter, BM-DBM was also incorporated (0.15%, w/w) into the lotion formulation. The lotion excipients were: sorbitan monostearate, polyoxyethylene sorbitan monostearate, butylated hydroxyanisole, *p*-hydroxybenzoic acid methyl ester or sodium benzoate, isopropyl isostearate (Henkel, Fino Mornasco, Italy), cetearyl isononanoate (Henkel), cetearyl alcohol (Henkel), D-sorbitol, dehydroacetic acid, EDTA and water (lotion pH, 5.0). A portion of the test sample (700–730 mg) containing free or complexed *trans*-EHMC, was transferred into a quartz cuvette (path-length, 2 mm) and then exposed to the solar simulator, as described in a previous study [20]. After the appropriate exposure interval (4 h), the cuvette was removed and its content quantitatively transferred into a 10-ml calibrated flask, diluted to volume with methanol and filtered (0.45-μm membrane filter). A portion (5 μl) of the resulting solution was analyzed by HPLC for both *trans*-EHMC and the photolytic degradant. All samples were protected from light both before and after irradiation. The degree of photodegradation was measured by comparing the peak areas of *trans*-EHMC from the irradiated samples, with those obtained by analysis of an equivalent amount of the unirradiated preparation.

2.10. In-vitro sun protection factor measurement

The in-vitro determination of the lotion sun protection factor (SPF) was carried out according to the Diffey and Robson [22] technique, with minor modifications as reported in an earlier study [20].

3. Results and discussion

3.1. HPLC study of the interaction of trans-EHMC with cyclodextrins

In a previuos report [23], the complexation of EHMC-related compounds, including methyl and ethyl cinnamate, with α- and β-CDs has been examined by phase–solubility diagrams. However, in the present study, a large variability was observed for the solubility values of *trans*-EHMC in the aqueous solutions of the different cyclodextrins examined. This can be traced to the fact that *trans*-EHMC is a liquid with a density of ca. 1 (1.007–1.012 at 25 °C) and hence it is difficult to separate from the aqueous phase without contamination. In order to overcome this problem, a different strategy was investigated based on reversed-phase HPLC. In this chromato-

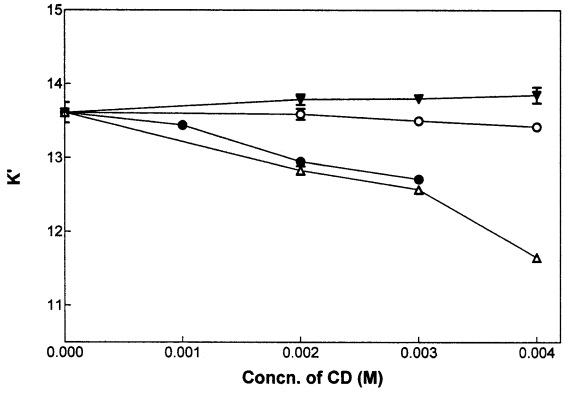

Fig. 2. Dependence of *trans*-EHMC capacity factor (k') on the concentration of different cyclodextrins in the eluent at 25 °C. Key: (▼) HP-α-CD; (○) HP-γ-CD; (●) β-CD and (△) HP-β-CD. Each point represents the mean ± S.D. of triplicate experiments. Lines connecting the data points are shown. ANOVA analysis of variance: HP-α-CD, $F = 3.52$, df = 11, $P > 0.05$; HP-γ-CD, $F = 3.28$, df = 11, $P > 0.05$; β-CD, $F = 93.97$, df = 11, $P < 0.001$; HP-β-CD, $F = 320.91$, df = 11, $P < 0.001$. Chromatographic conditions: column, Hypersil BDS Phenyl; mobile phase, 50% methanol in water; flow-rate, 0.4 ml/min.

graphic mode, it is known that the introduction of cyclodextrins in the mobile phase can cause a decrease in the retention of sample molecules owing to complex formation [24,25]. Since this modification of the retention characteristics is closely related to the complex stability, the extent of solute interaction with different cyclodextrins can be estimated from chromatographic data [24,25]. Fig. 2 illustrates the influence of various concentrations of the examined cyclodextrins, in methanol–water (50:50, v/v) mobile phase, on *trans*-EHMC retention factor (k'). Methanol was selected as the eluent organic modifier because of its limited interference with cyclodextrin inclusion activity [24] as compared to other organic solvents (e.g. acetonitrile, tetrahydrofuran) used in reversed-phase systems. No significant variation (ANOVA analysis of variance) in the k' values was observed with increasing concentrations of HP-α-CD and HP-γ-CD in the mobile phase (Fig. 2). On the other hand, the addition of β-CD or HP-β-CD to the eluent produced a marked decrease in the chromatographic retention of *trans*-EHMC (Fig. 2). These results indicate that β-CD and HP-β-CD interact more strongly with *trans*-EHMC than the other cyclodextrins and consequently they were selected for further experiments. Moreover, a linear relationship ($r > 0.97$) was found between $1/k'$ and β-CD or HP-β-CD concentrations in the eluent which suggests, in accordance to previously described equations [24,25], a 1:1 stoichiometry for the complexes.

3.2. Complex characterization

^{1}H-NMR spectral studies were carried out to gain further information on the interaction between *trans*-EHMC and the cyclodextrins. The effects of HP-β-CD and β-CD on the chemical shift values of selected protons of *trans*-EHMC are summarized in Table 1. The aromatic proton signals were shifted upfield (negative $\Delta\delta$ values) while a downfield shift (positive $\Delta\delta$ values) was observed for the protons belonging to the alkyl chain, with the exception of H1′ (see Fig. 1). These findings suggest that the aromatic portion of the sunscreen molecule is located inside the cyclodextrin cavity with the ester group close to the external surface of the macrocycle [26]. Moreover, the changes in the ^{1}H signals measured in the presence of HP-β-CD were of lower magnitude than those induced by β-CD (Table 1), indicating a stronger interaction of *trans*-EHMC with the latter cyclodextrin.

The characterization of the complexes in the solid state was performed by thermal analysis. The

Table 1
^{1}H-NMR chemical shift changes ($\Delta\delta$, ppm) for *trans*-EHMC in the presence of cyclodextrins

Protons	$\Delta\delta$ [a]	
	HP-β-CD	β-CD
H2, H6	−0.007	−0.008
H3, H5	−0.004	−0.004
OCH_3	−0.003	−0.004
H1′	−0.003	−0.004
H2′	+0.002	+0.010
H4′	+0.001	+0.007
H5′	+0.003	+0.014
H6′, H7′, H8′	+0.002	+0.012

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

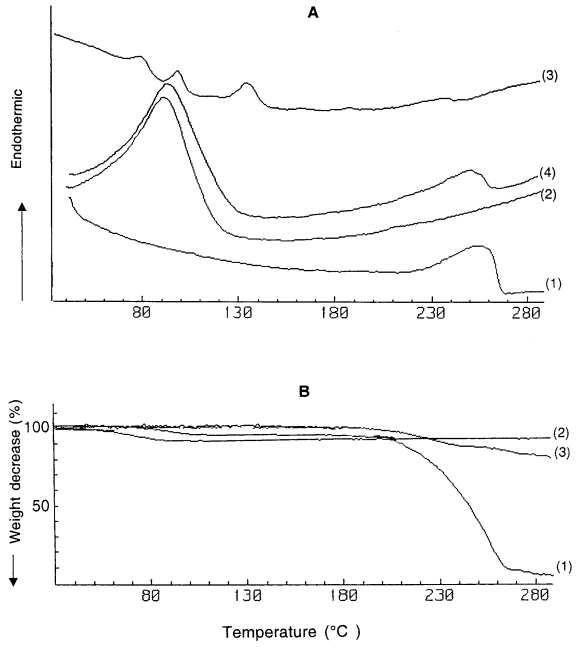

Fig. 3. DTA (A) and TGA (B) thermograms of *trans*-EHMC (1), β-CD (2), *trans*-EHMC–β-CD complex (3) and *trans*-EHMC–β-CD physical mixture (4).

DTA profiles and the corresponding TGA curves of β-CD, *trans*-EHMC and its complex with β-CD are illustrated in Fig. 3A and B, respectively. In addition, the thermogram of the physical mixture is reported in Fig. 3A. β-CD exhibited a broad peak near 90 °C (Fig. 3A) which can be traced to the release of water. *trans*-EHMC displayed an endothermic transition at about 255 °C (DTA curve) corresponding to its boiling temperature, as confirmed by the weight loss observed in the TGA trace (Fig. 3B). This transition disappeared in the DTA thermogram of the complex but was present in the physical mixture profile (Fig. 3A). Moreover, the TGA curve of complexed *trans*-EHMC (Fig. 3B) showed a marked reduction of the sunscreen volatility. These changes in thermal behavior indicated the inclusion of *trans*-EHMC into the β-CD cavity. Similar thermograms were obtained for the HP-β-CD–*trans*-EHMC complex.

3.3. Chemical degradation studies

Since *trans*-EHMC contains an ester functional group, its solvolysis represents one of the main degradation mechanisms of the sunscreen. Because of its poor aqueous solubility, the decomposition of *trans*-EHMC was studied in methanol. Moreover, base catalysis was used to accelerate the reaction. Under these conditions, methyl-*p*-methoxycinnamate (MMC) was identified as the only breakdown product by GC–MS analysis. The course of reaction was followed by HPLC measuring the remaining concentration of the parent compound. The data generated for the decompositon of *trans*-EHMC in the absence and presence of β-CD are depicted in Fig. 4. The obtained linear relationships ($r > 0.99$) indicated that the base-catalyzed degradation of *trans*-EHMC in methanolic solutions is described by a pseudo-first-order kinetic. Moreover, statistical analysis (unpaired *t*-test) of the rate constants calculated

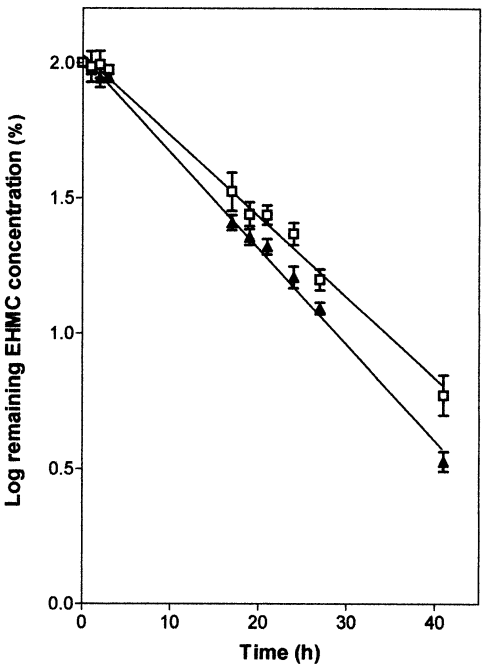

Fig. 4. Semilogarithmic plots of the degradation of free and cyclodextrin-complexed *trans*-EHMC in methanolic KOH (0.04 M) at 25 °C. Key: (▲) *trans*-EHMC; (□) *trans*-EHMC–β-CD. Values are means ± S.D. of at least three experiments.

for the free sunscreen ($k = 0.081 \pm 0.002$/h) and its cyclodextrin complex ($k = 0.069 \pm 0.002$/h) showed that the decomposition of *trans*-EHMC was significantly decreased ($P < 0.01$) when the sunscreen is complexed with β-CD. The pseudo-first-order kinetic behavior is also valid for the system with HP-β-CD (graph not shown). However, in this case the effect observed on the rate constant was not statistically significant (unpaired *t*-test, $P > 0.2$). The superior stabilizing properties of β-CD as compared to HP-β-CD can be explained in terms of a higher degree of interaction of the ester moiety with β-CD, as shown by the NMR data (Table 1). The degradation of *trans*-EHMC was not completely inhibited by β-CD complexation (Fig. 4). This can be ascribed to the location of the ester group which, according to the NMR studies reported above, is protruding from the cyclodextrin cavity and hence it is not efficiently shielded against the nucleophilic attack by CH_3O^-.

3.4. Photodegradation studies

In order to examine the effect of HP-β-CD and β-CD on the photochemical behavior of *trans*-EHMC, the photolysis experiments were performed on a lotion (oil-in-water emulsion) as a base. This system was selected as a model formulation since it represents the most common type of sunscreen preparation [27] and hence simulates the actual conditions in the finished suncare product [11]. Free or complexed *trans*-EHMC was incorporated into the lotion containing *p*-hydroxybenzoic acid methyl ester as one of the preservative and exposed for 4 h to the solar simulator. During the light-stability measurements, the applied UV-B energy was equivalent to 10 Minimal Erythemal Dose (MED) which is considered representative of daily sunlight irradiance [9,11]. The only product originated from the photodecomposition of *trans*-EHMC was identified by GC–MS and NMR as *cis*-EHMC, in accordance with previous reports in the literature [9–11]. Since the *cis*-isomer exhibits reduced UV absorptivity compared to *trans*-EHMC [9,10], the photo-induced isomerization of the sunscreen agent decreases its sun protection activity. Photodimers of *trans*-EHMC have been detected by

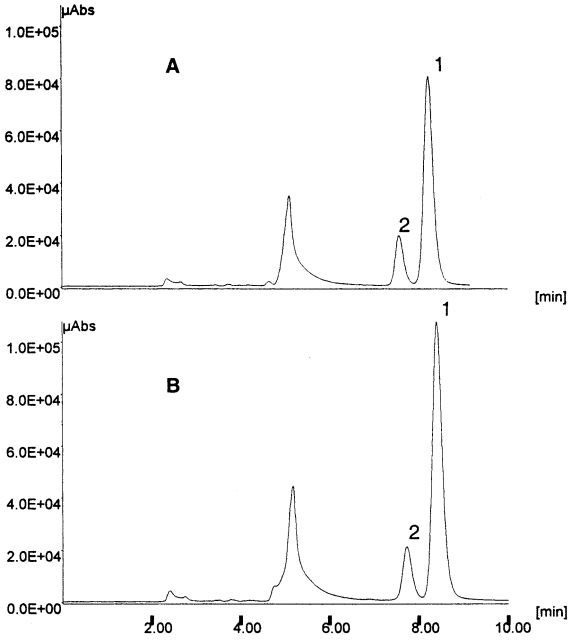

Fig. 5. HPLC chromatograms of sample lotions containing: (A) free *trans*-EHMC; or (B) *trans*-EHMC–β-CD complex, after 4 h irradiation with the solar simulator. Peaks: 1, *trans*-EHMC; 2, *cis*-EHMC. Operating conditions as described in Section 2.

Table 2
Comparative photodegradation data for free and complexed *trans*-EHMC after 4 h irradiation with the solar simulator

Formulation	% Sunscreen loss[a]		P[b]
	trans-EHMC alone	*trans*-EHMC/β-CD	
Lotion A[c]	35.2 ± 0.6	30.8 ± 2.5	< 0.003
Lotion B[d]	35.8 ± 2.0	26.1 ± 3.2	< 0.002
Lotion C[e]	30.3 ± 2.2	24.2 ± 2.9	< 0.02

[a] Each value represents the mean ± S.D. of six determinations.
[b] P values are calculated by unpaired *t*-test. Significance was taken as $P < 0.05$.
[c] Formulation containing *p*-hydroxybenzoic acid methyl ester.
[d] Formulation containing sodium benzoate and 5% (w/w) β-CD.
[e] Formulation containing BM-DBM (0.15%, w/w).

Broadbent et al. [7] employing rather drastic irradiation conditions (high-pressure mercury lamp, 13.5-h irradiation time) which do not

simulate natural exposure to the sunlight. The extent of photodegradation of the UV filter was measured by HPLC (representative chromatograms are shown in Fig. 5). In the preparation containing *trans*-EHMC alone, the percentage loss of the sunscreen agent reached 35.2% (Table 2). This is in good agreement with the data reported by Berset et al. [9]. A statistically significant reduction of the extent of degradation to 30.8% (Table 2) was attained in the lotion containing *trans*-EHMC complexed with β-CD. At variance with the data obtained for β-CD, the irradiation-induced decomposition of the sunscreen was not significantly affected by HP-β-CD. Hence, the photostability effects of the examined cyclodextrins correlate with their protective activity in alkaline conditions. In a previous study [20], we demonstrated that the effectiveness of cyclodextrin complexation in emulsion preparations could be hampered by the excipient competitive displacement of the guest molecule from the cavity of the macrocycle. Accordingly, in an attempt to maximize the photostability enhancement achieved by β-CD, special attention was paid in the present investigation to the design of the lotion vehicle. This approach led to the use of sodium benzoate instead of *p*-hydroxybenzoic acid methyl ester, which was selected initially as a preservative and which has been reported to interact with β-CD [26]. In addition, an excess (5%, w/w) of β-CD was included in the formulation. Under these conditions, a 26.1% decrease in the level of the UV filter was measured in the lotion containing the β-CD–*trans*-EHMC complex, whereas 35.8% of the sunscreen agent was lost following irradiation of the preparation containing free *trans*-EHMC (Table 2). These data demonstrated a further reduction of *trans*-EHMC photodegradation by β-CD complexation. In addition, the in-vitro determination of the SPF of the two formulations showed that complexation had no significant influence ($P > 0.05$, unpaired t-test) on the SPF (the SPF values ranged from 2.3 to 2.5).

Because of the important role of UV-A radiation in contributing to the harmful effects of sun exposure [1,10], sunscreen products effective against UV-B and -A rays are recommended [1]. In particular, the combination of *trans*-EHMC with one of the most widely used UV-A filters [19], BM-DBM, is frequently present in typical sun-care preparations [28]. In order to simulate these conditions, further studies were performed on lotions containing BM-DBM in conjunction with *trans*-EHMC or its complex with β-CD. The degree of *trans*-EHMC photodegradation decreased in the presence of BM-DBM (Table 2), due to the protecting effect of the latter against the additional decomposition caused by the UV-A radiation [28] of the simulated sunlight. Also in this case, the photo-instability of *trans*-EHMC was reduced (from 30.3 to 24.2%) by inclusion into the β-CD cavity (Table 2). Moreover, the percentage loss of BM-DBM following irradiation was smaller ($16.4 \pm 0.5\%$) in the formulation containing complexed *trans*-EHMC as compared to the lotion containing *trans*-EHMC alone ($22.6 \pm 0.8\%$). Since BM-DBM undergoes a photo-induced reaction with *trans*-EHMC leading to adduct formation [28], its improved stability in the presence of the β-CD–*trans*-EHMC complex can be traced to hindered interaction with the cyclodextrin enclosed UV-B filter.

4. Conclusions

The results obtained demonstrated that in addition to reducing the degradation of *trans*-EHMC under alkaline conditions, complexation with β-CD was effective in enhancing the photostability of the sunscreen agent. Moreover, the inclusion of *trans*-EHMC into the β-CD cavity limits unfavorable interactions with other formulation components. The *trans*-EHMC–β-CD complex can be considered a promising way to improve the physico-chemical properties of the UV filter.

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References

[1] National Institute of Health Consens Statement Online, Sunlight, Ultraviolet Radiation and the Skin 7 (1989) 1–29.
[2] M.A. Pathak, Skin Pharmacol. 4 (1991) 85–94.
[3] A. Ziegler, A.S. Jonason, D.J. Leffell, J.A. Simon, H.W. Sharma, J. Kimmelman, L. Remington, T. Jacks, D.E. Brash, Nature 372 (1994) 773–776.
[4] M. Hochberg, C.D. Enk, Photochem. Photobiol. 70 (1999) 766–772.
[5] C.G. Hayden, M.S. Roberts, H.A.E. Benson, Aust. N.Z. J. Med. 28 (1998) 639–646.
[6] A. Green, G. Williams, R. Neale, V. Hart, D. Leslie, P. Parsons, G.C. Marks, P. Gaffney, D. Battistutta, C. Frost, C. Lang, A. Russell, Lancet 354 (1999) 723–729.
[7] J.K. Broadbent, B.S. Martincigh, M.W. Raynor, L.F. Salter, R. Moulder, P. Sjoberg, K.E. Markides, J. Chromatogr. A732 (1996) 101–110.
[8] R. Stokes, B. Diffey, Int. J. Cosmet. Sci. 21 (1999) 341–351.
[9] G. Berset, H. Gonzenbach, R. Christ, R. Martin, A. Deflandre, R. Mascotto, J.D. Jolley, W. Lowell, R. Pelzer, T. Stiehm, Int. J. Cosmet. Sci. 18 (1996) 167–177.
[10] N. Tarras-Wahlberg, G. Stenhagen, O. Larkö, A. Rosén, A. Wennberg, O. Wennerström, J. Invest. Dermatol. 113 (1999) 547–553.
[11] A. Deflandre, G. Lang, Int. J. Cosmet. Sci. 10 (1988) 53–62.
[12] S.H. Dromgoole, H.I. Maibach, J. Am. Acad. Dermatol. 22 (1990) 1068–1078.
[13] European Economic Community Council Directive 76/768, Annex VII, 1976.
[14] US Food and Drug Administration, Federal Register 64, 1999, p. 27666.
[15] K. Kimura, T. Katoh, Contact Dermatitis 32 (1995) 304–305.
[16] T. Schmidt, J. Ring, D. Abeck, Dermatology 196 (1998) 354–357.
[17] T. Loftsson, M.E. Brewster, J. Pharm. Sci. 85 (1996) 1017–1025.
[18] R.A. Rajewski, V.J. Stella, J. Pharm. Sci. 85 (1996) 1142–1169.
[19] S. Scalia, S. Villani, A. Scatturin, M.A. Vandelli, F. Forni, Int. J. Pharm. 175 (1998) 205–213.
[20] S. Scalia, S. Villani, A. Casolari, J. Pharm. Pharmacol. 51 (1999) 1367–1374.
[21] T. Higuchi, K.A. Connors, Adv. Anal. Chem. Instrum. 4 (1965) 117–212.
[22] B.L. Diffey, J. Robson, J. Soc. Cosmet. Chem. 40 (1989) 127–133.
[23] K. Uekama, F. Hirayama, K. Esaki, M. Inoue, Chem. Pharm. Bull. 27 (1979) 76–79.
[24] K. Fujimura, T. Ueda, M. Kitagawa, H. Takayanagi, T. Ando, Anal. Chem. 58 (1986) 2668–2674.
[25] R.M. Mohseni, R.J. Hurtubise, J. Chromatogr. 499 (1990) 395–410.
[26] L.W. Chan, T.R.R. Kurup, A. Muthaiah, J.C. Thenmozhiyal, Int. J. Pharm. 195 (2000) 71–79.
[27] E. Siemer, in: W. Umbach (Ed.), Cosmetics and Toiletries, Ellis Horwood, New York, 1991, pp. 98–99.
[28] W. Johncock, Cosmet. Toil. 114 (1999) 75–82.