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Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 29–34
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Complexation of the sunscreen agent, 4-methylbenzylidene camphor with cyclodextrins: Effect on photostability and human stratum corneum penetration

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Received 20 September 2006; received in revised form 9 January 2007; accepted 10 January 2007
Available online 16 January 2007

Abstract

The interaction between the sunscreen agent, 4-methylbenzylidene camphor (4-MBC) and hydrophilic α-, β- and γ-cyclodextrin derivatives was investigated in water by phase-solubility analysis. Among the studied cyclodextrins, random methyl-β-cyclodextrin (RM-β-CD) had the greatest solubilizing activity. The complexation of the sunscreen agent with RM-β-CD was confirmed by nuclear magnetic resonance spectroscopy and powder X-ray diffractometry. The light-induced decomposition of 4-MBC in emulsion vehicles was markedly decreased by complexation with RM-β-CD (the extent of degradation, determined by HPLC, was 7.1% for the complex compared to 21.1% for free 4-MBC). The influence of RM-β-CD on the human skin penetration of the sunscreen was investigated in vivo using the tape stripping method, a useful procedure for selectively removing the outermost cutaneous layers. Considerable quantities (21.2–25.1% of the applied dose) of 4-MBC permeated in the stratum corneum. However, no significant differences in the amounts of UV filter in the 10 first strips of the horny layer were observed between the formulations containing 4-MBC free or complexed with RM-β-CD. Therefore, RM-β-CD complexation did not alter the retention of 4-MBC in the superficial layers of the stratum corneum, where its action is more desirable.

Keywords: Sunscreen agent; 4-Methylbenzylidene camphor; Cyclodextrin complexation; Photodegradation; Stratum corneum penetration; Tape stripping

1. Introduction

Exposure to the UV radiation from the sun (290–400 nm) is associated with several harmful effects on human skin including erythema, photoageing, immune suppression and skin-cutaneous cancer, the latter representing the most prevalent form of human neoplasia [1–3]. These findings have prompted the widespread use of topical sun protective preparations [1,4]. The most common active ingredients in these products are organic sunscreen agents which attenuate the transmission of the solar UV rays to the skin by absorbing the radiation [1,5]. Sunscreen photostability is an essential requirement for the efficacy and safety of UV filters, since their decomposition during sunlight exposure not only decreases the initial photoprotective capacity but can also generate harmful photolytic products [4,6,7]. Another important characteristic that sunscreens should have is minimal percutaneous penetration as they exert their effect on the skin surface [8,9].

The present study focuses on 4-methylbenzylidene camphor (4-MBC; Fig. 1) since it is an important organic UV filter, widely used in sunscreen and cosmetic products [4,6]. In addition, it has been shown to stabilize the photolabile sunscreen agent, butyl methoxydibenzoylmethane [10]. 4-MBC is classified as an UV-B filter since it absorbs most efficiently in the 290-320 nm range and it is included in the list of authorized UV filters in Europe, Australia and is being considered for use in the USA [10]. Although 4-MBC has been regarded as rather photostable [6,11], recent studies have demonstrated that this sunscreen agent undergoes marked degradation under sunlight exposure [12,13]. Therefore, new systems with enhanced 4-MBC photostability are required. Various strategies have been investigated to protect UV filters against light-induced degradation, including polymeric or lipid micro- and nanoparticles [14,15] and inclusion complexes with cyclodextrins

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doi:10.1016/j.jpba.2007.01.016

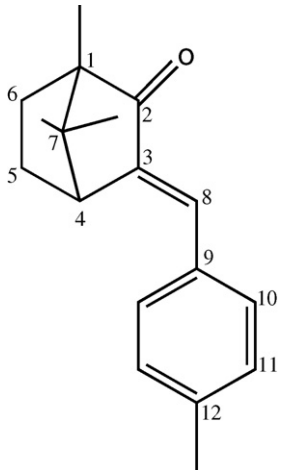

Fig. 1. Chemical structure of 4-MBC.

[16]. The latter are cyclic oligosaccharides which can entrap appropriately sized lipophilic compounds into their hydrophobic cavities by forming non-covalent inclusion complexes [17]. This complexation phenomenon can enhance the stability to air and light of the included molecule [17]. Accordingly, previous reports [7,16,18] demonstrated that complexation with cyclodextrins decreased the photolysis of the sunscreen agents, octyl-dimethylaminobenzoate, octyl-*p*-methoxycinnamate and phenylbenzimidazole sulphonic acid. Published studies on the interaction between cyclodextrins and 4-MBC [19] have not examined the influence of complexation on the photochemical stability of the UV filter, nor on its skin permeation. The current investigation was thus undertaken to assess the potential of cyclodextrins in reducing the decomposition of 4-MBC under simulated sunlight irradiation. Moreover, the effect of complexation on the in vivo penetration of the UV filter into human stratum corneum was also evaluated.

2. Materials and methods

2.1. Materials

The cyclodextrins used in this study included: hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-α-cyclodextrin (HP-α-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD) and random methyl-β-cyclodextrin (RM-β-CD). They were purchased from Aldrich Chimica (Milan, Italy). 4-Methylbenzylidene camphor (4-MBC) was a gift from Merck (Darmstadt, Germany). Methanol, acetonitrile and water were high-performance liquid chromatography (HPLC)-grade from Merck. The excipients for the cream preparation were obtained from Sigma (Steinheim, Germany) and Henkel (Fino Mornasco, Italy). All other chemicals were of analytical-reagent grade (Sigma).

2.2. High-performance liquid chromatography

The HPLC apparatus comprised a Model LabFlow 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 (Jasco, Tokyo, Japan) set at 298 nm. 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 701 syringe (10 μl; Hamilton, Bonaduz, Switzerland). Separations were performed according to the method of Simeoni et al. [20], with minor modifications. A 5-μm Zorbax SB-CN column (150 mm × 3.0 mm i.d.) fitted with a guard column (5-μm particles, 4 mm × 2 mm i.d.) was eluted isocratically, at a flow-rate of 0.4 ml/min, with methanol–acetonitrile–water (35:25:40, v/v/v). The identity of 4-MBC 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. 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) with helium as the carrier gas (inlet pressure, 70 kPa) was used. The GC operating conditions were: injector temperature, 250 °C; column temperature, 100 °C for 1 min, then programmed at 12 °C/min to 250 °C. 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 analyses were carried out according to Higuchi and Connors [21]. An excess amount of 4-MBC was added to aqueous solutions (4 ml) containing increasing concentrations (0–60 mM) of the examined cyclodextrins. The obtained suspensions were stirred in screw-capped vials at 25 ± 1 °C and shielded from light. After equilibrium was reached (3 days), the content of each vial was filtered through 0.20-μm membrane filters (Albet, Barcelona, Spain) and analysed for 4-MBC by HPLC as outlined above. Data were determined from the average of at least three determinations. Solubility diagrams were constructed for each cyclodextrin by plotting the molar concentration of 4-MBC 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 the sunscreen in the absence of cyclodextrins and slope is the slope of the obtained phase solubility diagram.

2.5. Inclusion complex preparation

The inclusion complexes were prepared at a 1:1 molar ratio of 4-MBC to HP-β-CD or RM-β-CD. The sunscreen agent (64.0 mg, 0.25 mmol) was dissolved in methanol (3.0 ml) and added to 6 ml of purified water containing an equimolar 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 amount of 4-MBC in each complex was determined by HPLC after proper dilution.

2.6. 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.

2.7. NMR spectroscopy

^{1}H NMR spectra were recorded on a Varian Mercury Plus spectrometer (400 MHz). Since the aqueous solubility of 4-MBC is too low for the recording of NMR spectra, samples were solubilized in CD_3OD, at a concentration of ca. 10 mM. 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.

2.8. UV spectrophotometry

UV spectra were recorded in methanol on a UV–vis/NIR Spectrometer (Lambda 19; Perkin-Elmer, Norwalk, USA).

2.9. Photodegradation studies

Photodecomposition experiments were performed in cream preparations (oil-in-water emulsion) containing 4-MBC (1.0%, w/w) alone or complexed with cyclodextrins. The formulation excipients were: sorbitan monostearate (2%), polyoxyethylene sorbitan monostearate (4.5%), butylated hydroxyanisole (0.02%), isopropyl isostearate (12.0%), cetearyl alcohol (10.0%), sodium benzoate (0.1%), glycerine (2.0%), dehydroacetic acid (0.1%), tetrasodium EDTA (0.1%) and water (68%). The creams were prepared according to the common procedure used in compounding practice. The UV filter dissolved in isopropyl isostearate, or its cyclodextrin complex dispersed in water, was added in the cooling phase of the emulsion preparation at about 40 °C. A portion (40–50 mg) of the cream containing uncomplexed or complexed 4-MBC was distributed, as a uniform as possible layer by circular movements of a gloved finger, onto a Transpore™ tape (3M Health Care, Neuss, Germany) at a level of 2 mg/cm^2. The samples were then irradiated for 1h with a solar simulator (Suntest CPS+, Atlas, Linsengericht, Germany) 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 750 W/m^2. After the exposure interval, the Transpore™ tape was cut into small pieces and extracted with ethanol (5 ml) under sonication (15 min). The extraction was repeated twice with methanol and the combined fractions were adjusted to volume (20 ml). The obtained samples were filtered (0.45 μm membrane filters) and analysed for 4-MBC by HPLC. The degree of photodegradation was evaluated by measuring the percentage of recovered sunscreen agent with respect to non-exposed samples. The results were the average of eight experiments.

2.10. Tape stripping method

Ethical approval was obtained by the Ethics Committee of the University of Modena. After informed consent, 6 female human volunteers aged 25–30 years and free of dermatological disorders participated in the study. The same sunscreen preparations utilized for the photostability experiments were applied at a dose of 2 mg/cm^2 to areas (2 cm × 5 cm) on the intern forearm which were previously wiped with ethanol and dried. The cream samples containing free 4-MBC (1.0%, w/w) or its equivalent amount of RM-β-CD complex were randomly allocated to previously marked areas, respectively on the upper and lower part of the forearm of each volunteer. The formulations were homogeneously distributed by means of a gloved finger. At 30 min after application, the remaining product was removed from the treated area by light cleaning with a cotton swab and the skin was tape-stripped 10 times using a transparent adhesive tape (Scotch® 600 crystal clear tape; 3 M) [22]. The tape strips were applied under control conditions by pressing them 20 times with a 500 g roller and then removed. The first strip was added to the cotton swab for the assay of the non-penetrated 4-MBC [9,22,23]. The following nine tape strips were pooled separately into three groups (group 1: strips 2–4; group 2: strips 5–7; group 3: strips 8–10) for analysis of the sunscreen content. The procedure was also carried out with a formulation without 4-MBC and a blank was determined. The obtained specimens were extracted with 3 × 5 ml of methanol-acetonitrile (90:10, v/v) under sonication, diluted to volume (20 ml) filtered and the resulting solutions were analysed for 4-MBC by HPLC. The penetration results were expressed as percentage of the applied dose.

2.11. Statistical analysis

Data were analysed for significance by use of 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

Solubility analysis was used initially for studying the interaction of 4-MBC with cyclodextrins in water. Because of

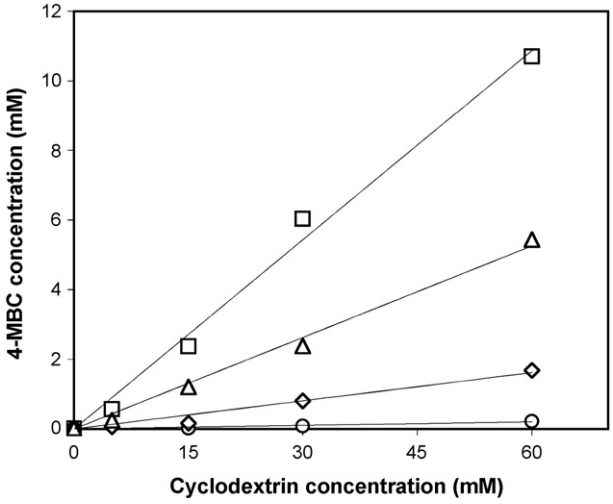

Fig. 2. Phase-solubility diagrams for 4-MBC with different cyclodextrins at 25 °C in purified water. Key: (◯) HP-α-CD; (◊) HP-γ-CD; (△) HP-β-CD; (□) RM-β-CD. Each point represents the mean of at least three experiments. The lines are the regression lines obtained using least squares linear regression analysis (RM-β-CD: $y = 0.174x + 0.109$, $r^2 = 0.993$; HP-β-CD: $y = 0.087 + 0.149$, $r^2 = 0.995$; HP-γ-CD: $y = 0.029x + 0.096$, $r^2 = 0.986$; HP-α-CD: $y = 0.003x + 0.013$, $r^2 = 0.948$).

the limited aqueous solubility of natural cyclodextrins [17], the highly water-soluble hydroxypropylated-(i.e., HP-α-CD, HP-β-CD and HP-γ-CD) and randomly methylated-(i.e., RM-β-CD) derivatives were selected for this investigation. Moreover, reduced interaction between the sunscreen agent and unmodified α-CD, β-CD and γ-CD has been previously reported [19]. Fig. 2 shows the influence of the examined cyclodextrins on the apparent aqueous solubility of 4-MBC. The diagrams obtained demonstrated that the major solubility enhancement was produced by RM-β-CD (from 0.011 to 10.710 mM), in accordance with the results of Fenyvesi et al. [19]. This indicated that this cyclodextrin exhibited stronger interactions with the sunscreen agent than HP-α-CD, HP-β-CD and HP-γ-CD. This effect may be ascribed to the extended hydrophobic surface of the RM-β-CD inner cavity [24]. The linear A_L-type relationship of the solubility profiles (Fig. 2) suggested a 1:1 stoichiometry for all complexes [21]. The stability constant ($K_{1:1}$) for the RM-β-CD/4-MBC inclusion complex, calculated according to the method of Higuchi and Connors [21], was $19{,}110.1 \pm 957.2\ M^{-1}$. The measured value is lower than that reported by Fenyvesi et al. [19] for the same system ($K_{1:1}$, $66{,}400\ M^{-1}$), this discrepancy being traceable to differences in the experimental conditions and RM-β-CD degree of substitution. For instance, the difference in the equilibrium times (1h versus 3 days) and the analytical techniques used for the 4-MBC assay (UV spectrophotometry versus HPLC), can affect the evaluation of the dissolved UV filter concentration and hence the stability constant value.

The interaction between 4-MBC and RM-β-CD was investigated in solution (CD_3OD) also by ^1H NMR spectroscopy, which provides the most conclusive evidence of complex formation [25]. Table 1 lists the major changes in the chemical shift values of 4-MBC protons (see Fig. 1 for 4-MBC structure and atom labels) induced by the presence of RM-β-CD. The largest variations were detected for the protons of the camphor moiety, thus indicating a stronger interaction of this portion of the sunscreen molecule with the cyclodextrin cavity.

Table 1
^1H NMR chemical shifts for 4-MBC in absence and presence of RM-β-CD

Protons	δ_{free}	$\delta_{complex}$	$\Delta\delta$[a]	$\delta_{complex}/\delta_{free}$
1-Me	0.782	0.809	0.027	1.034
7-Me	0.989	1.005	0.016	1.016
7-Me′	1.025	1.047	0.022	1.021
12-Me	2.352	2.363	0.011	1.005
H-4	3.134	3.148	0.014	1.004
H-8	7.154	7.167	0.013	1.002
H-11	7.224	7.236	0.012	1.002
H-10	7.397	7.407	0.010	1.001

[a] $\Delta\delta = (\delta_{complex} - \delta_{free})$.

The solid-state characterization of the 4-MBC/RM-β-CD complex was performed by powder X-ray diffractometry. As illustrated in Fig. 3, the 4-MBC crystalline peaks present in the X-ray diffraction pattern (Fig. 3A) of the physical mixture of the sunscreen with the cyclodextrin, were absent in the diffractogram of the complex (Fig. 3B). These results demonstrated the amorphous nature of this system, providing further evidence of the inclusion of the UV filter into the RM-β-CD cavity.

3.2. Photostability studies

In order to investigate the influence of RM-β-CD complexation on the photochemical reactivity of 4-MBC, the photostability experiments were performed on a cream (oil-in-

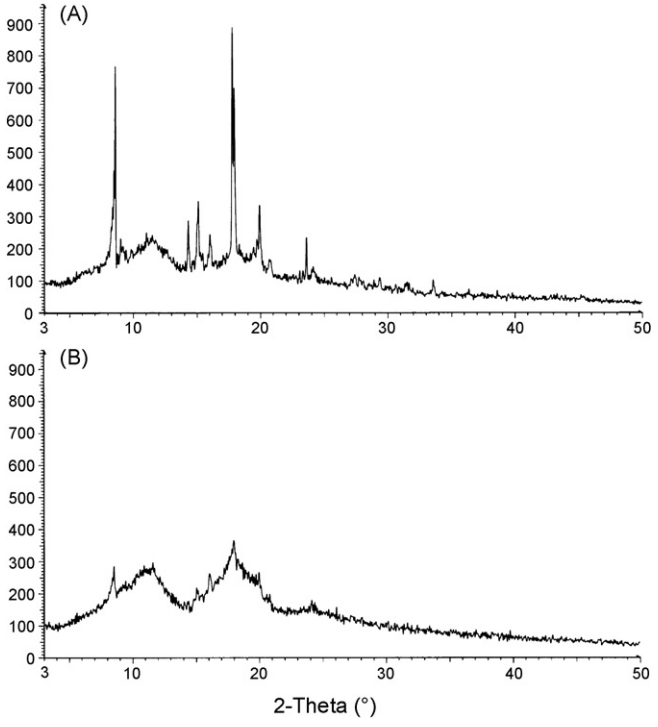

Fig. 3. Powder X-ray diffraction patterns of 4-MBC/RM-β-CD (1:1) physical mixture (A) and 4-MBC/RM-β-CD (1:1) complex (B).

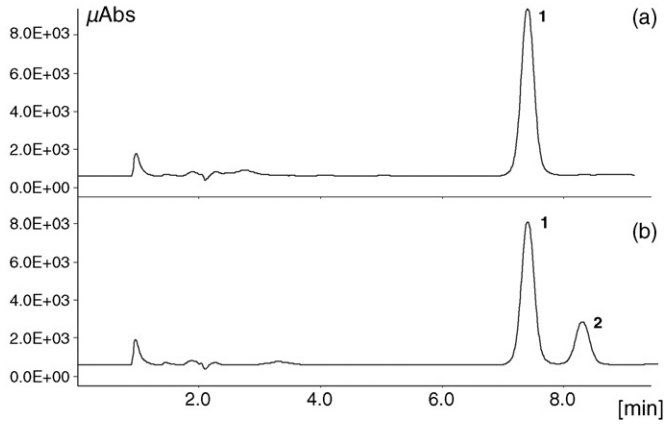

Fig. 4. HPLC chromatograms of a cream sample containing free 4-MBC, before (a) and after (b) 1h irradiation with the solar simulator. Other operating conditions as described in Section 2. Peaks: 1, *trans*-4-MBC; 2, *cis*-4-MBC.

water emulsion) as a topically applicable vehicle. This system was selected as a model formulation since it represents the most common type of sunscreen preparation [26] and hence simulate the conditions encountered in the real use of these products. The emulsions containing free 4-MBC or its complex with RM-β-CD were applied onto Transpore™ tapes (a surgical tape able to simulate the texture of human skin) and exposed to the solar simulator. The applied UV-B energy was equivalent to 10 minimal erythemal dose (MED) which is considered representative of daily solar emission [6]. GC–MS analysis showed that the only compound produced upon irradiation of 4-MBC exhibited the same mass spectrum as the original UV filter (spectra not shown) and thus it was assigned as a photoisomer. Since in commercial products 4-MBC consists entirely (>99%) of the *trans*-isomer [27], the photoproduct was identified as the *cis*-isomer. This is in accordance with a previously published study on the assessment of 4-MBC photostability [6]. The degree of the light-induced isomerization of the UV filter was measured by HPLC (representative chromatograms are shown in Fig. 4). In the formulation containing free 4-MBC, 21.1% of the sunscreen content was lost following irradiation (Table 2), in good agreement with the results of Gaspar and Campos [13]. Conversely, a marked reduction of the extent of degradation to 7.1% was attained in the cream containing the 4-MBC/RM-β-CD complex (Table 2). Additional photolysis experiments performed on an emulsion containing 4-MBC complexed with HP-β-CD (Table 2), showed that the photo-induced isomerization of the sunscreen agent was not decreased by HP-β-CD (the percentage loss of 4-MBC was 23.8%). Hence the photostabilization effect of the examined cyclodextrins correlated with their complexation strength ($K_{1:1}$, 19,110.1 M^{-1} for RM-β-CD and 8695.5 M^{-1} for HP-β-CD). Moreover, UV analysis of 4-MBC and its RM-β-CD complex indicated that the shape of the spectrum and the degree of UV absorption of the sunscreen were not affected by complexation (spectra not shown). The in vitro sun protection factor (SPF) of the creams was also determined, according to the Diffey and Robson technique [28]. The SPF values measured for the formulations containing the uncomplexed 4-MBC or its complex with RM-β-CD ranged from 2.9 to 3.1, the difference being not statistically significant ($P > 0.05$, unpaired *t*-test). This indicates that complexation does not affect the photoprotective capacity of the examined sunscreen preparations.

Table 2
Comparative photodegradation data for free and complexed 4-MBC in cream preparations, after 1 h irradiation with the solar simulator

Sample	4-MBC loss[a] (%)	P[b]
Free 4-MBC	21.1 ± 4.3	
4-MBC/RM-β-CD	7.1 ± 5.7	<0.001
4-MBC/HP-β-CD	23.8 ± 3.0	>0.1

[a] Each value is the mean ± S.D. of eight determinations.
[b] *P*-values (unpaired *t*-test) vs. free 4-MBC.

3.3. Tape stripping

Several studies have demonstrated that cyclodextrin complexation can influence the delivery of topically applied drugs, either enhancing or decreasing their percutaneous absorption [29]. Since sunscreens should be formulated to target the outermost cutaneous layers [8], the effect of RM-β-CD on the skin permeation of 4-MBC was also investigated. This aspect is particularly relevant since this sunscreen agent has been shown in vitro to be a potential endocrine disrupter [30]. The human skin penetration of 4-MBC was investigated in vivo, using the tape stripping technique which is a simple and minimal invasive procedure based on the removal of the horny layer step by step with an adhesive tape [31]. With this method the corneocyte aggregates as well as the topically applied substances are transferred to the tape strips. The quantitative determination of the amount fixed to each strip permits the in vivo evaluation of the stratum corneum distribution of dermatological products, useful for estimating their percutaneous absorption [31].

The same formulations submitted to the foregoing photolysis experiments and containing 4-MBC alone or complexed with RM-β-CD, were applied to the skin surface and the quantity of UV filter permeated into the stratum corneum was determined by tape stripping in combination with HPLC. The overall recoveries obtained as sum of unabsorbed 4-MBC and the sunscreen diffused into the horny layer removed by the tape strips were in the 76.7–96.4% range, which can be considered satisfactory taking into account that the stratum corneum was stripped only 10 times [23]. For both tested preparations, the majority of the applied sunscreen dose (57.0–65.3%) remained on the skin surface at the end of the experiment. However, a substantial amount of 4-MBC (21.2–25.1% of the applied dose) permeated into the stratum corneum, the highest proportion being found within the superficial layers (Fig. 5). The penetration profiles obtained for the UV filter in the horny layer (Fig. 5) were in good agreement with that reported by Weigmann et al. [32]. For each of the examined stratum corneum layers, the distribution of the UV filter was not significantly different ($P > 0.3$) between the emulsions containing free 4-MBC or its complex with RM-β-CD (Fig. 5). This indicated that cyclodextrin complexation did not modify the cutaneous availability of the sunscreen agent.

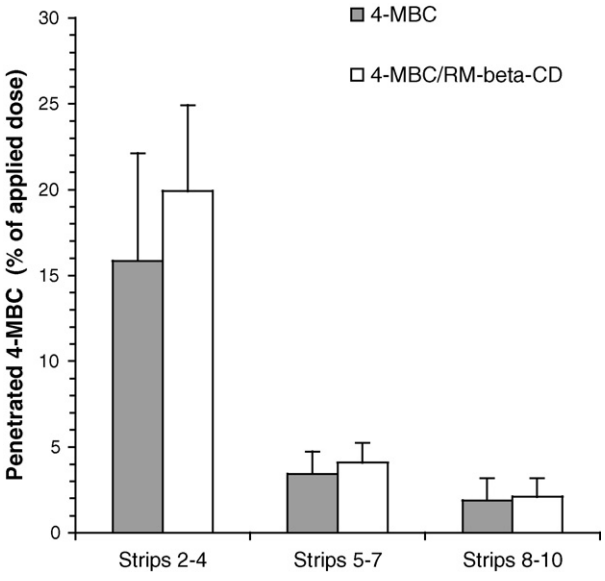

Fig. 5. Concentration profiles of 4-MBC in the stratum corneum in vivo, 30 min after application of creams containing free 4-MBC or its complex with RM-β-CD. The UV filter amounts in strips 2–4, 5–7 and 8–10 of the stratum corneum are shown (mean ± S.D., $n = 6$).

4. Conclusions

The results described in this study demonstrated that complexation of 4-MBC with RM-β-CD markedly reduced the degradation of the sunscreen agent without affecting its localized distribution on the skin surface. The 4-MBC/RM-β-CD complex can be considered a useful system to improve the UV filter efficacy.

Acknowledgement

The authors are grateful to MIUR for financial support.

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