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Microencapsulation of a cyclodextrin complex of the UV filter, butyl methoxydibenzoylmethane: In vivo skin penetration studies

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ABSTRACT

Lipid microparticles loaded with the complex between hydroxypropyl-β-cyclodextrin (HP-β-CD) and the sunscreen agent, butyl methoxydibenzoylmethane (BMDBM) were evaluated for their effect on the UV filter percutaneous penetration. The microparticles were prepared by the melt emulsification technique using tristearin as lipidic material and hydrogenate phosphatidylcholine as the surfactant. Human skin penetration was investigated in vivo by the tape stripping technique, a minimal invasive procedure based on the progressive removal of the upper cutaneous layers (stratum corneum) with adhesive tape strips. The amount of sunscreen fixed to each strip was determined by HPLC after solvent extraction. The recovery of the UV filter from spiked adhesive tapes was >94.4% and the precision of the method was better than 7.6% relative standard deviation. Non-encapsulated BMDBM, its complex with HP-β-CD, the lipid microparticles loaded with the sunscreen alone or the BMDBM/HP-β-CD complex were introduced into oil-in-water emulsions and applied to human volunteers. Compared to the cream with the non-encapsulated sunscreen agent (percentage of the applied dose penetrated, 9.7% ± 2.5), the amount of BMDBM diffusing into the stratum corneum was increased by the formulations containing the BMDBM/HP-β-CD complex (17.1% ± 3.2 of the applied dose) or the microparticles loaded with BMDBM only (15.1% ± 2.7 of the applied dose). On the contrary, a significant decrease in the level of UV filter penetrated into the stratum corneum was achieved by the cream containing the microencapsulated BMDBM/HP-β-CD complex (percentage of the applied dose penetrated, 6.0% ± 1.5). The reduced BMDBM percutaneous penetration attained by the latter system should enhance the UV filter efficacy and limit potential toxicological risks.

1. Introduction

Topical sunscreen products are widely used for protecting human skin against the harmful effects of exposure to the solar UV radiation, including sunburn, cutaneous photoageing, immunosuppression and skin cancers [1,2]. The active ingredients of these preparations, referred to as sunscreen agents or UV filters, decrease the dose of UV rays reaching the skin by absorbing, reflecting or scattering the radiation [3], the most common sunscreen agents being organic chemicals [4]. Ideally, a sunscreen product should provide effective protection against both the UVB (290–320 nm) and UVA (320–400 nm) radiation of sunlight, while exhibiting high photostability [4]. In addition, minimal percutaneous penetration of the UV filters is essential, since they exert their effect on the skin surface [3,5].

Transdermal absorption is strongly affected by the physicochemical properties of the substance, such as molecular weight and octanol/water partition coefficient ($\log P_{ow}$), as well as by the nature and properties of the formulation vehicle in which it is applied. The relatively low molecular weight, together with the lipophilic characteristics of most organic sunscreen agents promotes their partition/dissolution into the surface of the stratum corneum and the diffusion through its lamellar lipid domains. In order to prevent these phenomena and the possible subsequent risk of toxic effects and failure in sun protection, sun-care formulations should be designed to guarantee the localisation of UV filters at the skin surface or in the uppermost part of the stratum corneum [5]. Approaches aiming to inhibit or minimize sunscreen permeation through the skin include the use of vehicles in which UV filters are highly soluble [6], chemical modifications to increase their molecular weight [4], complexation with cyclodextrins [7] or incorporation in microparticles [8].

Butyl methoxydibenzoylmethane (BMDBM; Fig. 1) is the most widely used UVA absorber [4,9], being included in the list of authorized sunscreen agents in Europe, USA, Australia and Japan

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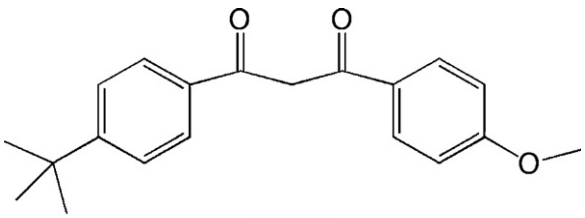


BMDBM

Fig. 1. Chemical structure of BMDBM.

[10]. However, this sunscreen agent has been shown to be photounstable [4] and to exhibit appreciable permeation into human stratum corneum and viable epidermis [5,11,12]. In earlier investigations we demonstrated that complexation of BMDBM with hydroxypropyl-β-cyclodextrin (HP-β-CD) reduced the sunscreen degradation under simulated sunlight [13,14], although no significant influence on the in vitro percutaneous penetration of the UVA filter was observed [7]. Cyclodextrins are cyclic oligosaccharides which can incorporate appropriately sized lipophilic compounds into their hydrophobic cavities, forming non-covalent inclusion complexes. They can enhance the aqueous solubility and chemical stability of the included active substance, control the release rate and increase or decrease their permeability into and through the skin [15,16].

However, the photostabilizing effect on BMDBM produced by its complexation with HP-β-CD decreased following its incorporation in an emulsion vehicle, probably due to the competitive displacement of the UV filter from the cyclodextrin cavity by the formulation excipients [17]. This is a major disadvantage, since emulsions represent the most common type of sunscreen preparations and hence simulate the actual conditions of use of sun-protective products [9]. This drawback was overcome by incorporation of the BMDBM/HP-β-CD complex in lipid microparticles, particles in the micrometer size-range consisting of a solid fat core stabilized by a layer of surfactant molecules at the surface [8,18]. In fact, after introduction in a cream preparation, the microparticle-entrapped BMDBM/HP-β-CD complex exhibited higher photostabilization efficacy than the non-encapsulated form [17], the observed effect being traced to the particle matrix limiting the formulation excipient interference on complexation.

The purpose of this study was to evaluate whether the encapsulation of the BMDBM/HP-β-CD complex into lipid microparticles could also affect the skin permeation of the sunscreen agent. The lipoparticles loaded with the complex between BMDBM and the cyclodextrin were incorporated in a model emulsion formulation, applied to human volunteers and their influence on the UV filter percutaneous penetration was assessed by the tape-stripping technique, a non-invasive in vivo procedure based on the selective removal of the stratum corneum with adhesive tapes. The amount of BMDBM fixed to each strip was determined by high-performance liquid chromatography, after solvent extraction. For comparison purposes, formulations containing the non-encaspulated BMDBM/HP-β-CD complex or lipoparticles loaded with uncomplexed BMDBM were also prepared and examined.

2. Materials and methods

2.1. Materials

Butyl methoxydibenzoylmethane was supplied by Merck (Darmstadt, Germany). Tristearin was purchased from Fluka Chemie (Buchs, Switzerland). Hydrogenated soybean phosphatidylcholine was a gift by Cargill (Hamburg, Germany). Hydroxypropyl-β-cyclodextrin (average molar substitution 0.6) was purchased from Sigma–Aldrich (Milan, Italy). Caprylic/capric triglyceride (Miglyol 812) was obtained from Polichimica (Bologna, Italy). Adhesive tapes (Scotch Crystal 600, 19 mm width) for the in vivo tape stripping were purchased from 3M France (Cergy-Pontoise Cedex, France). Methanol, acetonitrile and water of HPLC grade were from Merck. All other chemicals were of analytical grade (Sigma, St. Louis, MO, USA).

2.2. High-performance liquid chromatography

The high-performance liquid chromatographic (HPLC) apparatus consisted of a Model LabFlow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 10 μl sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable wavelength UV–vis detector (Jasco, Tokyo, Japan) set at 350 nm. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developments, 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 Scalia et al. [19], with minor modifications. A 5 μm Zorbax SB-CN column (150 mm × 3.0 mm i.d.; Agilent Technologies, Waldbronn, Germany), eluted isocratically at a flow-rate of 0.4 ml/min with methanol–acetonitrile–water (55:25:20, v/v/v), was used at ambient temperature. The identity of the BMDBM 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. Preparation and characterization of the BMDBM/HP-β-CD complex

The complex was prepared at a 1:2 molar ratio of BMDBM to HP-β-CD by the co-evaporation method, as described previously [17]. The complex was characterized by powder X-ray diffraction analysis (D5000 powder diffractometer; Siemens, Munich, Germany). The BMDBM content in the complex was determined by HPLC after proper dilution.

2.4. Microparticle preparation and characterization

Lipid microparticles were prepared according to Scalia et al. [17], by emulsifying at 70 °C melted tristearin (3.6 g) containing BMDBM (1.2 g) or the BMDBM/HP-β-CD complex (2.4 g), respectively, with 2% (w/v) hydrogenated soybean phosphatidylcholine in phosphate buffer (50 ml; 0.1 M, pH 7.4). The sample was mixed (13,500 rpm for 3 min) with an Ultra-Turrax T25 (IKA-Werk, Staufen, Germany) and then rapidly cooled at room temperature under magnetic stirring. The formed particles were recovered by centrifugation (6000 rpm for 15 min), freeze-dried and characterized by scanning electron microscopy (SEM, Cambridge Stereoscan 360, Cambridge Instruments, Bar Hill, UK) and optical microscopy (Nikon Diaphot inverted microscope, Tokyo, Japan) coupled with a computerized image analysis system (Micrometrics™ camera 122CU and software vision 1.0). In vitro release studies were performed by adding samples containing equivalent amounts of BMDBM (5 mg) to propylene glycol (100 ml) under mechanical stirring (50 rpm) at 37° C. At appropriate time intervals, 1-ml aliquots of the release medium were withdrawn and replaced with an equal volume of fresh medium. The samples were filtered (0.45 μm) and assayed for BMDBM by UV spectrophotometry at 330 nm (Lambda 3B, Perkin-Elmer, Norwalk, USA). The amount of BMDBM entrapped in the lipid microparticles was determined by HPLC after dissolution of the particles (30–40 mg) in ethanol (10 ml) under sonication (15 min).

2.5. Emulsion formulations

In vivo penetration studies were performed on creams (oil-in-water emulsions) containing BMDBM (0.7%, w/w) or its complex with HP-β-CD, non-encapsulated or loaded in microparticles. The emulsion excipients were: sorbitan monostearate (2%), polyoxyethylene sorbitan monostearate (4.5%), butylated hydroxyanisole (0.02%), isopropyl isostearate (9.0%), cetearyl isononanoate (8.0%), and cetearyl alcohol (7.0%) for the internal phase and sodium benzoate (0.1%), glycerin (2.0%), dehydroacetic acid (0.1%), EDTA (0.1%) and water (66%) for the external phase. The creams were prepared according to the common procedure used in compounding practice. The BMDBM/HP-β-CD complex and the lipid microparticles (3.5–14 g per 100 g of cream) were dispersed in water and added in the cooling phase of the emulsion preparation at about 40 °C.

2.6. In vivo skin permeation assay

The in vivo skin permeation studies were carried out by the tape stripping technique. Six healthy caucasian volunteers aged 20–50 years and free of dermatological disorders gave signed informed consent for the experimentation. Their forearms received the test emulsions to a delineated area of 2 cm × 5 cm, at a dose of 2 mg/cm^2, according to COLIPA standard [20]. The creams were homogeneously distributed by means of rubber gloves. After an application time of 60 min, the excessive product was removed by a cotton swab and the stratum corneum was stripped 10 times, as suggested by FDA for bioavailability and bioequivalence studies of topical drugs [21]. The tapes were applied to the skin with a constant pressure by a 500 g roller. The used part of the glove together with the cotton swab and the first stripped tape, representing unabsorbed UV filter, and three groups of tapes (group 1: strips 2–4; group 2: strips 5–7; group 3: strips 8–10) were collected separately. The obtained samples were extracted with 3 ×5 ml of methanol–acetonitrile (90:10, v/v) under sonication, followed by overnight extraction, under stirring, with fresh methanol–acetonitrile (5 ml). After dilution to volume (20 ml) and filtration, the resulting solutions were analysed for BMDBM by HPLC. The results were expressed in penetrated BMDBM percentage of the applied dose.

2.7. Assay validation

Adhesive tape samples of untreated stratum corneum were spiked with 5 and 20 mg of the tested emulsions and processed as outlined above. The percentage recoveries were calculated by comparing the peak areas of BMDBM extracted from the test samples with those obtained by direct injections of equivalent amount of the analyte dissolved in methanol–acetonitrile (90:10, v/v). The chromatographic precision was evaluated by repeated analyses ($n = 6$) of the same sample solution from a tape spiked with an emulsion containing microencapsulated BMDBM. The method precision was calculated by extraction and HPLC assay of independent tapes ($n = 6$) spiked with 5 mg of the same emulsion formulation.

2.8. Statistical analysis

Data were analysed for significance by analysis of variance (ANOVA) and Tukey's or Dunnett's post test. Differences were considered significant for $P < 0.05$. All computations were carried using the statistical software GraphPad Instat (Graphpad Software, San Diego, CA).

3. Results and discussion

3.1. Lipid microparticle preparation and characterization

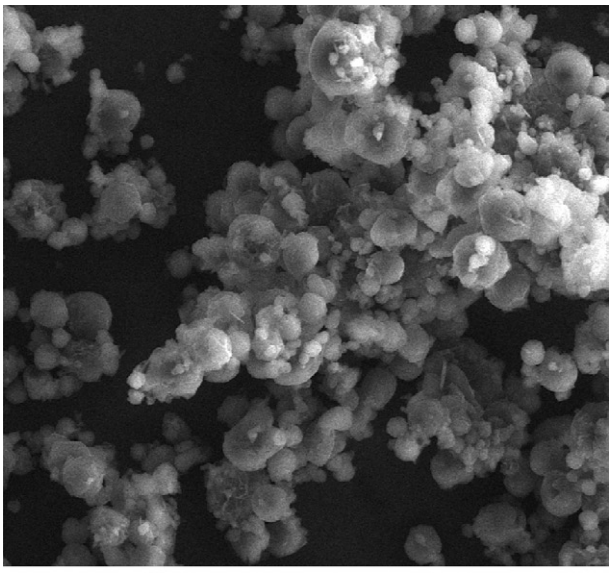
For sunscreen complexation, HP-β-CD was selected since it is one of the most useful cyclodextrin due to its safety and inclusion capacity [15,16]. The HP-β-CD complex was prepared and characterized by powder X-ray diffraction, as described earlier [14]. The disappearance of the BMDBM crystalline peaks in the X-ray diffractogram of the complex (data not shown) indicated the amorphous nature of the system, providing evidence of inclusion complex formation between BMDBM and HP-β-CD.

For the preparation of the lipid microparticles loaded with free BMDBM or its complex with HP-β-CD, a melt emulsification technique which avoids the use of organic solvents, was employed [17]. Moreover, hydrogenated phosphatidylcholine was selected as the surfactant due to its biocompatibility and tristearin as lipid material because it has been shown to achieve efficient incorporation of BMDBM [17]. SEM micrographs of the microparticles indicated a spherical shape for the lipospheres loaded with the sunscreen agent alone (Fig. 2a) while the ones containing the UVA filter complexed with HP-β-CD were irregular (Fig. 2b). The particle size, determined by computerized image analysis, was between 7 and 50 μm, which is appropriate when active substances should remain on the skin surface [22], as for the sunscreen agents. The quantity of BMDBM incorporated into the lipid particles was 19.4% ± 1.4 (w/w) for the particles loaded with the UVA filter only and 4.9% ± 0.8 for the lipoparticles containing the BMDBM/HP-β-CD complex. This difference in sunscreen agent content was due to the limited quantity of the complex which can be dispersed in the melted lipid phase for the preparation of the microparticles. Additional characterization of the lipoparticles was performed by in vitro release studies using a lipophilic medium (Miglyol 812) in which the sunscreen agent was sufficiently soluble (11%, w/w), whereas the particles remained intact [17]. Under these conditions (Fig. 3), more than 90% of non-encapsulated BMDBM dissolved in the medium after 10 min. On the other hand, the lipid microparticles produced a release modulation with 78% and 53% BMDBM released after 60 min for the particles containing the free UVA filter or its complex with HP-β-CD, respectively (Fig. 3). Hence, the slowest release rate was achieved by the microparticles loaded with complexed BMDBM, this effect being probably due to reduced diffusion of the HP-β-CD complex through the lipid particle matrix [17].

3.2. In vivo skin penetration studies

Although in vitro testing represents a useful and simple model for prediction of percutaneous absorption in humans, in vivo studies are necessary for a more realistic assessment of the degree of skin uptake in typical "in-use" conditions. Moreover, the use of human skin ex vivo, mounted in Franz cell devices, as an alternative to in vivo methods [23], is limited by the scarcity of skin explants available as well as the complex sample preparation [12,24]. In addition, as reported by the Organisation for Economic Cooperation and Development in Guideline 428 [25], it is especially difficult to examine in vitro the permeation of very lipophilic substances, such as the UV filter reported in this study (BMDBM log $P_{ow} = 4.68$), and, consequently, to find good in vitro–in vivo correlations, because of their low solubility in most physiological receptor fluids as well as their binding to skin components.

Therefore, in the present investigation, the transcutaneous penetration of BMDBM was examined in vivo on human volunteers. For these experiments, a cream (oil-in-water emulsion) was selected as topically applicable vehicle since it represents the most common type of sunscreen product [9] and hence simulates real conditions of use. Lipoparticles loaded with BMDBM, free or as HP-β-CD com-



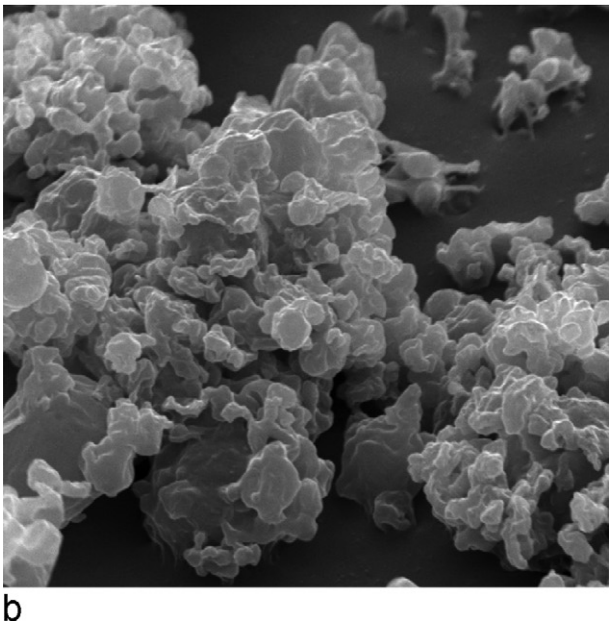


Fig. 2. Scanning electron microscopy (SEM) micrographs of lipid microparticles loaded with BMDBM (a) or the BMDBM/HP-β-CD complex (b).

plex, were incorporated into the creams. Emulsions containing the UVA filter or its cyclodextrin complex in the non-encapsulated forms were also prepared and examined. The in vivo permeation of BMDBM into human skin was studied using the tape stripping technique, based on the progressive removal of the stratum corneum layers by sequential stripping with adhesive tapes [5,12,21,26]. The amount of substance transferred to the individual tape strips, following topical application, is quantified to provide the in vivo stratum corneum penetration profile, which is predictive of skin absorption [21,26]. The formulations containing BMDBM or its HP-β-CD complex, free or entrapped in the lipid particles, were applied to the volar forearm of the human volunteers and the UVA filter level in the collected tapes was determined by HPLC after solvent extraction.

The accuracy of the method was evaluated by recovery experiments. The average recoveries of BMDBM from the adhesive tapes

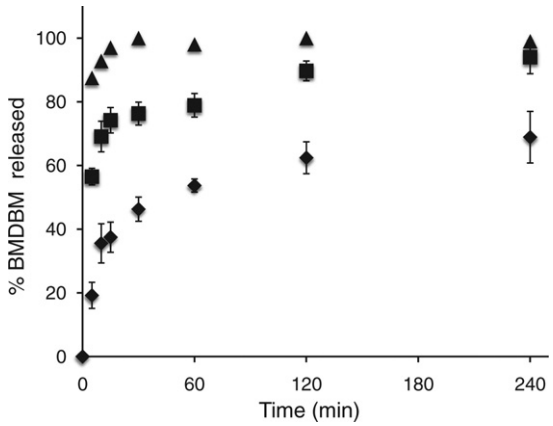


Fig. 3. BMDBM dissolution (▲) and release from lipid microparticles loaded with BMDBM (■) or the BMDBM/HP-β-CD complex (♦). Values are means ± SD ($n = 3$).

were higher than 94.4%. The method precision, as determined by replicate assays of spiked tapes, gave relative standard deviation values of 1.8% and 7.6% for the chromatographic and method precision, respectively.

The levels of BMDBM measured in the combined tapes (2–4, 5–7, 8–10), as a function of the strip number, are shown in Fig. 4. The overall recoveries obtained as sum of the BMDBM unabsorbed and diffused into the horny layers removed by the tape strips, were satisfactory (>82%) and complied with the COLIPA guideline for percutaneous absorption [20]. For all the examined formulations, the majority of the applied sunscreen dose (>70.3%) was not absorbed. Moreover, the main fraction (52–66.1%) of the permeated sunscreen agent was localised in the upper part of the horny layer (strips 2–4) with the UVA filter content decreasing with increasing stratum corneum depth (Fig. 4), in accordance with earlier investigations [5,12]. However, marked differences were observed in the total amounts of UVA filter diffused into the stratum corneum (strips 2–10) from the tested preparations (Fig. 4). Compared to the cream containing free BMDBM (penetrated percentage of applied dose, 9.7% ± 2.5) a significant ($P < 0.01$, Dunnett's test) enhancement in sunscreen permeation was produced by the formulations

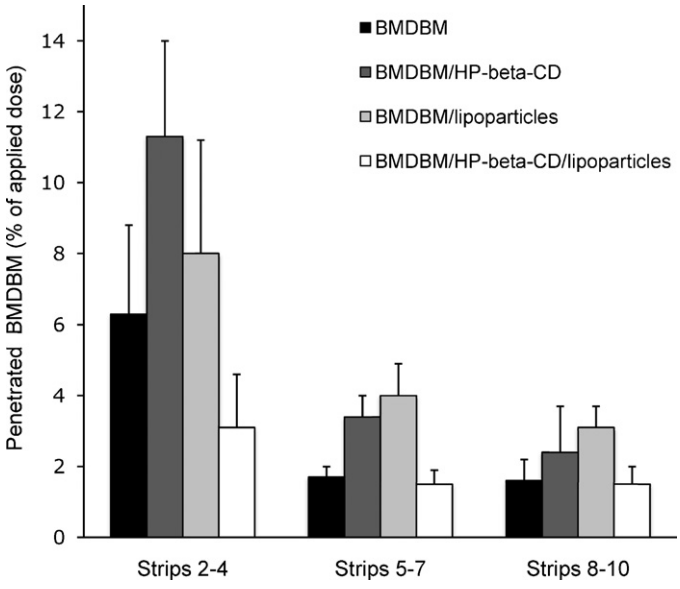


Fig. 4. Concentration profiles of BMDBM in the stratum corneum in vivo after application of its formulations. The UV filter amounts in strips 2–4, 5–7 and 8–10 are shown (mean ± SD, $n = 6$).

based on BMDBM-loaded lipoparticles (penetrated percentage of applied dose, 15.1% ± 2.7) or the cyclodextrin-complexed UVA filter (penetrated percentage of applied dose, 17.1% ± 3.2). Conversely, the penetration of the sunscreen in the horny layer was reduced to 6.0% ± 1.5, following application of the preparation containing the microencapsulated BMDBM/HP-β-CD complex, the difference with free BMDBM being statistically significant ($P < 0.05$, Dunnett's test). The values obtained for the in vivo human skin absorption of free BMDBM are consistent with those reported in previous studies [5,12,27]. Moreover, the distribution of BMDBM in the various parts of the stratum corneum (strips 2–4, 5–7 and 8–10) exhibited distinct variations among the examined cream preparations. Higher sunscreen levels were measured in each group of tapes, following application of the UVA filter as HP-β-CD complex or in the microparticle-entrapped form as compared to free BMDBM and the microencapsulated complexed sunscreen (Fig. 4). Differences were statistically significant ($P < 0.01$, Tukey's test) for the strip group 5–7.

The increased sunscreen accumulation into the stratum corneum produced by its complexation with HP-β-CD (Fig. 4) can be traced to improved solubility of the UVA filter which enhanced the amount of BMDBM delivered to the skin surface [16]. In fact, since under normal conditions neither the cyclodextrin molecules nor their complexes are able to permeate the skin, hydrophilic cyclodextrins can enhance percutaneous diffusion by increasing the thermodynamic activity in water containing vehicles [28]. In addition, the contributing effect of increased skin permeability, associated with the application of HP-β-CD, cannot be excluded [16]. The observed influence of HP-β-CD on BMDBM percutaneous penetration is in line with the results reported in a published study on mouse skin [29]. Moreover, in human volunteers the skin permeation of lipophilic drugs from hydrophilic formulations has been shown to be enhanced by complexation with HP-β-CD [30].

Also the promoting effect of the lipid microparticles on the uptake of the sunscreen by the skin (Fig. 4) is in good agreement with data obtained in a previous investigation [12]. This effect is not attributable to the carrier penetration, since intact microparticles do not diffuse into the stratum corneum [22,31]. Rather, the observed enhanced BMDBM delivery into the skin can be probably ascribed to increased concentration of the UVA filter in contact with the cutaneous surface and its efficient release from the lipoparticles into the horny layer [32].

Although lipid microparticles and HP-β-CD complexes have been reported to improve the efficacy of BMDBM by reducing its photodegradation [13,17,29], the obtained results indicated that they can also have a detrimental effect on the sunscreen activity. In fact, by increasing the UVA filter diffusion into the stratum corneum (Fig. 4), these systems diminished the sunscreen concentration on the skin surface, where it should act, and hence reduced its photoprotective activity. Therefore, for the development of new and efficient carriers for BMDBM, their influence on both photochemical behaviour and skin absorption should be examined.

On the other hand, the reduction (by 38.2%) in the quantity of UVA filter permeated into the stratum corneum, attained by the microencapsulated HP-β-CD/BMDBM complex (Fig. 4), can be explained in terms of reduced diffusion through the lipid particle matrix of the complexed sunscreen agent compared to its free form, as also indicated by the in vitro release curves (Fig. 3). This phenomenon will increase the sunscreen fraction entrapped in the microparticles and hence not available for permeation.

4. Conclusions

The results described in the present study indicated the suitability of the tape stripping technique for the in vivo evaluation of BMDBM skin permeation. Moreover, the data reported here demonstrated that the incorporation of BMDBM as HP-β-CD complex into lipid microparticles decreased the sunscreen penetration into the stratum corneum. Since the concentration present in the stratum corneum is related to the fraction that reaches the deeper viable skin tissues and the systemic circulation [21], the obtained results suggested that lipoparticles loaded with complexed BMDBM reduced the percutaneous absorption of the sunscreen agent. This effect not only enhances the protective power of the UVA filter by retaining it at the skin surface, but also limits potential toxic reactions. The latter factor is particularly relevant for BMDBM, since this sunscreen agent generates free radicals when exposed to sunlight [14].

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