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Encapsulation in lipospheres of the complex between butyl methoxydibenzoylmethane and hydroxypropyl-β-cyclodextrin

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Abstract

The aim of this study was to investigate the incorporation into lipospheres of the complex between hydroxypropyl-β-cyclodextrin (HP-β-CD) and the sunscreen agent, butyl methoxydibenzoylmethane (BMDBM) and to examine the influence of this system on the sunscreen photostability. The formation of the inclusion complex was confirmed by thermal analysis and powder X-ray diffraction. Lipid microparticles loaded with free BMDBM or its complex with HP-β-CD were prepared using tristearin as the lipid material and hydrogenated phosphatidylcholine as the emulsifier. The obtained lipospheres were characterized by scanning electron microscopy and differential scanning calorimetry. The microparticle size (15–40 μm) was not affected by the presence of the complex. Release of BMDBM from the lipospheres was lower when it was incorporated as inclusion complex rather than as free molecule. Unencapsulated BMDBM, its complex with HP-β-CD, the sunscreen-loaded lipospheres or the lipoparticles containing the BMDBM/HP-β-CD complex, were introduced into a model cream (oil-in-water emulsion) and irradiated with a solar simulator. The photodegradation studies showed that all the examined systems achieved a significant reduction of the light-induced decomposition of the free sunscreen agent (the BMDBM loss decreased from 28.9 to 17.3–15.2%). However, photolysis experiments performed during 3 months storage of the formulations, demonstrated that the photoprotective properties of the HP-β-CD complex and of BMDBM alone-loaded lipospheres decreased over time, whereas the microencapsulated HP-β-CD/BMDBM complex retained its photostabilization efficacy. Therefore, incorporation in lipid microparticles of BMDBM in the cyclodextrin complex form is more effective in enhancing the sunscreen photostability than the complex alone or the liposphere-entrapped free BMDBM.

Keywords: Lipospheres; Cyclodextrins; Complexation; Sunscreen agents; Butyl methoxydibenzoylmethane; Photostability

1. Introduction

The topical application of sunscreening preparations represents a widespreadly used strategy for protecting the skin against damage (e.g., erythema, cutaneous photoaging, immune suppression and various forms of skin cancers) induced by the sunlight UV rays (National Institute of Health, 1989; Gasparro et al., 1998; Green et al., 1999). The sunscreen agents attenuate the transmission of the solar energy to the skin by absorbing, reflecting or scattering the UV radiation (Gasparro et al., 1998; Chatelain and Gabard, 2001). Although the shorter wavelength region (UV-B, 290–320 nm) of the solar UV spectrum has been regarded as the most harmful (National Institute of Health, 1989), efficient screening of UV-A radiation (320–400 nm) has become increasingly important due to the accumulating evidence of their major role in sunlight-induced skin pathologies (Tarras-Wahlberg et al., 1999; Chatelain and Gabard, 2001; Agar et al., 2004).

Butyl methoxydibenzoylmethane (BMDBM; Fig. 1) is the most efficient and widely used UV-A filter (Tarras-Wahlberg et al., 1999; Damiani et al., 2000; Scalia et al., 2002). It is included in the list of authorized sunscreen agents in Europe, USA, Japan and Australia (Scalia et al., 2002). However, several studies have demonstrated that BMDBM undergoes marked decomposition under sunlight exposure leading to a decrease of its expected UV-protective capacity (Schwack and Rudolph, 1995; Scalia et al., 1998; Tarras-Wahlberg et al., 1999; Chatelain and Gabard, 2001). Moreover, the light-induced degradation of the sunscreen agent generates free radicals which have been shown in vitro to damage DNA and bovine serum albumin (Damiani et al., 2000;

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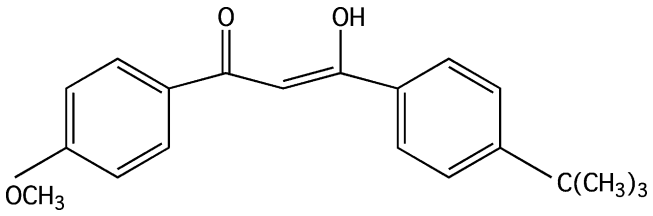

Fig. 1. Chemical structure of BMDBM.

Scalia et al., 2002). Therefore, there is a need for new systems able to reduce the photoinstability of BMDBM.

Cyclodextrins are cyclic oligosaccharides which can incorporate appropriately sized lipophilic compounds into their hydrophobic cavities, forming non-covalent inclusion complexes (Loftsson and Brewster, 1996; Rajewski and Stella, 1996). This complexation phenomenon can enhance the stability to air and light of the included molecule (Loftsson and Brewster, 1996; Rajewski and Stella, 1996; Uekama et al., 1998). Accordingly, in previous studies (Scalia et al., 1998, 2002), we demonstrated that complexation of BMDBM with hydroxypropyl-β-cyclodextrin (HP-β-CD) decreased the extent of decomposition and free radical formation observed upon exposure of the UV filter to simulated sunlight. However, the stabilising effect of HP-β-CD was more marked in solution than in lotion vehicles (oil-in-water emulsions), the difference being traced to the competitive displacement of the sunscreen from the cyclodextrin cavity by the emulsion excipients (Scalia et al., 1998; Loftsson and Masson, 2001). The reduced efficacy of complexation in emulsions is a major disadvantage, since they represent the most common type of sunscreen preparations (Scalia et al., 1998).

In order to overcome this problem, the present study reports on the incorporation of the complex between HP-β-CD and BMDBM into lipid microparticles (lipospheres). Lipospheres, based on naturally occurring lipids, consist of a solid fat core stabilized by a layer of surfactant molecules on the surface. They represent a biocompatible and appropriate carrier system for sunscreens, since the encapsulation in the lipid matrix enables increased photostability and modified release (Wissing and Müller, 2002; Yener et al., 2003). The lipospheres loaded with the BMDBM–cyclodextrin complex were then incorporated in emulsion formulations and their influence on the light-induced degradation of the UV filter was evaluated. For comparison purposes, lipospheres containing free BMDBM were also prepared and examined.

2. Experimental 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 Degussa Texturant Systems Italia (Padova, Italy). Hydroxypropyl-β-cyclodextrin (average molar substitution 0.6) was purchased from Aldrich Chimica (Milan, Italy). Caprylic/capric triglyceride (Miglyol 812) was obtained from Polichimica (Bologna, Italy). Methanol, acetonitrile and water of HPLC grade were from Merck. All other chemicals were of analytical grade (Sigma, St. Louis, MO, USA).

2.2. Quantitative determination of BMDBM

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 on 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). Chromatography was performed 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 of the inclusion complex

The inclusion complex was prepared at a 1:2 molar ratio of BMDBM to HP-β-CD. The complex was obtained by adding a solution of BMDBM (0.125 mmol) in methanol (4.0 ml) to 3.5 ml of purified water containing 0.250 mmol of HP-β-CD. The resulting 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 BMDBM in the complex was determined by HPLC after proper dilution.

2.4. Complex characterization

The BMDBM/HP-β-CD complex was subjected to thermal analysis on a differential scanning calorimeter (DSC-4, Perkin-Elmer, Norwalk, CT, USA). The samples (6–7 mg) were accurately weighed in crimped aluminium pans and heated from 30 to 130 °C, at a scanning rate of 10 °C/min under dry nitrogen flow (30 ml/min).

X-ray diffraction patterns were recorded on a D5000 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.

2.5. Liposphere preparation

Lipospheres were prepared by emulsifying (70 °C) melted tristearin (3.6 g) with 2% (w/v) hydrogenated soybean phosphatidylcholine in 0.1 M phosphate buffer solution (pH 7.4; 60 ml). Free BMDBM or its complex with HP-β-CD was added to the melted lipid phase at about 70 °C. After stirring (10,000 rpm; Ultra-Turrax T25, IKA-Werk, Staufen, Germany) the samples for 3 min, the oil-in-water emulsion was rapidly cooled under magnetic stirring to below 20 °C. The formed lipospheres were recovered by centrifugation (5000 rpm for 5 min), washed with water, filtered and freeze-dried.

2.6. Liposphere characterization

Liposphere morphological structure was examined by both optical videomicroscope (N-400FL, Optika Microscope, M.A.D. Apparecchiature Scientifiche, Bergamo, Italy) and scanning electron microscope (SEM) (Philips XL-40, Eindhoven, The Netherlands). The particle size was determined by computerized image analysis of at least 100 lipospheres on SEM micrographs.

Further characterization was performed by subjecting the lipospheres to DSC analyses, as outlined above. Measurements were made in triplicate.

The amount of BMDBM entrapped in the lipid microspheres was determined by HPLC analysis of the filtered (0.45 μm membrane filters) solutions obtained by dissolving the microparticles (30–40 mg) in ethanol (10 ml) under sonication (15 min). Each sample was analyzed in triplicate. The encapsulation efficiency was calculated as the percentage ratio between the quantity of BMDBM entrapped in the lipospheres and the amount of sunscreen added to the melted lipid phase.

2.7. In vitro release

The sunscreen dissolution and release were studied by adding previously sieved (100 μm) samples containing equivalent amounts (5 mg) of BMDBM to Miglyol 812 (100 ml) at 37 °C and under mechanical stirring (50 rpm). The experiments were performed comparing BMDBM alone, lipospheres loaded with the free sunscreen agent or with its HP-β-CD complex. At fixed time intervals, 1 ml aliquots of the medium were withdrawn and replaced with an equal volume of fresh release medium. The test sample was filtered and assayed for BMDBM by UV spectrophotometry on a UV/vis spectrometer (Lambda 3B, Perkin-Elmer, Norwalk, USA).

2.8. Photodegradation studies

Photodecomposition experiments were performed in cream preparations (oil-in-water emulsion) containing BMDBM (0.2%, w/w) or its equivalent amount of HP-β-CD complex, encapsulated in lipid microparticles. Emulsions containing the sunscreen agent (0.2%, w/w) alone or complexed with HP-β-CD were also examined. The formulation excipients were: sorbitan monostearate (2%), polyoxyethylene sorbitan monostearate (4.5%), butylated hydroxyanisole (0.02%), isopropyl isostearate (9.0%), cetearyl isononanoate (8.0%), cetearyl alcohol (7.0%), sodium benzoate (0.1%), glycerin (2.0%), dehydroacetic acid (0.1%), EDTA (0.1%) and water (67%). The cream was prepared according to the common procedure used in compounding practice (Martin, 1993). The lipid microparticles (1–4 g per 100 g of cream) or the complexed BMDBM were dispersed in water and added in the cooling phase of the emulsion preparation at ca. 40 °C. Portion (100 mg) of the test creams were transferred by means of a syringe onto the bottom of a beaker (surface area, 10 cm^2) and then irradiated for 2 h 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 500 W/m^2. After the exposure interval, the beaker was removed and its content quantitatively transferred into a 10 ml calibrated flask with methanol and the remaining BMDBM concentration 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 BMDBM from the irradiated samples with those obtained by the analysis of an equivalent amount of unirradiated preparations. The results are the average of at least six experiments.

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

3. Results and discussion

3.1. HP-β-CD/BMDBM complex preparation and characterization

The inclusion complex between BMDBM and HP-β-CD was prepared by the co-evaporation method and characterized by powder X-ray diffraction and DSC, as previously described (Scalia et al., 2002). The diffractogram of the complex (Fig. 2B) did not show the BMDBM crystalline peaks which were present in the diffraction pattern of the physical mixture of the two components (Fig. 2A). Moreover, the DSC thermogram of the complex (Fig. 3b) exhibited a transition (at about 60 °C) which can be referred to HP-β-CD dehydration and a weak endotherm at the melting point of BMDBM (Fig. 3a). Since the melting enthalpy (ΔH_f) is much lower (0.10 ± 0.02 J/g) (Fig. 3b) than that calculated for the BMDBM melting in its physical mixture with HP-β-CD ($\Delta H_f = 5.24 \pm 0.40$ J/g), this peak indicates the presence of a small fraction of free BMDBM along with the complex (Scalia et al., 1998).

3.2. Lipoparticles preparation and characterization

Lipid microparticles loaded with free BMDBM or its complex with HP-β-CD were prepared using tristearin as lipidic material and hydrogenated phosphatidylcholine as the emulsifier. The highest liposphere yield was obtained at a triglyceride/phospholipid ratio of 3:1. Investigations by SEM indicated that the lipospheres loaded with BMDBM alone showed a

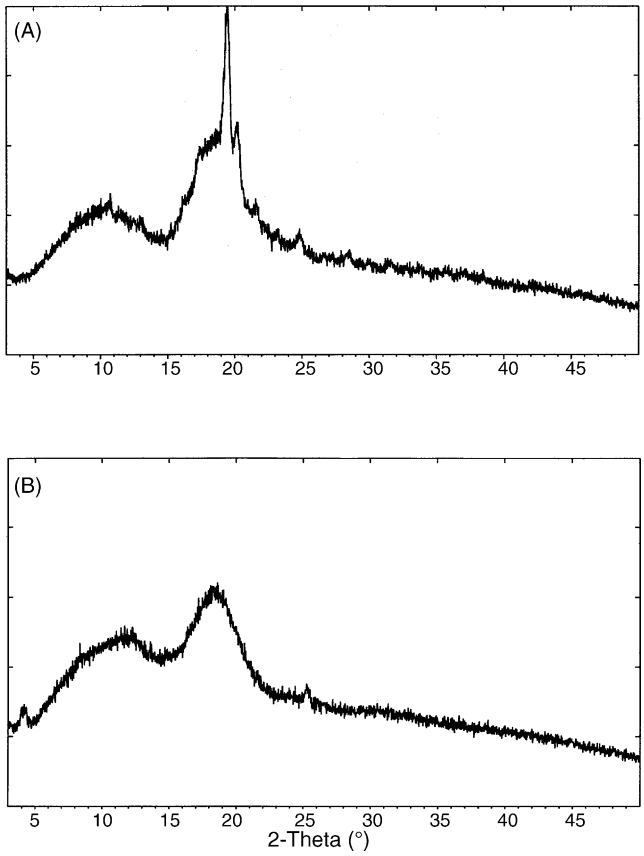

Fig. 2. Powder X-ray diffraction patterns of BMDBM/HP-β-CD (1:2) physical mixture (A) and BMDBM/HP-β-CD complex (B).

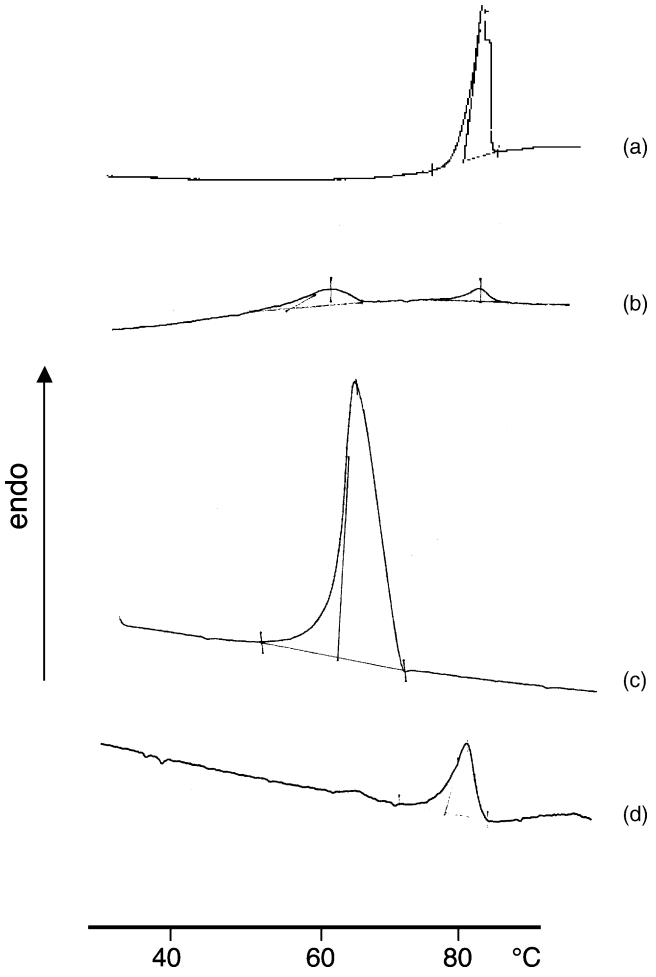

Fig. 3. DSC thermograms of: (a) BMDBM, (b) BMDBM/HP-β-CD complex, (c) tristearin and (d) phosphatidylcholine.

spherical shape and a smooth surface (Fig. 4a), while the ones containing the sunscreen agent complexed with HP-β-CD were mainly irregular and exhibited uneven surfaces (Fig. 4b). Hence, the microparticle morphology was affected by the incorporation of the cyclodextrin. On the other hand, dimensional analysis demonstrated that the obtained particles were in the 5–70 μm size range independently on the presence of HP-β-CD, with a main population of 15–40 μm, which is considered proper for topical application when minimal percutaneous absorption is desirable (Wiechers, 2000; Toll et al., 2004), as for sunscreen agents (Simeoni et al., 2004). Additional characterization of the lipid microparticles was carried out by thermal analysis. As illustrated in the thermograms reported in Figs. 3 and 5, the crystallization of tristearin in the lipospheres led to a partial polymorphic modification from stable β-form (T_{max} = 63–65 °C) (Fig. 3c) to unstable α-form (T_{max} = 48–51 °C) and β′-form (T_{max} = 61–62 °C) (Fig. 5). The endotherm at higher temperature (T_{max} = 81.2 ± 0.1 °C) in the unloaded liposphere thermogram (Fig. 5a), typical of phosphatidylcholine (Fig. 3d), indicates the presence of a small excess of emulsifier. The BMDBM melting peak (T_{max} = 83.8 ± 0.3 °C; Fig. 3a) shifted to lower temperature (T_{max} = 74.9 ± 0.2 °C) in the physical mixture of BMDBM and unloaded lipospheres (Fig. 5b) with a ΔH_f of 24.41 ± 2.88 J/g. A broad endotherm at the same temperature but with a lower ΔH_f value (7.94 ± 1.38 J/g) was evident in the thermogram of the lipospheres loaded with free BMDBM (Fig. 5c), indicating a nearly molecular dispersed state of the sunscreen inside the lipospheres. The BMDBM melting peak disappeared in the DSC profile of the microencapsulated complex (Fig. 5d), being replaced by an endotherm at slightly higher temperature (T_{max} = 81.0 ± 0.3 °C) ascribable to phosphatidylcholine. The thermal behaviour of the lipospheres loaded with the HP-β-CD complex suggests that the sunscreen is dispersed in an amorphous state.

The amount of BMDBM incorporated into the microparticles was higher (20.49 ± 1.23, w/w) for the lipospheres prepared with the sunscreen agent alone as compared to the lipid particles containing the BMDBM complexed with HP-β-CD (4.61 ± 0.61, w/w). This decrease in sunscreen loading was not due to differences in the encapsulation efficiency (89–93% for all examined systems), but to the limited quantity of complex which can be dispersed in the melted lipid phase during liposphere preparation.

3.3. In vitro release studies

To evaluate the influence of the microencapsulation process on the in vitro release of free or cyclodextrin-complexed BMDBM, a lipophilic medium (Miglyol 812) was selected in which the sunscreen agent was sufficiently soluble (11%, w/w)

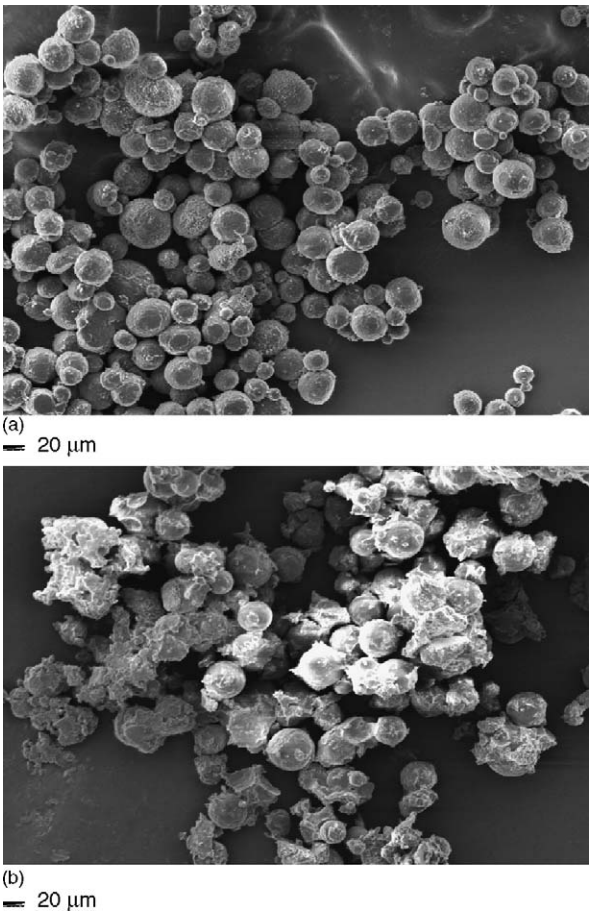

Fig. 4. SEM micrographs of lipospheres loaded with BMDBM (a) or the BMDBM/HP-β-CD complex (b).

to assure sink conditions, whereas lipospheres remain intact (Wissing and Müller, 2002). The release profiles of BMDBM from lipid microparticles obtained with or without HP-β-CD and the dissolution curve of the sunscreen alone are shown in Fig. 6. The dissolution of unencapsulated BMDBM was very fast up to a plateau, with more than 90% of the sunscreen dissolved in the first 10 min. Lipospheres gave rise to decreased BMDBM releases and did not exhibit burst-effect phenomena (Fig. 6), suggesting that the sunscreen is entrapped in the lipid particles and not adsorbed at their external surface. The slowest release rate was produced by the microparticles containing the UV filter complexed with HP-β-CD (Fig. 6). Hence, the liposphere retention capacity was enhanced by encapsulation of the sunscreen agent as inclusion complex rather than in the free form. This is in accordance with the study by Cavalli et al. (1999) on the incorporation of hydrocortisone and progesterone as complexes with β-cyclodextrin into solid lipid nanoparticles. The observed effect can be probably ascribed to reduced diffusion of the HP-β-CD complex through the lipid microparticle matrix.

3.4. Photodegradation studies

In order to investigate the effect of the lipid particles on the photochemical behaviour of BMDBM, the photolysis experi-

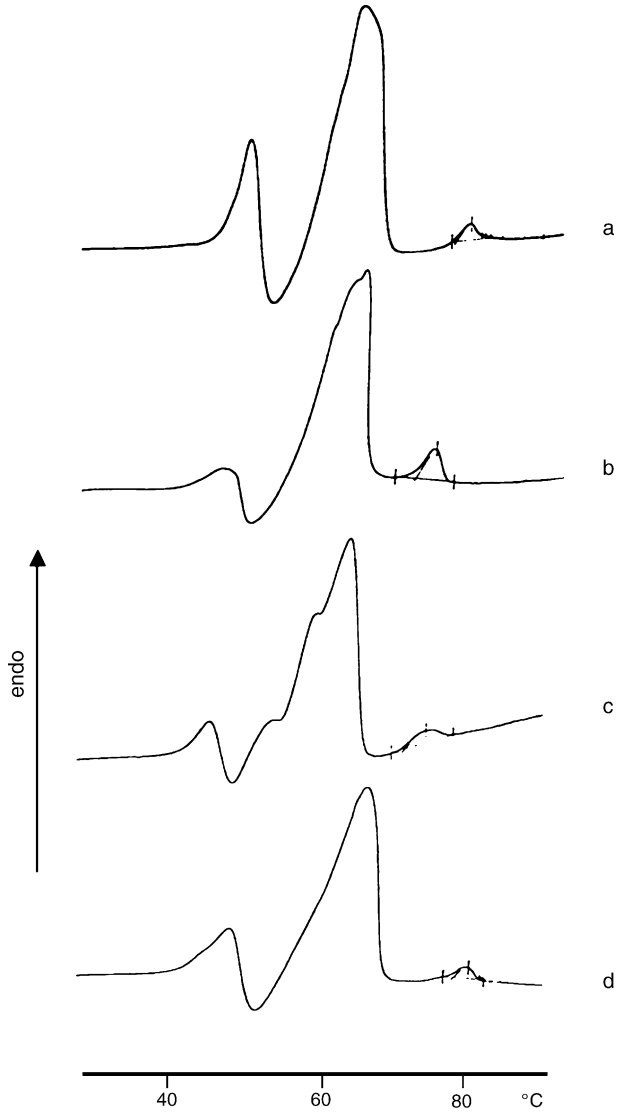

Fig. 5. DSC thermograms of: (a) unloaded lipospheres, (b) physical mixture (BMDBM with unloaded lipospheres), (c) lipospheres loaded with free BMDBM and (d) lipospheres loaded with the BMDBM/HP-β-CD complex.

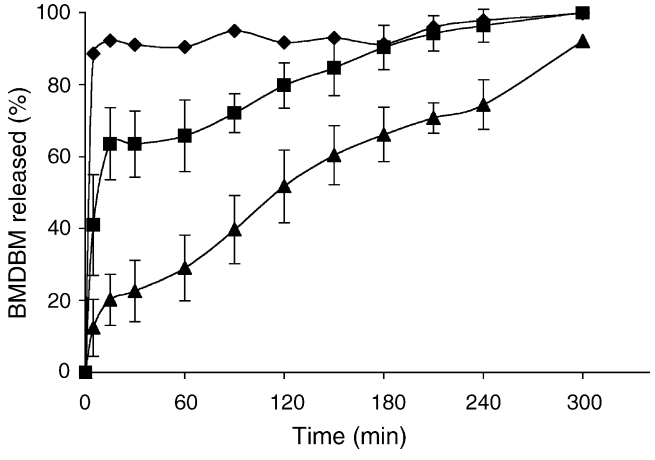

Fig. 6. BMDBM dissolution (♦) and release from lipospheres loaded with BMDBM (■) or the BMDBM/HP-β-CD complex (▲). Values are mean ± S.D. ($n = 3$).

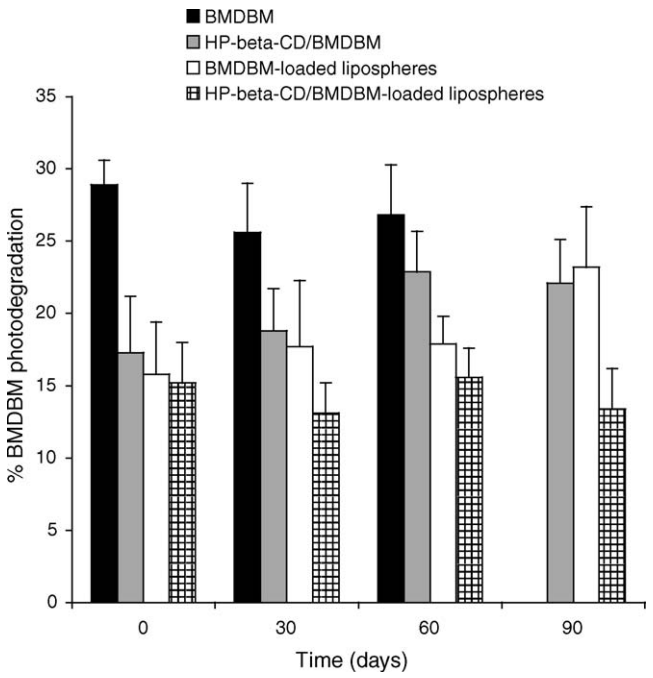

Fig. 7. BMDBM photodegradation (%) in its formulations immediately after preparation and at different storing times. Values are mean ± S.D. of at least six experiments.

ments were performed on a cream (oil-in-water emulsion) as a vehicle. This system was selected as a model formulation since it represents the most common type of sunscreen preparation (Scalia et al., 1998). Lipospheres loaded with the sunscreen agent either free or as HP-β-CD complex were incorporated into the cream. Creams containing non-encapsulated BMDBM or its complex with HP-β-CD were also prepared. The formulations were exposed to the solar simulator and the extent of photodecomposition was measured by HPLC. During the photostability experiments, the applied UV energy was equivalent to 10 minimal erythemal dose (MED) which is considered representative of daily solar emission (Tarras-Wahlberg et al., 1999). In the formulation containing plain BMDBM, 28.9 ± 3.4% of the sunscreen content was lost following irradiation (Fig. 7). The photoinduced decomposition of the sunscreen agent was decreased to 17.3 ± 3.9% by complexation with HP-β-CD (Fig. 7). Moreover, a reduction in the extent of BMDBM photodegradation to 15.8 ± 3.6% and 15.2 ± 2.8% was attained in the creams containing the microparticle-entrapped BMDBM or its complexed form, respectively (Fig. 7). These data indicate that, immediately after the preparation of the creams, there were not significant differences ($P > 0.05$) in the photostabilization effects between the cyclodextrin complex and the liposphere systems. To evaluate whether the enhancement of BMDBM photostability achieved by the examined systems varied with time, photolysis experiments were performed over 3 months storage of the cream samples at room temperature and in the dark and the generated data are depicted in Fig. 7. During the above time interval a statistically significant reduction in the stabilising properties of the HP-β-CD complex (the extent of BMDBM degradation after 3 months was 22.1 ± 3.0%) and of the lipospheres loaded with free BMDBM (the extent of BMDBM degradation after 3 months was 23.2 ± 5.2%) were observed (Fig. 7). Conversely, in the formulation containing the microencapsulated HP-β-CD/BMDBM complex, the photostabilization efficacy was retained, the percentage loss of the sunscreen agent being 13.4 ± 2.8% after 3 months of storage (Fig. 7). The higher effectiveness of the HP-β-CD complex following its encapsulation in lipospheres, could be traced to reduced competitive displacement of the UV filter from the cyclodextrin cavity by the emulsion excipients. Moreover, the decreased efficacy over time of the lipid microspheres containing the free UV filter (Fig. 7) could be ascribed to the release of BMDBM from the lipospheres into the oil phase of the emulsion, which reduces the sunscreen fraction protected by the lipid particle matrix. This phenomenon is slowered by encapsulation of BMDBM as complex with HP-β-CD, as indicated by the in vitro release curves (Fig. 6).

4. Conclusions

The results described in the present study demonstrate that the microencapsulated HP-β-CD/BMDBM complex is superior to both the complex alone and the lipospheres loaded with plain BMDBM, in enhancing the sunscreen agent photostability, particularly during storage of the formulations. A disadvantage of this system is the limited liposphere loading capacity. In order to improve the UV filter incorporation, the amount of complex dispersed in the tristearin melt could be increased by selecting cyclodextrins less hydrophilic than HP-β-CD (Uekama et al., 1998). However, if a higher BMDBM content in the final product is required, relatively large amounts of lipospheres (up to 10–15%, w/w) can be introduced in the formulation since they are based on well-tolerated, regulatorily accepted (i.e. lipids and surfactants used in creams) excipients (Müller et al., 2002; Wissing and Müller, 2002).

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