ELSEVIER

International Journal of Pharmaceutics 175 (1998) 205–213

international journal of pharmaceutics

Complexation of the sunscreen agent, butyl-methoxydibenzoylmethane, with hydroxypropyl-β-cyclodextrin.

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Received 15 April 1998; received in revised form 12 August 1998; accepted 18 August 1998

Abstract

The interaction between the sunscreen, butyl-methoxydibenzoylmethane (BM-DBM), and parent and modified α-, β- or γ-cyclodextrins was investigated in water by phase-solubility analysis. Among the available cyclodextrins, only hydroxypropyl-β-cyclodextrin (HP-β-CD) produced a significant increase in the aqueous solubility of BM-DBM. The complexation of the sunscreen agent with HP-β-CD was studied by circular dichroism, differential scanning calorimetry and X-ray diffractometry. The data from the solubility and the circular dichroism studies suggested the formation of a 1:2 (sunscreen:cyclodextrin) complex. The photodegradation of BM-DBM was reduced by inclusion complexation with HP-β-CD. Therefore the complex can be used to improve the photostability of the sunscreen agent.

Keywords: Butyl-methoxydibenzoylmethane; Complex characterization; Hydroxypropyl-β-cyclodextrin; Inclusion complexation; Photodegradation

1. Introduction

The harmful effects of sunlight, especially the solar UV radiation, on human skin are well recognised (National Institute of Health, 1989). While acute exposure to the sun's UV rays promotes an inflammatory response (sunburn erythema), chronic exposure can lead to skin photoageing and to certain forms of skin cancer (National Institute of Health, 1989; Pathak, 1991; Ziegler et al., 1994). Public awareness of the hazards of exposure to sunlight has resulted in an increasingly widespread use of sunscreen agents (National Institute of Health, 1989; Dromgoole and Maibach, 1990; Schrader et al., 1994). Chemical

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0378-5173/98/$ - see front matter
PII S0378-5173(98)00283-X

sunscreens are compounds which absorb UV light thereby decreasing the amount of the solar radiation energy reaching the stratum corneum. The sunlight UV wavelengths that reach the earth's surface include UV-B (290–320 nm) and UV-A radiation (320–400 nm) (National Institute of Health, 1989). While some of the sunscreen preparations on the market are appropriate only for UV-B rays, protection also against UV-A wavelengths has assumed increasing importance since more has become known about their adverse effects on human skin (National Institute of Health, 1989; Schwack and Rudolph, 1995; Pollet et al., 1996).

Photochemical stability is the most important characteristic of an effective UV filter, since the light-induced decomposition of the sunscreen agent not only reduces its photoprotective power, but can also promote phototoxic or photoallergic contact dermatitis (Dromgoole and Maibach, 1990; DeLeo et al., 1992; Schrader et al., 1994; Rieger, 1997).

Butyl-methoxydibenzoylmethane (BM-DBM) is one of the most widely used UV-A filters in sunscreen preparations as well as in the photoprotection of cosmetic products (Roscher et al., 1994; Schwack and Rudolph, 1995). BM-DBM is included in the list of authorized sunscreen agents both in Europe (EEC Directive, 1976) and in the USA (US Food and Drug Administration, 1993). The photodecomposition of BM-DBM has been demonstrated by several authors both in solution (Roscher et al., 1994; Schwack and Rudolph, 1995) and in a model emulsion (Deflandre and Lang, 1988). Hence, in order to enhance the effectiveness and safety of this sunscreen agent, there is a need for new systems exhibiting improved BM-DBM photostability.

Cyclodextrins are capable of modifying some physicochemical properties of active substances by forming noncovalent inclusion complexes (Duchêne, 1987; Loftsson and Brewster, 1996). The complexation can result in increased aqueous solubility and dissolution rate of the guest molecule (Rajewski and Stella, 1996). Moreover, there is generally an improvement in the stability of the included molecule to air and light (Uekama et al., 1983; Duchêne, 1987; Loftsson and Brewster, 1996). To the best of our knowledge, there are no reports in the literature on the use of cyclodextrins for the enhancement of sunscreen agent photostability.

In this report we investigated the inclusion of BM-DBM in hydroxypropyl-β-cyclodextrin (HP-β-CD) both in solution and in the solid state. The effectiveness of the inclusion complexation in reducing the BM-DBM photodecomposition was also studied.

2. Materials and methods

2.1. Materials

BM-DBM (Fig. 1) was supplied by Givaudan (Geneva, Switzerland). The cyclodextrins used in this study included: α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (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 and acetonitrile were HPLC grade from Baker (Phillipsburg, NJ, USA). Water was purified by a Modupure Plus system (Continental Water Systems, San Antonio, TX, USA). All other chemicals were of analytical-reagent grade (Sigma, Milan, Italy).

2.2. Solubility studies

Solubility studies were carried out according to Higuchi and Connors (1965). An excess amount of BM-DBM was added to various concentrations of the different cyclodextrins examined (0–120 mM for HP-β-CD and 0–15 mM for the other cyclodextrins) in 5 ml of purified water. The sus-

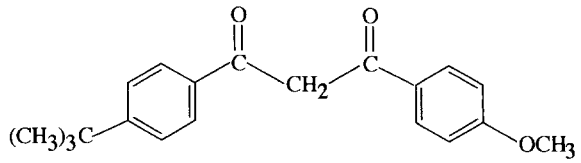

Fig. 1. Chemical structure of BM-DBM.

pensions were stirred in 10 ml screw-capped vials at 25 ± 2°C and shielded from light. When equilibrium has been reached (about 3 days), the content of each vial was filtered through 0.45-μm membrane filters (Millipore, Bedford, MA, USA) and analyzed by HPLC to determine the BM-DBM concentration. All the data are the average of at least three determinations (relative standard deviations were within 5%).

2.3. Physical mixture preparation

Fine-powdered physical mixture of BM-DBM and HP-β-CD with guest/host molar ratio of 1:2 was prepared by simple dry mixing of exactly weighed amounts of the two components, adopting the geometric dilution method.

2.4. Preparation of the inclusion complex

The inclusion complex was prepared at a 1:2 molar ratio of BM-DBM to HP-β-CD. To obtain the ethyl alcohol complex (EA complex), both BM-DBM (38.8 mg, 0.125 mmol) and HP-β-CD (344.6 mg, 0.250 mmol) were dissolved at room temperature in 6.5 ml of 95% ethanol. To obtain the water/ethyl alcohol complex (W/EA complex), BM-DBM (38.8 mg, 0.125 mmol) was dissolved at room temperature in 3.0 ml of 95% ethanol, to which 3.5 ml of purified water containing 344.6 mg (0.250 mmol) of HP-β-CD were added. The obtained solutions were 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. The solid complex was kept under vacuum in a desiccator for 3 days before analysis.

2.5. Thermal analysis

Differential scanning calorimetric (DSC) curves were recorded on a Perkin-Elmer DSC-4 differential scanning calorimeter equipped with a computerised data station (Perkin-Elmer, Norwalk, USA). Indium (99.99%; m.p. 156.6, ΔH_f 28.45 J/g; Perkin-Elmer) was used to check the instrument. All samples (about 5 mg) were heated in crimped aluminium pans (Perkin-Elmer) at a scanning rate of 5°C/min using dry nitrogen flow (30 ml/min).

2.6. X-ray diffractometry

X-ray diffraction patterns were recorded on a PW 3710 powder diffractometer (Philips, Eindhoven, The Netherlands) using a voltage of 40 kV and a current of 20 mA for the generator, with Cu anode material. The wavelengths of the Ni filtered radiation were: $\lambda_1 = 1.5406$ Å and $\lambda_2 = 1.5444$ Å. The diffractograms were recorded from 3° (2θ) to 40° (2θ) at an angular speed of 1° (2θ) per minute using 1-0.2-1 mm slits.

2.7. Circular dichroism

Circular dichroism spectra were carried out at room temperature using a Model J-500 A automatic recording circular dichrograph (Jasco, Tokyo, Japan) interfaced with an IBM-AT computer. The instrument was calibrated with an aqueous solution of D(+)camphorsulfonic acid and a dioxane solution of epiandrosterone (Schipper and Dekkers, 1981). A cylindrical fused quartz cell of 1 cm pathlength was used for the circular dichroism measurements. The value for BM-DBM/HP-β-CD spectra are given in molar elipticity. The BM-DBM concentrations ranged from 2×10^{-5} to 7.3×10^{-5} M.

2.8. High-performance liquid chromatography (HPLC)

The HPLC apparatus consisted of a modular chromatographic system (Model 980-PU pump and Model 975-UV variable wavelength UV-Vis detector; Jasco, Tokyo, Japan) linked to an injection valve with a 20 μl sample loop (Model 7125; Rheodyne, Cotati, Ca, USA). The detector was set at 355 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 802 RN syringe (10 μl; Hamilton, Bonaduz, Switzerland). Separations were performed on a 5-μm Zorbax SB-Phenyl column (250 × 4.6 mm I.D.; Rockland Technologies, Newport, Delaware, USA) fitted with a guard column (LiChrospher RP-18, 5-μm particles, 4 × 4 mm I.D.; Merck, Darmstadt, Germany) and

eluted with methanol/acetonitrile/water (65:20:15, v/v) containing 0.5% (v/v) acetic acid. The mobile phase was deaerated on-line by a Model ERC-3311 automatic solvent degasser (Erma, Tokyo, Japan). The column temperature was maintained at 35°C using a Model 7990 Space Column Heater (Jones Chromatography, Hangoed, UK). Chromatography was performed under isocratic conditions, at a flow-rate of 0.8 ml/min. The identity of BM-DBM 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.

2.9. 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 270°C and 250°C, respectively. A SE-54 fused silica capillary column (25 m × 0.25 mm I.D.; CE Instruments) with helium as the carrier gas was used. The GC operating conditions were: injector temperature, 270°C; column temperature, 100°C for 2 min, then programmed at 10°C/min to 270°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).

2.10. Photodegradation

Photodegradation studies were carried out in solution (70% ethanol in propylene glycol) and in a lotion (oil-in-water emulsion) both containing free or complexed BM-DBM (0.15%, w/w).

The lotion excipients were: sorbitan monostearate, polyoxyethylene sorbitan monostearate, butylated hydroxyanisole, *p*-hydroxybenzoic acid ethyl ester, isopropyl isostearate (Henkel, Fino Mornasco, Italy), cetearyl isononanoate (Henkel), cetearyl alcohol (Henkel), D-sorbitol, dehydroacetic acid, EDTA, water. To prepare the lotion, the emulsifiers and the free BM-DBM were first incorporated into the oil phase. Both the oil- and the aqueous-phase were heated to approximately 60°C and the aqueous phase was slowly added to the oil phase while stirring with a Silverson mixer (Chesham, England). Mild agitation was continued until the emulsion cooled at room temperature. Butylated hydroxyanisole and complexed BM-DBM were added in the cooling phase of the production process at about 40°C.

A portion of the test preparation (10 μl for the solution, 30–40 mg for the lotion) containing the free or complexed BM-DBM, was spread on a glass plate and then exposed to the solar simulator which consisted of a 200 W Xenon-Mercury lamp (Hanovia 901-B1) fitted with focusing lens to center the light on the sample and with a WG 320 filter ($\lambda > 290$ nm). At the appropriate irradiation interval (4 h), the plate was removed and immersed in methanol under sonication (10 min). The resulting sample was diluted to volume (20 ml), filtered (0.45-μm membrane filter) and a portion (10 μl) of the clear solution was analysed by HPLC. The degree of degradation was measured by comparing the peak areas of BM-DBM from the irradiated samples, with those obtained by analysis of an equivalent amount of the non-exposed preparation. Statistical analysis of the results was carried out by Student's *t*-test.

3. Results and discussion

3.1. Solubility studies

Fig. 2A illustrates the influence of the cyclodextrins examined in this study, on the water solubility of BM-DBM which is almost insoluble in water. The obtained phase-solubility diagrams indicated major variations in the interaction between BM-DBM and α-, γ- or β-cyclodextrins. This can be ascribed to the different cavity dimensions of the macrocycles. Although β-CD and HP-β-CD produced the best results, only HP-β-CD achieved a remarkable increase in the water solubility of the UV-A filter. The reduced effectiveness of β-CD compared to HP-β-CD is probably due to its low water solubility which limits the solubility of the complex itself.

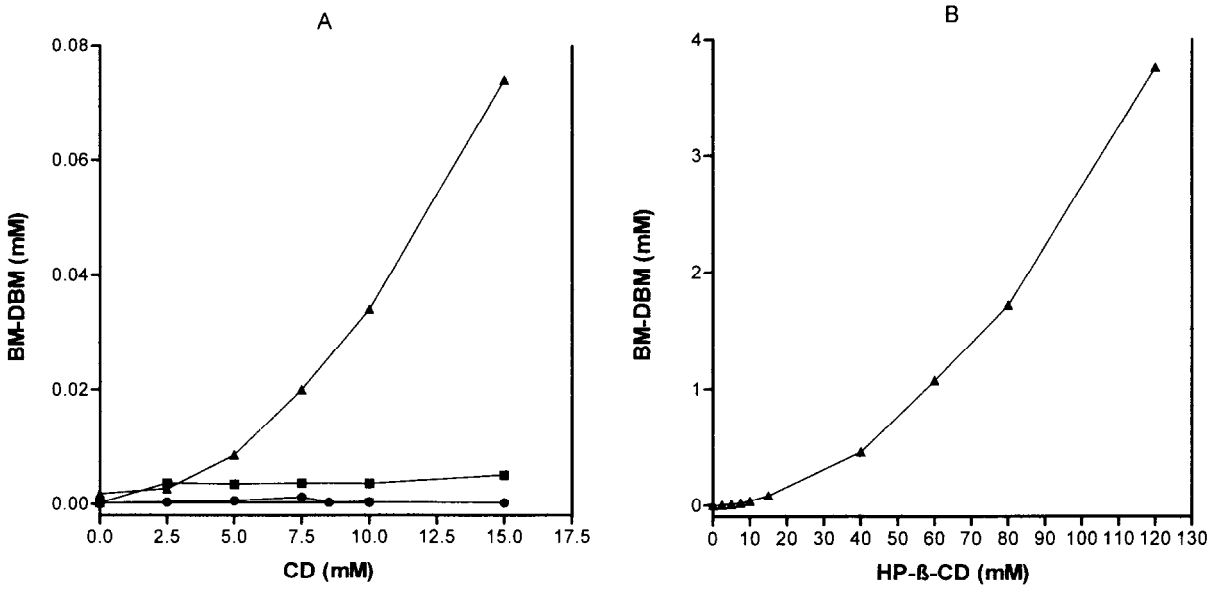

Fig. 2. (A) Phase-solubility diagrams for BM-DBM with different cyclodextrins (0–15 mM) in purified water at 25°C. (—▼—) α-CD; (– ◆ –) HP-α-CD; (– • –) HP-γ-CD; (– ■ –) β-CD and (—▲–) HP-β-CD. (B) Phase-solubility diagram for BM-DBM in the presence of HP-β-CD (0–120 mM) in purified water and at 25°C.

Based on the foregoing results, HP-β-CD was selected for further experiments. Over the concentration range used in this study (Fig. 2B) the solubility of BM-DBM increased as the HP-β-CD concentration increased. The solubility enhancement can be attributed to the formation of an inclusion complex with a greater solubility than BM-DBM alone. The phase diagram (Fig. 2B) exhibited a positive curvature which can be classified, according to the literature (Higuchi and Connors, 1965), as an Ap-type phase behaviour and which suggests (Higuchi and Connors, 1965) that the complex is formed to a higher order than one in BM-DBM. Thus, the assumption was made that only two complexes were formed, i.e. BM-DBM/HP-β-CD, 1:1 mole ratio (BM-DBM · HP-β-CD) and BM-DBM/HP-β-CD, 1:2 mole ratio (BM-DBM · HP-β-CD_2). The two inclusion complexes are in equilibrium in solution.

The apparent stability constants of the inclusion complexes ($K_{1:1}$ and $K_{1:2}$):

$$K_{1:1} = [\text{BM-DBM} \cdot \text{HP-}\beta\text{-CD}]/[\text{BM-DBM}] \times \cdot [\text{HP-}\beta\text{-CD}]$$

$$K_{1:2} = [\text{BM-DBM} \cdot \text{HP-}\beta\text{-CD}_2]/[\text{BM-DBM}] \times [\text{BM-DBM} \cdot \text{HP-}\beta\text{-CD}]$$

were calculated from the solubility values according to the method of Higuchi and Connors (1965) using a simple computer program. The values for $K_{1:1}$ and $K_{1:2}$ were found to be 2233 M^{-1} and 13 M^{-1}, respectively.

3.2. Circular dichroism studies

Since BM-DBM lacks a chiral center (Fig. 1), it does not exhibit any dichroic activity. After interaction with HP-β-CD, which possesses a chiral cavity, BM-DBM showed an induced circular dichroism spectrum in the 300–400 nm range (Fig. 3). This can be traced to the perturbation of the electronic transitions of the guest molecule caused by inclusion into the assymetric cavity of the cyclodextrin (Hirayama and Uekama, 1987; Yoshida et al., 1994; Puglisi et al., 1996). The

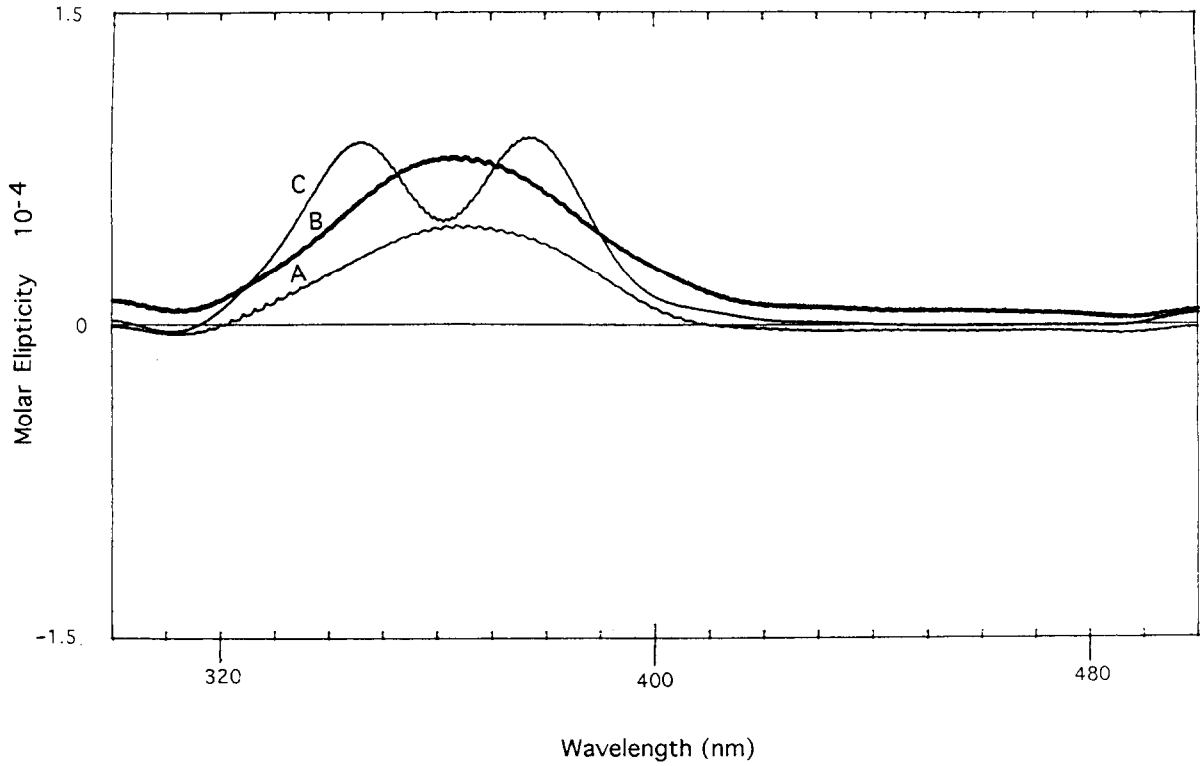

Fig. 3. Circular dichroism absorption spectra of BM-DBM in the presence of various concentrations of HP-β-CD in water. (A) 7.5 mM HP-β-CD; (B) 10 mM HP-β-CD; (C) 15 mM HP-β-CD.

induced circular dichroism spectra reported in Fig. 3 show an interesting feature: the profiles are dependent on the HP-β-CD concentration. In fact, at 7.5 and 10 mM HP-β-CD, the spectra are characterized by a positive band centered at 363 nm, while an increase in HP-β-CD concentration to 15 mM produces a split-type doubly positive signal at 348 and 375 nm, this pattern being maintained at higher cyclodextrin molarities (data not shown). The split of the induced circular dichroism band with increasing HP-β-CD concentrations, could be attributed to the formation of a BM-DBM/HP-β-CD complex with a 1:2 molar ratio, in agreement with the data of the phase-solubility diagram (Fig. 2B). Moreover, the positive sign of the induced circular dichroism spectra indicates that the electric dipole moment in the chromophore is parallel to the long axis of the HP-β-CD (Yoshida et al., 1994; Puglisi et al., 1996).

3.3. Solid state studies

In order to obtain the inclusion complex between BM-DBM and HP-β-CD, ethanol or ethanol–water (46:54, v/v) were used as solvents. Although ethanol is necessary to dissolve the sunscreen agent, reducing the concentration of this organic solvent in the solution phase may enhance the complexation efficiency. In fact, ethanol has been shown to act as a competing guest molecule (Pitha and Hoshino, 1992) and also to decrease the formation of inclusion complexes through non-specific solvent effects (Pitha and Hoshino, 1992; Loftsson and Brewster, 1996).

DSC revealed some information on solid state interactions of BM-DBM with HP-β-CD (Fig. 4). BM-DBM crystals showed the endothermic peak at about 80°C ($\Delta H_f = 59.0$ J/g), corresponding to the product melting. The DSC profile of HP-β-CD exhibited a typical endothermic peak (be-

tween about 60 and 140°C) due to water loss (not shown). The appearance of two endothermic peaks corresponding to BM-DBM melting and to HP-β-CD dehydration was also evident in the thermogram of the physical mixture. The BM-DBM melting peak disappeared in the thermogram of W/EA inclusion complex, suggesting the complete inclusion of BM-DBM molecules in the HP-β-CD cavity. On the contrary, a broad peak due to the BM-DBM melting was evident in the thermogram of the EA inclusion complex. As the ΔH_f value (1.4 J/g) is lower than that calculated for the BM-DBM melting in the physical mixture thermogram ($\Delta H_f = 5.2$ J/g), this peak could indicate the presence of free BM-DBM along with the complex (Palmieri et al., 1997).

The X-ray diffractometry studies (Fig. 5) revealed the crystalline nature of BM-DBM and the amorphous state of HP-β-CD. The X-ray diffraction pattern of the physical mixture was the superimposition of BM-DBM and amorphous HP-β-CD patterns. The diffractogram of the W/EA inclusion complex showed no BM-DBM signals, demonstrating the complete amorphousness of the product obtained by the evaporation process of the water/ethyl alcohol solution. The absence of both the BM-DBM melting peak in the

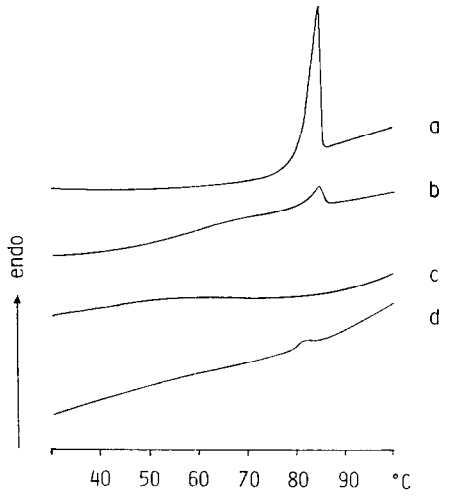

Fig. 4. DSC thermograms of BM-DBM (a), BM-DBM/HP-β-CD physical mixture (b), BM-DBM/HP-β-CD water-ethyl alcohol complex (c) and BM-DBM/HP-β-CD ethyl alcohol complex (d).

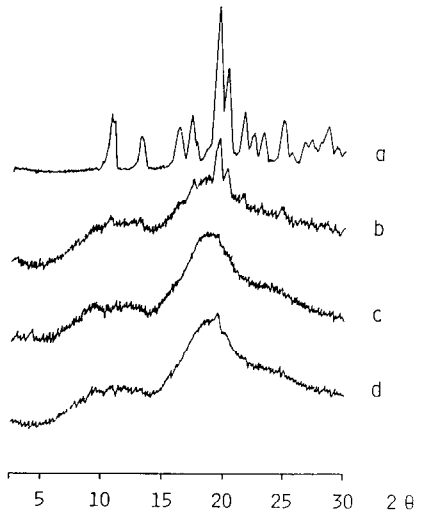

Fig. 5. X-ray diffraction patterns of BM-DBM (a), BM-DBM/HP-β-CD physical mixture (b), BM-DBM/HP-β-CD water-ethyl alcohol complex (c) and BM-DBM/HP-β-CD ethyl alcohol complex (d).

DSC thermogram and of the crystalline peaks in the X-ray diffraction pattern of the W/EA complex can be attributed to the inclusion of the guest molecule within the cyclodextrin cavity (Rajagopalan et al., 1986). Also the X-ray diffraction pattern of the EA complex suggested inclusion of BM-DBM in the HP-β-CD cavity, although the main peak of BM-DBM ($d = 4.55$ Å) appeared with a very low intensity. This indicates incomplete inclusion of the molecule into the HP-β-CD cavity, in accordance with the results of the thermal analysis.

Therefore, the inclusion of BM-DBM is more efficient when HP-β-CD and BM-DBM are solubilized in a mixture of water and ethyl alcohol (54:46, v/v), rather than in ethyl alcohol alone. This can be traced to increased complexation efficiency at lower ethanol concentration in the solution phase, in accordance with the work of Pitha and Hoshino (1992) on the formation of the testosterone/HP-β-CD complex.

3.4. Photostability studies

Initial photolysis experiments were carried out in solution using ethanol-propylene glycol (70:30,

v/v) as the solvent since this system represents a simple in vitro model simulating conditions of actual use (Berset et al., 1996). Solutions containing free or complexed BM-DBM (0.15%, w/w) were exposed for 4 h to the Xenon solar simulator (Deflandre and Lang, 1988; Schrader et al., 1994) and the degree of BM-DBM photodegradation measured by HPLC (see Table 1). The major products originated from the photodecomposition of BM-DBM were identified by GC-MS as 4-*t*-butyl benzoic acid, 4-*t*-butyl acetophenone and 4-methoxy acetophenone. The percentage loss of the sunscreen agent reached 70.4% in the solution containing BM-DBM alone. This result is in good agreement with the data reported by Berset et al. (1996). A lower degree of degradation (49.2%) was observed in the solution containing BM-DBM complexed with HP-β-CD, which indicates that this system achieves improved photostability for the UV-A filter. On the other hand, the BM-DBM/HP-β-CD physical mixture did not affect significantly the photochemical behaviour of the sunscreen in the tested solution.

In order to simulate more realistic conditions, further studies were performed on a lotion (oil-in-water emulsion) which was selected as a model formulation since it represents the most widely used type of sunscreen preparation (Siemer, 1991). Free or complexed BM-DBM was incorporated (0.15%, w/w) into the lotion and irradiated with the solar simulator. In the preparation containing BM-DBM alone, 26.6% of the sunscreen content was lost following irradiation, as determined by HPLC (Table 1). This is in accordance with previous studies (Deflandre and Lang, 1988). Under the same conditions, the lotion containing the BM-DBM/HP-β-CD complex exhibited a 17.3% decrease of the UV-A filter level (Table 1). Higher dispersion of the photodegradation results was observed in the lotion compared to the solution medium (Table 1). This can be ascribed to sunscreen interaction with lotion excipients, emulsion heterogeneity and absorption changes due to scattered light. However, statistical analysis demonstrated that the difference between the formulations containing free or complexed BM-DBM is significant ($p < 0.025$). The obtained data indicate that the inclusion of BM-DBM into the HP-β-CD cavity reduces the photodecomposition of the UV-A filter both in the solution and in the emulsion vehicles.

Table 1
Comparative photodegradation data for free and complexed BM-DBM, in solution or in a lotion, after 4 h irradiation with the solar simulator

Sample	Percentage sunscreen loss[a]	
	BM-DBM	BM-DBM/HP-β-CD complex
Solution	70.4 ± 6.6	49.2 ± 3.4
Lotion	26.6 ± 7.7	17.3 ± 3.8

[a] Each value is the mean ± S.D. of six determinations.

4. Conclusions

The inclusion complex of BM-DBM with HP-β-CD was characterized in the solid state by DSC and X-ray diffractometry, while circular dichroism was used in solution. The complexation of BM-DBM enhanced the photostability of the sunscreen agent. In addition, the inclusion of BM-DBM into the HP-β-CD cavity should limit the interaction of the UV filter with the skin, thus decreasing its irritation potential.

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