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European Journal of Pharmaceutical Sciences 22 (2004) 241–249
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
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# Complexation of the sunscreen agent, phenylbenzimidazole sulphonic acid with cyclodextrins: effect on stability and photo-induced free radical formation

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Received 10 October 2003; received in revised form 18 February 2004; accepted 17 March 2004

Available online 14 May 2004

**Abstract**

The interaction between the sunscreen agent, phenylbenzimidazole sulphonic acid (PBSA) and hydrophilic α-, β-, and γ-cyclodextrin derivatives was investigated under acidic conditions (pH 4.0) by phase-solubility analysis. Among the available cyclodextrins, hydroxypropyl-β-cyclodextrin (HP-β-CD) and random methyl-β-cyclodextrin (RM-β-CD) had the greatest solubilizing activity. The complexation of the sunscreen agent with HP-β-CD and RM-β-CD was confirmed by nuclear magnetic resonance spectroscopy. Solid-phase characterization of the PBSA/cyclodextrin systems by X-ray diffractometry defined the most appropriate method (co-evaporation) and cyclodextrin concentration (10-fold molar excess) for the preparation of a stable complexed form of PBSA. Long-term stability studies demonstrated that the decrease of the sunscreen level in emulsion preparations (pH 4.0) was almost completely suppressed by HP-β-CD, RM-β-CD being less effective. Moreover, the irradiation-induced decomposition of PBSA in the emulsion vehicle was markedly reduced by complexation with HP-β-CD (the extent of degradation was 3.9% for the complex compared to 9.1% for uncomplexed PBSA), whereas RM-β-CD had no significant influence. In addition, electron paramagnetic resonance (EPR) spin-trapping studies showed that the inclusion of the sunscreen agent into the HP-β-CD cavity completely inhibited the formation of free-radicals generated by PBSA on exposure to simulated sunlight, thereby suppressing its photosensitising potential.


*Keywords:* Sunscreen agent; Phenylbenzimidazole sulphonic acid; Cyclodextrin; Complexation; Stability; Photo-induced free radicals

## 1. Introduction

It is well-documented that exposure to the UV radiation (290–400 nm) from the sun plays a causal role in skin photodamage including acute inflammatory responses (i.e., erythema, oedema) and long-term adverse reactions such as cutaneous photoaging, immune suppression and various forms of skin cancers (National Institute of Health, 1989; Ziegler et al., 1994; Serre et al., 1997; Tarras-Wahlberg et al., 1999). The increasing knowledge on the hazards of exposure to sunlight has fuelled the widespread use of topical sunscreening preparations (National Institute of Health, 1989; Schauder and Ippen, 1997; Gasparro et al., 1998; Green et al., 1999). The active sunscreen constituents are of two types, inorganic substances that act mainly by reflecting or scattering the UV radiation and organic agents which decrease the dose of UV rays reaching the skin by absorbing the radiation, the latter being used most commonly (Gasparro et al., 1998). When exposed to sunlight, the sunscreen molecule is photoactivated and the excitation energy is eventually dissipated in the form of thermal energy, fluorescence, phosphorescence or transferred to the surrounding molecules. In addition, photo-induced decomposition of the sunscreen agent can also occur leading to a decrease of its UV-protective capacity and to the accumulation on the skin of potentially harmful photolytic products (Berset et al., 1996; Schauder and Ippen, 1997; Tarras-Wahlberg et al., 1999; Maier et al., 2001). Consequently, a high photochemical stability is an essential requirement for an effective UV filter (Berset et al., 1996; Maier et al., 2001). Another

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doi:10.1016/j.ejps.2004.03.014

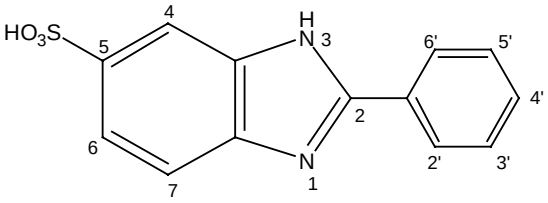



Fig. 1. Chemical structure of PBSA.

important characteristic that sunscreens should have is complete solubilization in the finished preparation, since the precipitation of the UV filter will lead to its uneven distribution over the skin surface and consequently to a reduction of the protective effect of the sun-care product (Johncock, 1999).

Phenylbenzimidazole sulphonic acid (PBSA; Fig. 1) is a widely used sunscreen agent (Inbaraj et al., 2002; Hayden et al., 1998) which absorbs most efficiently in the 290–320 nm region (UV-B) of the solar UV radiation. It is included in the list of authorized UV filters in Europe (EEC Directive, 1976), USA (US Food and Drug Administration, 1999) and Australia (Hayden et al., 1998). PBSA is used, after conversion into a salt, as a water-soluble sunscreen in aqueous preparations and in the hydrophilic phase of emulsions. In the latter case, the association of PBSA with an oil-soluble UV absorber achieves a synergic increase of the sun protection factor (SPF) which makes it possible to employ a lower concentration of UV filters (Siemer, 1991; Johncock, 1999). However, as PBSA is poorly soluble in acidic media, the pH of the formulations should remain at values higher than 7 (Siemer, 1991; Johncock, 1999) and hence above the desirable acid range of 4–6 (Block, 2000). Moreover, although PBSA is considered photostable (Berset et al., 1996), recent studies have demonstrated that this sunscreen agent under solar-simulated radiation generates a variety of free radicals and active oxygen species that cause photo-induced damage to DNA in vitro (Stevenson and Davies, 1999; Inbaraj et al., 2002).

Because of the growing interest in cyclodextrins and their ability to improve both the solubility and stability of active ingredients (Duchêne et al., 1999), the present study was carried out to evaluate the influence of cyclodextrin-based systems on the performance of PBSA. Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and an apolar cavity. They can encapsulate appropriately sized lipophilic molecules into their hydrophobic interior by forming non-covalent complexes (Loftsson and Brewster, 1996). This phenomenon affects the physico-chemical properties of the included substance and can increase its stability to air and light and the apparent aqueous solubility (Loftsson and Brewster, 1996; Rajewski and Stella, 1996).

The present work reports on the preparation and characterization of the complexes between PBSA and hydroxypropyl-β-cyclodextrin (HP-β-CD) or random methyl-β-cyclodextrin (RM-β-CD) and investigates the effect of a third component (i.e., water-soluble polymers or L-lysine) on the complexation efficiency. The influence of complexation on the light-induced free radical generation by the sunscreen and on its photostability and solubility at acidic pH are also presented.

## 2. Materials and methods

### 2.1. Materials

Phenylbenzimidazole sulphonic acid was supplied by Merck (Darmstadt, Germany). The cyclodextrins used in this study included hydroxypropyl-β-cyclodextrin, hydroxypropyl-α-cyclodextrin (HP-α-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD) and random methyl-β-cyclodextrin. They were purchased from Aldrich Chimica (Milan, Italy). Polyvinylpyrrolidone (PVP; MW ca. 55,000), hydroxyethyl cellulose (HEC; MW ca. 90,000), L-lysine and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) were obtained from Aldrich Chimica. Methanol, acetonitrile and water were high-performance liquid chromatography (HPLC)-grade from Merck. All other chemicals were of analytical-reagent grade (Sigma Milan, Italy).

### 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 305 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 on a 5 μm Zorbax SB-CN column (150 mm × 4.6 mm i.d.) fitted with a guard column (5 μm particles, 4 mm × 2 mm i.d.) and eluted isocratically, at a flow-rate of 1.0 ml/min, with aqueous triethylammonium phosphate (pH 2.8)–methanol–acetonitrile (70:25:5, v/v/v). The column temperature was maintained at 40 °C using a Model 7990 Space Column Heater (Jones Chromatography, Hangoed, UK). The identity of PBSA 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. Phase-solubility studies

Solubility analyses were carried out according to Higuchi and Connors (1965). An excess amount of PBSA was added to 5 ml acetate buffer solutions (50 mM; pH 4.0) containing the different cyclodextrins examined (0–90 mM) with or without a water-soluble polymer (0.25%, w/v) or L-lysine (90 mM). The samples with the polymers were heated in a sealed container at 120 °C for 20 min. The obtained suspensions were stirred in 10 ml screw-capped vials at 25 ± 1 °C

and shielded from light. After equilibrium had been reached (3 days, as demonstrated by a constant PBSA concentration of three successive samples at 3, 5 and 7 days), the content of each vial was filtered through 0.45 μm membrane filters (Whatman, Clifton, NJ, USA) and analysed for PBSA 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 PBSA 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.

### 2.4. Inclusion complex preparation

The inclusion complexes were prepared at a 1:1 and 1:10 molar ratios of PBSA to cyclodextrin using two different methods, namely kneading and co-evaporation. For kneading, the calculated amounts of PBSA and corresponding cyclodextrin were weighed, wetted with a small volume (5 ml) of methanol/water solution (30:70, v/v) and the slurry was kneaded throughly for ca. 30 min. After evaporation of the solvent, the samples obtained were dried under low pressure at room temperature for 3 days and stored in a desiccator. In the co-evaporation method, the calculated amount of PBSA was dissolved in methanol (4 ml) and added to 6 ml of an aqueous solution containing an equimolar or a 10-fold molar 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 by rotary evaporation and the residue was kept in a desiccator until used. The amount of PBSA incorporated into each complex was determined by HPLC after proper dilution.

### 2.5. 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° (2$\theta$) to 50° (2$\theta$) at an angular speed of 1° (2$\theta$) per minute using 1–1–1–0.15° slits.

### 2.6. NMR spectroscopy

$^1$H NMR spectra were recorded on a Varian Mercury Plus spectrometer (400 MHz). Since the aqueous solubility of PBSA is too low for the recording of NMR spectra, samples were solubilized in DMSO-$d_6$, at a concentration of ca. 10 mM. Chemical shifts are reported in ppm ($\delta$) relative to TMS. Typical parameters for the $^1$H NMR spectra were 0.35 Hz/pt resolution, 16 scans, 18 s relaxation delay, 90° pulse.

### 2.7. UV spectrophotometry

UV spectra were recorded in MeOH:DMSO:$H_2O$ (50:35:15, v/v) on a UV-Vis/NIR Spectrometer (Lambda 19; Perkin-Elmer, Norwalk, USA).

### 2.8. EPR measurements

Electron paramagnetic resonance (EPR) measurements were performed with a X-band Bruker 220 SE spectrometer (Bruker, Karlsruhe, Germany) equipped with a TE 201 resonator (Bruker OR 4104, 100% optical transmittance). Spectra were recorded with the following instrumental settings: 2 mW microwave power, 1 G modulation amplitude and 100 kHz field modulation. A quartz flat cell was used as a reaction vessel and samples were irradiated directly inside the microwave cavity employing a 350 W medium pressure Hg lamp. Irradiation wavelengths were selected using an Oriel 59814 (Oriel Corporation, USA) band pass filter (290 nm $< \lambda <$ 410 nm) coupled with an Oriel IR-block filter to avoid thermal effects. EPR spin-trapping experiments were performed in oxygen saturated phosphate buffer solutions (pH 7.4), containing DMPO (100 mM) as spin-trap and free or cyclodextrin-complexed PBSA (10 mM). The samples were irradiated in the EPR cavity for 25 min and spectra of the paramagnetic adducts were accumulated and recorded at appropriate time intervals. EPR signal intensities of the obtained paramagnetic adducts were measured at a fixed field position. Each series of experiments was repeated at least three times.

### 2.9. Long-term stability studies

The stability of PBSA was tested in cream preparations (oil-in-water emulsion) containing the sunscreen (0.5%, w/w) alone (after neutralization with NaOH) or complexed with cyclodextrins. The cream excipients were: sorbitan monostearate, polyoxyethylene sorbitan monostearate, butylated hydroxyanisole, isopropyl isostearate (Henkel, Fino Mornasco, Italy), cetearyl isononanoate (Henkel), cetearyl alcohol (Henkel), sodium benzoate, glycerin, dehydroacetic acid, EDTA, water. To prepare the cream, butylated hydroxyanisole, free or complexed PBSA were dissolved at room temperature in isopropyl isostearate, aqueous sodium hydroxide or deionized water, respectively. The other oil- and aqueous-soluble components were separately 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, free or complexed PBSA were added in the

cooling phase of the production process at about 40 °C. The pH of the formulations was adjusted to 4.0 with citric acid. The emulsions were placed into stoppered containers and stored at room temperature for 6 months, in the dark. At appropriate time intervals, aliquots (300–320 mg) were withdrawn from the emulsions and transferred into 10 ml calibrated flasks, diluted to volume with methanol and filtered (0.45 μm membrane filter). A portion (5 μl) of the resulting solution was analysed by HPLC for the assay of the remaining amount of PBSA.

### 2.10. Photodegradation studies

Photodecomposition experiments were performed in the same formulations utilized for the long-term stability studies. A portion (200–220 mg) of the test sample containing uncomplexed or complexed PBSA (0.5%, w/w) was transferred by means of a syringe onto the bottom of a beaker 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 (2 h), the beaker was removed and its content quantitatively transferred into a 10 ml calibrated flask with methanol and the remaining PBSA 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 PBSA from the irradiated samples, with those obtained by analysis of an equivalent amount of the unirradiated preparations.

### 2.11. In vitro sun protection factor measurement

The in vitro determination of the cream sun protection factor was carried out according to the Diffey and Robson (1989) technique, with minor modifications as reported in an earlier study (Scalia et al., 1999).

## 3. Results and discussion

### 3.1. Solubility studies

The solubility method was used initially for studying the interaction of PBSA with cyclodextrins. Because of the limited aqueous solubility of natural cyclodextrins (Loftsson and Brewster, 1996), the highly hydrophilic hydroxypropylated (i.e., HP-α-CD, HP-β-CD, HP-γ-CD) and randomly methylated (i.e., RM-β-CD) derivatives were selected for this investigation. Fig. 2A illustrates the phase-solubility curves of the UV-filter with the examined cyclodextrins in pH 4.0 acetate buffer (0.05 M). The solubilizing activity of cyclodextrins was evaluated at this pH, because it is under acidic conditions that precipitation of the water-insoluble

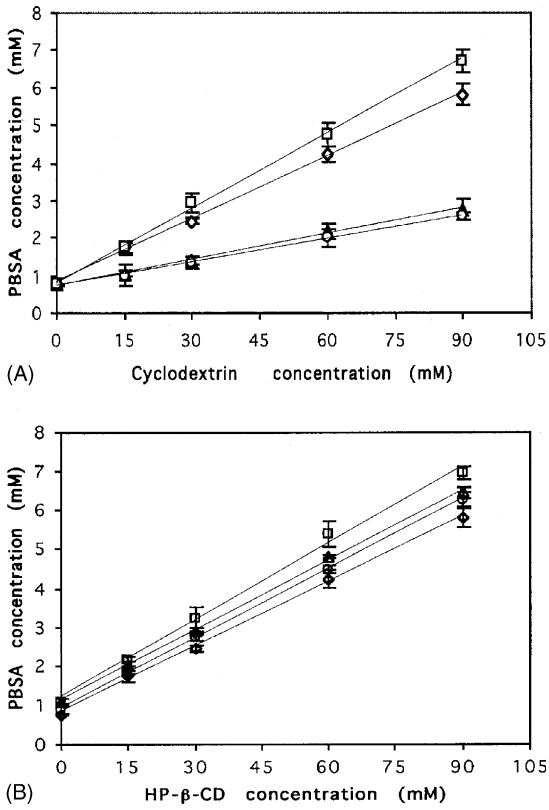



Fig. 2. (A) Phase-solubility diagrams for PBSA with different cyclodextrins in pH 4.0 acetate buffer at 25 °C. (△) HP-α-CD; (○) HP-γ-CD; (□) RM-β-CD; (◇) HP-β-CD. (B) Phase-solubility diagrams in pH 4.0 acetate buffer at 25 °C of PBSA in the presence of HP-β-CD without (◇) and with 0.25% (w/v) PVP (△), 0.25% (w/v) HEC (○) or 90 mM L-lysine (□). Each point represents the mean ± S.D. of at least three experiments. The lines are the regression lines obtained using least squares linear regression analysis.

free acid form of PBSA (p$K_a$ 4.5) occurs with loss of screening efficacy (Johncock, 1999). Moreover, 4.0 represents the lowest value in the normal pH range for cosmetic products and for the skin surface (Block, 2000). The diagrams obtained (Fig. 2A) demonstrated that the major solubility enhancements were produced by RM-β-CD (8.9-fold increase) and HP-β-CD (7.7-fold increase) which indicated that these cyclodextrins exhibited stronger interactions with PBSA than HP-α-CD and HP-γ-CD. Over the concentration range used in this study, the apparent solubility of PBSA increased linearly ($r > 0.99$) with increasing RM-β-CD or HP-β-CD concentrations showing AL-type profiles (Higuchi and Connors, 1965). These plots (Fig. 2A) with a slope <1 suggested that the solubility enhancement can be attributed to the formation of a complex with a 1:1 stoichiometry (Higuchi and Connors, 1965). The stability constants ($K_{1:1}$) calculated according to the method of Higuchi and Connors (1965) were 91.9 ± 2.1 and 78.6 ± 2.5 M$^{-1}$ for the RM-β-CD/PBSA and HP-β-CD/PBSA complex, respectively. Published studies (Loftsson and Brewster, 1996; Loftsson, 1998) have demonstrated that the efficiency of complexation can be improved by formation of ternary complexes between

the cyclodextrin, the guest molecule and a third component. Accordingly, the influence of the combined use of HP-β-CD and the water-soluble polymers, HEC and PVP, on the hydrosolubility of PBSA was evaluated at pH 4.0 after activation by heating (Loftsson, 1998). In the presence of HEC (0.25%, w/v) or PVP (0.25%, w/v) the solubilization of the sunscreen induced by HP-β-CD was only slightly improved (Fig. 2B), the enhancement being not synergistic. The $K_{1:1}$ calculated from the AL-type curves (Fig. 2B) for the HEC- and PVP-systems were $70.5 \pm 1.4$ and $58.2 \pm 1.2\,M^{-1}$, respectively. The lower values for the stability constants of these ternary systems as compared to the PBSA/HP-β-CD binary complex ($K_{1:1}$, $78.6\,M^{-1}$) can be ascribed to the solubilizing activity of the polymers themselves (Fig. 2B). Basic aminoacids can be used for ternary cyclodextrin complexation of acidic drugs due to their potential ability to interact simultaneously with the guest molecule (via salt formation) and the cyclodextrin (Piel et al., 1997). The addition of L-lysine (90 mM) to the pH 4.0 buffered medium produced a greater increase of the HP-β-CD solubilizing effect as compared to the polymer systems, although the differences were small (Fig. 2B). The stability constant value for the PBSA/L-lysine/HP-β-CD ternary complex was $66.3 \pm 1.0\,M^{-1}$. The decrease of the $K_{1:1}$ value observed in the presence of L-lysine can be traced to the higher initial drug solubility due to salt formation. Based on the results of the solubility studies, the systems with the highest stability constants (i.e., PBSA/RM-β-CD and PBSA/HP-β-CD) were selected for further experiments.

### 3.2. Complex characterization

The interaction between PBSA and the cyclodextrins was ascertained in solution by $^1$H NMR spectroscopic studies which provide the most conclusive evidence of complex formation (Hedges, 1998). Table 1 lists the variations in the chemical shift values of the aromatic protons of PBSA (see Fig. 1 for PBSA structure and atom labels) induced by the presence of RM-β-CD or HP-β-CD. All the $^1$H NMR signals were shifted upfield (negative $\Delta\delta$ values) indicating that the aromatic rings were located inside the cyclodextrin cavity (Chan et al., 2000). The largest chemical shift changes (Table 1) were detected for the protons of the phenyl substituent (see Fig. 1), suggesting that this moiety is deeply inserted into the cyclodextrin hollow cone.

Table 1
$^1$H NMR chemical shift changes ($\Delta\delta$, ppm) for PBSA in the presence of cyclodextrins

| Protons | $\Delta\delta$[a] | |
|---|---|---|
| | HP-β-CD | RM-β-CD |
| H4 | −0.029 | −0.034 |
| H6 | −0.017 | −0.014 |
| H7 | −0.011 | −0.008 |
| H2′, H6′ | −0.057 | −0.065 |
| H3′, H4′, H5′ | −0.041 | −0.044 |

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

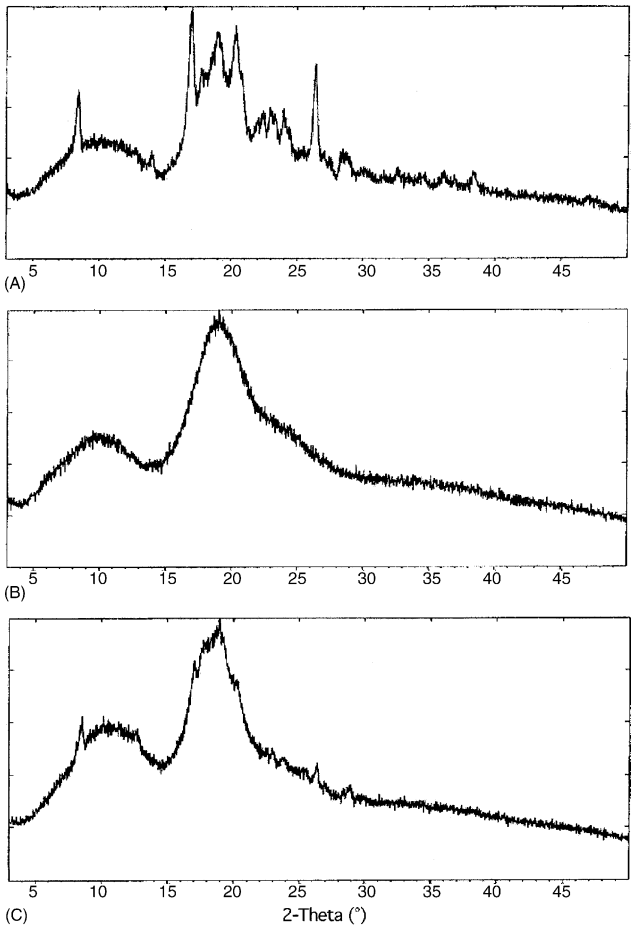



Fig. 3. Powder X-ray diffraction patterns of PBSA/HP-β-CD (1:10) physical mixture (A), PBSA/HP-β-CD (1:10) co-evaporated complex (B) and PBSA/HP-β-CD (1:10) kneaded complex (C).

The complexes of PBSA with HP-β-CD and RM-β-CD were characterized in the solid-state by powder X-ray diffraction. The solid complexes were prepared in molar ratios of 1:1 and 1:10 (guest:host), using two different methods, kneading and co-evaporation. As illustrated in Fig. 3, the diffractogram of the HP-β-CD complex (1:10, sunscreen:cyclodextrin) obtained by the co-evaporation process (Fig. 3B) did not show the PBSA crystalline peaks which were present in the X-ray diffraction pattern of the physical mixture (Fig. 3A). This finding provided evidence of the inclusion of PBSA into the cyclodextrin cavity. Conversely, in the diffractogram of the kneaded HP-β-CD complex (1:10, guest:host), the main PBSA signals appeared, though with a very low intensity (Fig. 3C). This indicated that the complexation of the sunscreen agent is less efficient when the kneading procedure is adopted and consequently the co-evaporation method was utilized to prepare the complexes for further studies. The same results were obtained from the X-ray diffraction analysis of the PBSA/RM-β-CD complexes (1:10 molar ratio). In contrast with the foregoing

data, the systems with a 1:1 molar proportion displayed distinct PBSA peaks at 8.5, 17, 20.3, 26.2° (diffraction patterns not shown). Hence, the X-ray diffraction studies suggest that complete inclusion of the sunscreen into the cyclodextrin cavity is achieved when a molar excess (10-fold) of the host is used. This result is not surprising given the low values for the equilibrium complexation constants (91.9 $M^{-1}$ for RM-β-CD and 78.6 $M^{-1}$ for HP-β-CD).

### 3.3. Stability studies

Since PBSA has a very low solubility both in water and oil, it must be neutralized completely with a suitable base in order to be used as a water-soluble UV-B filter. Moreover, to prevent crystallization, the pH of the finished sun-care preparation must be adjusted to values above 7.0 (Johncock, 1999). This is a disadvantage because the topical application of such a product can alter the skin physiological acidic pH, causing irritation (Schneider and Thor, 1991). In addition, it is important to ensure that no compound with an acidic reaction is used in the sunscreen formulation since this could lead to precipitation of the water-insoluble free acid form of PBSA with loss of efficacy (Johncock, 1999). In order to evaluate the effect of cyclodextrin complexation on the chemical–physical stability of PBSA, an aging study was performed using a cream (oil-in-water emulsion) as a medium. This vehicle was selected as a model formulation since it represents the most commonly used type of sunscreen preparation (Siemer, 1991). In addition, the cream pH was adjusted to 4.0 because it is under acidic conditions that product stability is reduced via precipitation. Free PBSA (as sodium salt) or its complexed form was incorporated (0.5%, w/w) into the cream and subjected to the stability-indicating assay. We have reported above that for efficient complexation of PBSA a 10-times molar excess of cyclodextrin is required. Accordingly, the complexes in a molar ratio of 1:10 (PBSA:cyclodextrin) were used in the subsequent studies The higher proportions of cyclodextrins should also reduce the competitive displacement of the guest molecule from the host cavity by the various emulsion excipients (Scalia et al., 1999; Scalia et al., 2002). A number of studies have demonstrated that an excess amount of cyclodextrin can lead to decreased drug absorption through skin (Loftsson and Masson, 2001 and references therein). Accordingly, the excess of cyclodextrin present in the test formulations could reduce the percutaneous penetration of PBSA, thereby enhancing its retention at the skin surface where the sunscreen action is highly desirable (Treffel and Gabard, 1996).

The emulsions containing the sodium salt of the free sunscreen agent or its cyclodextrin complex were analysed for PBSA over 6 months at room temperature and in the dark and the data generated are depicted in Fig. 4. The plot for the cream containing the uncomplexed sunscreen agent showed the PBSA level falling below 81.0% of the initial concentration within 6 months (Fig. 4). Under the same storage conditions, 89.9% of the UV filter initial

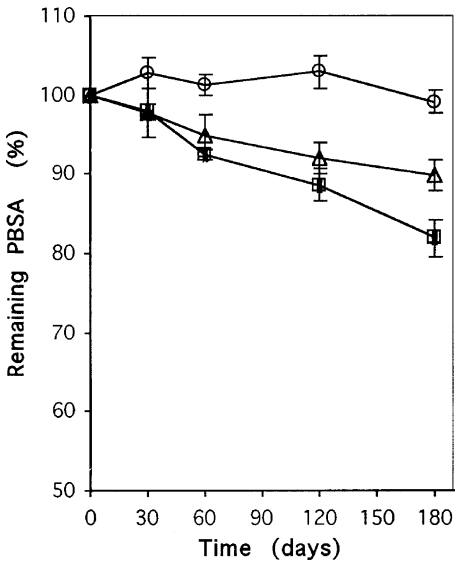



Fig. 4. Plots of PBSA concentration vs. time in its formulations with or without cyclodextrin. (□) PBSA; (△) PBSA/RM-β-CD; (○) PBSA/HP-β-CD. Values are mean ± S.D. of at least three experiments.

content was recovered after 6 months in the PBSA/RM-β-CD preparation (Fig. 4). Although this result indicated a reduction in the loss of PBSA, the differences observed between the samples without or with RM-β-CD were not statistically significant ($P > 0.1$, unpaired *t*-test), with the exception of the values measured at day 180 ($P < 0.01$, unpaired *t*-test). However, the formulation containing the PBSA/HP-β-CD complex retained 99.1% of the original sunscreen concentration after the test period (Fig. 4), indicating a higher effectiveness of HP-β-CD as compared to RM-β-CD in enhancing the stability of the sunscreen agent at acidic pH. Therefore, the efficacy of the examined complexes in long-term stability studies was not related to the corresponding complex formation constants (91.9 $M^{-1}$ for RM-β-CD and 78.6 $M^{-1}$ for HP-β-CD).

To investigate the influence of the cyclodextrin systems on the photochemical behaviour of PBSA, photolysis experiments were also performed on the formulations submitted to the foregoing aging study. The creams were exposed for 2 h to the solar simulator, the applied UV-B energy being equivalent to 20 minimal erythemal dose (MED) which is considered representative of daily solar emission (Tarras-Wahlberg et al., 1999). Following irradiation, 9.1% of the sunscreen content was lost in the preparation containing PBSA alone (Table 2), in good agreement with previous studies (Berset et al., 1996). The photo-induced decomposition of the sunscreen agent was not significantly affected (Table 2) by complexation with RM-β-CD (8.0% degradation). Conversely, under the same experimental conditions, a statistically significant reduction of the extent of photodegradation to 3.9% was attained in the cream containing the PBSA/HP-β-CD system (Table 2). Hence, the photostabilization effects of the examined cyclodextrins correlate with their activity in the aging study. Moreover, photolysis

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

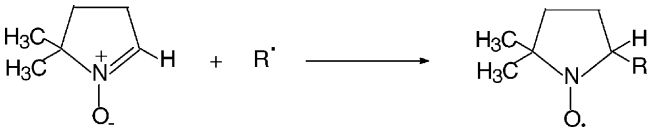
| Sample | %PBSA loss[a] | $P$[b] |
|---|---|---|
| Free PBSA | 9.1 ± 2.2 | |
| PBSA/RM-β-CD | 8.0 ± 1.5 | >0.1 |
| PBSA/HP-β-CD | 3.9 ± 1.7 | <0.001 |

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

experiments performed after 6 months storage of the cream samples at room temperature and in the dark, demonstrated that the sunscreen photostability enhancement achieved by HP-β-CD complexation was retained during the above time interval (the percentage loss of PBSA upon irradiation was 4.2 ± 1.0% in the formulation containing the complexed UV filter as compared to 10.8 ± 1.1% for the preparation containing free PBSA). The in vitro determination of the sun protection factor of the creams containing the uncomplexed PBSA or its complex with HP-β-CD showed that complexation had no significant influence on the photoprotective capacity of the examined sunscreen preparations (the SPF values ranged from 2.5 to 2.8; $P > 0.05$, unpaired $t$-test). In addition, UV spectrophotometric analysis of PBSA and its HP-β-CD complex showed that the shape of the spectrum and the degree of UV absorption of the sunscreen agent were not affected by complexation (spectra not shown).

### 3.4. EPR studies

Recent reports in the literature (Stevenson and Davies, 1999; Inbaraj et al., 2002) have demonstrated that when exposed to solar UV radiation, PBSA generates a variety of free radicals and active oxygen species that are capable of causing in vitro damages in single- and double-stranded DNA. Accordingly, the next aspect of the present investigation was to examine the influence of the cyclodextrin complex microenvironment on the photosensitising properties of the sunscreen agent. Aqueous solutions (pH 7.4) containing PBSA free or complexed with HP-β-CD were illuminated for 25 min with simulated sunlight and the photo-induced free radicals were characterized by the EPR spin-trapping technique, using DMPO as spin-trap. Radical species ($R^{\bullet}$) generated during sunscreen irradiation were monitored by detection of the more stable paramagnetic adduct formed according to the following equation (Janzen and Haire, 1990):




On exposure to UV light of the sample containing uncomplexed PBSA and the spin-trap, a spectrum was obtained consisting of four lines with a 1:2:2:1 intensity ratio and with hyperfine splitting constants $a_N = a_H = 14.9$ G (Fig. 5) which can be ascribed to the formation of the adduct between

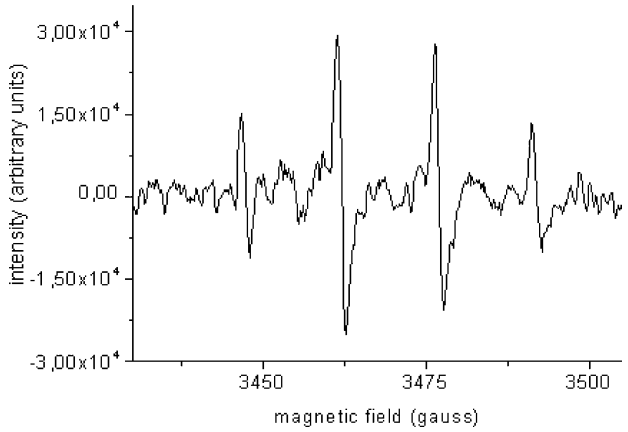



Fig. 5. EPR spin-trapping spectrum of the adduct (DMPO-OH•) obtained after irradiation of buffered (pH 7.4) aqueous solutions containing DMPO (100 mM) in the presence of PBSA (10 mM). $a_N = a_H = 14.9$ G.

DMPO and OH• radicals (Buettner, 1987). When the irradiation was carried out in the absence of PBSA, no EPR signal was detected. The observation of the spectrum of the adduct DMPO-OH• is a clear indication that a photosensitisation process from PBSA to $O_2$ occurred (Inbaraj et al., 2002). The adduct can be expected to derive from direct trapping (Beauchamp and Fridovich, 1970), very fast decomposition of DMPO-$O_2^{\bullet-}$ formed via an electron transfer process (Finkelstein et al., 1979) or singlet oxygen generated via an energy transfer reaction from photogenerated PBSA triplet state to $O_2$ (Jones and Wilson, 1980). The increase of the DMPO-OH• adduct EPR signal intensity during PBSA irradiation is illustrated in Fig. 6. Under the same experimental conditions, no EPR signal was detected (see Fig. 6) in the solution containing PBSA complexed with HP-β-CD (1:10, guest:host). The results shown in Fig. 6 clearly demonstrated that the photo-induced production of oxygen-centered radicals by PBSA is completely inhibited by inclusion complexation of the sunscreen agent with HP-β-CD. This

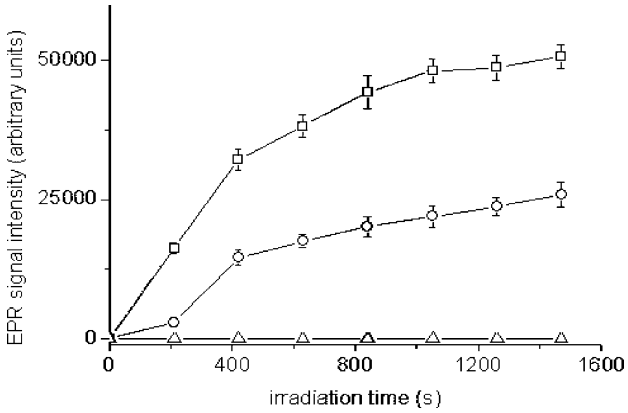



Fig. 6. EPR signal intensity at a fixed field position of the paramagnetic adduct (DMPO-OH•) during irradiation of buffered (pH 7.4) aqueous solutions containing DMPO (100 mM) in the presence of PBSA (10 mM). (□) Free PBSA; (△; 1:10) PBSA/HP-β-CD; (○; 1:1) PBSA/HP-β-CD. Each point is the mean ± S.D. of at least three experiments.

remarkable effect can be traced to hindered interaction of the photoactivated complexed PBSA with oxygen or to a reduction of the life-time of the photogenerated sunscreen triplet state. Additional measurements were performed on sample containing the complex in a 1:1 (sunscreen:cyclodextrin) molar ratio. On UV illumination of this system, the paramagnetic adduct between DMPO and $OH^{\bullet}$ was detected, though its formation rate was significantly lower as compared to free PBSA (see Fig. 6). These data indicated that the influence of HP-β-CD on the sunscreen photosensitizing activity is much greater for the complex with a 1:10 (PBSA:cyclodextrin) molar proportion, in line with the powder X-ray results on the efficiency of the inclusion process.

## 4. Conclusions

The results described in this study demonstrate that the complexation of PBSA with HP-β-CD extends the stability of the sunscreen agent to the acidic pH conditions optimal for topical formulations, such as the sun-care products. Moreover, the inclusion of PBSA into the HP-β-CD cavity represents an effective strategy to enhance the sunscreen photostability as well as to suppress the light-induced production of free radicals by the UV filter. Hence, the PBSA/HP-β-CD system should also minimize the damage inflicted by the sunscreen to DNA following illumination with simulated sunlight.

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