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Preparation and Evaluation of Inclusion Complexes of Commercial Sunscreens in Cyclodextrins and Montmorillonites: Performance and Substantivity Studies

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Herein we describe inclusion complexes of commercial sunscreens in cyclodextrins and montmorillonites to generate new sunscreen derivatives with optimized functional properties such as water resistance and skin adherence. Four cyclodextrins (-, -, and -cyclodextrin, and -dimethyl cyclodextrin) and two montmorillonites (sodium and alkylammonium) were investigated for encapsulating some commercial sunscreens. Our results reveal a good yield and inclusion products with functional properties obtained by using kneading technique on Eusolex® 2292 and Eusolex® 6007 in -cyclodextrin and solubilization method on Eusolex® 6007 and NeoHeliopan® MA in montmorillonite. In addition, molecular modeling studies indicated flexibility as important for the intercalation of the host molecule.

Keywords coprecipitation; solubilization; kneading techniques; cyclodextrin; montmorillonites

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INTRODUCTION

The widespread use of topical sunscreen preparations has increased due to the knowledge about the harmful effects of sunlight ultraviolet (UV) radiation (290 nm–400 nm), including erythema, cutaneous photoaging, immune suppression, and skin cancer (Del Hoyo, 1996; National Institutes of Health, 1989; Serre, Cano, Picot, Meynadier & Meunier, 1997; Ziegler et al., 1994). Organic chemicals that absorb UV radiation are the most common active constituents in these products used for attenuating the sun effects on the skin (Chatelain & Gabard,

2001; Gasparro, Mitchnick, & Nash, 1998; Jiang, Hayden, Prankerd, Roberts, & Benson, 1996).

Substantivity represents the sunscreen's ability of maintaining its features on the skin under stressful conditions, including continual and repetitive water exposure or sweating. Thus, the ideal sunscreen formulation should present a high photostability since the light-induced decomposition may decrease its screening effects and generate toxic degradation products. In addition, in order to achieve the highest protective effect and minimize the toxicological risk derived from the percutaneous penetration, the ideal agent should also display a high degree of retention in the outermost cutaneous layers and resist removal by sweat or water, without needing frequent reapplication (Hayden, Roberts, & Benson, 1997, 1998; Horwitz, Frost, & Steven, 1982; Maier, Schauberger, Brunnhofer, & Hönigsmann, 2001).

The literature describes several approaches for improving the substantivity of sunscreen formulations, such as natural and synthetic complex polymer preparation, proper solvent selection, and encapsulation processes (Lowe, Shaath, & Pathak, 1997). Among the encapsulation processes, liposome, liposphere, and cyclodextrin present the advantage of increasing sunscreen concentration on the application area as a result of the high level of adherence to the stratum corneum (Benita, 1996; Motwani & Zatz, 1997; Scalia, Villanis, Scatturin, Vandelli, & Forni, 2007; Wienner et al., 1994).

Cyclodextrins (CDs) comprise a series of α-1,4-linked cyclic oligosaccharides formed by six (α-cyclodextrin [α-HCD]), seven (β-cyclodextrin [β-CD]), eight (γ-cyclodextrin [γ-CD]) or more glucose units leading to a cup-like geometry. CDs show a rigid structure with a singular hydrophobic cavity due to the absence of hydroxyl groups. This specific structure allows CDs to enhance solubility, chemical stability, and bioavailability of poorly soluble drugs, also reducing toxicity and controlling the rate of release (Uekama, Hirayama, & Irie, 1998). Moreover, complexation using cyclodextrin may also: (a) enhance drug stability to air and light, (b) affect the availability of topically applied sunscreens, either increasing or decreasing their permeability into and through the skin, and (c) delay the photodegradation process (Rajewski & Stella, 1996; Loftsson & Brewster, 1996; Simeoni, Scalia, & Benson, 2006; Uekama et al., 1998). Therefore these CD's structural and biological features are of pharmaceutical interest and have been widely used in pharmaceutical applications (Scalia, Molinari, Casolari, & Maldotti, 2004; Simeoni, Scalia, & Benson, 2004; Ventura et al., 2005).

The use of the intercalation process of organic guest species into clays is a way of constructing ordered inorganic–organic assembly with unique microstructures controlled by host–guest and guest–guest interactions (Theng, 1974). Organic clay intercalation compounds may produce a novel photofunctional supramolecular system and improve sunscreen substantivity. Meanwhile, physical and chemical sunscreen associations may be obtained using the intercalation process of commercial sunscreens and clays. Organophilic smectites prepared by cation exchange of the interlayer cations with cationic surfactants (i.e., long chain alkylammonium cations) mix into organic solvents and adsorb organic molecules in the interlayer space by hydrophobic interactions (Dekany, Szanto, & Nagy, 1986; Dekayn, Szanto, & Weiss, 1989; Dekany, Szanto, Weiss, & Lagaly, 1986; Ogawa & Kuroda, 1997; Simeoni et al., 2004). These features allow introducing ionic and nonionic organic compounds into the interlayer space of smectite clays, which is useful for the intercalation of organic sunscreens (Ogawa, Aono, Kuroda, & Kato, 1993; Ogawa, Hama, & Kuroda, 1999; Ogawa, Kimura, Kuroda, & Kato, 1996; Ogawa, Shirai, Kuroda, & Kato, 1992; Ogawa, Wada, & Kuroda, 1995; Sasaki & Fukuhara, 1997). The main advantage of the inclusion of sunscreens in montmorillonite cavities and its lipophilic derivative is their functional similarity with β-CD, but with a lower cost profile. Considering the larger volume of the interlayer space, the montmorillonite could minimize the negative effect of volume and molecular flexibility in the inclusion process.

Herein we investigate the sunscreen inclusion process using CDs and montmorillonites as carrier to create new cosmetic formulations with differential release and substantivity profiles. To that purpose, six commercial sunscreens, Eusolex® 232 (2-phenyl-benzimidazole-5-sulfonic acid), Eusolex® 2292 (3-[4-metoxyphenyl]-2-propenoic acid 2-ethyl hexyl ester), Eusolex® 6007 ([4-dimethylamino] benzoic acid 2-ethyl hexyl ester), Eusolex® 6300 (3-[4´-methylbenzylidene]-boran-2-one or 3-[4-methylbenzylidene] camphor), benzophenone-4 (5-benzoyl-4-hydroxy-2-methoxy-benzenesulfonic acid), and NeoHeliopan® MA (menthyl anthranilate), four CDs (α-CD, β-CD, γ-CD, and γ-diethyl-cyclodextrin [γ-DCD]), and two montmorillonites derivatives (sodium and alkylammonium montmorillonites) were tested by using coprecipitation, solubilization, and kneading techniques. In addition the sunscreen-CDs, inclusion complexes were analyzed in a solid state by differential scanning calorimetry (DSC), infrared spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM), and the inclusion yields were determined by UV analysis. The structures of the inclusion and nanocomposites yields were characterized by XRD, thermogravimetric analysis (TGA), and TEM. Finally, the formulated inclusion complexes were evaluated by in vitro and in vivo sun protection factor (SPF) assays and molecular modeling techniques (Pinto et al., 2004).

MATERIALS AND METHODS

Materials

All commercial sunscreens were at analytical grade including Eusolex® 232 [2-phenyl-benzimidazole-5-sulfonic acid], Eusolex® 2292 [3-(4-metoxyphenyl)-2-propenoic acid 2-ethyl hexyl ester], Eusolex® 6007 [(4-(dimethylamino) benzoic acid 2-ethyl hexyl ester], Eusolex® 6300 [3-(4-methylbenzylidene)-boran-2-one or 3-(4-methylbenzylidene) camphor]. Eusolex®

is a trade name of Merck KGaA, Darmstadt, Germany. Benzophenone-4 [5-benzoyl-4-hydroxy-2-methoxy-benzenesulfonic acid], and NeoHeliopan® MA (methyl anthranilate). γ-CD, β-CD, and β-dimethyl cyclodextrins (β-DCD; Wacker GmbH, Munich, Germany) were at pharmaceutical grade. Sodium montmorillonite and its alkylammonium derivative (Viscogel B8) were from Bentec (Livorno, Italy) at pharmaceutical grade. Purified water used in all solution preparations was obtained using a Milli-Q system (Millipore, Massachusetts, USA).

Formulation of Sunscreens

The oily phase was prepared using isooctyl stearate (4%), Chembase® (10%; fatty alcohols and nonionic emulsificants mixture), and Phenova® (0.5%; liquid conservant containing methyl, ethyl, propyl, and butyl parabens and phenoxyethanol). The aqueous phase was prepared using glycerol (4%) and distillated water q.s.p. 100%. Triethanolamine (0.1%) was added to the aqueous phase only in the Eusolex® 232 formulation. Usual sunscreen concentrations of 6% w/v (U.S. Food and Drug Administration [FDA], 1993) were incorporated in a nonionic emulsion using corrected inclusion complex mass (dispersion) and sunscreen alone (dissolution). The preparation of the initial emulsion consisted of the mixture of the oily phase, heated at 80°C and completely homogenized, over the aqueous phase, also heated at 80°C. The emulsion mixture was stirred until it reached the optimal consistence at room temperature.

Preparation of the Sunscreen-CD Complexes Using the Solubilization Method

In order to establish the optimal β-CD/sunscreen ratio (Scalia, Villanis, S.; Scatturin, Vandelli, & Forni, 1998; Higuchi & Connors, 1965), a concentrated solution of β-CD (1.15 g diluted in 100 ml of purified water) was prepared and then distributed to obtain solutions with a β-CD/sunscreen mass ratio of 2:1, 3:1, 4:1, 5:1, 6:1, and 7:1 (weight/weight). For control purposes, a "0" point including only the original sunscreen without cyclodextrin was also prepared. Then, 50 mg of sunscreen was dissolved in 5 ml of an ethanol/water mixture (70/30, respectively), except for benzophenone-4, where water as used instead, and added to the reaction medium containing the β-CD solution (q.s.p. 20 ml). Due to the higher solubility of β-dimethyl cyclodextrin (β-DCD) in water (57 g in 100 ml), the best β-DCD/sunscreen ratio for inclusion was experimentally determined by mixing the sunscreen (50 mg) in ethanol/water (70:30) solution (20 ml) and adding the amount of β-DCD necessary to reach the CD/sunscreen molar ratio of 2:1 to 8:1 until total solubilization. After that, all CD solutions were stirred during 72 hours at 25°C, kept without stirring for decantation of any precipitate, and finally filtered in Wathman 42 analytical paper. In order to reach a higher homogeneity, the experiments using α-CD or γ-CD were performed as described for β-CD, except for temperature (55°C) and stirring (300 rpm). The inclusion products were frozen at −70°C and lyophilized for further analysis if no precipitate was observed.

Inclusion of Sunscreens Into CD by Kneading Method

The calculated amounts of sunscreens and corresponding cyclodextrin were weighted and moistened with a small volume (1 ml) of ethanol/water solution (70:30 v/v). The mixtures were ground thoroughly for a approximately 5 minutes in a mortar with a pestle until the mixture reached a granulation aspect (Scalia et al., 1998). The resulting product was transferred and charged for 1 hour in a ball mill (11 cm:8cm l/d), containing 27 g of porcelain ball (1 cm mean diameter). The resulted samples were dried in an oven at 55°C for 30 minutes and stored in desiccators.

Intercalation of Sunscreens in the Interlayer Space of Alkylammonium and Sodium Montmorillonite

Alkylammonium or sodium montmorillonites were dispersed in an acetic acid/water mixture (1:1) with magnetic stirring. The sunscreen solubilized in the same solvent was added, and the suspensions were allowed to react for 1 to 30 hours with magnetic stirring at room temperature. The suspension was centrifuged and the solids were dried under reduced pressure. We tested the molar ratio of sunscreen to montmorillonite from 1:0.5 to 1:2 (sunscreen/clay).

Characterizations

The concentrations of sunscreen in the solution in the cyclodextrin complex and in the interlayer of montmorillonite were determined by using a UV spectrometer (Perkin-Elmer, Norwalk, Connecticut, USA). The concentration in the calibration curve was in concentrations of 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 μg/ml for the studied compound, with an R^2 value of 0.995 to 0.998 in all studied cases (International Conference on Harmonization, 1995). Fourier transformed infrared (FTIR) spectra for all samples were recorded using KBr pellets (1 mg sample/300 mg KBr) on a PerkinElmer FTIR Spectrometer Paragon 1000 grating instrument with slow scan and normal slit width. All inclusion complexes were also analyzed by DSC (with measurements performed in a PerkinElmer DSC 7 apparatus).

XRD

An XRD pattern of the sunscreen/montmorillonite composites was determined by using a Rigaku X-ray diffractometer. The diffraction angle (2 θ) of the patterns was recorded from 2° to 10° at 1°/minute; with a total scanning speed of 41/minute. CuKa radiation was used as the X-ray source, which was operated at 40 kV and 30 mA at room temperature and a 0.15418 nm wavelength. Silicon powder was used as the standard sample for calibrating the peak position and viscogel B8 for internal comparison.

DSC Analysis

The samples were analyzed at 22.5 ml/minute in a PerkinElmer DSC 7 apparatus with an aluminum support and nitrogen flow. The samples were heated from 0°C to 150°C (10°C/minute).

TEM Analysis

The samples were prepared by placing a small amount of powder in the copper grating covered with special carbon film and analyzed in a vacuum of 10 to 4 Torr, tungsten filament at 2127°C, and using the transmission electron microscope JEOL 1200EX as described by Kornmann (2000).

Ocular Irritability

Initially, we determined the pH of each formulation and adult New Zealand albino rabbits were used to test each formulation (two animals/test). Briefly, one gram of each formulation was diluted with 2 ml of purified water. This solution (0.1 mL) was applied in the conjunctiva of one eye of each animal, after raising the inferior eyelid of the ocular globe. Then both eyelids were joined per 10 seconds for preventing substance loss. The other eye acted as control with no treatment.

The irritation level was evaluated at 0, 4, 24, 48, and 72 hours after application. We evaluated the experiment based on the presence or absence of injury in the cornea (opacity), iris (inflammation), and redness of conjunctive eyelid, bulb, cornea, and iris (Brito, 1994).

Primary Cutaneous Irritability

Four adult New Zealand albino rabbits were used to test each formulation. The dorsal region of the animal body was shaved 24 hours before the application of the sample. Four small sites of application were randomly chosen and two were submitted to abrasion as described by Brito (1994). Then the formulation (0.5 g) was applied in two sites (one submitted to the abrasion and the other not), while the other two acted as controls for the assay. The application area was covered with gauze fixed by a hypoallergic adhesive tape for 4 hours. Then the gauze and the formulation sample were removed and the sites were washed with water for residue elimination. The evaluation of the primary cutaneous irritability (edema and erythema) was performed 4, 24, 48, and 72 hours after the formulation application (Brito, 1994). The positive symptoms of primary cutaneous irritability lead to not testing cumulative ocular and cutaneous irritability.

Cumulative Cutaneous Irritability

This test indicates the feasible effects of the repeated expositions of the formulation on human skin. The assay was performed similarly to the primary irritability test, except that each formulation was reapplied 24, 48, and 72 hours after the first application (Brito, 1994).

Ethical Approval of In Vivo Tests

The Ethics Research Committee of Clementino Fraga Filho Hospital University of the Federal University of Rio de Janeiro, Brazil, approved all in vivo test protocols and the whole project, which was numbered as process number 115/04.

In Vitro and In Vivo SPF Analysis

The in vitro analysis of these sunscreens was performed as described by Mansur (1986). Briefly, each preparation was diluted in ethanol at a final concentration of 0.2 μL/ml and its UV absorbance was determined in triplicate. The in vivo SPFs were determined as described by the FDA (1993), where 20 human volunteers (skin types I, II, and III) and solar UV simulators from Multiport 601-Solar Light Company were used. The water-resistant property was determined by evaluating the subject after two 20-minute immersions with moderate activity in water. The cyclodextrin complexes sunscreen (6%) were incorporated in a nonionic emulsion by dissolution or dispersion (montmorillonite sunscreen complexes) and compared with the negative control (Eusolex® 2292, 6% alone). A lotion with homosalate (8%) was used as the standard preparation. All assays were made by Alergisa-SP according to Brazilian ethical protocols. The inclusion products were also analyzed in the irritability test as described elsewhere (Brito, 1994). In all formulations, the pH value ranged form 6.8 to 7.5 whereas product viscosity, determined by using Brookfield viscometer (model LVT), ranged from 12,000 to 18,000 cps.

Statistical Data Analysis

One-way analysis of variance and Wilcoxon match pairs tests were used to analyze all statistical data obtained in this study.

Molecular Modeling Analysis of the Sunscreens

The conformational analysis of all the sunscreens was performed using the AM1 Hamiltonian with the SPARTAN 1.0.5 program (Wavefunction, Inc. Irvine, California, USA, 2000). The dihedral angles were independently searched between 0° and 360° in 30° steps. After the reoptimization of the minimal energy conformations with the keywords GNORM = 0.2 and PRECISE, Hessian matrix analyses were employed to characterize them as true minima of the potential energy surface. The minimum dimensions of the drugs were estimated using the van der Waals radii and the molecular structures.

In the absence of guest and under dehydratation conditions, the X-ray diffraction d001 spacing for montmorillonite was 9.6 Å, which indicated that no interlayer molecules (i.e., water or guest) were present in the control sample. Adsorption of

guest molecules between the layers of montmorillonite increased the d001 spacing. The difference between the d001 spacing of the expanded montmorillonite-guest complex and the d001 spacing of the collapsed montmorillonite-control system was designated as the △d001 value.

RESULTS AND DISCUSSION

Cyclodextrins

Initially, two sunscreens of widespread commercial use (Eusolex® 2292 and Eusolex® 6300) were tested for inclusion into β-CD by using the coprecipitation or solubilization techniques. This method is described in the literature as an easy technique for including sunscreens such as a butylmethoxydibenzoylmethane that provides a significant yield and allows the determination of the ideal carrier/guest molecule ratio for inclusion (Giordano, Novak, & Moyano, 2001).

The DSC and UV analysis (Pitha & Hoshino, 1996) of the inclusion products revealed only the Eusolex® 2292 inclusion in β-CD (Figure 1). This complex formation presented a nonlinear behavior and insoluble sunscreen residues, which prevented the determination of the ideal ratio of these molecules (Table 1). These results pointed to the low or no solubilization of the guest molecule in the inclusion carrier as a factor involved in the inclusion assay's low reproducibility and yield observed in both sunscreen experiments. The ethanol competition with the guest molecule may also interfere in the formation of the inclusion complex as described by the literature (Wade & Weller, 1999).

Importantly the increase of temperature (55°C) and stirring speed (300 rpm) was not able to solve the solubility issue, which infers that the inclusion carrier solubility is a restriction factor for the inclusion process. Therefore, in order to confirm solubility as an issue for this inclusion process, β-DCD was used as the molecular encapsulation agent due to its higher solubility in water (Lach & Chin, 1964). Our results showed neither an alteration in yield nor in response linearity when using β-DCD (Table 1) that may exclude the inclusion carrier solubility as a restriction factor in this case.

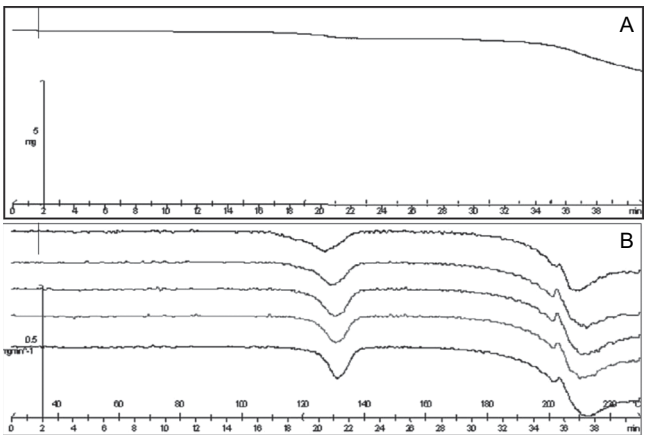

FIGURE 1. DSC analysis of Eusolex® 2292 and β-CD inclusion: (A) Eusolex® 2292 alone and (B) five samples analyses (ratio of 2:1 to 7:1 CD/sunscreen)—approximately 120°C inclusion complex and approximately 210°C sunscreen degradation.

TABLE 1
Evaluation of the Inclusion Process of Eusolex® 2292 Using β-CD and β-DCD Using the Solubilization Technique

Ratio (CD/sunscreen)	Precipitate Absorbance	Inclusion Yield (%)	
		β-CD	β-DCD
0:1	—	—	—
2:1	0.660	16.95	8.44
3:1	0.661	16.97	6.66
4:1	0.662	17.00	9.18
5:1	0.682	17.51	6.61
6:1	0.751	19.28	5.77
7:1	0.754	19.30	5.64

In order to analyze the influence of the guest molecule water solubility in the inclusion process, as described by Largen and colleagues (1998), benzophenone-4, a sunscreen with a high solubility in water (1 g in 4 ml of water), was used to replace the Eusolex® 2292 in the β-CD inclusion experiments (Largen, Endo, Ueda, & Zimmermann, 1998; Loftsson & Brewster, 1996). In spite of the absence of insoluble residues at the end of the inclusion process, the DSC results showed no inclusion complex formation in this experiment.

The literature describes sunscreen molecular volume as a major restrictive factor for an inclusion process (Dodziuk, 2002). The molecular volumes of the more stable conformations of both Eusolex® 2292 and Eusolex® 6300 calculated by using a molecular modeling approach revealed that both sunscreen structures were bigger (367.53 $Å^3$ and 318.54 $Å^3$) than the cavity presented by β-CD (cavity volume = 262 $Å^3$; Figure 2). However further experimental assays with Eusolex® 2292 using α-CD (174 $Å^3$) revealed even lower inclusion yields and reproducibility, while γ-CD (472 $Å^3$) increased yields approximately 20% (Table 2). Thus, these data inferred no prevalence of the molecular volume over the encapsulation process and other restraining parameters.

In the case of benzophenone-4, the conformational analysis revealed two stable conformations (A and B, DH = −182.39 Kcal/mol and 179.27 Kcal/mol, respectively), which in agreement with the negative inclusion result, present a larger molecular volume (A = 309,64 $Å^3$, B = 310,14 $Å^3$) than the β-CD cavity (262 $Å^3$; Figure 2). Furthermore, the conformational flexibility of the benzophenone-4 was lower than that of Eusolex® 2292 and Eusolex® 6300, which probably compromised the suitable interaction of the smallest portions of the sunscreen molecule with the β-CD lipophilic cavity. Taken together, these data suggest that the ideal sunscreen for the

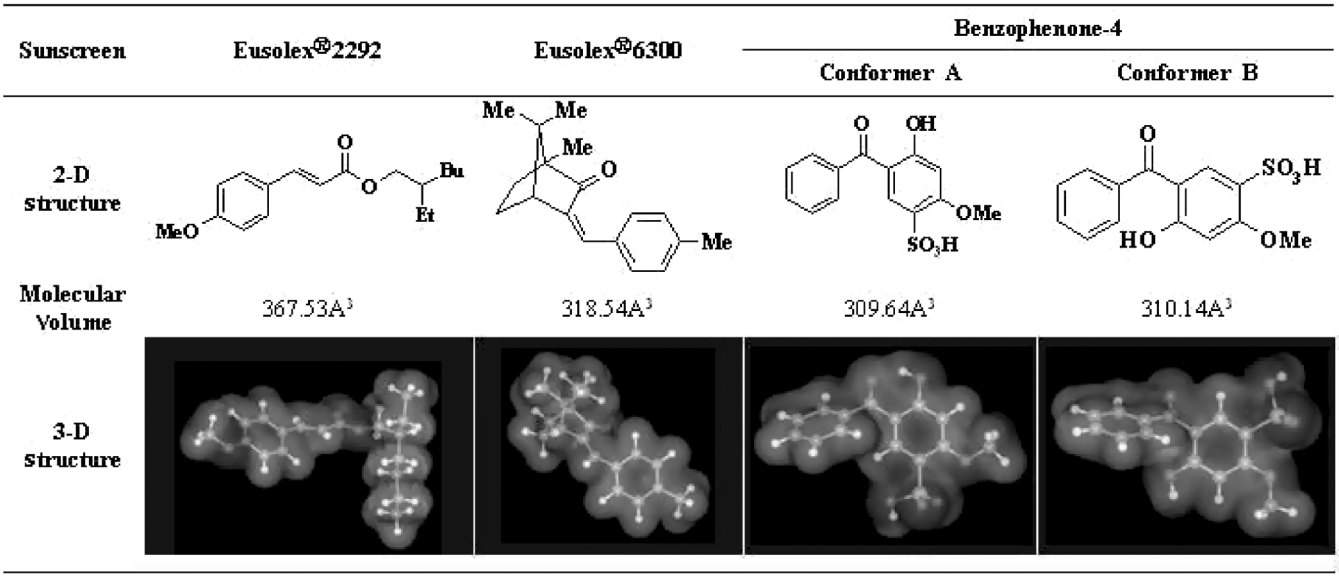

FIGURE 2. Theoretical analysis of the most stable conformations of Eusolex® 2292, Eusolex® 6300, and benzophenone-4, showing 2-D structure, molecular volume, and 3-D structure (ball-and-stick and van der Waals surface representation).

TABLE 2
Evaluation of the Eusolex® 2292 Inclusion Process by Solubilization Technique Using α-CD, β-CD, and γ-CD

Ratio (CD:Sunscreen)	α-CD Yield (%)	β-CD Yield (%)	γ-CD Yield (%)
0	—	—	—
2:1	9.98	16.95	18.89
3:1	8.89	16.97	17.61
4:1	11.21	17.00	20.28
5:1	9.97	17.51	18.10
6:1	13.41	19.28	23.56
7:1	7.59	19.30	23.25

inclusion process tested should present a high significant solubility, a proper molecular volume, and an optimal conformational flexibility in order to be included into the cyclodextrin (Cramer, 1982; Garcia, Mayoral, & Alvira, 1997; Scalia et al., 1998). The investigation of new prototypes for inclusion of sunscreens such as Eusolex® 232 and Eusolex® 6007 reinforced the importance of these features as they showed no or low inclusion yield (0% and 23%, respectively), whereas they were similar to Eusolex® 2292 in molecular volume and flexibility.

Due to the negative coprecipitation results, and to evaluate different and industrially feasible inclusion techniques, the kneading process was used in the inclusion of sunscreens into β-CD. This method is described in the literature as an easy procedure, with low cost and high reproducibility (Loftsson & Brewster, 1996). The kneading technique results (2:1 CD/sunscreen) showed a successful inclusion process with Eusolex® 2292 and Eusolex® 6007, and a considerable yield for Eusolex® 6300 (Table 3). In addition, these new sunscreen preparations have a better interaction with the skin (SPF in vivo test) and significantly decreased the UV radiation–induced erythema in the irritability test, compared with the formulation without β-CD (Table 3). Interestingly, the DSC results revealed an evident endothermic peak for the included Eusolex® 6007 at 121ºC and water at 101ºC, in contrast to Eusolex® 2292 (Figure 3). Since the Eusolex® 2292 inclusion product did not show any conclusive DSC result using the initial experimental conditions, double heating was adopted during the DSC procedure to completely remove the water present in β-CD. The data obtained with the double heating showed the sunscreen inclusion product at 115ºC to 120ºC (Figure 3). Interestingly, the comparison of the kneading products with the previous solubilization technique results (Figure 1) indicated an increase in the thermal stability of the molecule since the degradation product observed for Eusolex® 2292 at 210ºC was not observed using the kneading process (Figure 3).

Montmorillonite Nanocomposites

Based on the CD results and some chemical features, Eusolex® 6007 was chosen as a prototype for inclusion. In fact, its basic site for protonation is probably favorable for the inclusion in the interlayer space of montmorillonite in cation form. Meanwhile, the use of NeoHeliopan® MA, a product structurally related to Eusolex® 6007 and also included in cationic form, was investigated. In this study, Eusolex® 2292 was used as a neutral host molecule (Figure 4).

TABLE 3
Inclusion Complex (IC) Yield, SPF Values from In Vitro and In Vivo Assays, and Skin Irritability Tests of Five Sunscreens (Eusolex® 232, 2292, 6007, and 6300, and Benzophenone-4) Free or Complexed with β-CD by the Kneading Technique

		In vitro SPF			In Vivo SPF			Irritability (h)[a]	
Sunscreen	IC yield (%)	Free	IC	*SD*	Free	IC	*SD*	Free	IC
Eusolex® 232	0	14.40	13.10	1.00	11.70	11.70	0.14	72	48
Eusolex® 2292	70	7.50	9.00	0.55	10.10	10.70	0.19	72	48
Eusolex® 6007	43	12.80	13.10	0.70	12.30	11.80	0.26	48	24
Eusolex® 6300	16	11.90	10.60	1.50	11.90	10.60	0.10	72	48
Benzophenone-4	0	6.90	6.70	0.80	5.80	5.90	0.12	48	48

[a]All sunscreens tested (free or complexed with β-CD) were not irritable for the subjects.

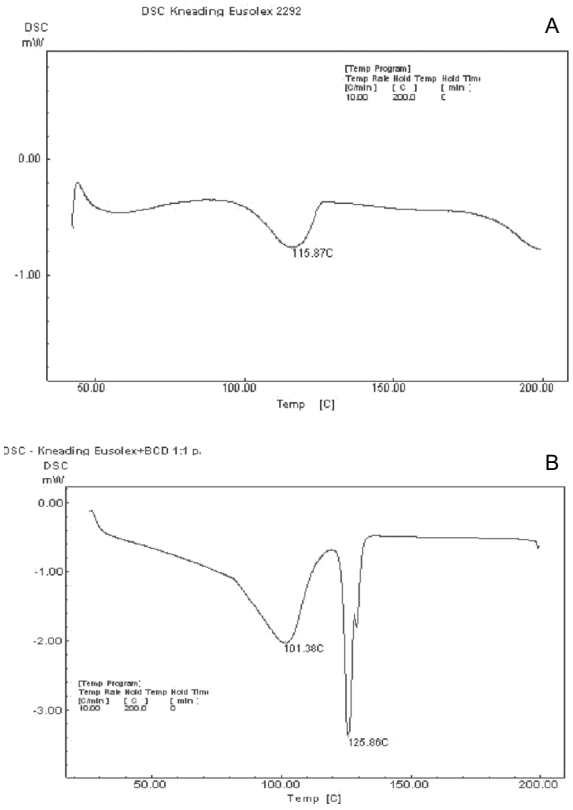

FIGURE 3. DSC analysis of (A) Eusolex® 2292 and (B) Eusolex® 6007 into β-CD.

As expected, Eusolex® 6007 and NeoHeliopan® MA effectively interacted with both montmorillonites (sodium and organophilic), probably due to their cationic characteristics. These features probably allow these molecules to interact with the clays both through ionic exchange and electrostatic interactions as well as through adsorption and lipophilic interactions (Porubcan, Born, White, & Hem, 1979). The optimal assay conditions were the time reaction of 1 hour at rt. and a molar ratio of 1:1 (sunscreen/clay). For both sunscreens, the concentration of guest molecules observed was higher than the cationic exchange capacity defined for the sodium bentonite (Viseras Iborra et al., 2006). Thus, in addition to a cationic exchange, there is also a process of sunscreen–clay interaction inside the layers (Table 4).

The use of Eusolex® 2292 with alkylammonium and sodium montmorillonite showed an inclusion yield below 20 meq/100 g and near to zero, respectively (Table 4). This result suggested the requirement of cationic species for interacting with sodium clay, and the occurrence of dipole-dipole or hydrophobic interactions inside the layer when it is intercalated with alkylammonium ions (alkylammonium montmorillonite). The alkylammonium derivative presents an interlayer distance (28.95 Å) higher than sodium montmorillonite due to the presence of the quaternary ammonium salt.

By analyzing the XRD results of the inclusion complexes, a significant increase in interlayer spacing in all experiments indicated the insertion of guest molecules inside the layers (Table 5). The infrared spectrum showed an important displacement of the diffractograms of the inclusion products of the sunscreens in alkylammonium montmorillonite, which confirmed the success of the process (Figures 5 and 6). As expected and in agreement with the literature, the analysis of the DSC and TGA of the nanocomposites showed no conclusive results (Lee & Kim, 2002). Thus, to determine the volume and height of the most stable conformations (monolayer, bilayer, trilayer, pseudolayer, or paraffinic) of each inclusion complex, we used a molecular modeling approach (Lee & Kim, 2002). Our theoretical data using the SPARTAN program showed a lower height of Eusolex® 6007 (4.7 Å) than the movement caused by it (5.47–6.35 Å). Thus, we may suggest that the photoprotector molecule is located in a bilayer or pseudo trilayer conformation inside the layer space of the studied clays, probably due to its significant molecular flexibility.

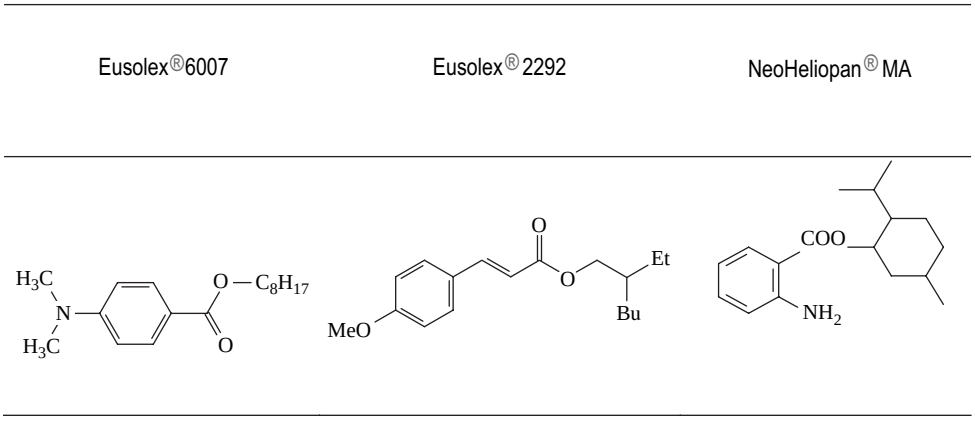

FIGURE 4. Sunscreens used in montmorillonite inclusion process.

TABLE 4
Inclusion Complex Yields of Three Sunscreens (Eusolex® 2292, 6007, and NeoHeliopan® MA) in Two Montmorillonite Derivatives (Sodium and Alkylammonium)

	Montmorillonite Inclusion Complex Yield (meq/100 g)	
Sunscreen	Sodium	Alkylammonium
Eusolex® 2292	8	20
Eusolex® 6007	240	250
NeoHeliopan® MA	220	220

TABLE 5
Interlayer Distance (ID) of the Montmorillonite Structure, Calculated by XRD of the Free Montmorillonite (Sodium or Alkylammonium) and the Corresponding Inclusion Complex of Eusolex® 6007 and NeoHeliopan® MA

Samples of Montmorillonite	Structure	ID (Å)	Δ (Å)
Sodium montmorillonite (SM)	Free	9.50	—
SM and Eusolex® 6007	Inclusion complex	14.97	5.47
SM and NeoHeliopan® MA	Inclusion complex	14.50	5.00
Alkylammonium montmorillonite (AAM)	Free	28.95	—
AAM and Eusolex® 6007	Inclusion complex	35.30	6.35
AAM and NeoHeliopan® MA	Inclusion complex	30.90	1.95

[a]$\Delta = ID_{inclusion\ complex} - ID_{free}$.

Interestingly, the movement observed for NeoHeliopan® in the sodium bentonite was higher (5 Å) than that of alkylammonium montmorillonite (1.95 Å), whereas the basal spacing

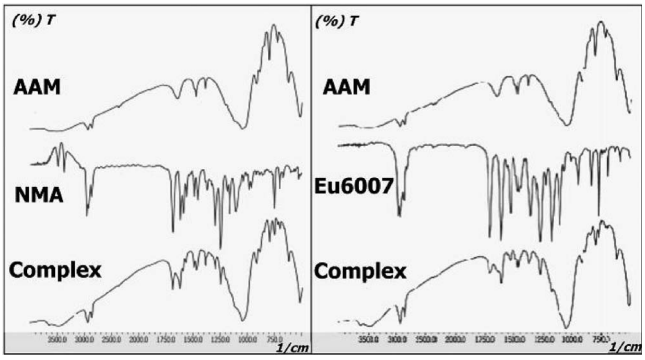

FIGURE 5. Infrared spectra of free alkylammonium montmorillonite (AAM), free NeoHeliopan® MA (NMA), and Eusolex® 6007 (Eu6007), and the correspondent inclusion complexes (Complex).

of this guest is 14.5 Å. This spacing represents an increase of 4.9 Å compared with dehydrated montmorillonite (9.6 Å). This interlayer spacing is somewhat smaller than the 5.9 Å minimum dimensions as the height calculated of the NeoHeliopan® minimized structure by molecular modeling (Figure 7). Green-Kelley and colleagues (1955) studied a number of organic molecules adsorbed onto the interlayer surface of montmorillonite and noted that the increase in interlayer space could be smaller than the dimension of the adsorbed molecule by as much as 1 Å. Further studies showed that keying or a different geometric packing can occur for the adsorbed molecule, resulting in smallest basal spacing than expected. Thus the observed interlaminar spacing is possibly due to the adsorption of the guest and suggests that a single layer has been adsorbed in a parallel orientation. Importantly, and in agreement with the molecular modeling results, we observed an evident layer structure in the inclusion of Eusolex® 6007 and montmorillonite. This revealed the formation of an intercalated structure similar to that observed for viscogel B8 and clay intercalated with octadecylamine (Figure 8).

The new sunscreens obtained were then tested for in vivo assay to determine the SPF and the influence of the clay in the functionality of the evaluated filters. Similar to the CD results,

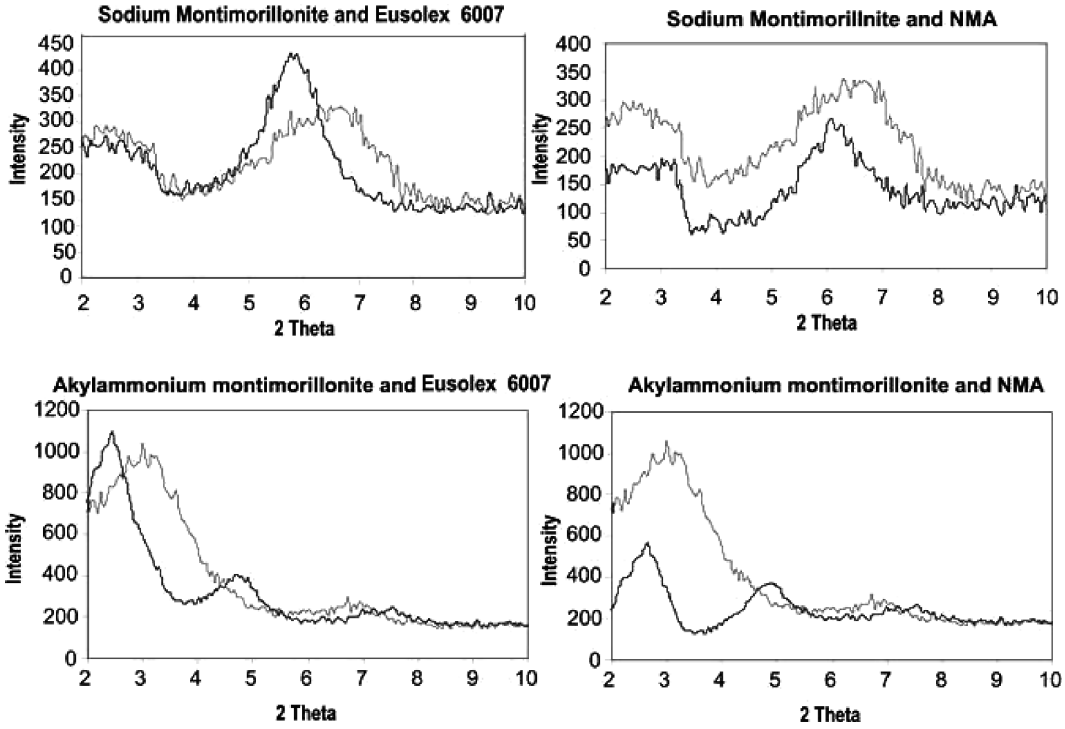

FIGURE 6. Diffractograms of free sodium montmorillonite and alkylammonium montmorillonite, showed in gray, and the correspondent inclusion complexes of Eusolex® 6007 and NeoHeliopan® MA (NMA), showed in black.

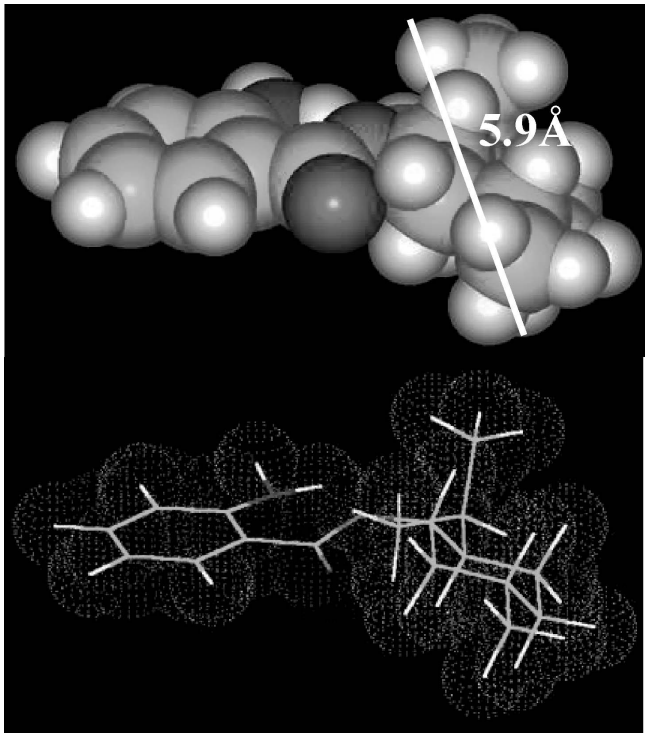

FIGURE 7. Theoretical analysis of the most stable conformation for NeoHeliopan® MA showing the 5.9 Å minimum dimension, as calculated by Van der Waals radii.

there was a significant increase in both formulations' SPF value compared with the solar filter alone in both cases. These data infer a synergistic profile of the clay with the inclusion material in the layers, acting as a physical solar filter (Table 6). Interestingly, all products were safe when tested in dermal and ocular irritability, presenting no irritability.

CONCLUSION

In summary, different inclusion techniques were evaluated to determine the optimal conditions for inclusion of known sunscreens into CDs and montmorillonites. Our results indicated that Eusolex® 2292, Eusolex® 6007, and NeoHeliopan® MA inclusion in β-CD or montmorillonite could improve their functionality and commercial use.

The study of different techniques of inclusion pointed to the molecular volume and solubility as important to the success of the process. It also showed the viability of using the kneading technique on an industrial scale for preparing sunscreens enclosed into CDs. The montimorillonite and its derivatives had shown an efficient profile as carriers for preparing more efficient sunscreens of combined action (chemical and physical) than those obtained with isolated molecules. Therefore, we presented new sunscreen complexes as suitable sun protector formulations, since they presented a reduced systemic absorption, low dermal irritability, and higher water resistance. In addition, the scale up feasibility of the inclusion process for

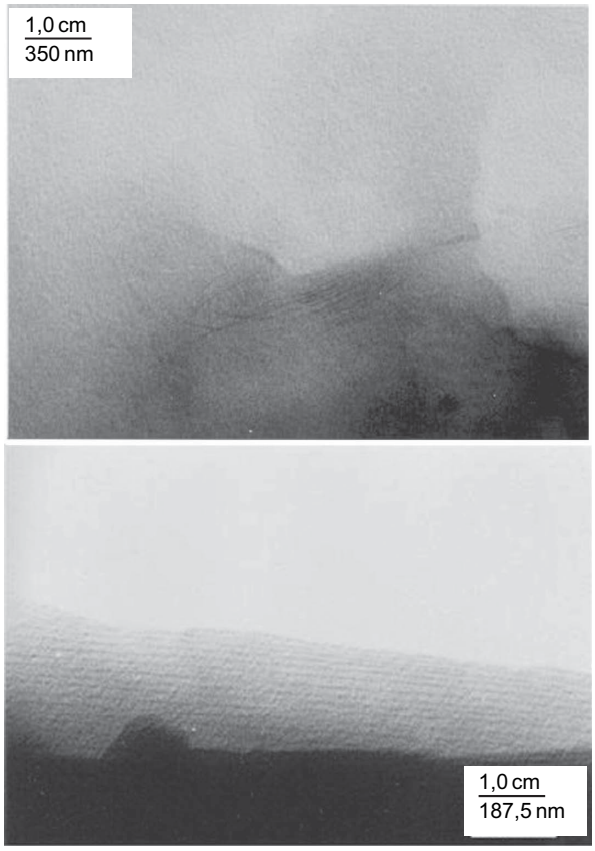

FIGURE 8. Layer structure of Eusolex 6007 and alkylammonium montmorillonite nanocomposites.

TABLE 6
SPF Values from In Vivo Assays of the Free Sunscreens Eusolex® 6007 and NeoHeliopan® MA, or in Inclusion Complexed Form with Sodium or Alkylammonium Montmorillonite

Sample	Form	SPF	*SD*
Eusolex® 6007	Free	12.30	± 0.26
Eusolex® 6007 and sodium montmorillonite	Inclusion complex	14.20	± 0.33
Eusolex® 6007 and alkylammonium montmorillonite	Inclusion complex	13.00	± 0.55
NeoHeliopan® MA	Free	9.80	± 0.44
NeoHeliopan® MA and sodium montmorillonite	Inclusion complex	11.10	± 0.25
NeoHeliopan® MA and alkylammonium montmorillonite	Inclusion complex	10.60	± 0.38

structurally related solar filters in CDs and similar encapsulation systems were achieved in this work.

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