Drug Development and Industrial Pharmacy, 2010; 36(6): 688–697

informa
healthcare

ORIGINAL ARTICLE

Physicochemical study on microencapsulation of hydroxypropyl-β-cyclodextrin in dermal preparations

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Abstract

Objective: To investigate the effect of hydroxypropyl-β-cyclodextrin (HP-β-CD) concentration on the physicochemical properties of the sunscreen agents, namely oxybenzone (Oxy), octocrylene (Oct), and ethylhexyl-methoxy-cinnamate (Cin), in aqueous solution and cream formulations. *Methods*: The inclusion complexes of sunscreen agents with hydroxypropyl-β-cyclodextrin (HP-β-CD) in aqueous solution and solid phase were studied by UV-vis spectrophotmetery, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and ^{13}C-NMR techniques. The photodegradation reaction of the sunscreen agents' molecules in lotion was explored using UV-vis spectrophotometry and high-performance liquid chromatography (HPLC). *Results*: The formation of the inclusion complexes was confirmed experimentally using DSC, SEM, and ^{13}C-NMR. The results of spectrophotometric and HPLC studies have shown that the inclusion complexation with HP-β-CD has the potential to enhance the photostability of the selected sunscreen agents in lotion. HPLC results indicated that HP-β-CD has approximately increases the photostability of Oct by six- to eightfold. Moreover, the presence of HP-β-CD in lotion controlled the isomerization process of Cin to a certain degree, which was found to be a function of the amount of HP-β-CD added. *Conclusions*: It has been demonstrated that the photostability of the tested sunscreen agents has been enhanced upon forming inclusion complexes with HP-β-CD in lotion. The results of this study demonstrate that HP-β-CD can be utilized as photostabilizer additive for enhancing the photostability of the sunscreen agents' molecules.

Key words: *Dermal preparations; hydroxypropyl-β-cyclodextrin; inclusion complex; photostability; sunscreen filters*

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucopyranose units linked together by oxygen bridges at the 1 and 4 positions (α,1,4-glycoside bonds)[1]. This class of organized media possesses a hydrophilic exterior and a hydrophobic cavity because of C^3H, C^5H, and C^6H hydrogens and O^4 ether oxygen that enables the CDs to extract a variety of organic guest molecules of appropriate size and hydrophobicity from the bulk aqueous solution[2–4]. The most familiar members are α-, β-, and γ-CDs consisting of six, seven, and eight glucose units, respectively.

Hydroxypropyl-β-cyclodextrin (HP-β-CD) is produced from β-CD by the addition of propylene oxide to some of the hydroxyl group of β-CD. This modification results in a different chemical structure of β-CD but can form complexation as β-CD. Chemical modification of CDs can alter their physical properties, mainly their solubility. HP-β-CD is partially substituted poly(hydroxypropyl) ether of β-CD (Figure 1)[5].

Complexation of various compounds with CDs leads to an enhancement in some of the characteristics of the guest molecules, such as stability, bioavailability, membrane permeability, and solubility[6]. Thus, CDs have been employed in a variety of fields such as catalysis, pharmaceutical and food industries[7–9], separation sciences[10–15], and biotechnology[16,17]. In cosmetics the use of CDs is still short in literature information in comparison

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(Received 4 Jul 2009; accepted 28 Oct 2009)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.3109/03639040903449738

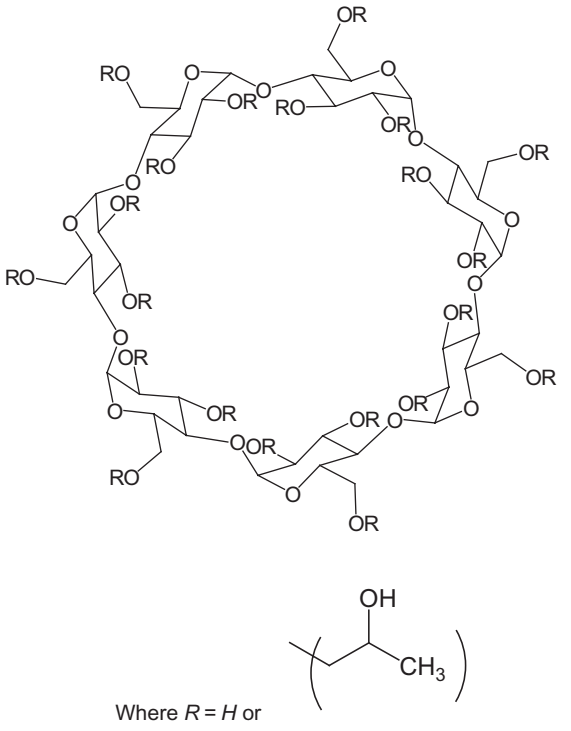

Figure 1. Chemical structure of HP-β-CD.

with other areas, whereas most publications are patents. It is worth mentioning that significant alterations in the physicochemical properties of the included molecule have been observed upon forming the inclusion complex with CDs[1–3,9].

CD encapsulation of a drug will affect many of the drug's physicochemical properties without affecting its intrinsic pharmacologic properties[4]. One of the most unique properties of CDs is their ability to enhance dermal and transdermal drug delivery without affecting the skin barrier, the stratum corneum. The skin has a much lower affinity for hydrophilic CD molecules, and therefore they remain in the aqueous vehicle system[5,8,9].

CDs have been used for a variety of reasons in cosmetic preparations, such as reducing the odor in mercaptan systems, improving the stability of hair dyes, controlling volatility, and as an active ingredient in antiacne treatments[18]. They may affect the permeation properties of certain active ingredients and have been used to improve fragrance stability. CDs may also be used as emulsifiers in creams or lotions and in an underarm deodorants[18]. CDs have been shown to modify cutaneous absorption of active ingredients although their overall benefit in this regard has yet to be proven[18]. As they do not absorb UV or Vis light, CDs can protect a guest molecule from oxidation and photodegradation[19]. Therefore, microencapsulation of sunscreen agents has become recently an interesting area of study[20].

The interaction between ethylhexyl-*p*-methoxycinnamate with unmodified and modified α-, β-, and γ-CDs was studied by water phase solubility analysis. Although photodegradation of sunscreen agent was significantly reduced by the formation of inclusion, comparative studies of the influence of CDs on stability showed that β-CD increases stability and limits adverse interactions on the UV filter with other formulation ingredients[21].

The effect of HP-β-CD concentration on the transdermal permeation and skin accumulation has been studied by Felton et al.[22] It has been observed that upon increasing the concentration of HP-β-CD from 0% to 10%, the transdermal permeation and skin accumulation of oxybenzone (Oxy) was decreased. They suggested that high concentrations of HP-β-CD may create a drug reservoir on the skin surface[22].

HP-β-CD is one of the most useful CDs in pharmaceutical applications because of its high aqueous solubility and inclusion capacity. This compound is too large and hydrophilic to readily penetrate the skin[23]. HP-β-CD has been subjected to extensive toxicological studies and is considered safe, with no tumor-initiating or tumor-promoting effects[24]. In this study, the inclusion complexation of selected sunscreen filters, Oxy, octocrylene (Oct), and ethylhexyl-methoxy-cinnamate (Cin) (Figure 2) with HP-β-CD in aqueous solution and solid phase was investigated by UV–Vis spectrophotometry, differential scanning calorimetry (DSC), and scanning electron microscopy (SEM) techniques. The photostability of dermal preparations in the presence and absence of HP-β-CD was investigated by UV–Vis spectrophotometry and high-performance liquid chromatography (HPLC) techniques.

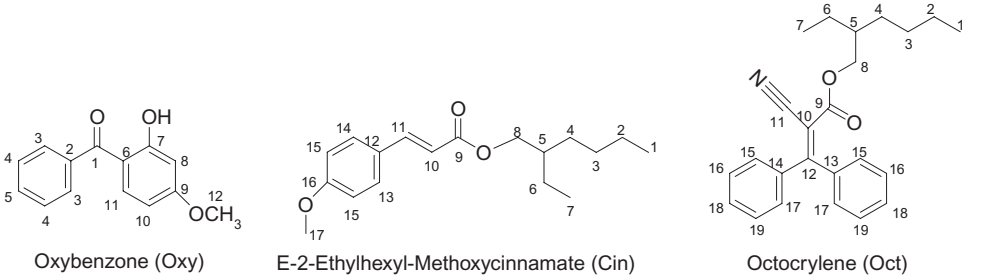

Figure 2. Chemical structures of oxybenzone (Oxy), octocrylene (Oct), and ethylhexyl-methoxy-cinnamate (Cin).

Materials

HP-β-CD (97+% purity) was purchased from Acros Organics (Geel, Belgium). HP-β-CD has the following specifications: the average substitution degree 2.8 (±5%), hydroxylpropyl group per CD ring, and mainly the substitution at positions C2 at the glucopyranose unit. The sunscreen filters Oxy, Cin, and Oct (98% purity) were kindly provided by Alfa Chemical Manufacture (Amman, Jordan), supplied from Haarmann and Reimer (H&R), Holzminden, Germany. Emulgade 1000 (cetostearyl alcohol), Emulgin B1 (polyglycerol poly-12-hydroxystearate), Lanette 16 (cetyl alcohol), Eutanol G (octyl dodecanol), crodamol IPM (isopropyl myristate), glycerin, methyl paraben (MP), and propyl paraben (PP) were also kindly provided by Alfa Chemical Manufacture. Methanol, ethanol, and acetonitrile of HPLC grade were purchased from Scharlau (Sentmenat, Barcelona, Spain). For photostability, formulation, and HPLC experiments, in solid and liquid inclusion complexes, all solutions were freshly prepared with deionized water. All chemicals were used without further purification. Lotion with sun protection factor 30 (SPF 30) was provided by Alfa Chemical Manufacture.

Measurements and methods

Preparation of the inclusion complexes

All samples were prepared by dissolving about 200 mg with different molar ratios, 0:1, 1:1, and 2:1 of [HP-β-CD]:[guest]. Complexations with HP-β-CD and sunscreen agents were done in methanol/water mixture (85:15, v/v) with stirring in solution for 48 hours at room temperature. The solid form of the inclusion complexes was prepared by freeze-drying method. The solution was frozen by immersion in shell freezer and freeze-dried over 24 hours in a Lyph-lock 6 freeze dryer (Labconco, Kansas City, MO, USA). For SEM measurements the crystals of free Oxy and HP-β-CD were obtained by crystallization from a 8:2 methanol–water solution; within 1 week, colorless (Oxy) and white (HP-β-CD) crystals precipitated. The product was filtered and washed with diethyl ether.

Inclusion in formulation

Dry mixing method was used in the preparation of inclusion paste. The inclusion paste was formed by mixing the three sunscreen agents, Oxy, Cin, and Oct, and preservatives MP and PP with different amounts of HP-β-CD. The complexation took place by mixing the paste for more than 15 minutes. The thickness of the paste seems to depend almost on the amount of HP-β-CD. Unfortunately, the guests (Oxy, Cin, Oct, MP, and PP) need a large amount of CDs to form equimolar ratio. Most formulations used in the pharmaceutical industry are usually only a few percent by weight of CDs added as additives. Accordingly, in this study the CDs were added in a few weight percent.

Sunscreen lotion formulation

The ingredients of the sunscreen lotion with SPF 30 are shown in Table 1.

Procedure

Parts A and C were mixed as described in 'Inclusion in formulation'. Part B was melted at 60°C. Then, parts A and C were mixed with part B, and hot water (60°C) was added to the mixture. Then the mixture was cooled to room temperature. Note that a continuous stirring should be carried out through all steps. Lotion sample was prepared by dissolving about 150 mg of lotion using a mixture of ethanol and water (85:15, v/v), and then the solution was diluted to 500 mL.

UV–Vis spectrophotometry measurements

UV–Vis spectra were recorded with a Shimadzu single beam UV–Vis spectrophotometer (UV-2401 (PC) S). The spectra of the sunscreen agent inclusion complexes were recorded in a mixture of alcohol (methanol or ethanol) and water in 1-cm cuvette; for inclusion experiments the concentration of sunscreen agent was ~30 μM, and HP-β-CD was added in different molar ratios (one- to fourfold). Stirring time ranged from 2 hours to 4 days.

High-performance liquid chromatography measurements

HPLC chromatograms were recorded by Shimadzu Auto Sampler. The separation was achieved with a LiChroCart 125-4 RP-18 end-capped (5-μm) column

Table 1. Sunscreen lotion SPF 30 formula ingredients.

Part	Ingredient name	Weight (g)
A	Cin	8
	Oxy	8
	Oct	6
	MP	0.2
	PP	0.15
B	Emulgade 1000	8
	Emulgin B1	3
	Lanette 16	3
	Eutanol G	3
	IPM	1
	Glycerin	5
C	HP-β-CD	0–10
D	Water	70

(Merck, Darmstadt, Germany). The separation and detection parameters were as follows: the mobile phase consisted of isocratic methanol/water (85:15, v/v), a flow rate of 1 mL/min, an equal sample volume of 100 µL was injected, and the wavelength of absorption detection was set at 308 nm.

Thermal analysis

DSC measurements were carried out using Shimadzu DSC-50 system equipped with a computerized data station TA-5 WS/PC and with vented aluminum pans. Thermograms of ~10-mg samples were obtained by scanning within a temperature range of 50–400°C and a scanning rate of 10°C/min. An empty pan was used as reference.

Scanning electron microscopy

The morphology of Oxy, HP-β-CD, Oxy:HP-β-CD physical mixture (1:1 molar ratio), and solid Oxy:HP-β-CD inclusion complex (1:1 molar ratio) was analyzed by SEM. The samples were vacuum coated with gold for 150 seconds, and the images were taken on a LEO 1530 SEM operating at 20 kV.

^{13}C-NMR study

The ^{13}C-NMR spectra were recorded at 25°C using Bruker Model AC-200E spectrometer with Me_4Si as an internal standard, operating at nominal ^{13}C frequencies 63 MHz. In all measurements the solvent was DMSO-d_6.

Photostability study

The photolysis experiments were performed by using a 150-watt Mercury UV immersion lamp (Heraeus, Hanau, Germany). Photostability of all sunscreen agents was studied separately and as a mixture in lotion. The photolysis cell temperature was controlled by a flow of tap water through the photolysis jacket. The temperature was always less than 40°C. At this temperature there is no thermal degradation expected for the three sunscreen agents. The photostability of sunscreen lotion has been studied in the absence and presence of HP-β-CD. The sunscreen lotions were prepared according to the common procedure described previously in this section. The photostability experiments were performed in cream formulations (oil-in-water emulsion) containing sunscreen lotions (2%, w/w) alone or complexed with HP-β-CD. The concentration of sunscreen lotions was held constant for all the cream formulations. One hundred microliters of the lotions or cream formulations was spread as a layer, as uniform as possible by circular movements of a gloved finger, onto a 50-cm^2 glass plate (2 mg/cm^2). Ten minutes after applications, the plate was exposed to UV radiation for 1 hour. For each UV radiation dose, another plate was kept in dark as a control. After exposure, the samples were ultrasonically dissolved in 10^3 µL of methanol for 2 minutes. Then the solution was transferred to a 10×100-mm glass tube and dried under nitrogen at room temperature. The dried extract was reconstituted with 2 mL of methanol analyzed by UV–Vis spectrophotometer (diluted to 15 µL/mL) and stored at −10°C until analyzed by HPLC. The results were the average of five experiments.

The sunscreen assay was monitored by HPLC and UV–Vis spectrophotometer. The UV–Vis spectra of the sample have been recorded before and after 3 hours of irradiation in time interval of 1 hour. The HPLC chromatograms were done for the same samples scanning above. The half-life ($t_{0.5}$) is about 3 hours for the lotion SPF 30.

Results and discussion

UV–Vis spectrophotometry study

It is worth mentioning that assay determination for Cin was done by UV spectroscopy and the absorption was at λ_{max} = 308 nm. It is well known that the Cin has two isomers (E- and Z-isomers). E-isomer has a molar absorption coefficient (ε) of 24,000 M^{-1}/cm at λ_{max} = 310 nm in methanol/water (90:10, v/v), whereas ε for Z-isomer at λ_{max} = 310 nm is 12,600 M^{-1}/cm in the same solvent. The photoisomerization in solution is always in equilibrium[25,26]. The purchased raw material is assumed to have 100% of E-isomer, and the isomerization is known to be observed by exposing the sample to irradiation especially in dilute solutions at low concentrations. Thus, the photoisomerization is expected to occur through the long time of stirring (3 days).

Figure 3 shows the UV–Vis absorption spectra of the sunscreen filter (Cin) in the absence and presence of different HP-β-CD concentrations at room temperature (25°C). Looking at Figure 3, we conclude that the absorption intensity decreases with the concentration of HP-β-CD and two isosbestic points were observed. The observation of two isosbestic points in the UV–Vis absorption spectra proposed the presence of two equilibria. These equilibria are attributed to two possibilities; the first is between Cin and HP-β-CD in 1:1 and 1:2 molar ratios (Cin:HP-β-CD), and the second between two configurations of Cin (E- and Z-isomers), whereas the two configurations of Cin have different values of extinction coefficient (ε). Figure 4 shows the two Cin configurations.

Oxy and Oct show small changes in absorption upon the inclusion by HP-β-CD in methanol/water mixture. It is well known that through inclusion of the molecule

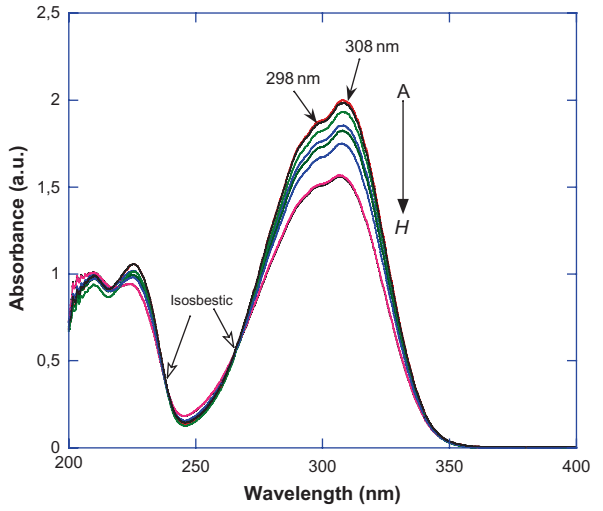

Figure 3. Absorption spectra of Cin (3.62 mM) in methanol/water mixture (85:15, v/v) as a function of HP-β-CD's concentration at 25°C. [HP-β-CD] (M): (A) 0, (B) 1.61, (C) 3.21, (D) 4.82, (E) 6.43, (F) 8.03, (G) 9.64, (H) 11.25. Stirring time is 6 hours.

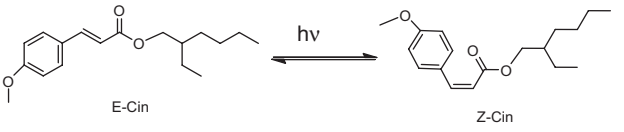

Figure 4. Chemical structure of E- and Z-Cin configuration.

within a CD cavity, its UV–Vis spectrum usually changes, due to partially or totally replacement of the solvation shell of the molecule by the CD molecule, which leads to new solute environment interactions[3]. In most studied cases, when the CD is added to an aqueous solution of an organic or inorganic substance, significant changes in the position and intensity of the absorption spectra are observed. Thus, the small changes in absorption upon the inclusion of Oxy and Oct by HP-β-CD suggest that the new solute environment interactions by HP-β-CD are not significantly effective in the case of Oxy and Oct.

Thermal analysis

Thermal analysis methods, particularly DSC, are widely used in pharmaceutical fields, ranging from control of raw materials to stability, and preformulation studies for development of new formulation[27]. In this study, DSC is utilized to evidence the complexation between sunscreen agents and CD. Typically, a shift or disappearance of the endothermic peak that corresponds to the melting or sublimation points of pure guest molecule is observed upon performing similar DSC analysis of the inclusion complex[28]. Figure 5 shows the DSC thermograms of solid complexes (1:1 molar ratio), physical mixture (1:1 molar ratio), pure sunscreen agent (Oxy), and pure HP-β-CD.

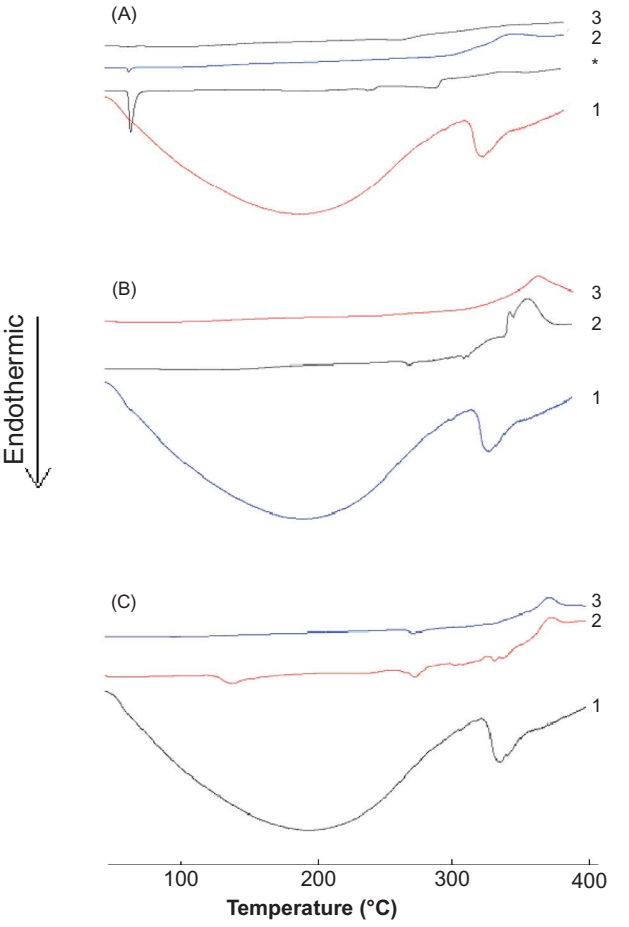

Figure 5. DSC thermograms of Oxy (A), Cin (B), and Oct (C); curves 1, 2, and 3 correspond to HP-β-CD, physical mixture of sunscreen agent and HP-β-CD, and sunscreen agent:HP-β-CD inclusion complex, respectively. '*' in (a) corresponds to pure Oxy.

As can be noticed from Figure 5, the DSC thermogram of HP-β-CD has two peaks: one broad endothermic peak above 100°C, corresponding to the release of water from HP-β-CD[29], and another sharp endothermic peak above 300°C, corresponding to the decomposition of HP-β-CD. Furthermore, the DSC thermogram of Oxy (Figure 5, frame A, curve *) exhibits a sharp endothermic peak at approximately 65°C, corresponding to the melting of Oxy, which hardly appears in the physical mixture of Oxy and HP-β-CD (curve 2) and completely disappears for the inclusion complex (curve 3). This observation confirms the formation of the inclusion complex of Oxy with HP-β-CD. Frames B and C (Figure 5) show the thermograms for the Cin and Oct sunscreen agents, respectively. As can be observed from these two frames, the thermograms of the physical mixtures of Cin:HP-β-CD (frame B, curve 2) and Oct:HP-β-CD (frame C, curve 2) were similar to that of inclusion complexes of Cin:HP-β-CD (frame B, curve 3) and Oct:HP-β-CD (frame C, curve 3), respectively. This indicated that inclusion of Cin and Oct with

HP-β-CD could properly take place in both types of preparations, by physical mixing and by solution mixing followed by removal of solvent. These sunscreen agents are known to be affected by both mixing time and delay time before doing the thermal analysis. As both Cin and Oct sunscreen agents are liquid below room temperature (25°C), the DSC thermograms of pure Cin and Oct could not be measured.

Scanning electron microscopy

Figure 6 shows the SEM images of a sample of free Oxy, free HP-β-CD, physical mixture of Oxy and HP-β-CD (1:1 molar ratio), and inclusion complex of Oxy with HP-β-CD (1:1 molar ratio). As can be noticed from Figure 6, the surface morphology of the inclusion complex (Figure 6D) was completely different from those of the free Oxy (Figure 6A) and free HP-β-CD (Figure 6B). The physical mixture of Oxy and HP-β-CD (Figure 6C) revealed similarities in surface morphology with the crystal of the free Oxy and free HP-β-CD. The inclusion complex was seen as a compact and homogeneous powder-like structure (Figure 6D), which was smaller than that observed for the crystals of free Oxy and free HP-β-CD. It is worth mentioning that the results of SEM and DSC measurements confirm the formation of an inclusion complex of the Oxy with HP-β-CD. On the other hand, there was no complexation observed in the physical mixture of the compounds.

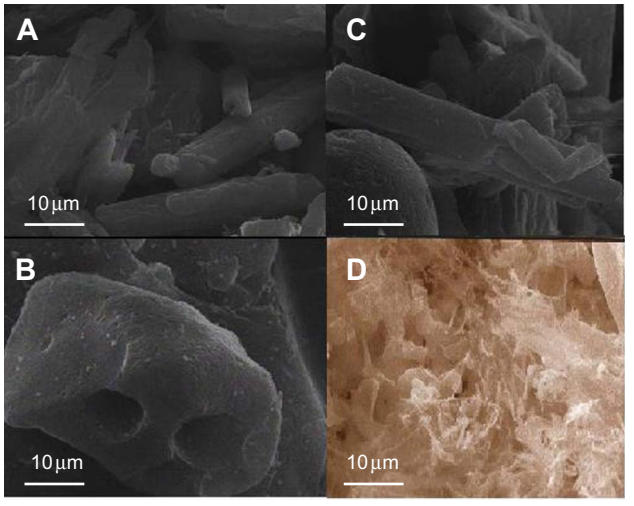

Figure 6. Scanning electron micrographs of Oxy (A), HP-β-CD (B), Oxy:HP-β-CD (1:1 molar ratio) physical mixture (C), and Oxy:HP-β-CD (1:1 molar ratio) inclusion complex (D). Magnification 2500×.

^{13}C-NMR study

Here, we have addressed the possible complexation/inclusion of the three sunscreens (Oxy, Cin, and Oct) in HP-β-CD with ^{13}C-NMR spectroscopy, combined with other complementary characterization techniques (such as DSC and SEM). The ^{13}C-NMR spectra for the pure sample of sunscreen agents (Oxy, Cin, and Oct) were compared to the spectra of their complexes with HP-β-CD to identify the shifts that could provide evidence for complexation phenomena. The results of this study were summarized in Table 2.

Table 2 shows the ^{13}C chemical shifts of the free sunscreen agents in the presence of complex-forming agent (HP-β-CD), as well as the shift differences between equivalent spectral points of the free sunscreen agents and their inclusion complexes with HP-β-CD. From these results it can be seen that both positive and negative shielding effects occur, in which the magnitude of the shielding changes was larger for the carbon atoms of the phenyl groups of the sunscreen agents. These shifts may be the result of either a higher shielding of carbon atoms of phenyl group in the HP-β-CD cavity or a larger stability in the equilibrium between complexes and free sunscreen agents (or both)[30]. It is hard to determine the exact structure of the complexes based on NMR data,

Table 2. ^{13}C chemical shifts of pure sunscreen agents (Oxy, Cin, and Oct), their inclusion complex with HP-β-CD, and the differences between the free sunscreens and the inclusion complexes.

Oxy atom	Oxy (ppm)	Oxy:HP-β-CD (ppm)	Δδ (ppm)	Cin atom	Cin (ppm)	Cin:HP-β-CD (ppm)	Δδ (ppm)	Oct atom	Oct (ppm)	Oct:HP-β-CD (ppm)	Δδ (ppm)
1	199.7	199.0	−0.7	9	166.3	166.9	0.6	12	168.5	169.0	0.5
9	165.7	165.7	0	16	161.2	161.4	0.2	9	162.5	162.0	−0.5
7	164.3	164.3	0	11	143.8	144.5	0.7	14	139.0	138.5	−0.5
2	138.4	138.1	−0.3	12	129.5	130.4	0.9	13	139.0	138.5	−0.5
11	134.9	134.8	−0.1	13	129.6	131.0	1.4	11	116.8	116.8	0
5	132.2	132.4	0.2	14	129.6	131.0	1.4	10	104.6	104.3	−0.3
3	128.0	129.0	1.0	10	115.1	115.6	0.5	8	68.1	68.0	−0.1
4	128.4	128.7	0.3	15	114.1	114.9	0.8	15	128.3	129.8	1.5
6	114.0	114.3	0.3	8	62.2	62.2	0	17	128.3	129.8	1.5
10	107.3	107.3	0	17	54.8	55.6	0.8	16	128.6	130.4	1.8
8	101.5	101.5	0					19	128.6	130.4	1.8
12	55.9	55.9	0								

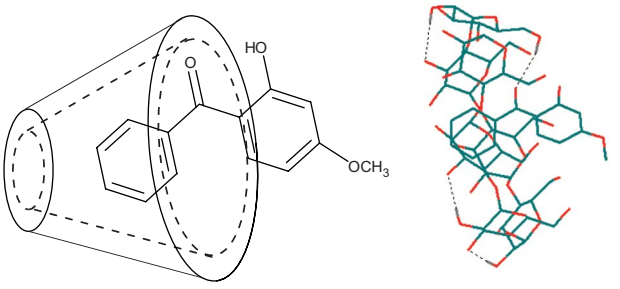

Figure 7. Hypothetical structure of Oxy:HP-β-CD inclusion complex.

especially the orientation of HP-β-CD rims. However, Figure 7 shows a typical 1:1 inclusion complex structure for Oxy with HP-β-CD based on ^{13}C-NMR shifts.

Inclusion in formulation and photostability study

The main goal of complexation of sunscreen agents with CDs is to enhance the stability, photostability, and wettability of sunscreens agents and to reduce its skin penetration. Loftsson and Masson showed that the effect on skin penetration may be related to CD concentration, with reduced flux generally observed at relatively high CD concentrations[31]. At higher CD concentrations, the excess CD would be expected to complex free sunscreen agents and hence reduce skin penetration.

In this study, sunscreen lotion formulation has been prepared with an aim to enhance the performance of the sunscreen products. It has been noticed that the prepared lotion containing HP-β-CD has gained an excellent physical compatibility. In the first observation, it looked similar to the lotion without HP-β-CD and it was difficult to distinguish between them. By increasing the amount of HP-β-CD, it becomes thicker. On the other hand, the other physical properties such as spreadability, color, and application were indistinguishable. Figure 8 shows the resistance of lotion containing HP-β-CD to dehydration. It was observed that the loss in lotion's weight was decreased as the amount of HP-β-CD increased. The percentage losses because of dehydration after 10 days at room temperature were 49%, 39%, and 34% with the addition of 0, 5, and 10 g of HP-β-CD, respectively. The result of this study suggested that 10 g of HP-β-CD shifted the equilibrium of the complexation reaction toward the complexed form (less dehydration percentage), thereby reducing the amount of free sunscreen agent available for permeation and slowing the rate of skin permeation. Therefore, the higher concentrations of HP-β-CD may create a drug reservoir on the skin surface. This is of great importance for the performance of sunscreen products as the UV-absorbing agents must remain in the outermost layer of the skin to be effective.

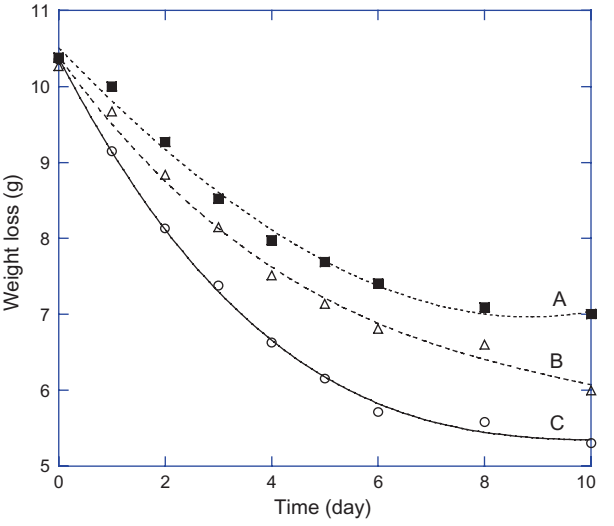

Figure 8. Loss in mass of sunscreen lotion along 10 days with different amounts of HP-β-CD: (A) 10 g of HP-β-CD, (B) 5 g of HP-β-CD, (C) free of HP-β-C.

The influence of HP-β-CD on the photodegradation process of cream formulations was investigated by UV–Vis spectrophotometry (Figure 9); hence, inclusion complexation with HP-β-CD has significantly retarded the rate of the photodegradation reaction of cream formulations. The spectrum obtained from the cream formulations in methanol solution with free HP-β-CD exhibited an absorption maxima at 287 and 309 nm (Figure 9A). These characteristic absorption maxima of sunscreen agents were also observed from the cream formulations with 5 and 10 g of HP-β-CD solution samples at 285 and 307 nm (Figure 9B and C). The slightly blue shift could be attributed to the formation of inclusion complexes in the sample and the more hydrophilic environment surrounding sunscreen agents. Furthermore, it is clear that, after the addition of 5 and 10 g of HP-β-CD (Figure 9B and C), the photostability of cream formulations was significantly increased compared to that in the absence of HP-β-CD (Figure 9A). The spectra shown in Figure 9 indicate that there is a synergetic effect between HP-β-CD and other lotion ingredients.

Moreover, reverse phase HPLC separation process was performed for each sample that has been exposed to irradiation. Figure 10 illustrates typical chromatograms for cream formulations before and after 3 hours of irradiation in the absence of 5 g of HP-β-CD (frames A and B), presence of 5 g of HP-β-CD (frames C and D), and 10 g of HP-β-CD (frames E and F). According to the relative polarity of the sunscreen agents and based on the standard chromatograms for pure sunscreen agents, we believe that the peaks that are observed in Figure 10 correspond to Oxy (a, retention time 2.8 minutes), Oct (b, retention time 5.6 minutes), Z-Cin (c,

retention time 7.7 minutes), and E-Cin (d, retention time 9.3 minutes), where Z- and E-Cin are the two possible isomers of Cin. It is worth mentioning that HPLC results revealed approximately related retention times for all sunscreen agents after the irradiation in the presence and absence of HP-β-CD, indicating identical photodegradation pathways.

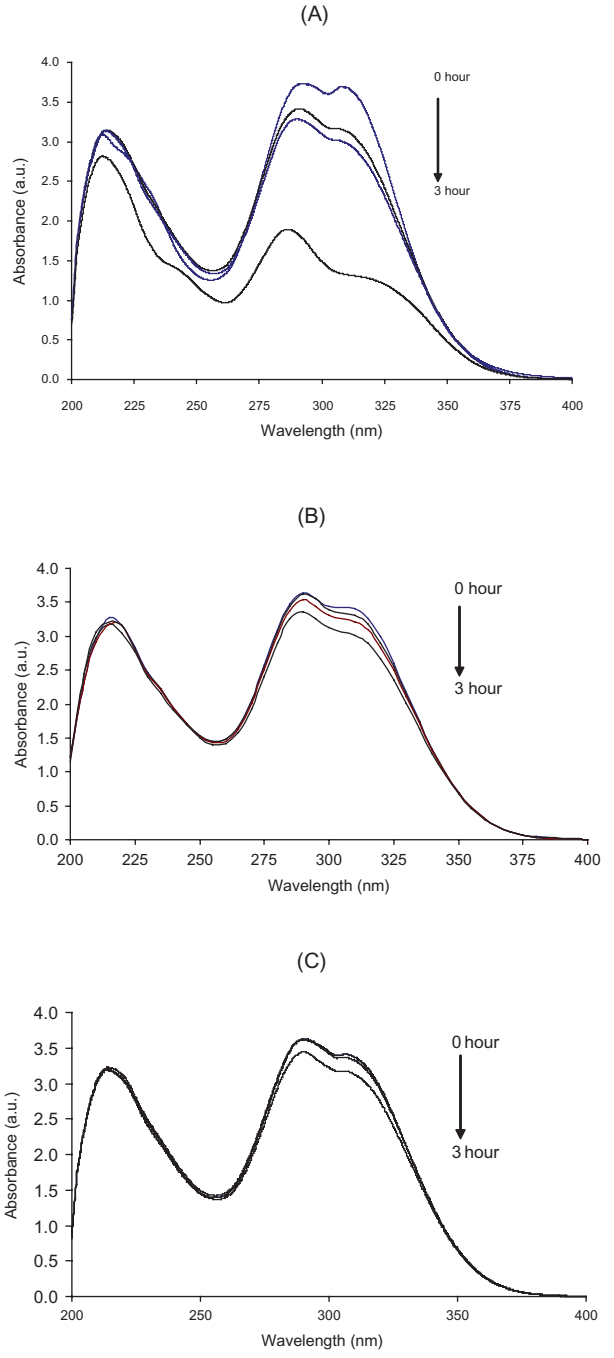

Figure 9. Change in absorption spectra of cream sample after different time of irradiation in the absence and presence of HP-β-CD. Irradiation time 0, 1, 2, and 3 hours. (A) Free of HP-β-CD, (B) 5 g HP-β-CD, (C) 10 g HP-β-CD.

Based on the results obtained from the reverse phase HPLC, a quantitative evaluation was conducted. Quantification was carried out by integration of the peak areas using external standardization method. The integrated peak value for each sunscreen agent before irradiation was set as a reference, where the extent of degradation was estimated based on Equation (1):

$$\%\,\text{remain} = \frac{A_a}{A_b} \times 100\%, \tag{1}$$

where A_a and A_b correspond to peak area after and before irradiation, respectively. The percent remain results after 3 hours of irradiation for the sunscreen agents in the presence and absence of HP-β-CD were summarized in Table 3. Interestingly, the percent remain of Oct in the presence of HP-β-CD is approximately six- to eightfold the value in the absence of HP-β-CD. Moreover, the results obtained for all sunscreen agents using HPLC as a quantitative method (Table 3) are consistent with those obtained using the spectrophotometric methods.

It is worth mentioning that Oxy is a photostable sunscreen agent because of its intramolecular rearrangements; the percent remain after 3 hours of irradiation in the absence of HP-β-CD was 66.3%, even though the presence of HP-β-CD increases the photostability of Oxy where the percent remain under same conditions of irradiation was 97.2–99.1% (Table 3).

Moreover, the presence of HP-β-CD increases the photostability of Cin and controlled its isomerization process to a certain degree, which is a function of the amount of HP-β-CD added. Typically, the enhancement of the photostability of the molecules in the presence of CDs can be attributed to the inclusion of the guest molecule inside the CDs cavity, either partially or entirely. Hence the photostabilization effect of the examined CDs (HP-β-CD) correlated with their complexation strength with the tested sunscreen agents.

The stability of the preservative in these samples could not be studied because of the unsuitable detection limit of HPLC method. Sunscreen agents were highly concentrated, whereas preservatives were much diluted. The preservatives, MP and PP, have retention times lower than 2 minutes; in this part of chromatograms the degradable materials were also observed even though the addition of CDs to the preservative had a good effect on the stability in dermal and transdermal effects. We believe that the results of this study demonstrate that HP-β-CD can be utilized as photostabilizer additive for enhancing the photostability of sunscreen agents' molecules. Moreover based on what has been proposed by Felton et al.[22], HP-β-CD can act as a reservoir for the sunscreen agents on the skin surface, which will reduce its transdermal permeation.

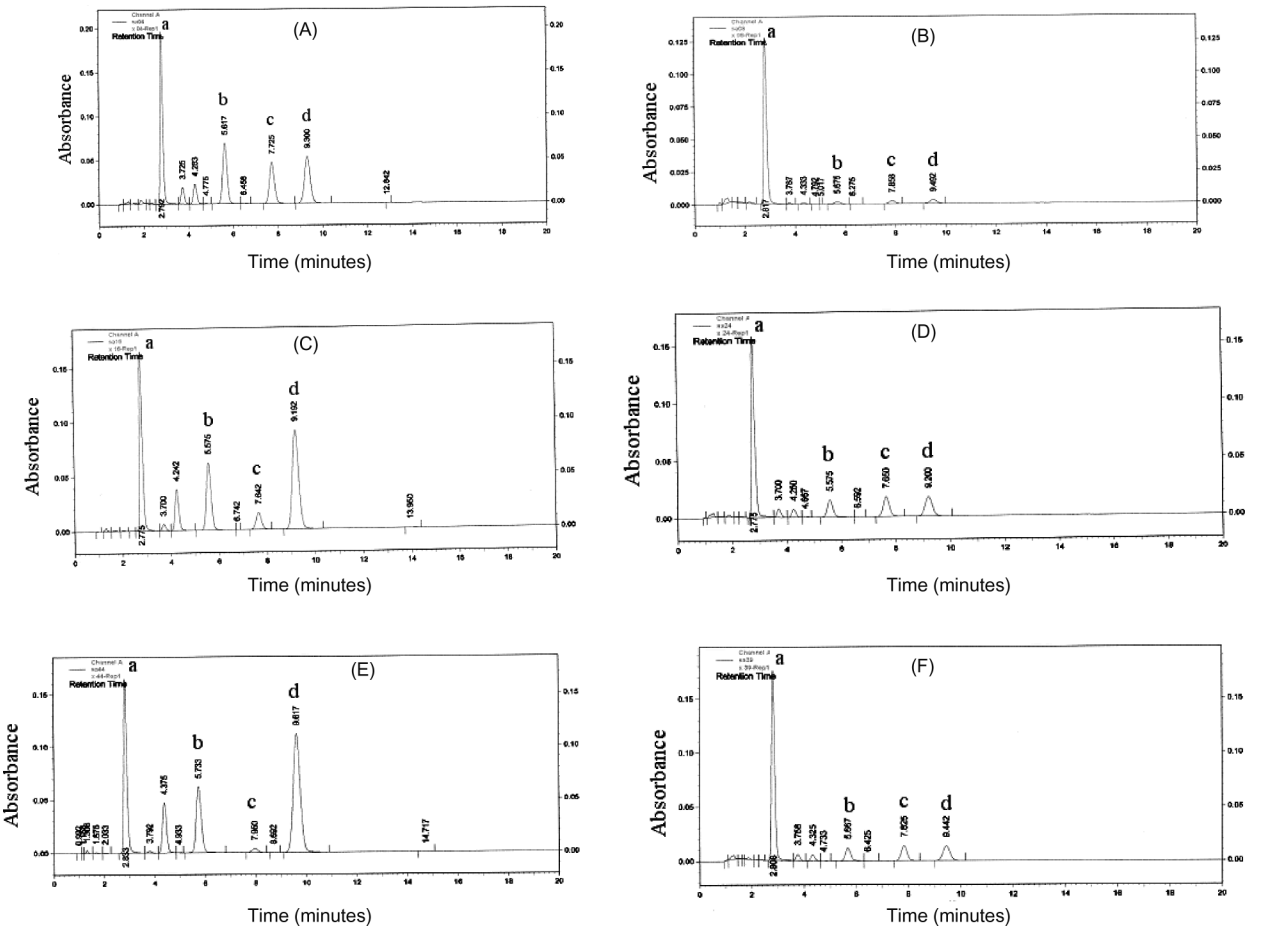

Figure 10. Chromatograms of cream sample before (A, C, and E) and after irradiation for 3 hours (B, D, and F) in the absence of HP-β-CD (A and B), in the presence of 5 g HP-β-CD (C and D), and 10 g of HP-β-CD (E and F).

Table 3. Percent remain of sunscreen agents (Oxy, Cin, and Oct) in cream formulations, after 3 hours of irradiation in the absence and presence (5 and 10 g) of HP-β-CD.

	Oxy (%)	Oct (%)	E-Cin (%)	Z-Cin (%)
Free of HP-β-CD	66.3 ± 5.1	3.3 ± 1.0	5.2 ± 1.1	5.3 ± 1.2
HP-β-CD (5 g)	97.2 ± 6.1	25.4 ± 3.2	11.4 ± 2.9	18. 5 ± 3.7
HP-β-CD (10 g)	99.1 ± 6.2	18.9 ± 3.1	19.1 ± 3.1	11.5 ± 3.5

Each value is the mean ± SD of five determinations.

Conclusion

In this study, we have demonstrated the ability of HP-β-CD to form inclusion complexes with selected sunscreen agents, namely, Oxy, Cin, and Oct. The formation of the inclusion complexes was confirmed experimentally using DSC, SEM, and ^{13}C-NMR. The results of spectrophotometric and HPLC studies have shown that the inclusion complexation with HP-β-CD has the potential to enhance the photostability of the selected sunscreen agents in lotion. Retarding the photodecomposition of the sunscreen agents' molecule in the presence of HP-β-CD can be attributed mainly to the protection role of HP-β-CD upon including the sunscreen agents' molecule inside its cavity. The results of both techniques were highly consistent for Oxy and Cin photostability studies. HPLC results indicated that HP-β-CD has approximately increased the photostability of Oct by six- to eightfold. Moreover, the presence of HP-β-CD in the lotion controlled the isomerization process of Cin to a certain degree, which was found to be a function of the amount of HP-β-CD added. This study provides an insight on the importance of investigating the photostability of sunscreen agents' molecules and proposes a remedy for enhancing such imperative property.

Acknowledgments

Financial support by Deanship of Research at Jordan University of Science and Technology (grant number

30/2003) is deeply appreciated. The authors are indebted to Physikalische Chemie I, Ruhr-University at Bochum, Germany, for their help in SEM measurements.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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