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ORIGINAL ARTICLE

Inclusion of cinnamaldehyde in modified γ-cyclodextrins

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Abstract This work deals with the formation of inclusion complexes between cinnamaldehyde and two synthetic alkylcarbonates of γ-cyclodextrin, namely ethylcarbonate-γ-CD and octylcarbonate-γ-CD. Complexation was monitored by phase-solubility studies, by FT-IR spectroscopy and by DSC and TG analysis.

The diffusion of cinnamaldehyde through a cellulose membrane was investigated in the absence and in the presence of each of the two γ-CD alkylcarbonates. Studies on photostability and stability over time were carried out on different cosmetic formulations containing pure cinnamaldehyde or an equivalent amount of cinnamaldehyde complexed with the two alkylcarbonates.

Phase-solubility diagrams, DSC, FTIR and TGA analysis suggested the formation of inclusion complexes. The diffusion of cinnamaldehyde through the cellulose membrane decreased in the presence of the two alkylcarbonates confirming the interaction of this molecule with the inclusion agents.

Moreover the stability of cinnamaldehyde to light and heat resulted increased by complexing this fragrance material with the two alkylcarbonates.

Keywords Cinnamaldehyde · γ-cyclodextrin alkylcarbonates · Complexation · Photo-stability · Thermo-stability

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Introduction

Cinnamic aldehyde (CIN) more precisely *trans*-CIN, the only naturally-occurring form, derives from the bark of cinnamon trees, *Cinnamomum zeylanicum (Lauraceae)*. The predominant application for this molecule is in the flavoring and fragrance industries. In fact, it is used as a flavoring agent for food items or as fragrance in perfumes and cosmetic products. To a lesser extent CIN is also used as fungicide for agriculture or as insecticide. The essential oil of cinnamon also has antimicrobial properties allowing to extend the shelf life of foods. The restrictive factors for fragrance ingredients are their high volatility and irritant effect that make difficult to formulate preparations containing them [1]. The ability of cyclodextrins to form an inclusion complex with a guest molecule could overcome these phenomena [2]. In fact, inclusion complexation exerts a strong effect on the guest's physicochemical properties; among which solubility enhancement of highly insoluble guests, stabilization of labile guests against the degradative effects of oxidation e.g. visible or UV light and heat, control of volatility and sublimation, physical isolation of incompatible compounds, etc...[3]. The aim of this study was to achieve increased photostability and thermostability for CIN, by preparing inclusion complexes of this molecule with two different alkylcarbonates of γ-cyclodextrin: ethylcarbonate-γ-cyclodextrin (E-CD) and octylcarbonate-γ-cyclodextrin (O-CD). Alkyl carbonates of γ-cyclodextrin were chosen for their low toxicity and their high complexing ability [4]. In this work each of these two complexes was tested in order to assay the protective effect of the host molecules towards the photo- and thermo-degradation of the guest compound. Phase solubility diagrams were obtained according to

Higuchi and Connors method [5], moreover CIN inclusion complexes were characterized by FTIR spectroscopy, DSC and TG analyses.

Experimental

Material

Trans-cinnamaldehyde or 3-phenyl-2-propenal (C_9H_8O, FW 132,15) was purchased from Sigma-Aldrich. Tegosoft®EE (octyl octanoate) and Natrosol®MR (hydroxy ethyl cellulose, HEC) were supplied by A.C.E.F., TWEEN®20 (Polysorbitan) and sodium dodecyl sulphate (SDS) by Fluka while Montanov®68 (Cetearyl alcohol/Cetearyl glucoside) and Sepigel®305 were kind gifts from Seppic. Ethyl carbonate of γ-cyclodextrin (E-CD) and octyl carbonate of γ-cyclodextrin (O-CD) were prepared in our laboratories according to the following procedure. Briefly the selected alcohol was activated by reaction with excess of carbonyldiimidazole in alcohol free chloroform. In the second step the imidazolyl derivative was allowed to react with anhydrous γ-CD in anhydrous pyridine at 80°C for 4 h. Once the reaction was over, the residual precipitate was filtered off and distilled water was added to the organic solution. The solid was recovered by filtration, washed many times with water and then liophylized [4]. The alkylcarbonates prepared as described have an average degree of substitution (DS) of three. The average molecular weights of the two alkylcarbonates were calculated on the basis of the average substitution degree and resulted to be 1513 Da for E-CD and 1765 Da for O-CD. A semi-permeable membrane (Spectra/Por® CE Dialysis membrane from cellulose esters) of MWCO 1000 was purchased from Spectrum Laboratories (Huston, Texas). All other solvents were of analytical grade and were used without further treatments.

Preparation of the complexes of CIN with the alkylcarbonates of γ-cyclodextrin

Inclusion complexes of CIN with the two different derivatives γ-cyclodextrin were prepared by the freeze-drying method in 1:1 molar ratio. A weighted quantity of CIN was stirred in water containing the modified CD for 24 h at room temperature; their dispersion was lyophilized overnight to yield a powder.

The complexes were also prepared by mixing directly appropriate amounts (molar ratio 1:1) of the components in a glass mortar.

The content of CIN in each complex was determined by HPLC after proper dilution: 5 mg of complex was dispersed in 5 ml of methanol. The suspension was filtered and centrifuged; the supernatant was injected for analysis. The chromatographic conditions were as follows: CIN absorbance was measured at $\lambda = 289$ nm; injection volume 20 μl, isocratic flow rate at 1.0 ml × min^{-1}; the mobile phase consisted of a mixture methanol/water (45/55 v/v).

Phase solubility studies

Solubility analysis were carried out according to Higuchi and Connors method [5]. An excess amount of CIN was added to aqueous solutions containing increasing concentration (0–20 μM) of E-CD or O-CD. The samples were stirred in 10 ml screw-capped vials at 25°C ± 1°C and shielded from light. After 2 days agitation the content of each vial was filtered and then assayed for CIN determination by HPLC. Data were determined from the mean of at least three tests and stability constants (k_{st}) were calculated from the curves using the following equation:

$$k_{st} = \text{slope}/S_0(1 - \text{slope})$$

were the slope is obtained from the least squares linear regression of the molar concentrations of CIN in solution versus the molar concentration of cyclodextrin in the solvent and S_0 is the intrinsic CIN solubility (15 mM) in the absence of cyclodextrin.

CIN membrane diffusion

The diffusion of CIN through a semi-permeable membrane (cut off 1000 Da) was studied using glass horizontal diffusion cells at room temperature. The receptor phase consisted of water while the donor phase consisted of aqueous solutions of CIN (1.0×10^{-3}M), free or complexed with each of the two γ-CD alkyl carbonates.

Spectroscopic determination of stability constant (k_{st})

The stability constant of the CIN:E-CD complex was also determined spectrophotometrically. The stability constant (k_{st}) was determined by analyzing changes in the maximum peak (at 289 nm) intensity of CIN. The absorption spectra of CIN in aqueous solutions containing increasing concentration of E-CD, keeping the concentration of probe molecule fixed (~10^{-5} M), were registered.

Characterization of the solid complexes

DSC studies

DSC measurements were performed on a Perkin-Elmer differential scanning calorimeter. All samples accurately weighed (1 mg of CIN or its equivalent as complex) were placed in sealed aluminum pans, before being heated under nitrogen flow at a scanning rate of 10°C min^{-1}, from 25 to 200°C. All samples were weighed in order to have the same amount of CIN, free or complexed.

FTIR studies

Infrared (IR) spectra were recorded on a Perkin Elmer System 2000 spectrometer and obtained from KBr pellets in the 4000–400 cm^{-1} regions. All samples were weighed in order to have the same amount of CIN, free or complexed.

TG analysis

Thermogravimetric (TG) curves were recorded on a Shimadzu TGA-50 thermogravimetric analyzer with heat rate of 10 K min^{-1} under a nitrogen atmosphere in the temperature range of 0–500 K. Also in this case all samples were weighed in order to have the same amount of CIN, free or complexed.

CIN stability studies

UV irradiation test The photostability of CIN was assessed in different samples prepared dispersing the aldehyde (1.0×10^{-3} M), free or complexed, in 2% w/w HEC gel or in an O/W emulsion (3% w/w Montanov®68, 20% w/w Tegosoft®EE, 77% w/w water) or in a gel emulsion (1% w/w Sepigel®305, 7% w/w Tegosoft®EE, 92% w/w water).

Each sample was placed at 10 cm from a light source (UVB lamp with an intensity of 2.6×10^{-4} W/cm^2). The CIN concentration was determined by HPLC assay and the results were expressed as percentages of the remaining CIN. Each test was carried out in triplicate.

Thermo-stability test An accelerated stability test was performed storing the samples (prepared as previously described for the photo-stability study) in an oven set at 40°C ± 2°C over a period of 10 days. All samples were analyzed in triplicate and at the specified time intervals (every 24 h) the concentration of CIN was assayed by HPLC. The results were expressed as percentages of the remaining CIN.

Skin penetration studies

This study was carried out on porcine ear skin pieces in order to determine the amount of CIN which permeates and accumulates into the deeper layer of the skin from different aqueous systems containing CIN, free or complexed, at the concentration of 1.0×10^{-3} M. An aliquot (1 g) of each sample was deposited on a Franz-type cell: the receptor phase contained 0.5% SDS aqueous solution. The flux of CIN through the skin was monitored over the first 7 h. After 24 h each cell was dismantled, the excess sample on the surface was removed and the CIN was extracted by solubilization in methanol then filtered and assayed by HPLC.

Results and discussion

The percentage of CIN contained in the complex with E-CD was 6.46% w/w, while the percentage contained in the complex with O-CD was 4.56%.

The solubility profiles of CIN:E-CD and CIN:O-CD complexes are reported in Fig 1. The diagrams showed that the inclusion of CIN in both CD derivatives improved its aqueous solubility displaying a typical A-type solubility curve [5]. The stability constants of the two complexes were calculated from the slope and the intercept of the initial portion of the curve, assuming a 1:1 stoichiometry for both the complexes and resulted to be respectively 73 M^{-1} for CIN:E-CD and 31 M^{-1} for CIN:O-CD. These results showed different CIN complexing ability of E-CD and O-CD: it seems that CIN is more favourable positioned or fitted inside the E-CD cavity than the O-CD one. Octyl groups may negatively affect the availability of the cavity for the guest molecule.

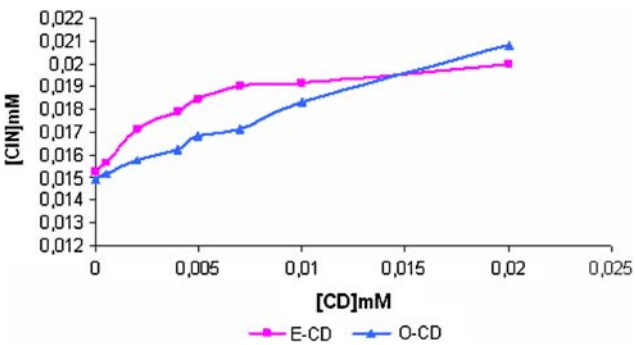

Fig. 1 Solubility of CIN in water solutions containing increasing amount of γ-CD alkylcarbonates

CIN and alkylcarbonates association was also studied by a diffusion method using an artificial membrane with a cut-off of 1000 Da. The choice of this barrier was justified by the structural characteristics of the drug: this membrane allows the diffusion of CIN (MW = 132.15) while blocks the diffusion of the γ-CD alkylcarbonates (MW > 1500).

Diffusion profiles of CIN, free or complexed with one of the two cyclodextrin derivatives, through a cellulose membrane are reported in Fig. 2.

Figure 2 showed that the diffusion of CIN complexed with both the alkylcarbonates was significatively delayed compared to that obtained from free CIN, confirming the interaction with the two alkylcarbonates.

The absorption spectra of CIN in aqueous solution containing varying concentration of E-CD were also studied. A gradual increase of the maximum peak intensity (at λ = 289 nm) was observed by the addition of E-CD, indicating the formation of CIN:E-CD complex.

The equilibrium constant may be described from the following equation [4]:

$$k_{st} \cdot A_\lambda = A_\lambda - A_\lambda^0 / C_x$$

where C_x is the concentration of E-CD while A_λ^0 and A_λ are absorbance at 289 nm for fixed concentration of CIN, in the absence and in the presence of E-CD, respectively (Fig. 3).

The calculated equilibrium constant k_{st} for CIN:E-CD complex resulted to be 76 M^{-1}, that is in agreement with the constant value obtained previously employing Higuchi and Connors method.

The formation of an inclusion complex between CIN and γ-CD alkylcarbonates was also demonstrated by DSC, FT-IR and TGA in the solid state. The DSC profiles of the two complexes, compared to the thermogram of the pure CIN, confirmed that there was an interaction. In fact the formation of an inclusion

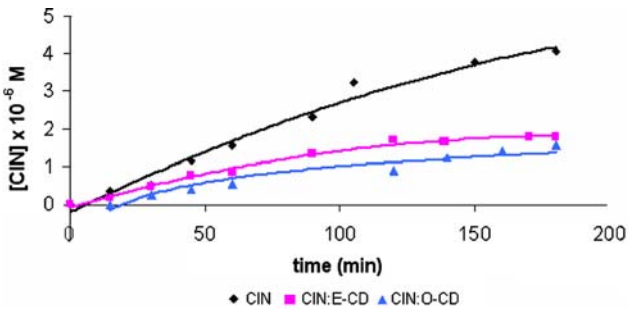

Fig. 2 Diffusion profiles through a cellulose membrane of CIN, free or complexed with one of the two γ-cyclodextrin carbonates

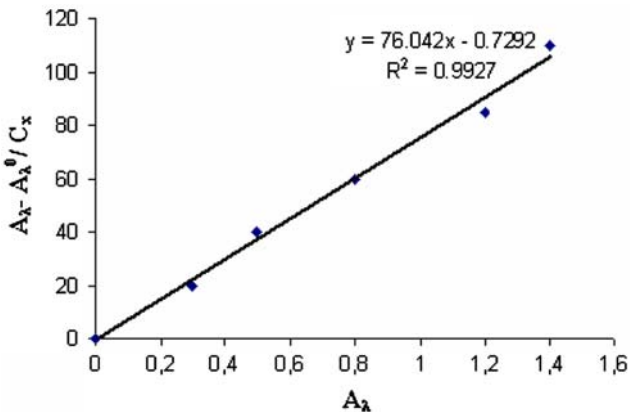

Fig. 3 Plot of $A_\lambda - A_\lambda^0 / C_x$ versus A_λ

complex is suggested by the absence of melting endotherm of CIN in the DSC thermograms of the complexes (data not shown).

The FTIR spectra of CIN, γ-CD alkylcarbonates and their inclusion compounds are reported in Fig. 4. Pure CIN spectrum exhibited prominent bands around 1600–1700 cm^{-1} that correspond to C = O and C-O stretching vibrations, whereas the spectra of E-CD and of O-CD were characterized by bands at 3600–3200 cm^{-1} due to the O-H stretching vibrations. In Fig. 4 the E-CD spectra are reported. The intense bands at 1600–1700 cm^{-1}were significantly reduced in the complexes suggesting that the CIN might be included into the hydrophobic cavity of CDs.

TGA of E-CD, O-CD and CIN inclusion compounds were performed. The O-CD curves are reported, as example, in Fig. 5. The first weight loss of pure octylcarbonate-γ-CD is around 100°C which can be explained as the release of water from the CD cavity, while the second weight loss was observed

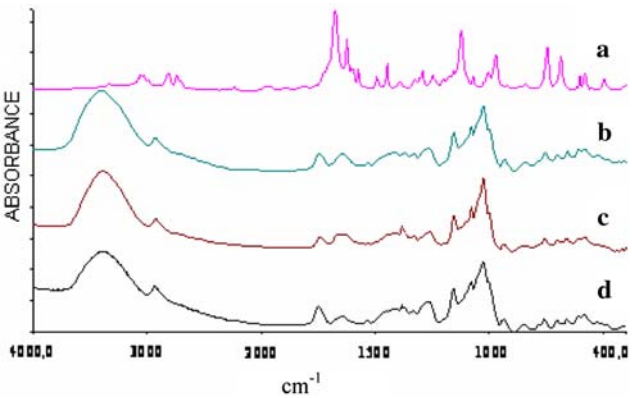

Fig. 4 FT-IR spectra of CIN (a), E-CD (b) and CIN:E-CD complex prepared by Freeze-drying (c) or in glass mortar (d)

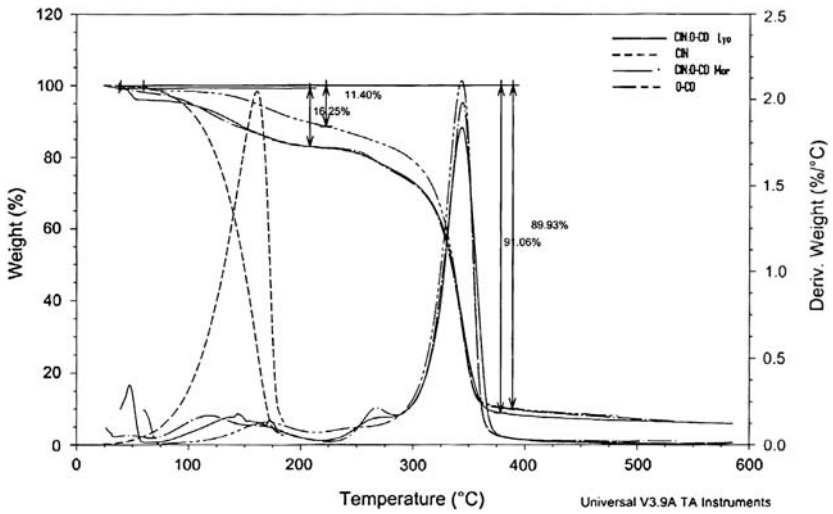

Fig. 5 TG curves of CIN (- - - -), O-CD (— - -) and CIN:O-CD complex prepared Freeze-drying (—) or in glass mortar (—·)

around 300°C due to the decomposition of the γ-CD octylcarbonate. TGA of CIN:O-CD complexes showed a further weight loss after 100°C that could be related to the CIN evaporation suggesting the formation of the inclusion complexes. The same behavior was observed for CIN:E-CD complexes.

The photostability of CIN, free or complexed, was examined in different cosmetic formulations (2% HEC gel, O/W emulsion and gel emulsion). As example the results of UVB irradiation of 1.0×10^{-3}M CIN dispersions in 2% HEC gel are shown in Fig. 6. The data indicated that the two γ-CD alkylcarbonates delayed the photo-degradation of CIN increasing its stability in the presence of light. The degradation of the aldehyde in all the cases followed a zero-order kinetic.

Data of thermo-stability studies are reported in table 1: it should be noticed that, after 10 days at 40°C, in all the sample examined CIN is less stable when is free than when is complexed. This result could be justified by the ability of the CDs to reduce fragrances' volatility.

Table 1 Trend of thermo-degradation of CIN, free and complexed, in different cosmetic formulations, after 10 days storage at 40°C (results are expressed as percentages of the remaining CIN)

Vehicle	CIN (%)	CIN:E-CD (%)	CIN:O-CD (%)
2% HEC gel	72	100	100
O/W emulsion	75	100	100
Gel emulsion	93	100	100

Figure 7 shows the fluxes (mg cm^{-2} h^{-1}) through the porcine ear skin of CIN, free or complexed with each of the two considered alkylcarbonates, while in table 2 data of skin accumulation (μg/cm^2) after 24 h are listed.

CIN complexation significantly affected the permeation behavior of this molecule through the porcine skin. In particular CIN permeation rate resulted notably slower from complex suspensions than from the control solution prepared with free CIN. Otherwise the entrapment into the skin was slightly enhanced by the complexation, as shown in table 2. This finding

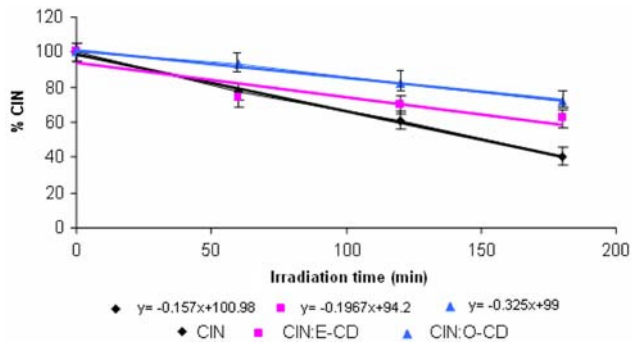

Fig. 6 Photo-degradation curves, under UVB irradiation, of CIN (◆), CIN:E-CD (■) and CIN:O-CD (▲) in HEC gel

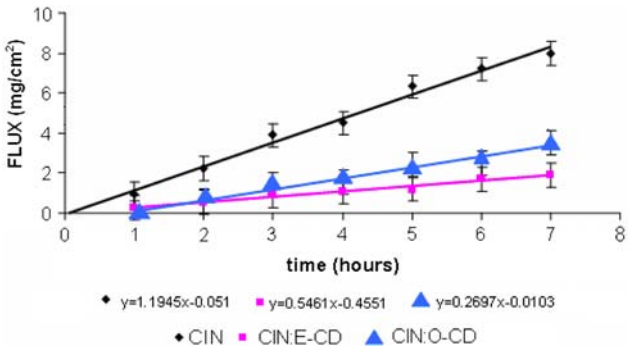

Fig. 7 Fluxes through the ear porcine skin, of CIN free or complexed with E-CD or O-CD

Table 2 Data of skin accumulation in the deeper layer of the porcine skin, after 24 h

Sample	CIN accumulated in the skin (μg/cm^2)
CIN	6,92
CIN:E-CD	18,12
CIN:O-CD	28,81

could be correlated with the ability of the CDs to interact with the skin components [6].

Conclusion

The possibility of using the complexation with γ-CD alkylcarbonates in order to increase the stability to light and heat of CIN has been investigated. Inclusion in E-CD and in O-CD of CIN was successfully obtained as proved by DSC, FTIR and TG analyses and also by phase solubility and membrane diffusion studies.

Both the two alkylcarbonates-γ-CD, considered in this present work, appeared to be effective for enhancing the photo- and thermo-stability of the CIN.

Moreover it was noticed that the complexation with these CD derivatives significantly affected the penetration of the CIN through porcine skin.

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