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Improvement of water solubility and dissolution rate of ursodeoxycholic acid and chenodeoxycholic acid by complexation with natural and modified β-cyclodextrins

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Abstract

The inclusion complexes of ursodeoxycholic and chenodeoxycholic acid with β-cyclodextrin, heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin and soluble polymerized β-cyclodextrin were investigated in solution (^{1}H-NMR spectrometry) and solid state (FT-IR spectroscopy and differential scanning calorimetry). Stability constants were determined at pH 7.4 and different temperatures and consequently thermodynamic parameters were obtained. All cyclodextrins are able to increase water solubility of the bile acids, particularly polymerized β-cyclodextrin. All complexes show high dissolution rate at 37°C and pH 1.1 and in particular freeze-dried complexes. © 1997 Elsevier Science B.V.

Keywords: Bile acids; β-Cyclodextrin; Dimethyl-β-cyclodextrin; Polymerized β-cyclodextrin; Characterization of the complexes; Solubility studies; Dissolution rate

1. Introduction

The major bile acids present in humans are cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), and ursodeoxycholic acid (UDCA) which occur primarily as glycine or taurine conjugates (Setchell and Matsui, 1983; Rossi et al., 1987; Scalia, 1990). In addition to their physiological role, two of the foregoing bile acids, namely CDCA and UDCA (see figures in Tables 3 and 4) have been introduced for the treatment of cholesterol gallstone diseases (Dazinger et al., 1972; Ward et al., 1984). However, because of the side-effects associated with CDCA therapy (Ward et

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al., 1984), UDCA is the most commonly administered drug (Tint et al., 1982). Furthermore, the introduction of UDCA for the treatment of bile reflux gastritis (Scalia et al., 1988) and especially of cholestatic liver diseases (Poupon et al., 1991) has expanded its use as a therapeutic agent.

Studies have shown that the bioavailability of UDCA and CDCA in vivo is poor and erratic, and are generally believed to result from the poor aqueous solubility of bile acids at physiological pH (Igimi and Carey, 1980; Moroi et al., 1992).

Many methods were used to increase water solubility of apolar drugs, but not always positive results were obtained (Sugimoto et al., 1981). It was reported (Duchêne, 1987) that cyclodextrins (CyDs) are able to modify some physicochemical properties of active substances by means of the inclusion into their hydrophobic cavity. After complexation the solubility, dissolution rate and bioavailability of the included drugs were increased (Ventura et al., 1994; Puglisi et al., 1995).

The ability of β-CyD to include bile salts in solution was proved by Miyajima et al. (1986). Nakanishi et al. (1989) showed that drug absorption from the rat small intestinal lumen was modified by β-CyD administration as inclusion complex following the possibility to exchange the guest molecule with bile salts present here. Vandelli et al. (1995) include UDCA into hydroxypropyl-β-CyD.

In this study we investigate both in solution and in the solid state the inclusion of UDCA and CDCA into the cavity of dimethyl-β-CyD and polymerized-β-CyD, comparing the obtained results with the UDCA and CDCA-β-CyD complexes. Our purpose is to enhance the dissolution rate of the two bile acids, improving their bioavailability.

FT-IR spectroscopy and differential scanning calorimetry (DSC) were used to characterize the complexes at the solid state, while ^{1}H-NMR spectra of the complexes in solution were performed to obtain information on the inclusion mode.

The influence of CyDs on water solubility of UDCA and CDCA was evaluated at various pH values and temperature, using the method of Higuchi and Connors (1965). Bile acids concentration in solution was evaluated by HPLC using electrochemical detection (Scalia et al., 1995). In vitro studies were performed to determine dissolution rate of the complexed drugs with respect to UDCA and CDCA alone.

2. Materials and methods

2.1. Chemicals

Ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) were supplied by Fluka Chemical (Buchs, Switzerland). β-Cyclodextrin (β-CyD) was provided by Roquette (Cassano Spinola, Italy). Heptakis-(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) and soluble β-cyclodextrin polymer (Poly-β-CyD) ($M_w = 3000-5000$; β-CyD content 50–60%) are Cyclolab R&D Laboratory (Budapest, Hungary) products.

All other chemicals and solvents were analytical reagent grade. Deionized, double distilled water was used.

2.2. Preparation of the solid inclusion complexes

The inclusion complexes between UDCA, CDCA and different CyDs were prepared by

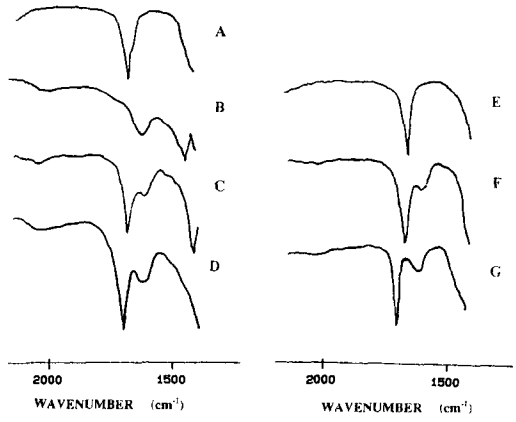


Fig. 1. FT-IR spectra of UDCA- and CDCA-DM-β-CyD systems. (A) UDCA; (B) DM-β-CyD; (C) UDCA-DM-β-CyD physical mixture (1:1 mol ratio); (D) UDCA-DM-β-CyD complex (1:1 mol ratio); (E) CDCA; (F) CDCA-DM-β-CyD physical mixture (1:1 mol ratio); (G) CDCA-DM-β-CyD complex (1:1 mol ratio).

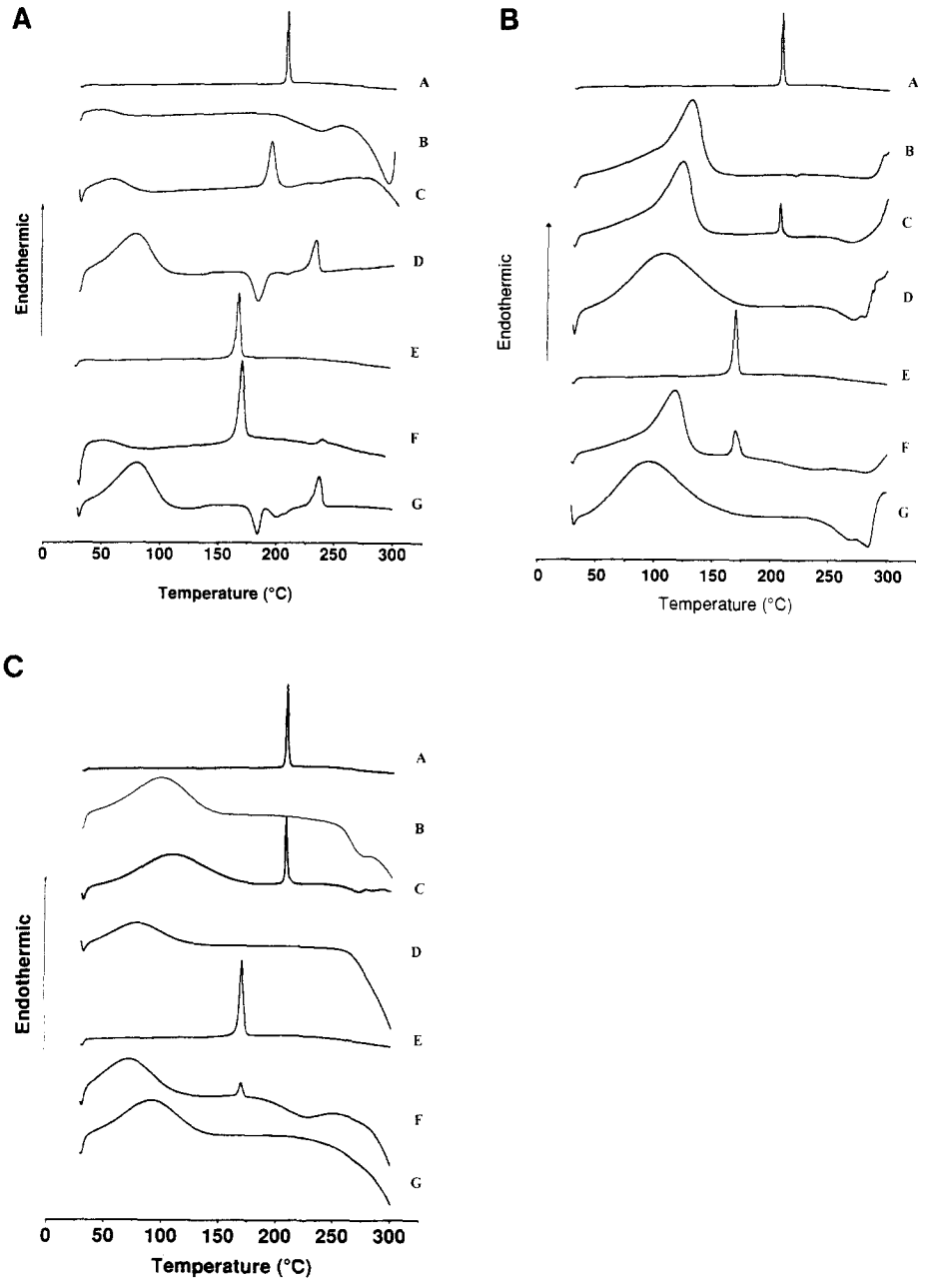


Fig. 2. (**A**) DSC curves of UDCA- and CDCA-DM-β-CyD freeze-drying systems. (A) UDCA; (B) DM-β-CyD; (C) UDCA-DM-β-CyD physical mixture (1:1 mol ratio); (D) UDCA-DM-β-CyD complex (1:1 mol ratio); (E) CDCA; (F) CDCA-DM-β-CyD physical mixture (1:1 mol ratio); (G) CDCA-DM-β-CyD complex (1:1 mol ratio). (**B**) DSC curves of UDCA- and CDCA-β-CyD freeze-drying systems. (A) UDCA; (B) β-CyD; (C) UDCA-β-CyD physical mixture (1:2 mol ratio); (D) UDCA-β-CyD complex (1:2 mol ratio); (E) CDCA; (F) CDCA-β-CyD physical mixture (1:2.5 mol ratio); (G) CDCA-β-CyD complex (1:2.5 mol ratio). (**C**) DSC curves of UDCA- and CDCA-Poly-β-CyD freeze-drying systems. (A) UDCA; (B) Poly-β-CyD; (C) UDCA-Poly-βy-P-CyD physical mixture (1:1 mol ratio); (D) UDCA-Poly-β-CyD complex (1:1 mol ratio); (E) CDCA; (F) CDCA-Poly-β-CyD physical mixture (1:1 mol ratio); (G) CDCA-Poly-β-CyD complex (1:1 mol ratio).

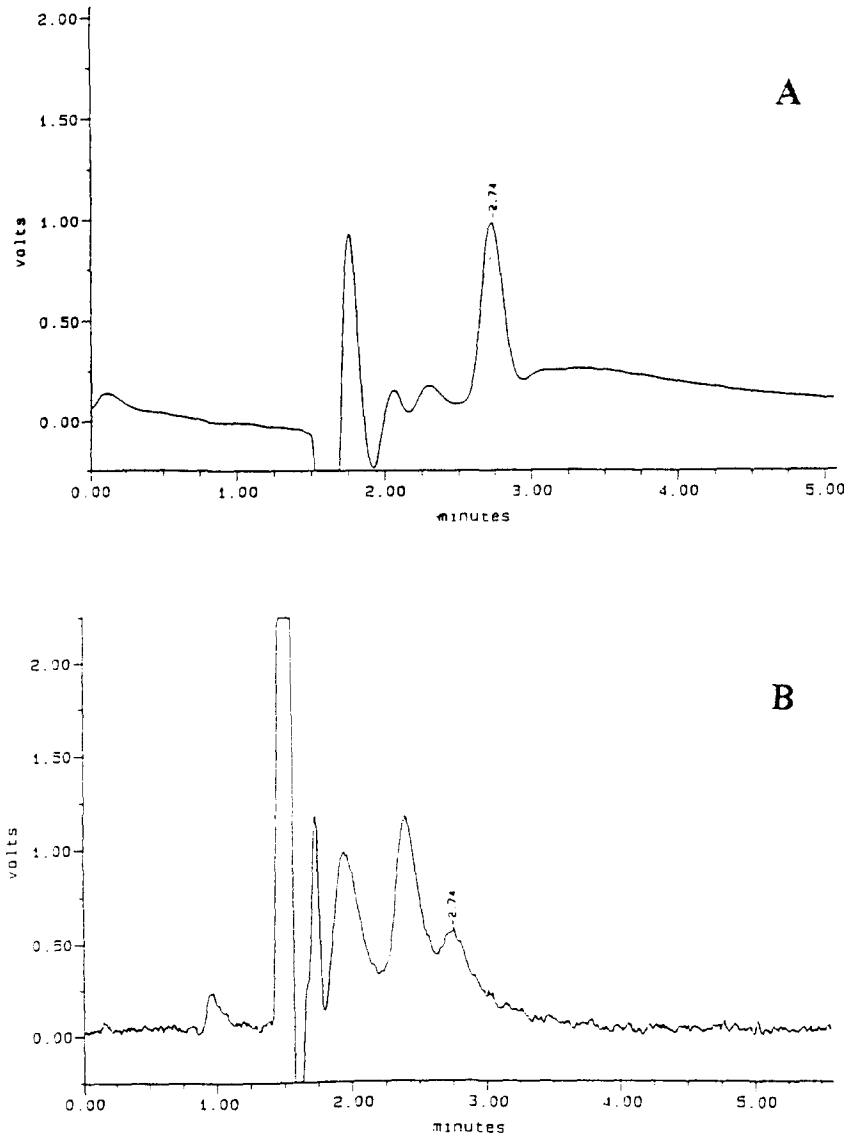
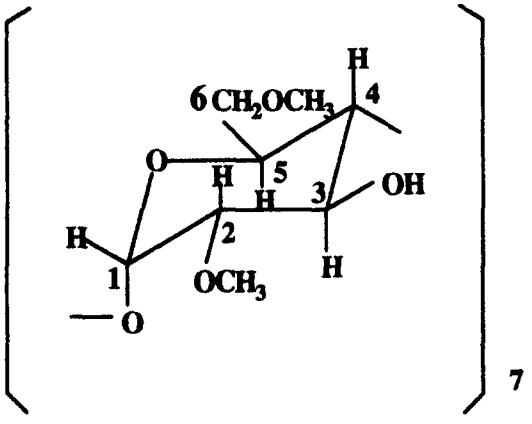


Fig. 3. (A) Typical chromatogram of UDCA (RT = 2.74) and (B) chromatogram of UDCA and its byproducts.

freeze-drying method (Puglisi et al., 1996). Amounts (1 g) of each CyDs were solubilized in water (60 ml) at room temperature and added of an excess amount of UDCA and CDCA. The suspensions were stirred at room temperature for 1 day, than they were filtered through a 0.45-μm Millipore filter and the solutions were freeze-dried (Edwards, Modulyo 4K).

Kneading method was also used as complexing technique for UDCA and CDCA with β-CyD and DM-β-CyD. The CyDs and the drugs (1:1 and 2:1 mol ratio for DM-β-CyD and β-CyD, respectively), were wetted with a methanol/water (50:50 v/v) mixture and kneaded vigorously for 15 min. Each sample was dried at 60°C at reduced pressure for 1 day.

Table 1
^{1}H-Chemical shifts of DM-β-CyD and its shifts in the presence of UDCA and CDCA acids (1:1 mol ratio)



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H	Without bile acids	With UDCA	$\Delta\delta^a$	With CDCA	$\Delta\delta^a$
1	5.494	5.540	−0.046	5.408	−0.086
2	—	—	—	—	
3	4.282	4.367	−0.085	4.155	−0.127
4	—	—	—	—	—
5	4.184	4.263	−0.079	4.041	−0.143
6	4.064	4.126	−0.062	3.950	−0.114
2′	3.902	3.944	−0.042	3.796	−0.106
6′	3.694	3.718	−0.024	3.621	−0.073

[a] δ complex − δ free.

2.3. Determination of the composition of the UDCA and CDCA-CyDs solid complexes

Amount of freeze-dried UDCA- and CDCA-CyDs complexes (30 mg) were solubilized in 50 ml of methanol. The obtained solutions were analyzed by HPLC method to determine the drugs concentration. Due to low solubility of β-CyD and Poly-β-CyD in methanol, the samples were filtered (0.5 μm Millipore filter) before the HPLC analysis. The amount of CyDs was determined by difference. In this way it was possible to calculate mol ratio.

2.4. FT-IR spectroscopy

FT-IR spectra of the pure components, the inclusion complexes prepared by both methods, and the physical mixtures, in the same molar ratio, were performed on KBr disks using a FT-IR spectrophotometer Perkin-Elmer mod. 1600.

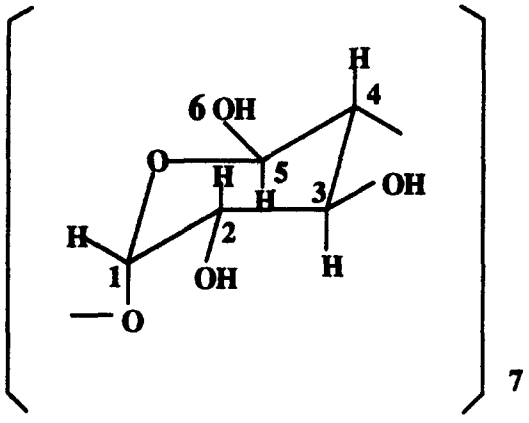
2.5. Differential scanning calorimetry (DSC)

DSC scans of the powder samples were recorded on a Mettler DSC 12E differential scanning calorimeter equipped with a Haake D8-G thermocryostate. A Mettler TA89E and FP89 system software was used for data acquisition. Each sample was scanned at a speed of 10°C/min from 30 to 300°C. Nitrogen was used as gas purge.

2.6. NMR spectroscopy

^{1}H-NMR experiments were performed at 300 MHz using a Bruker AC 300 spectrometer, the probe temperature was regulated to 303 K using Eurotherm control system. Spectra were collected by co-addition of 16 scans. TMS was used as external standard. Samples were solubilized in D_2O, adding drops of NAOD to facilitate drug solubilization.

Table 2
^{1}H-Chemical shifts of β-CyD and its shifts in the presence of UDCA and CDCA (1:1 mol ratio)



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H	Without bile acids	With UDCA	$\Delta\delta^a$	With CDCA	$\Delta\delta^a$
1	5.169	5.112	−0.057	5.124	−0.045
2	3.742	3.694	−0.045	3.712	−0.037
3	4.061	4.008	−0.053	3.993	−0.068
4	3.680	3.630	−0.050	3.648	−0.032
5	3.946	3.907	−0.039	3.868	−0.078
6	3.977	3.942	−0.035	3.909	−0.070

[a] δ complex − δ free.

2.7. Solubility studies

An excess amount of CDCA and UDCA was added to buffer solutions at different pH values (1.1 and 7.4) containing various CyDs concentrations ($0–16 \cdot 10^{-3}$ M for β-CyD and $0–38 \cdot 10^{-3}$ M for DM-β-CyD and Poly-β-CyD). The pH 1.1 suspensions were stirred at 37 ± 0.5°C for 2 days, until the equilibrium was reached. Two ml of each sample were than filtered and analyzed by HPLC to determine the drug concentration. The pH 7.4 suspensions were submitted at the same procedure but using different temperatures (25, 37 and 45 ± 0.5°C).

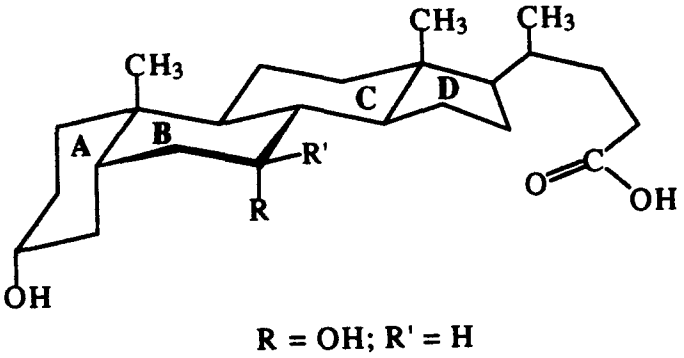
Apparent 1:1 stability constants (K_c) of drug-β-CyD systems were calculated from the straight portion of the phase solubility diagrams obtained at pH 7.4 according to Higuchi and Connors (1965) equation.

2.8. HPLC analysis

The HPLC apparatus (Waters Assoc., Milford, MA) comprised two Model 510 pumps, a Model 712 WISP auto-injector and an electrochemical detector (Model 5100A Coulochem; ESA, Bedford, MA) which consisted of a control module and an analytical cell (Model 5010) containing two in-line porous graphite coulometric electrodes. The analysis was performed in the oxidative mode. The guard cell was set at + 1.45 V. The applied potentials of the two electrodes in the analytical cell were + 0.60 V and + 1.40 V, respectively. The ED sensitivity range and response time were set at 1 μA and 4 s, respectively.

Separations were performed, at room temperature, on a 5-μm Ultrasphere ODS column (150 × 4.6 mm I.D.; Beckman, Berkeley, CA) fitted with a guard column (LiChrospher RP-18, 5 mm particles, 4 × 4 mm I.D.; Merck, Darmstadt, Germany)

Table 3
^{1}H-Chemical shifts of UDCA and its shifts in the presence of β-CyD and DM-β-CyD (1:1 mol ratio)



R = OH; R' = H

CH_3	Without CyDs	With β-CyD	$\Delta\delta^a$	With DM-β-CyD	$\Delta\delta^a$
18	0.777	0.865	0.088	0.810	0.033
19	1.035	1.049	0.014	1.020	−0.015
21	1.034	1.049	0.015	0.938	−0.096

[a] δ complex − δ free.

and eluted, isocratically, with methanol/acetonitrile/0.07 M aqueous sodium acetate adjusted to pH 5.0 with phosphoric acid (55:20:25, v/v). The flow rate was fixed at 0.9 ml/min. Peak areas were quantified by the external standardization method.

2.9. Dissolution studies

In order to evaluate the dissolution behaviour of free and complexed UDCA and CDCA, a test method was devised based on a USP 23 paddle method. Each sample was suspended in 900 ml of pH 1.1 buffer solution and stirred at 100 rpm at 37 ± 0.5°C. At fixed intervals of time, 2 ml of each sample were filtered and the solution was assayed by HPLC analysis to evaluate the drug concentration.

3. Results and discussion

Freeze-drying method produces amorphous samples with 1:1 mol ratio for both UDCA and CDCA with DM-β-CyD, 1:2 and 1:2.5 for UDCA-β-CyD and CDCA-β-CyD, respectively. Both UDCA and CDCA acids were present in drug-Poly-β-CyD systems in 8% (w/w) amount.

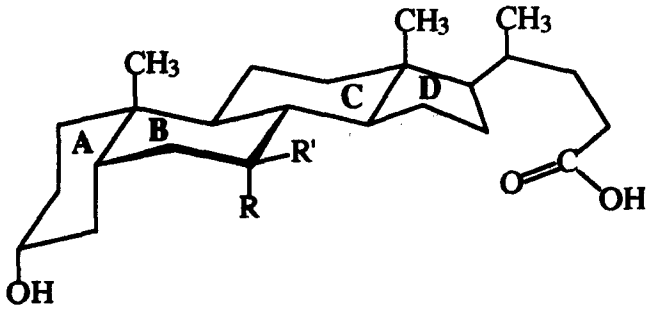
To verify the existence of a new solid phase freeze-dried and kneaded samples were characterized by IR diffractometry and DSC analysis.

FT-IR spectra of the freeze-dried UDCA- and CDCA-DM-β-CyD systems are shown in Fig. 1. The stretching band of carboxylic group observed at 1715 and 1718 cm^{-1} for CDCA and UDCA, respectively, was shifted to lower frequency in the complexes (1732 and 1728 cm^{-1}). The spectra of physical mixtures were the simple superposition of the spectra of the pure components.

The observed shift indicates the break of the intermolecular hydrogen bonding of the carboxylic group in the dimer molecule of the drug and consequently the monomeric dispersion of UDCA and CDCA in the macrocyclic matrix. Similar shifts were observed in the FT-IR spectra of the kneaded UDCA- and CDCA-DM-β-CyD samples. All other systems show a shift of carboxylic band in a similar manner of that observed in the presence of DM-β-CyD but with a less extent (spectra are not reported).

Other techniques, such as DSC, were employed to study our systems. The obtained calorimetric curves are shown in Fig. 2A–C.

Table 4
^{1}H-Chemical shifts of CDCA and its shifts in the presence of β-CyD and DM-β-CyD (1:1 mol ratio)



CH_3	Without CyDs	With β-CyD	$\Delta\delta^a$	With DM-β-CyD	$\Delta\delta^a$
18	0.738	0.783	0.045	0.771	0.033
19	0.982	1.015	0.033	0.956	−0.026
21	1.008	0.999	−0.009	0.956	−0.052

[a] δ complex − δ free.

The freeze-dried UDCA- and CDCA-CyD samples show the disappearance of the fusion peak of the two drugs, indicating the existence of an interaction between the two components (Cabral Marques et al., 1990). The endothermic peak observed around 235°C in the thermograms of UDCA- and CDCA-DM-β-CyD samples, probably, indicates the fusion of a new solid phase. In these thermograms is also evident an exothermic peak, probably due to amorphous nature of the samples that became crystalline after heating (Hanawa et al., 1993).

DSC curves of physical mixtures are the superposition of DSC thermograms of the pure components. In the physical mixture of CDCA-DM-β-CyD system a little endothermic peak is observed at 237°C. Probably this peak is referred to the fusion of a complex that is formed during preparation of physical mixture.

DSC thermograms of kneaded UDCA-DM-β-CyD, CDCA-DM-β-CyD and CDCA-β-CyD show a similar trend to the one observed for freeze-dried sample. In the case of UDCA-β-CyD sample the thermogram shows a peak corresponding with fusion peak of UDCA, showing that none or partial interaction is present between the two components (thermograms are not reported). Then, for this sample the kneading procedure was repeated for a longer time (40 min) than 15 min. In this condition, the solid makes brown and the HPLC analysis of this sample shows the presence of some byproducts with a minor retention time of the not treated UDCA (see chromatograms in Fig. 3). The byproducts were not identified, but their presence indicates that kneading method cannot be used to prepare UDCA-β-CyD complex.

^{1}H-NMR gives sure information about the inclusion of the bile acids into CyD cavity in aqueous solution. Even though there is no π electron on bile acid molecules, significant shifts of CyD protons were observed in the presence of UDCA and CDCA (Xinyi Tan and Lindenbaum, 1991) (Tables 1 and 2), particularly in the case of DM-β-CyD.

In the presence of two bile acids upfield shifts were observed not only for internal protons (H-3 and H-5) of the CyD cavity, but also for external ones. This trend indicates the formation of an inclusion complex, but also the existence of a probable interaction between the drugs and the external surface of the macrocycles.

The larger shift observed in the presence of CDCA for H-5 with respect to H-3, and the

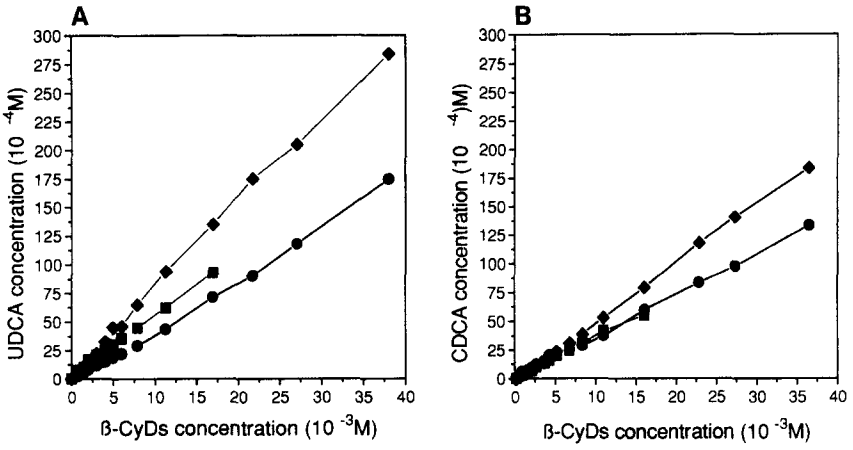


Fig. 4. (A) Phase solubility diagrams for UDCA in the presence of different β-CyDs in pH 1.1 buffer solution and at 37 ± 0.5°C. (■) UDCA-β-CyD; (◆) UDCA-Poly-β-CyD; (●) UDCA-DM-β-CyD. (B) Phase solubility diagrams for CDCA in the presence of different β-CyDs in pH 1.1 buffer solution and at 37 ± 0.5°C. (■) CDCA-β-CyD; (◆) CDCA-Poly-β-CyD; (●) CDCA-DM-β-CyD.

significant shift of H-6, indicates that this drug penetrates into the CyD cavity from the primary hydroxyl site of the macrocycle. Similar results have been obtained by Xinyi Tan and Lindenbaum (1991) for CDCA and β-CyD. The opposite magnitude of H-3 and H-5 shifts in the presence of UDCA indicates a probable opposite penetration of the drug.

In both cases, because of the high shifts observed for internal protons, it is possible to suppose that the drugs penetrate deeply in the cavity, establishing strong interaction with the host molecules.

Tables 3 and 4 reports the ^{1}H-chemical shifts of UDCA and CDCA in the presence of the CyDs. Only the shifts of methyl protons have been reported, because other signals present in the spectra are overlapped between them or partially overlapped with CyD signals. However, because methyl groups are distributed in all the parts of the molecule, their shifts can give information about the probable inclusion mode.

In the presence of both CyDs, higher shifts were observed for UDCA compare to CDCA, showing a stronger interaction and confirming our K_c values.

The magnitude of proton shifts of UDCA and CDCA evidences a different disposition of the drugs into the different CyD cavity.

The interaction of UDCA with β-CyD produces a significant downfield shift of CH_3-18, while similar small positive shifts have been observed for other two methyl groups. In the case of CDCA, CH_3-19 was also shifted downfields with a similar magnitude of CH_3-18, while a very little upfield shift was observed for CH_3-21. It is presumable that UDCA penetrates into β-CyD cavity from its hydrophilic part and forms a single 1:1 complex in which CyD is located between CH_3-19 and CH_3-21, then, interesting C and D rings of the drug.

In the case of CDCA it is supposable the existence in solution of two different complexes with 1:1 mol ratio, one concerning C and D rings of the drug such as UDCA and the other concerning the opposite part of the drug molecule.

In the presence of DM-β-CyD similar trends were observed for UDCA and CDCA: downfield shifts for CH_3-18 and upfield shifts for CH_3-19 and particularly for CH_3-21. This trend shows a similar orientation of the bile acids into DM-β-CyD cavity. Both bile acids deeply penetrate into DM-β-CyD cavity from their carboxylic group (higher CH_3-21shift than other CH_3), forming a single 1:1 complex. DM-β-CyD produces with its electronegative oxygen a shielding effect on peripheral CH_3 of the drug molecules.

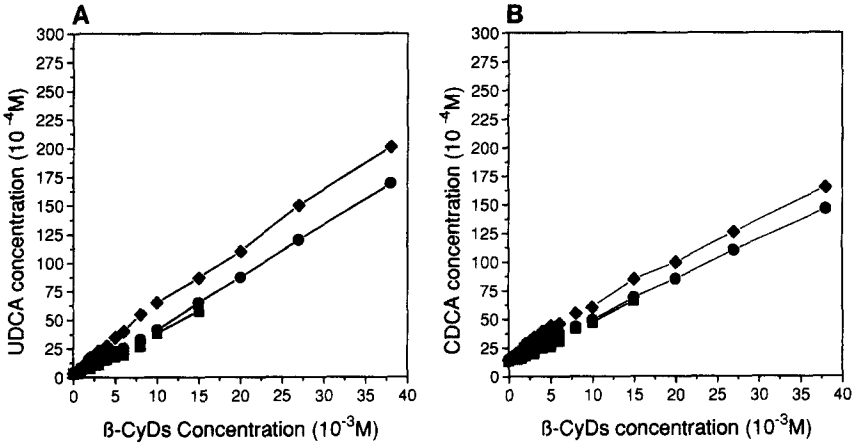


Fig. 5. (A) Phase solubility diagrams for UDCA in the presence of different β-CyDs in pH 7.4 buffer solution and at 2.5 ± 0.5°C. (■) UDCA-β-CyD; (◆) UDCA-Poly-β-CyD; (●) UDCA-DM-β-CyD. (B) Phase solubility diagrams for CDCA in the presence of different β-CyDs in pH 7.4 buffer solution and at 25 ± 0.5°C. (■) CDCA-β-CyD; (◆) CDCA-Poly-β-CyD; (●) CDCA-DM-β-CyD.

The influence of CyDs on water solubility of CDCA and UDCA was evaluated at different pH buffer solution (1.1 and 7.4) and at various temperatures (25, 37 and 45°C).

The solubility phase diagrams obtained at 37°C and at pH 1.1 for both drugs are shown in Fig. 4A–B. They present a linear increase of drug solubility at the increase of CyDs concentration (A_L type curve), showing that soluble complexes were formed and no precipitation was observed in the range of CyDs concentration used. The slope values are in all cases less than 1, indicating the existence in solution of complexes with 1:1 stoichiometry (Higuchi and Connors, 1965). Because CyDs were present in excess amounts with respect to free acids, probably both inclusion complexes and free CyDs were present in solutions.

It is observed a different affinity of three CyDs with regard to bile acids. All CyDs show a higher solubilizing ability for UDCA than CDCA. This trend is probably due to the spatial disposition of the O(7)H in two bile acids: axial in CDCA and equatorial in UDCA. It is presumable that both bile acids assume into CyDs cavity an axial disposition with respect to the z-axis of the macrocycle, hence the axial disposition of O(7)H of CDCA, probably, limits the complexation by sterical hindrance.

The solubility of free acids was not detectable at pH 1.1, due to their high lypophylicity and for this reason we cannot determine the stability constants (K_c) of these systems.

At pH 7.4 the solubility studies were performed at different temperatures (25, 37 and 45°C). The solubility diagrams obtained for UDCA and CDCA at 25°C are shown in Fig. 5A, B. An A_L type isotherm was obtained and in both cases the complexing ability of CyDs decreases in the following order: Poly-β-CyD > DM-β-CyD $\geq$ β-CyD. The slope values of these curves decrease with respect to pH 1.1. At pH 7.4 UDCA and CDCA are in their ionic form, showing their own water solubility. Probably, in this condition, salvation competes with complexation or limits itself.

At the increase of the temperature a different trend was observed for two bile acids. In the case of UDCA (Fig. 6A, B) linear increase of drug solubilization was shown such as 25°C, and a decrease of slope values (data not reported) was observed, showing that complexation is an exothermal process.

CDCA acts as a surfactant forming micellar aggregates (Cheng et al., 1992) with supersaturation of solution. The presence of different CyDs produces the desegregation of these micelles as a

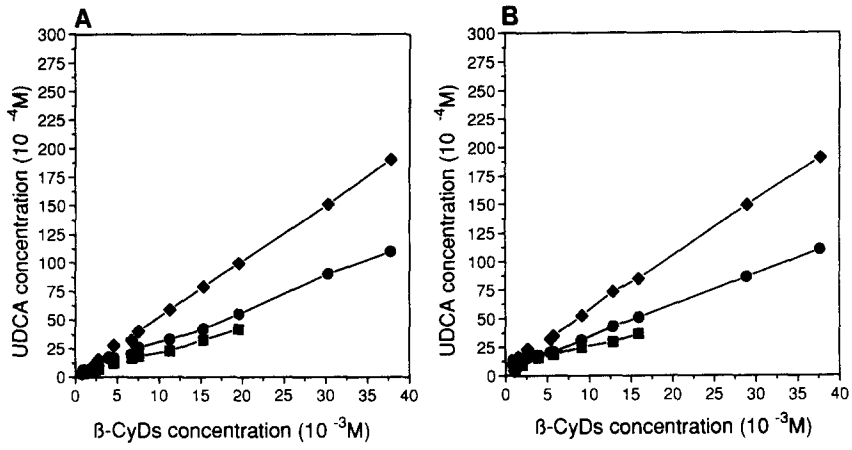


Fig. 6. (A) Phase solubility diagrams for UDCA in the presence of different β-CyDs in pH 7.4 buffer solution and at 37 ± 0.5°C. (■) UDCA-β-CyD; (◆) UDCA-Poly-β-CyD; (●) UDCA-DM-β-CyD. (B) Phase solubility diagrams for UDCA in the presence of different β-CyDs in pH 7.4 buffer solution and at 45 ± 0.5°C. (■) UDCA-β-CyD; (◆) UDCA-Poly-β-CyD; (●) UDCA-DM-β-CyD.

result of the complexation and probably of the interaction between the hydrophilic head group of the bile salt and the external surface of the CyD molecules. Other studies are in progress to evaluate this effect.

The apparent 1:1 stability constants (K_c) of the systems were determined by phase solubility diagrams and the obtained values were reported in Tables 5 and 6.

In all cases Poly-β-CyD shows the higher K_c values for both UDCA and CDCA when compared to other CyDs, showing a higher solubilizing capacity than other CyDs.

The thermodynamic parameters of the interaction of UDCA with CyDs at pH 7.4 were determined on the basis of the dependence of K_c values on temperature (Table 7).

Complexation is an exothermal process. In fact, the higher the temperature, the smaller the K_c value, and the ΔH values are always negative. It is observed that complexation is favoured by an enthalpic contribution, rather than an entropic one. The energetic level of the systems is dependent on the water release from the CyD cavity. Then, the more favourable ΔH change in the UDCA-DM-β-CyD system with respect to the other systems is probably due to a weaker interaction of the macrocycle with water molecules due to the lower presence of free hydroxyl groups and therefore the splitting of this water molecules requires less energy. The binding process between UDCA and CyDs occurs with negative ΔS values, showing more ordered systems. This trend probably reflects an increased conformational rigidity of the CyD ring, induced by complexation and a decrease in the degree of rotational and translational freedom of the drugs after complexation (Puglisi et al., 1996).

Table 5
Stability constant (K_c) values of UDCA-CyD systems determined at pH 7.4 at different temperatures

Temperature (°C)	UDCA-β-CyD (M^{-1})	UDCA-Poly-β-CyD (M^{-1})	UDCA-DM-β-CyD (M^{-1})
25	3549	7059	4306
37	1312	4989	1924
45	456	2546	625

Table 6
Stability constant (K_c) values of CDCA-CyD systems determined at pH 7.4 and at 25°C

Compounds	K_c (M^{-1})
CDCA-β-CyD	594
CDCA-Poly-β-CyD	680
CDCA-DM-β-CyD	566

Table 7
Thermodynamic parameters of the interaction between UDCA and CyDs

	UDCA-β-CyD	UDCA-Poly-β-CyD	UDCA-DM-β-CyD
ΔH	−9.47	−9.28	−19.33
ΔS	−48.54	−13.27	−47.51
ΔG	−48.97	−52.97	−50.90

ΔH and ΔG are in kJ mol^{-1} and ΔS is in J K^{-1} mol^{-1}.

4. Dissolution studies

The dissolution profiles of complexed UDCA and CDCA obtained at 37°C and pH 1.1 are shown in Fig. 7A, B.

Due to the insolubility of free bile acids at this pH, it is not possible to determine their dissolution profiles.

All complexes show high dissolution rates because of the increase of the water solubility and wettability of drugs and of the reduction of molecular crystallinity following complexation. The freeze-drying method produces amorphous drug-CyD complexes with reduced particle size and consequently they exhibit instantaneous dissolution compared to complexes prepared by kneading method.

The obtained solutions were stable and no precipitation of the free acids were observed although high concentrations of drugs were present.

5. Conclusion

The results obtained demonstrate that all CyDs used in this study are able to interact with UDCA and CDCA at the solid state. Only freeze-drying can be used as a general method to prepare the solid complexes with all CyDs, in fact, in the case of β-Cyd and UDCA the kneading method produces a simple physical mixture or the formation of drug byproducts. NMR spectroscopy shows that both bile acids have been deeply included in all CyDs, but the complexes have a different structure, depending on different CyDs.

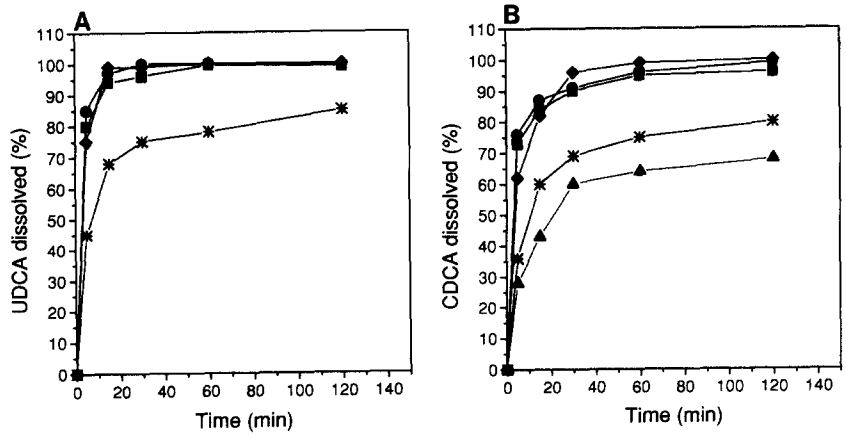


Fig. 7. (A) Dissolution profiles of UDCA-CyDs complexes in pH 1.1 buffer solution and at 37 ± 0.5°C. (◆) UDCA-Poly-β-CyD; (●) UDCA-DM-β-CyD freeze-dried complex; (■) UDCA-β-CyD freeze-dried complex; (*) UDCA-DM-β-CyD kneading complex. (B) Dissolution profiles of CDCA-CyDs complexes in pH 1.1 buffer solution and at 37 ± 0.5°C. (◆) CDCA-Poly-β-CyD; (●) CDCA-DM-β-CyD freeze-dried complex; (■) CDCA-β-CyD freeze-dried complex; (*) CDCA-DM-β-CyD kneading complex; (▲) CDCA-β-CyD kneading complex.

The inclusion complexes have a high stability constant values and they are favoured by an enthalpic contribution, rather that an entropic one.

Aqueous solubility of the drugs significantly increases when complexed with different CyDs, and particularly in the presence of Poly-β-CyD.

All drug-CyD complexes have a high dissolution rate, in particular, 100% of the UDCA-CyD freeze-dried complexes dissolve within 15 min. In vivo studies of the complexes are in progress to evaluate the effect of CyDs on bioavailability and pharmacological activity of the drug.

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