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# Complexation of ursodeoxycholic acid with β-cyclodextrin–choline dichloride coprecipitate

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**Abstract**

The inclusion complexes of ursodeoxycholic acid (UDCA) with β-cyclodextrin (βCD) coprecipitated with choline dichloride (CDC) or β-cyclodextrin were investigated to evaluate the effect of the presence of choline for UDCA inclusion in βCD. The inclusion complexes were investigated in solution by phase solubility diagrams and $^1$H NMR spectrometry and in solid state (kneading, freeze-drying, sealed heating and spray-drying) by DSC, SEM, HSM, XRD and IR spectroscopy. Stability constants were determined at pH 5.5 and 7.0 to simulate the environmental pH of the first intestinal tract and at different temperatures (25, 30 and 37°C) to obtain the thermodynamic parameters of inclusion. Both βCD–CDC and βCD increased the water solubility of UDCA particularly βCD–CDC. All complexes showed a high dissolution rate particularly the spray-dried complexes obtained in the presence of βCD–CDC. 

*Keywords:* Ursodeoxycholic acid; β-Cyclodextrin; β-Cyclodextrin–choline dichloride coprecipitate; Characterization of the complexes; Solubility studies; Dissolution rate

## 1. Introduction

Ursodeoxycholic acid (UDCA) is one of the major bile acids in bear bile, whereas it is contained only in trace amounts in human bile (Vandelli et al., 1995). It is the 7β epimer of chenodeoxycholic acid (CDCA) and is a white, odourless, crystalline powder with a bitter taste.

UDCA is used as a drug to dissolve cholesterol gallstones (Roda et al., 1993), to treat biliary cirrhosis (Simoni et al., 1995; Vandelli et al., 1995) and bile reflux gastritis (Scalia et al., 1988).

Owing to its low solubility at intestinal pH, its bioavailability is poor after oral administration (Igimi and Carey, 1980; Moroi et al., 1992; Giunchedi et al., 1996). UDCA solubility increases only above pH 8.4, but this high pH is usually reached only postprandially with sustained duodenal and pancreatic secretion (Roda et

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al., 1994). Many investigations have been carried out to increase the water solubility of UDCA by means of complexation with βCD or its derivatives. Miyajima et al. (1986) showed the ability of βCD to include bile salts. Vandelli et al. (1995) investigated the inclusion of UDCA in hydroxypropyl-βCD and Ventura et al. (1997) in dimethyl-βCD and polymerized-βCD showing improvement in the dissolution properties of UDCA particularly in the presence of hydroxypropyl-βCD and polymerized-βCD.

The possibility to increase the complexing ability of βCD by their prior coprecipitation with molecules able to increase the aqueous solubility of lipophilic drugs seemed to us to represent a further approach to the utilization of βCD as aqueous solubility increasing agents.

In this study, we investigated the inclusion of UDCA with βCD coprecipitated with choline dichloride (CDC) in a 1:1 molar ratio both in solution and in the solid state. Our purpose was to evaluate the influence of the hydrophilic molecule CDC on UDCA complexation with βCD and to study the main physico-chemical parameters influencing the functional properties of the UDCA–βCD–CDC complexes.

DSC, HSM, XRD, IR and SEM were used to characterise the complexes in the solid state, while $^1$H NMR spectra of complexes in solution were performed to obtain information on the inclusion mode.

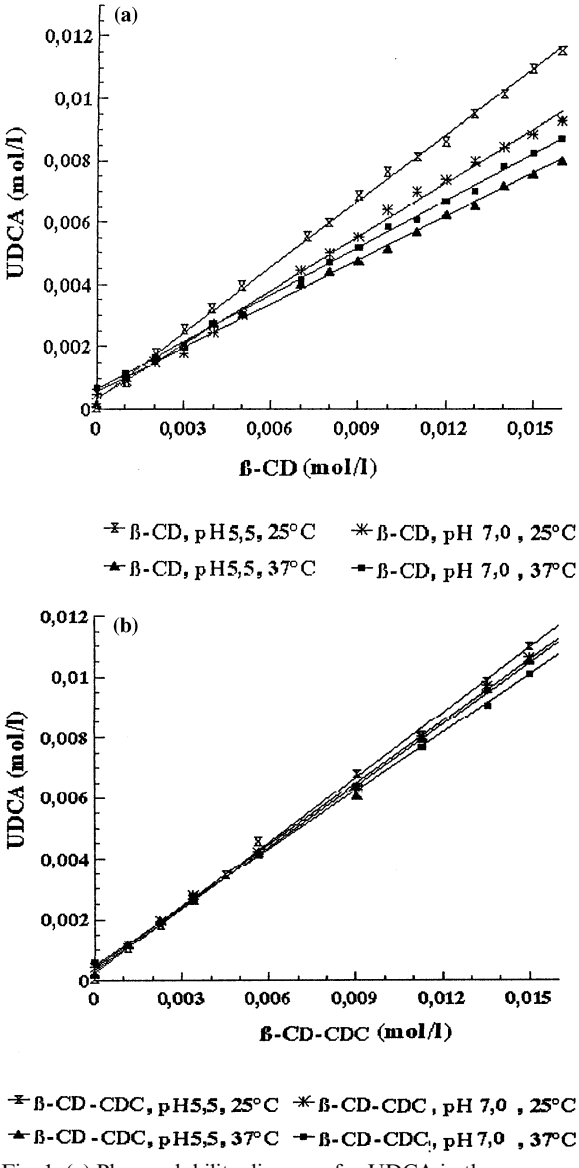



Fig. 1. (a) Phase solubility diagrams for UDCA in the presence of βCD. (b) Phase solubility diagrams for UDCA in the presence of βCD-CDC.

## 2. Materials and methods

### 2.1. Materials

UDCA (3α, 7β-dihydroxy-5β-cholan-24-oic acid; mol. wt. 392.6; Fluka Chemika, CH-Buchs), βCD (mol. wt. 1135; $H_2O$ 10–13%) (Fluka Chemika, CH-Buchs) and CDC (2-chloroethyltrimethyl-ammonium chloride) (mol. wt. 158.07; Fluka Chemika, CH-Buchs) were used to prepare the solid inclusion complex. All products were used as received from the manufacturer.

### 2.2. Analytical method

The concentration of UDCA in the samples was determined by HPLC (Scalia et al., 1989). The HPLC apparatus included an HPLC Pump 420 (Kontron Instruments) and an HPLC Detector 432 (Kontron Instruments). Chromatographic separation was performed on a Lichrospher 100 RP 18 column (240 mm long, 4.6 mm i.d.; 5 μm particle size) (Merck, Darmstadt, Germany) with

Table 1
Thermodynamic parameters of the inclusion process of UDCA in βCD or βCD–CDC

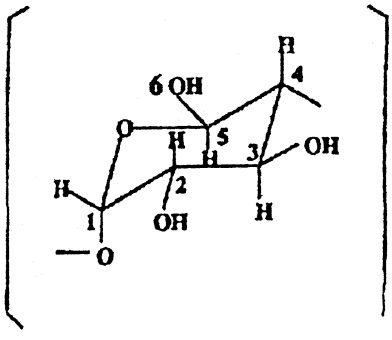
| | pH | $T$ (°C) | $K_a$ ($M^{-1}$) | $\Delta G$ (J/mol) | $\Delta H$ (J/mol) | $\Delta S$ (J/mol per K) |
|---|---|---|---|---|---|---|
| UDCA–βCD | 5.5 | 25 | 7696.3 | −22 170.5 | | |
| | | 30 | 5139.2 | −20 821.9 | −102 203.2 | −268.6 |
| | | 37 | 1558.8 | −18 934.5 | | |
| | 7.0 | 25 | 4263.7 | −20 691.5 | | |
| | | 30 | 3167.3 | −20 014.3 | −61 461.3 | −136.8 |
| | | 37 | 1632.5 | −19 066.8 | | |
| UDCA–βCD–CDC | 5.5 | 25 | 10 268.9 | −22 885.0 | | |
| | | 30 | 8734.0 | −22 792.2 | −28 446.9 | −18.7 |
| | | 37 | 6584.9 | −22 661.4 | | |
| | 7.0 | 25 | 5150.4 | −21 175.4 | | |
| | | 30 | 4494.1 | −21 138.5 | −23 370.9 | −7.4 |
| | | 37 | 3575.2 | −21 087.2 | | |

a mobile phase composed of methyl alcohol–0.02 M sodium acetate in water (80:20 w/w); the pH was adjusted to 4.3 with phosphoric acid; 20 μl volumes were eluted isocratically (flow rate, 1 ml/min) at room temperature. Quantitative detection of UDCA was performed at a wavelength of 210 nm using deoxycholic acid (3α, 12α-dihydroxy-5β-cholan-24-oic acid) (Sigma) as internal standard. The standard curve with concentrations ranging from 0.2 to 15 mg/ml was linear, with a correlation coefficient (mean ± S.D.) of 0.995 ± 0.001 ($n = 7$).

### 2.3. Solubility studies

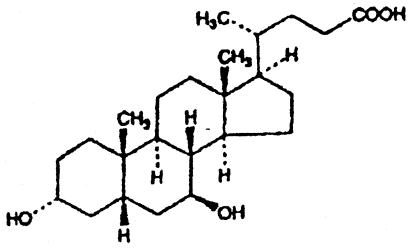
Solubility studies were carried out according to Higuchi and Connors (1965). To determine the solubility of UDCA, excess drug (50 mg) was added to 10 ml of deionized water containing different concentrations of βCD (from 0 to 0.016 mol/l). The suspension was shaken in 25-ml screw-capped vials at 30 strokes/min at 25, 30 and 37 ± 1°C and at different pH values (5.5 and 7.0). When equilibrium had been reached (7 days), the

Table 2
$^1$H-Chemical shifts of βCD and its shifts in the presence of UDCA




| | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ |
|---|---|---|---|---|---|---|
| δβCD | 5.043 | 3.632 | 3.938 | 3.558 | 3.829 | 3.852 |
| δβCD–UDCA | 5.062 | 3.662 | 3.888 | 3.614 | 3.761 | 3.853 |
| Δδ | 0.019 | 0.030 | −0.050 | 0.056 | −0.068 | 0.001 |

Table 3
$^1$H-Chemical shifts of methyl protons of UDCA and its shifts in the presence of βCD




| | $Met_{18}$ | $Met_{19}$ | $Met_{21}$ |
|---|---|---|---|
| δUDCA | 0.915 | 1.392 | 1.249 |
| δUDCA–βCD | 0.821 | 1.033 | 0.936 |
| Δδ | −0.094 | −0.359 | −0.313 |

content of each vial was filtered through a cellulose nitrate membrane (Millipore, 0.22 μm). UDCA concentration was determined in the filtered solutions. All the data are the average of three determinations.

### 2.4. Thermodynamic inclusion studies

The thermodynamic parameters of the inclusion process of UDCA in βCD or βCD–CDC were obtained by the relations between the apparent stability constant of the inclusion complexes ($K_a$) obtained from the solubility studies and temperature.

### 2.5. Preparation of the samples

#### 2.5.1. βCD–choline dichloride (βCD–CDC) coprecipitate

βCD (10 g; 8.81 mmol) was dissolved in 100 ml of DMSO. CDC (1.4 g; 8.81 mmol) was added.

Table 5
$^1$H-Chemical shifts of CDC and its shifts in the presence of βCD

| | $C_1$ | $C_2$ | $(Met)_3$ |
|---|---|---|---|
| δCDC | 4.016 | 3.783 | 3.214 |
| δCDC–βCD | 4.019 | 3.783 | 3.284<br>3.204 |
| Δδ | 0.003 | 0.000 | 0.070<br>−0.010 |

The solution was stirred for 24 h at 50°C. After this period a diethyl ether–acetone (2:1 v/v) mixture was added to the solution to induce coprecipitation of βCD and CDC. The precipitate obtained was subsequently washed first with the diethyl ether–acetone mixture and subsequently with diethyl ether and dried under vacuum.

#### 2.5.2. UDCA–βCD and UDCA–βCD–CDC physical mixtures

The physical mixtures were prepared by simple dry mixing of UDCA and βCD in amounts corresponding to 1:1 or 1:2 molar ratio or UDCA and βCD–CDC in amounts corresponding to 1:1 molar ratio. The mixing was performed adopting the geometric dilution method.

#### 2.5.3. Kneaded mixtures of UDCA–βCD or UDCA–βCD–CDC

Kneaded products were prepared from physical mixtures in a mortar by wetting the powder with the minimum volume of water and kneading vigorously for 45 min. Each sample was dried at 37°C for 1 day (Selecta 204).

#### 2.5.4. Heating of mixtures UDCA–βCD or UDCA–βCD–CDC in a sealed container

UDCA–βCD or UDCA–βCD–CDC were

Table 4
$^1$H-Chemical shifts of βCD and its shifts in the presence of βCD–CDC

| | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ |
|---|---|---|---|---|---|---|
| δβCD | 5.043 | 3.632 | 3.938 | 3.558 | 3.829 | 3.852 |
| δβCD–CDC | 5.049 | 3.643 | 3.902 | 3.587 | 3.761 | 3.848 |
| Δδ | 0.006 | 0.011 | −0.036 | 0.029 | −0.068 | −0.004 |

weighed in a molar ratio in 2-ml glass ampoules (400 mg total) and water was added (125 μl). The ampoules were sealed and heated at a defined temperature (100°C) for a specific time (1, 2 and 4 h for UDCA–βCD 1:1; 1 h for UDCA–βCD 1:2 and UDCA–βCD–CDC).

Table 6
$^1$H-Chemical shifts of βCD and its shifts in the presence of CDC and UDCA

| | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ |
|---|---|---|---|---|---|---|
| $\delta$βCD | 5.043 | 3.632 | 3.938 | 3.558 | 3.829 | 3.852 |
| $\delta$βCD–UDCA–CDC | 5.056 | 3.643 | 3.996 | 3.606 | 3.761 | 3.848 |
| $\Delta\delta$ | 0.013 | 0.011 | −0.058 | 0.048 | −0.068 | −0.004 |

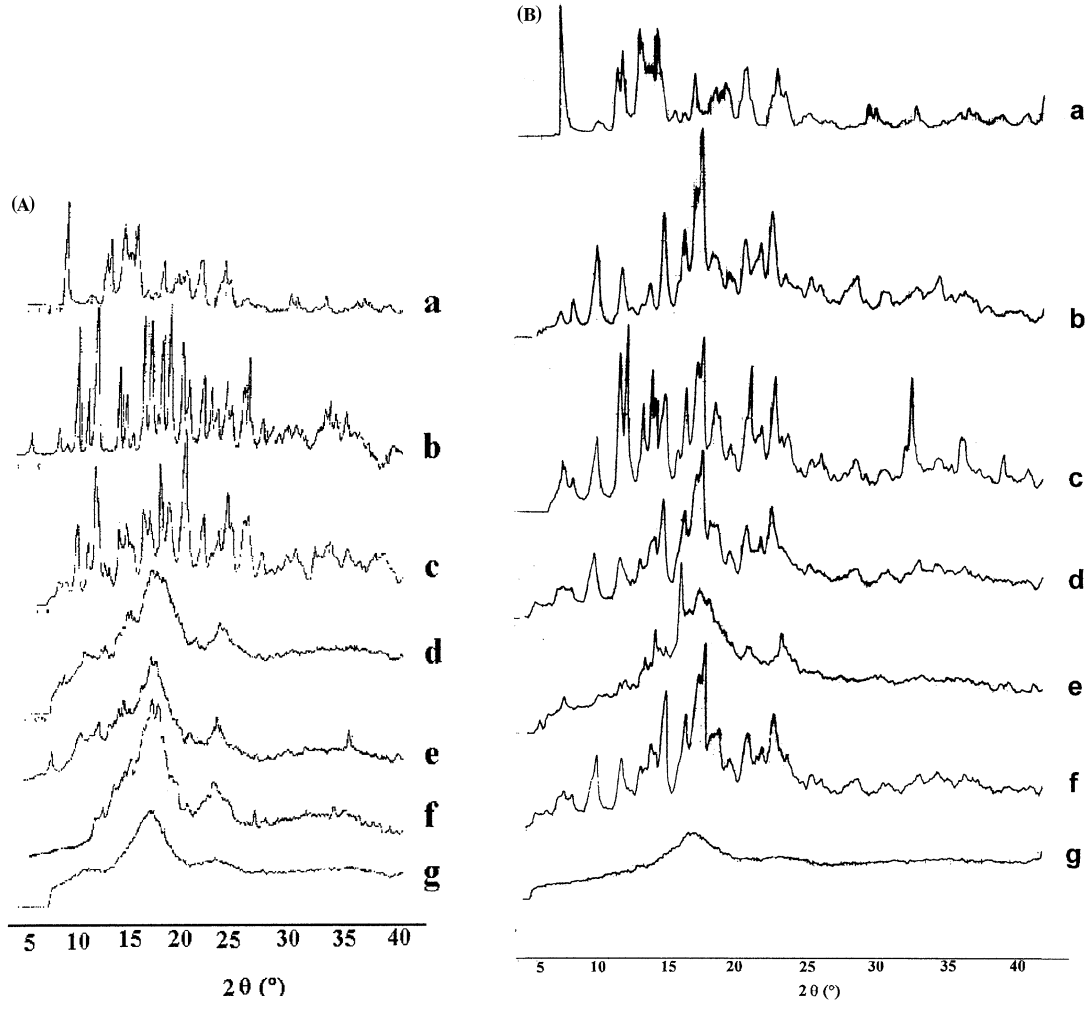



Fig. 2. (A) X-Ray diffraction patterns of UDCA and βCD: (a) UDCA; (b) βCD; (c) Physical mixture; (d) Kneading; (e) Freeze-drying; (f) Sealed heating; (g) Spray-drying. (B) X-Ray diffraction patterns of UDCA and βCD–CDC: (a) UDCA; (b) βCD–CDC; (c) Physical mixture; (d) Kneading; (e) Freeze-drying; (f) Sealed heating; (g) Spray-drying.

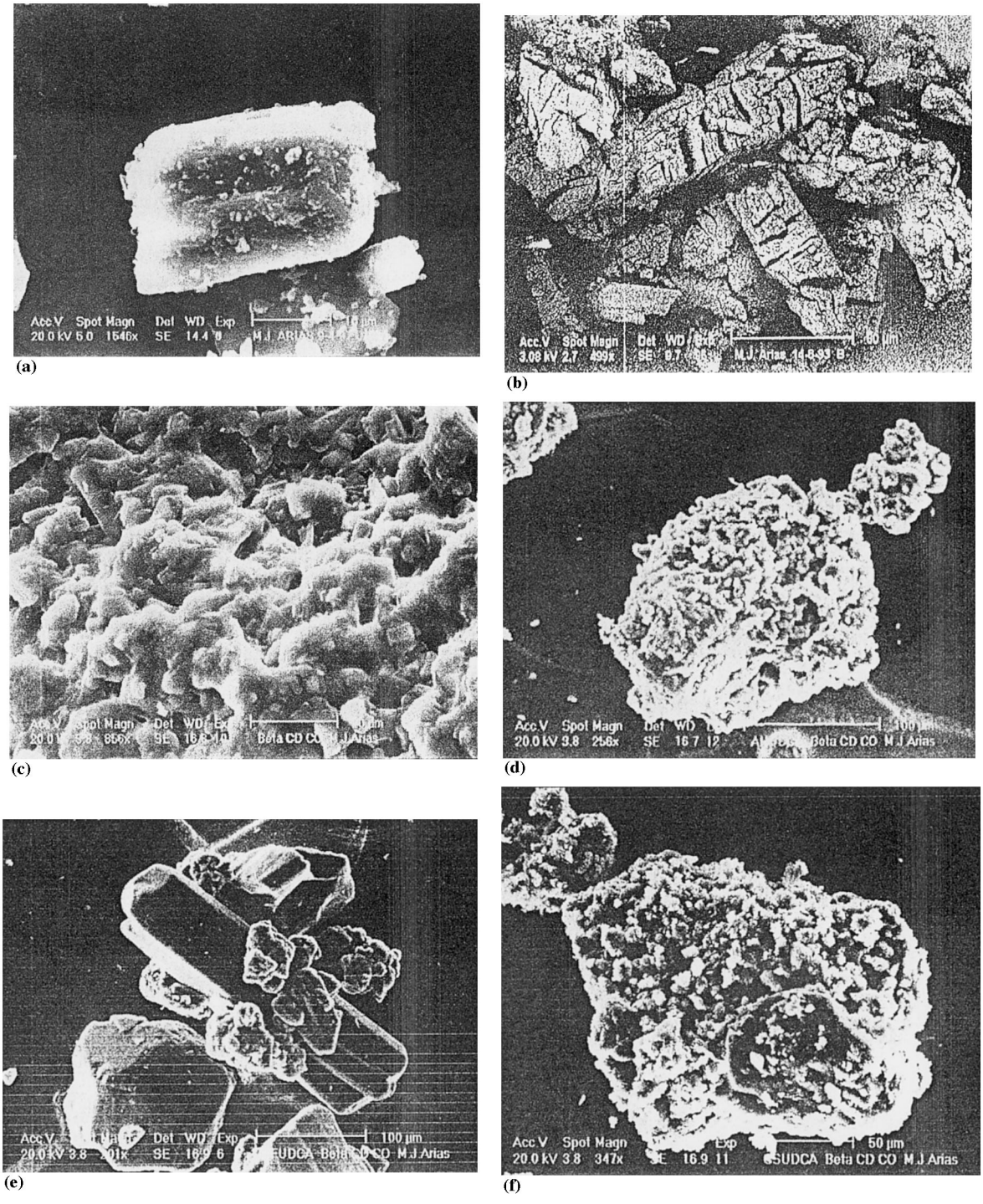
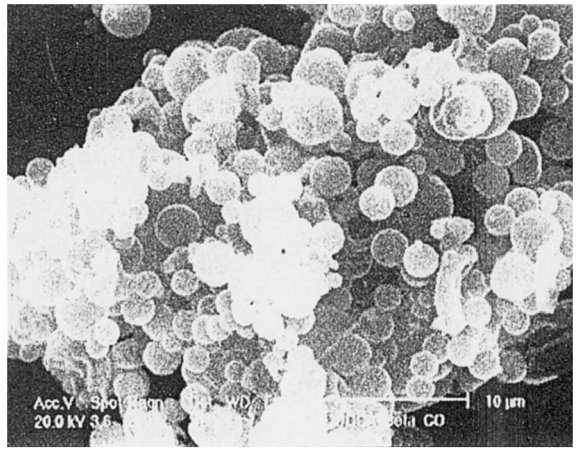



Fig. 3. SEM of UDCA, βCD and βCD–CDC. (a) UDCA; (b) βCD; (c) βCD–CDC; (d) Kneaded mixtures of UDCA–βCD–CDC; (e) Freeze-dried mixture of UDCA–βCD–CDC; (f) Sealed heated mixture of UDCA–βCD–CDC; (g) Spray-dried mixture of UDCA–βCD–CDC.




(g)

Fig. 3. (*Continued*)

### 2.5.5. Spray-dried mixtures of UDCA–βCD or UDCA–βCD–CDC

UDCA and βCD or βCD–CDC were dissolved into 250 ml of ethanol and 250 ml of water respectively, to obtain a solution. After a few minutes, the solution was evaporated with a spray-dryer (Mini Spray-Dryer, Buchi 190 M) under the following conditions: flow rate 315.8 ml/h, inlet temperature 156°C, outlet temperature 60°C.

### 2.5.6. Lyophilised mixtures of UDCA–βCD or UDCA–βCD–CDC

The lyophilised mixtures were prepared at a 1:1 and 1:2 molar ratio of UDCA to βCD and 1:1 of UDCA to βCD–CDC. The physical mixtures were dissolved at room temperature with the minimum volume of deionized water. The solution was cooled for 1 day and then freeze-dried (Telstar mod. Cryodos).

### 2.6. $^1H$ NMR studies

$^1H$ NMR experiments were performed at 500 MHz using a Bruker AC-500 spectrometer. TSP was used as external standard. Samples were solubilized in $D_2O$, adding drops of NaOD to facilitate drug solubilization.

### 2.7. Scanning electron microscopy (SEM)

The shape and morphological characteristics of UDCA, βCD, βCD–CDC particles and of all the systems prepared with the different techniques were analysed by scanning electron microscopy (Philips XL-30) at 20 kV.

### 2.8. Differential scanning calorimetry (DSC)

DSC curves were recorded on a Mettler FP 80 HT differential scanning calorimeter. Mettler FP 85 and FP 89 HT system software was used for data acquisition. All samples (7–10 mg) were heated in crimped aluminum pans at a scanning rate of 10°C $min^{-1}$ in the temperature range 80–280°C.

### 2.9. Hot stage microscopy (HSM)

Different observations were made during heating of all the systems prepared with the different techniques, using a hot stage device (Mettler model FP 82 HT and FP 89 HT) attached to an Olympus BH-2 light microscope.

### 2.10. X-Ray diffractometry (XRD)

X-Ray diffraction patterns were recorded using a Philips PW 1130/90 powder diffractometer. Ex-

perimental settings were: Cu Kα radiation; angular speed 1° (2θ)/min.

### 2.11. IR spectroscopy studies (IR)

IR spectra were recorded using an Infrared Fourier Spectrometer Bomen MB-120 employing the potassium bromide disk method.

### 2.12. Drug dissolution studies

Drug dissolution studies were based on a USP 23 paddle method. Each sample was suspended in 1000 ml of phosphate buffer ($Na_2HPO_4/KH_2PO_4$) pH 7.0 and stirred at 50 rev./min at $37 \pm 1$°C. At fixed intervals of time, 4 ml of each sample were filtered and the solution was assayed by HPLC analysis to evaluate the drug concentration. All experiments were carried out using either 500 mg of UDCA crystals or an equivalent amount of drug in the physical mixture or solid inclusion complex. All experiments were run in triplicate.

## 3. Results and discussion

### 3.1. Solubility studies

The phase-solubility phase diagrams obtained for UDCA–βCD and UDCA–βCD–CDC, re-

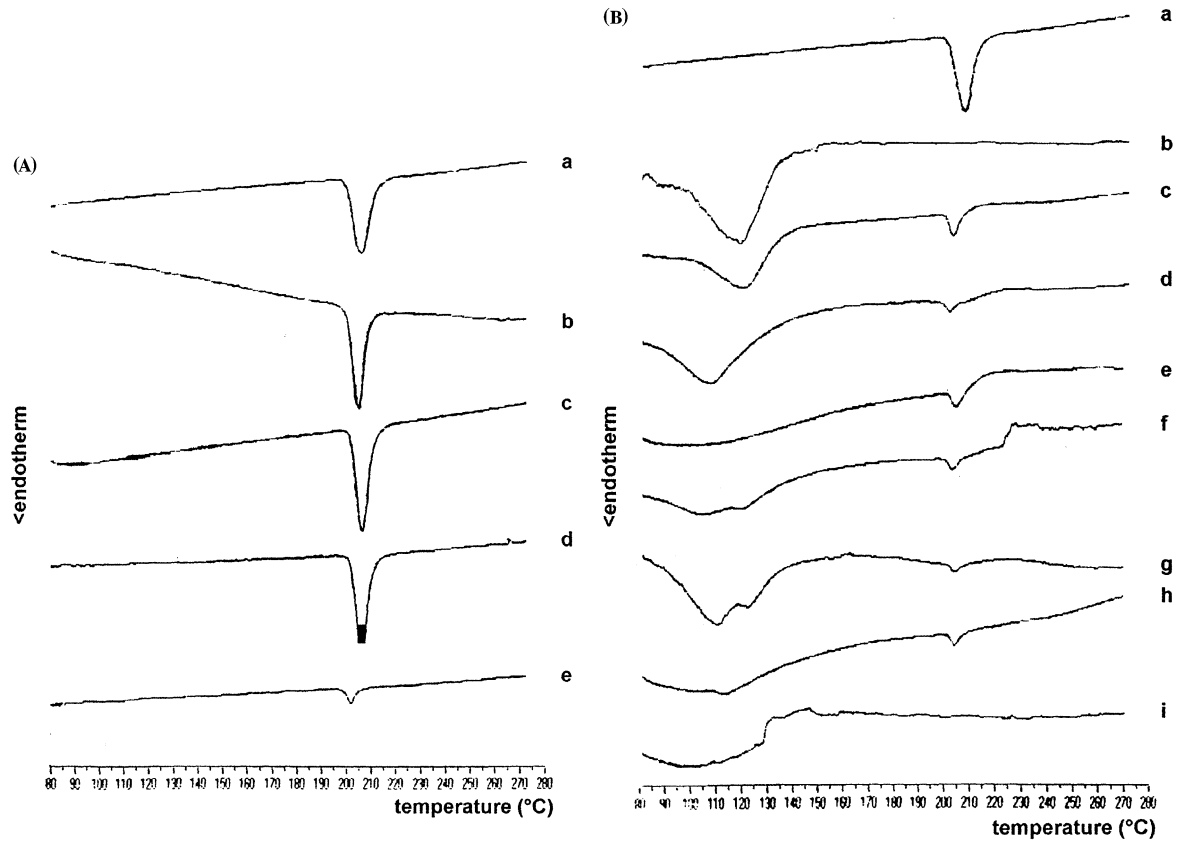



Fig. 4. (A) DSC curves of UDCA: (a) UDCA; (b) Kneading; (c) Freeze-drying; (d) Sealed heating; (e) Spray-drying. (B) DSC curves of UDCA and βCD: (a) UDCA; (b) βCD; (c) Physical mixture; (d) Kneading; (e) Freeze-dried; (f) Sealed heating 1 h; (g) Sealed heating 2 h; (h) Sealed heating 4 h; (i) Spray-drying. (C) DSC curves of UDCA and βCD–CDC: (a) UDCA; (b) βCD–CDC; (c) Physical mixture; (d) Kneading; (e) Freeze-drying; (f) Sealed heating; (g) Spray-drying.

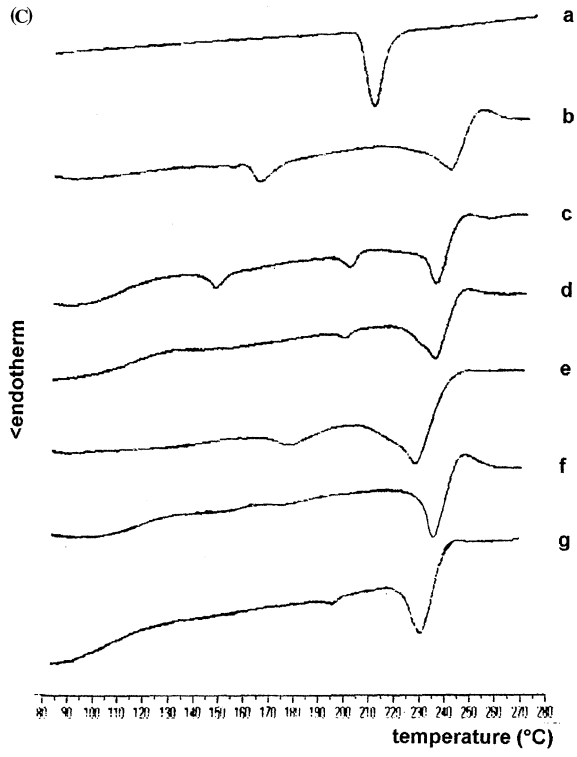



Fig. 4. (*Continued*)

ported in Fig. 1 present a linear increase in drug solubility at the increase of βCD or βCD–CDC concentration ($A_L$ type curve), showing that soluble complexes were formed. The slope values are in all cases less than one, indicating the existence in solution of complexes with 1:1 stoichiometry (Higuchi and Connors, 1965). Table 1 lists the 1:1 apparent stability constants of the inclusion complexes ($K_a$) calculated from the straight line of the diagrams according to the following equation:

$$K_a = S/[C_s(1 - S)]$$

where $C_s$ (the intercept) is the solubility of UDCA in the absence of the complexing agent and $S$ denotes the slope of the straight line. Table 1 and Fig. 1 show a higher affinity of UDCA for βCD–CDC than for βCD. Moreover the $K_a$ values are higher, for both systems, at 25°C and pH 5.5 than at 37°C and pH 7.0. At pH 7.0, UDCA is prevalently in its ionic form, under this condition solvation probably competes with complexation, weakly affecting the complexation tendency of UDCA.

### 3.2. $^1H$ NMR studies

Table 2 reports the $^1$H-chemical shifts of βCD and its shifts in the presence of UDCA. The high shifts observed for internal protons (H-5 and H-3) indicate that the UDCA penetrates deeply into the cavity strongly involving H-5 protons.

The shifts of UDCA methyl protons are reported in Table 3. The methyl groups are distributed throughout the molecule, their shifts can give information on the probable inclusion mode (Ventura et al., 1997). The interaction of UDCA with βCD produced a significant upfield shift of $CH_3$-19 and $CH_3$-21, while a smaller negative shift was observed for the $CH_3$-18. Presumably, UDCA penetrates into the βCD cavity from its hydrophilic part and forms a single 1:1 complex in which the CD is located between $CH_3$-19 and

Table 7
DSC data

| | | MP (°C) | $\Delta H_f$ (J/g) | % Amorphous |
|---|---|---|---|---|
| UDCA | Commercial | 210.4 | −50.6 | – |
| | Kneading | 201.5 | −44.7 | 11.66 |
| | Freeze-drying | 209.0 | −50.6 | – |
| | Sealed heating | 207.2 | −49.7 | 1.78 |
| | Spray-drying | 203.2 | −6.18 | 87.79 |

$CH_3$-21, then involving the C and D rings of the UDCA.

Table 4 reports the H-chemical shift of βCD and βCD-CDC. Upfield shifts were observed for internal protons (H-3 and H-5) of the βCD cavity indicating the existence of an interaction between the CDC and the βCD, but also the complexation of CDC in the βCD cavity. Moreover the methyl groups of the CDC are divided into two peaks in the presence of βCD (Table 5) supporting the CDC inclusion in the βCD cavity.

Table 6 reporting the chemical shifts of βCD in the presence of CDC and UDCA shows upfield shifts not only for internal protons (H-3 and H-5) of the CD cavity, but also for the external protons (H-6) indicating a strong inclusion of UDCA in βCD–CDC. A comparison of both the chemical shifts of UDCA in the presence of βCD–CDC (Table 6) or βCD (Table 2) disclosed the stronger inclusion of UDCA in βCD–CDC than βCD as the chemical shifts are higher in the presence of βCD–CDC.

### 3.3. Thermodynamic parameters of inclusion

The thermodynamic parameters of inclusion, reported in Table 1, indicate that UDCA complexation with βCD and βCD–CDC is spontaneous (negative $\Delta G$). The slightly higher $\Delta G$ absolute values obtained for UDCA complexation with βCD–CDC rather than with βCD suggest more spontaneous complexation between UDCA and βCD–CDC. The negative $\Delta H$ and $\Delta S$ values, obtained for all the systems indicate that complexation is an exothermal process producing more ordered systems with respect to the free drug in the presence of the complexing agent. The lower $\Delta H$ and $\Delta S$ absolute values obtained for UDCA complexation with βCD–CDC than βCD indicate that in the presence of βCD–CDC the more spontaneous complexation is driven by an entropic rather than enthalpic contribution.

Table 8
DSC data

| | | MP (°C) | $\Delta H_f$ (J/g) | % Amorphous |
|---|---|---|---|---|
| UDCA–βCD | Physical mixture | 204.2 | −6.29 | – |
| | Kneading | 199.6 | −5.56 | 88.39 |
| | Freeze-drying | 205.2 | −5.21 | 82.83 |
| | Sealed heating (1 h) | 202.5 | −5.68 | 90.30 |
| | Sealed heating (2 h) | 200.3 | −5.69 | 90.46 |
| | Sealed heating (4 h) | 205.0 | −5.92 | 94.12 |
| | Spray-drying | – | – | 100 |
| UDCA–βCD–CDC | Physical mixture | 198.9 | −2.35 | – |
| | Kneading | 198.4 | −1.01 | 42.98 |
| | Freeze-drying | 175.2 | −2.34 | 99.57 |
| | Sealed heating | – | – | 100 |
| | Spray-drying | – | – | 100 |

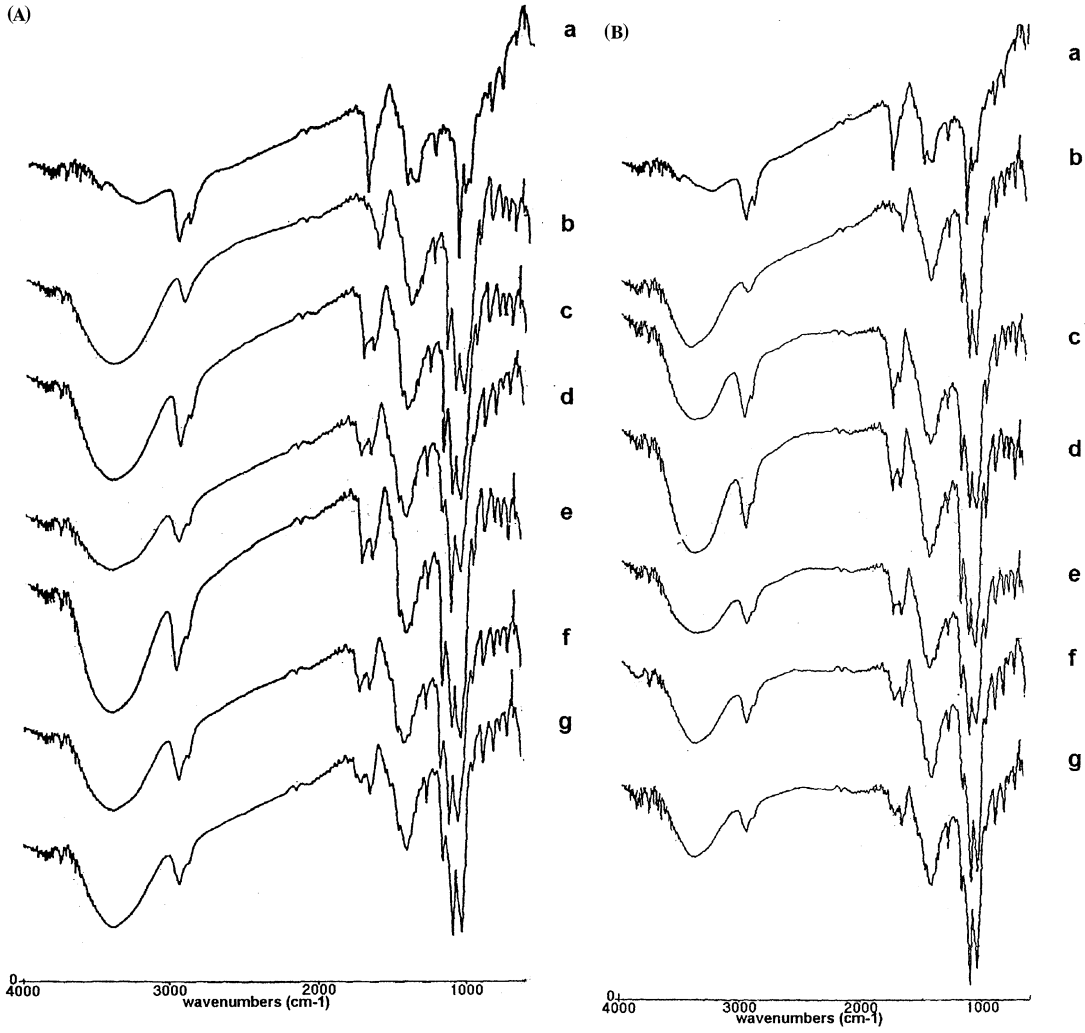



Fig. 5. (A) IR spectra of UDCA and βCD: (a) UDCA; (b) βCD; (c) Physical mixture; (d) Kneading; (e) Freeze-drying; (f) Sealed heating; (g) Spray-drying. (B) IR spectra of UDCA and βCD–CDC: (a) UDCA; (b) βCD–CDC; (c) Physical mixture; (d) Kneading; (e) Freeze-drying; (f) Sealed heating; (g) Spray-drying.

### *3.4. Solid-state studies*

#### *3.4.1. XRD*

The X-ray patterns (Fig. 2A,B) of the physical mixtures are the superposition of UDCA and βCD or βCD–CDC patterns. Some differences are present in the patterns obtained from UDCA and the UDCA–βCD or βCD–CDC mixtures treated differently. There is a gradation in the disappearance of the peaks of the two crystalline molecules (UDCA and βCD or UDCA and βCD–CDC) during kneading, freeze-drying, sealed heating and spray-drying, suggesting the same gradation in the percentage of the effective inclusion complex. This means that the kneaded powders, freeze-dried powders and the sealed heating powders are a mixture of inclusion complexes and free molecules, while the spray-dried powders contain only amorphous inclusion complexes.

*3.4.2. SEM*

Analysis by scanning electron microscope revealed the crystal state of UDCA as irregular shapes (Fig. 3a–g) and the crystallization of βCD in polyhedral forms.

The βCD–CDC coprecipitate occurs in the form of an amorphous crystalline mass.

The UDCA–βCD–CDC and UDCA–βCD products obtained by kneading and sealed heating occur in the form of large masses showing the existence of a single component in the mixtures obtained and thus indicating a possible complexation. In the case of freeze-drying, crystals of UDCA are evident surrounded by βCD or βCD–CDC indicating the existence of two components. The spray-dried products are substantially amorphous, therefore it is difficult to distinguish the complexes from the free components made amorphous during the spray-drying process.

*3.4.3. DSC*

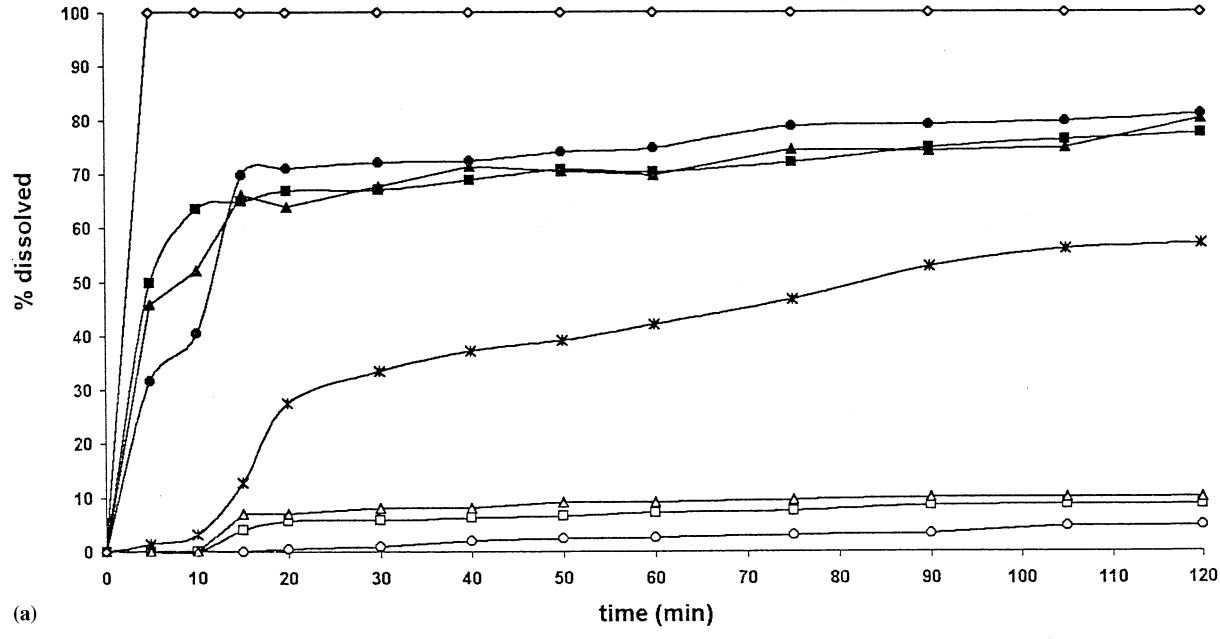
The DSC curves revealed some information on solid-state interactions of UDCA with βCD or βCD–CDC.

UDCA crystals showed the characteristic endothermic peak at 210°C, corresponding to drug melting. Fig. 4A reporting the DSC curves and Table 7 reporting the enthalpy values obtained by kneading, freeze-drying, sealed heating and spray drying of UDCA disclosed the smallest exothermic peak and the lowest enthalphy values for UDCA spray dried due to the amorphous nature of the spray-dried samples. The values relative to freeze-dried UDCA indicate that the product obtained was more crystalline than commercial UDCA.

The DSC profile of βCD exhibited a broad endothermic peak (about 60 and 140°C) due to water loss. The DSC curves of UDCA–βCD physical mixtures are the superposition of the DSC thermograms of the pure components (Fig. 4B). The βCD–CDC DSC curves show two endothermic peaks (about 155 and 230°C) corresponding to βCD and CDC respectively (Fig. 4C). The DSC curves of the UDCA–βCD products obtained by kneading, freeze-drying and sealed heating showed a small exothermic peak near to 210°C corresponding to free UDCA. The spray-dried UDCA–βCD showed the disappearance of the exothermic peak of UDCA, indicating complete complexation and amorphous nature (Fig. 4A). The freeze-dried UDCA–βCD product showed an enthalpy value lower than the kneading, sealed heating products indicating a higher degree of complexation with respect to the latter products but not a complete complexation (Table 8).

Table 9
Dissolution parameters of UDCA–βCD and UDCA–βCD–CDC mixtures

| | | Dissolution efficacy (DE) | | Dissolution percentage (DP) | |
|---|---|---|---|---|---|
| | | 60 | 120 | 60 | 120 |
| UDCA | Commercial | 0.012 | 0.023 | 2.53 | 4.72 |
| | Kneading | 0.051 | 0.067 | 7.07 | 9.49 |
| | Freeze drying | 0.070 | 0.083 | 9.00 | 10 |
| UDCA–βCD | Physical mixture | 0.27 | 0.39 | 42.1 | 57.0 |
| | Kneading | 0.69 | 0.71 | 70.2 | 77.4 |
| | Freeze-drying | 0.67 | 0.71 | 69.7 | 80.0 |
| | Sealed heating | 0.69 | 0.73 | 74.8 | 81.0 |
| | Spray drying | 1.04 | 1.02 | 100 | 100 |
| UDCA–βCD–CDC | Physical mixture | 0.48 | 0.54 | 54.0 | 65.9 |
| | Kneading | 0.79 | 0.83 | 86.0 | 90.2 |
| | Freeze-drying | 0.84 | 0.88 | 90.1 | 94.2 |
| | Sealed heating | 0.94 | 0.95 | 93.6 | 100 |
| | Spray drying | 100 | 100 | 100 | 100 |



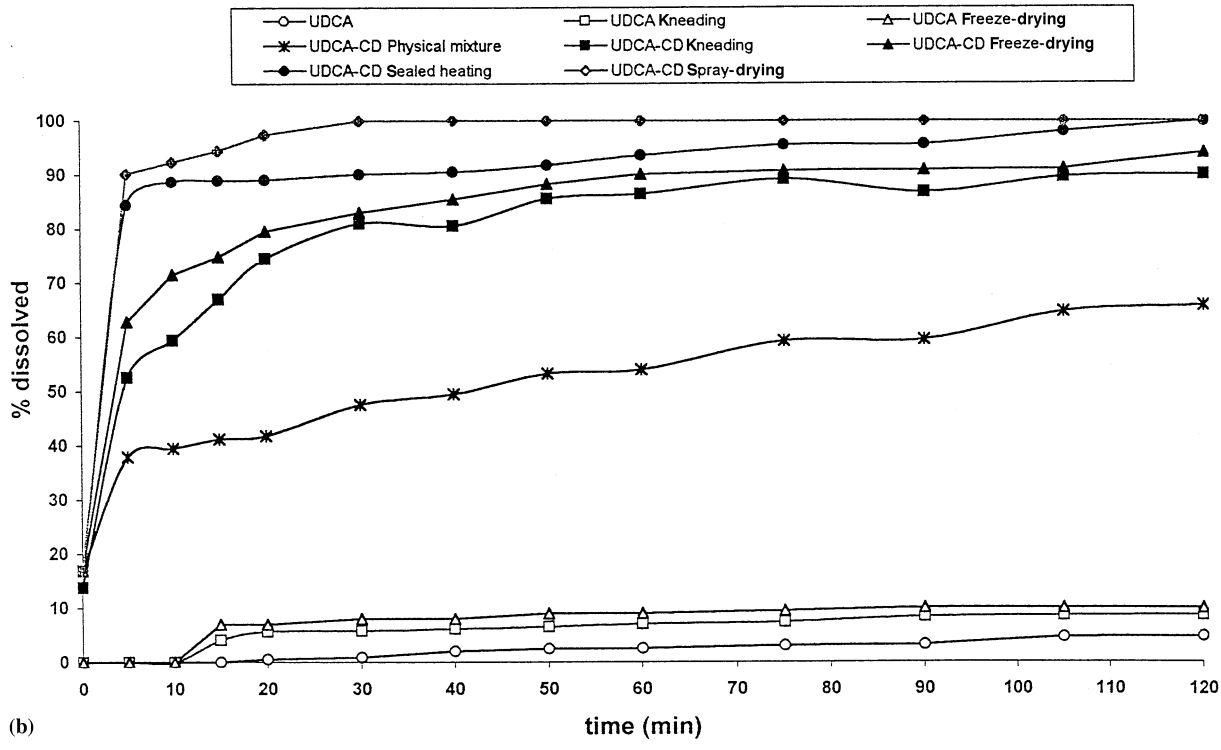




Fig. 6. (a) Dissolution profiles of UDCA and UDCA–βCD mixtures in phosphate buffer (pH 7.0) at $37 \pm 0.1$°C. (b) Dissolution profiles of UDCA and UDCA–βCD–CDC mixtures in phosphate buffer (pH 7.0) at $37 \pm 0.1$°C.

The physical mixture between UDCA and βCD–CDC was the superposition of DSC thermograms of the pure components (Fig. 4C). The DSC curves of UDCA–βCD–CDC products obtained by kneading and freeze-drying revealed a small exothermic peak near to 210°C corresponding to UDCA melting point, while the sealed heated and spray-dried products showed the disappearance of the UDCA fusion peak indicating complete complexation and, in the spray-dried product, amorphous nature.

*3.4.4. HSM*

Analysis by HSM confirmed the DSC studies. The UDCA–βCD and UDCA–βCD–CDC products were evaluated at various temperatures up to 210°C, showing the melting point of free UDCA. At this temperature the melting of UDCA was observed for all the products except the spray-dried ones indicating, in this case complete UDCA complexation.

*3.4.5. IR*

The IR spectra of physical mixtures (Fig. 5A,B) were the simple superposition of the spectra of the pure components. In the spectrum of the spray-dried UDCA–βCD or UDCA–βCD–CDC systems, the stretching band of the carboxylic group observed for free UDCA at 1716 $cm^{-1}$, was shifted to lower frequency in the complexes (1739 $cm^{-1}$). The samples obtained by kneading, sealed heating and freeze-drying showed no apparent displacement in the carboxylic absorption band. In these spectra no new peak appears, so that no chemical bonds were created in the compounds formed (Anguiano-Igea et al., 1992).

*3.4.6. Dissolution studies*

The dissolution profiles of UDCA and UDCA–βCD or UDCA–βCD–CDC products obtained by kneading, freeze-drying, sealed heating and spray-drying are shown in Fig. 6A,B. The dissolution data are reported in Table 9.

The dissolution of UDCA was slow and incomplete. All UDCA–βCD and UDCA–βCD–CDC showed higher dissolution rates than UDCA due to the increase in water solubility and the reduction in crystallinity. In particular, the spray-drying method produced completely amorphous solid products (Table 9) which consequently are characterised by instantaneous dissolution. The presence of CDC remarkably improved the dissolution rate of all UDCA–βCD–CDC products due to the strong increase in drug solubility as evidenced by the high stability constant values. The spray-drying method further enhanced the UDCA–βCD–CDC dissolution rate due to the amorphous effect induced on the solid product.

## 4. Conclusions

The results obtained demonstrate that βCD/choline dichloride 1:1 (mol/mol) coprecipitate interacts with UDCA better than βCD. This could be attributed to the formation of a new complexing structure (βCD–CDC) characterized by a higher affinity for UDCA than βCD as supported by the higher stability constant values and the favourable entropic contribution.

The UDCA–βCD–CDC products obtained by kneading, freeze-drying, sealed heating and spray-drying were characterized by enhanced dissolution rates with respect to the UDCA–βCD products obtained by the same methods. Among the methods used, only spray-drying provided complete complexation both in the presence of βCD–CDC and βCD while the other methods provided mixtures of inclusion complexes and free molecules. The UDCA–βCD–CDC complex obtained by the spray-drying method was the system with the highest dissolution rate among those examined.

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