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2-Hydroxypropyl-β-cyclodextrin complexation with ursodeoxycholic acid

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Abstract

The complexation in aqueous medium and in the solid phase of ursodeoxycholic acid (UDCA) with a highly soluble cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, was studied by means of solubility methods, IR and ^{13}C-NMR spectroscopy, X-ray diffractometry and thermal analysis. UDCA inclusion took place with 1:1 stoichiometry. ^{13}C-NMR analysis suggested that the side chain was introduced into the cyclodextrin cavity. The UDCA/cyclodextrin complex showed better dissolution properties than plain drug crystals. Therefore, the complex may be used to improve the delivery and bioavailability of ursodeoxycholic acid.

Keywords: Ursodeoxycholic acid; 2-Hydroxypropyl-β-cyclodextrin; Inclusion complexation; Solubility study; Solid-phase study; Drug dissolution

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. As a drug this bile acid is used to dissolve cholesterol gallstones (Nakagawa et al., 1977; Makino and Nakagawa, 1978) and to treat biliary cirrhosis (Poupon et al., 1994).

UDCA is a weak acid poorly soluble in water; its critical micelle concentration, pH of micellar aggregation as well as pH at which it precipitates are higher than those of other bile acids (Igimi and Carey, 1980; Carey, 1982; Hofmann and Roda, 1984). As UDCA is absorbed according to a passive diffusion mechanism (Aldini et al., 1992), these physico-chemical properties restrict its absorption. Thus, methods increasing drug solubility and dissolution rate could successfully be used to enhance the bioavailability of UDCA.

Complexation with cyclodextrins has been widely used (Corrigan and Stanley, 1982; Uekama et al., 1982; Otagiri et al., 1983) to improve both the dissolution rate and absorption of poorly soluble drugs. It is known that biliary acids form complexes with β-cyclodextrin (Miyajima et al., 1986; Tan and Lindenbaum, 1991), and these have been proposed to mask the bitter taste of bile acids (Nakazawa et al., 1988).

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As natural cyclodextrins show low aqueous solubility and toxic effects when used in parenteral application, many efforts have been directed to the development of new cyclodextrin derivatives with better properties (Duchêne and Wouessidjewe, 1990). Among the derivatives 2-hydroxypropyl-β-cyclodextrin is highly water soluble, and its amorphous structure leads to highly water soluble amorphous inclusions.

Therefore, it appears worthwhile to characterise the inclusion of UDCA with 2-hydroxypropyl-β-cyclodextrin. Knowledge of the interaction between the bile acid and the cyclodextrin derivative may provide useful information on the development of pharmaceutical forms for oral administration.

2. Materials and methods

2.1. Materials

Ursodeoxycholic acid (UDCA) (3α,7β-dihydroxy-5β-cholan-24-oic acid; Mol. Wt 392.6; Sigma, St. Louis, USA) and 2-hydroxypropyl-β-cyclodextrin (HPβCD) (average molar substitution, 0.6; average Mol. Wt, 1380; Aldrich, Milwaukee, USA) were used to prepare the solid inclusion complex. All products were used as received from the manufacturers.

2.2. Analytical method

The concentration of UDCA in the samples was determined by HPLC (Scalia et al., 1989). The HPLC apparatus included a liquid chromatographic system equipped with a 112 Solvent Delivery Module and a 166 UV/Vis Programmable Detector Module (Beckman, Fullerton, USA). 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 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^{-1}) 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. Standard curve with concentrations ranging from 0.2 to 15 mg ml^{-1} was linear, with a correlation coefficient (mean ± SD) of 0.998 ± 0.001 ($n = 5$).

2.3. Solubility studies

Solubility studies were carried out according to Higuchi and Lach (1954). To determine the solubility of UDCA, excess drug (250 mg; 0.64 mM) was added to 5 ml of deionized water containing different concentrations of HPβCD (from 0 to 40 mM l^{-1}). The suspensions were shaken in 10 ml screw-capped vials at 30 strokes min^{-1} at 25 ± 1°C. When equilibrium had been reached (about after 48 h), the content of each vial was filtered through a cellulose nitrate membrane (pore size 0.45 μm; Sartorius, Göttingen, Germany). UDCA concentration was determined in the filtered solutions. All the data are the average of three determinations.

2.4. Preparation of the UDCA-HPβCD physical mixture

The physical mixture was prepared at a 1:1 molar ratio by simple dry mixing of equimolar amounts of UDCA and HPβCD, adopting the geometric dilution method.

2.5. Preparation of lyophilised inclusion complex of UDCA and HPβCD

The lyophilised inclusion complex was prepared at a 1:1 molar ratio of drug to HPβCD. Practically, 487 mg (1.24 mM) of UDCA were dissolved at room temperature in 5 ml of ethyl alcohol (Carlo Erba, Milan, Italy) to which 15 ml of deionized water containing 1.7 g (1.24 mM) of HPβCD were added. The solution was cooled to −18°C for 30 min (Shell Freezer; Edwards, Crawley, UK) and then freeze-dried at 2 mbar and −40°C for 24 h (Liovac GT2; Leybold-Heraus, Hanau, Germany).

2.6. ^{13}C-NMR studies

^{13}C data were obtained at 300 K using an AMX-400 WB spectrometer (Bruker, Rheinstetten, Germany) operating at 106.61 MHz, on 10 mM CD_3OD solutions which were left to equilibrate for several days. ^{13}C δ values refer to internal CD_3OD set to 49.3 ppm. Typical parameters for ^{13}C-NMR ^{1}H-decoupled spectra were: 0.7 Hz/pt resolution, 2048 scans, 5 s relaxation delay, 45° read pulse. Exponential multiplication was applied before Fourier transformation.

2.7. DSC

DSC curves were recorded on a Perkin-Elmer DSC-4 differential scanning calorimeter equipped with a computerised data station. Indium (99.99%; Perkin-Elmer, Norwalk, USA) (m.p. 156.6; ΔH_f 28.45 J g^{-1}) was used to check the instrument. All samples (8–10 mg) were heated in crimped aluminum pans (Perkin-Elmer) at a scanning rate of 10°C min^{-1} using dry nitrogen flow (30 ml min^{-1}).

2.8. X-ray diffractometry

X-ray diffraction patterns were recorded using a PW 1050 powder diffractometer (Philips, Eindhoven, The Netherlands). Experimental settings were: Ni-filtered Cu radiation ($\lambda = 1.5418$ Å); tube settings 40 kV, 20 mA; angular speed 1° (2θ) per min; 1–0.1–1 slits.

2.9. IR spectroscopy studies

IR spectra were recorded using an Infrared Fourier Spectrometer (model IFS 113 v; Bruker) employing the potassium bromide disk method at a pressure of 400 kg cm^{-2} (Carver; model M, Menomonee Falls, USA).

2.10. Drug dissolution studies

Drug dissolution studies were carried out using USP XXII dissolution apparatus 4 (Dissotest CE-1; Sotax, Basel, Switzerland) in 100 ml of phosphate buffer (Na_2HPO_4/KH_2PO_4) pH 7.0 at a flow rate of 25 ml min^{-1}. All experiments were carried out at a temperature of 37 ± 0.2°C using either 150 mg of UDCA crystals or an equivalent amount of drug in the physical mixture or solid inclusion complex. Drug content in the solution was determined as reported above. All experiments were run in triplicate.

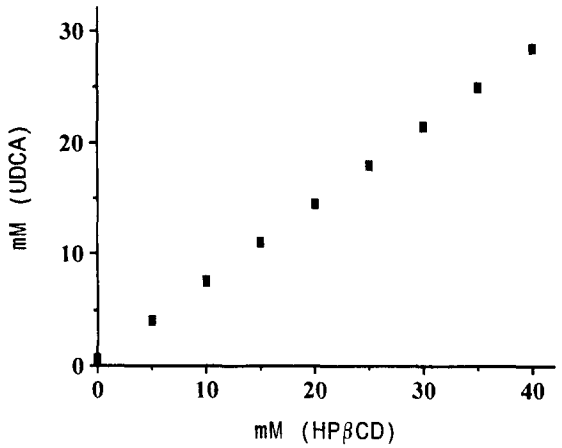

Fig. 1. Phase-solubility diagram of ursodeoxycholic acid with 2-hydroxypropyl-β-cyclodextrin in deionized water at 25 ± 1°C.

3. Results and discussion

3.1. Solubility studies

The phase solubility diagram of UDCA in aqueous HPβCD solutions at 25°C was plotted according to Higuchi and Kristiansen (1970) (Fig. 1). The solubility of UDCA increased linearly as the concentration increased. Therefore, the solubility curve could be classified as type A_L. The increase in solubility can be attributed to the formation of an inclusion complex between UDCA and HPβCD characterised by having a greater solubility than that of UDCA alone.

The (1:1) apparent stability constant of the inclusion complex (k') was calculated from the straight line of the diagram according to the following equation:

$$k' = S/[C_s(1 - S)] \quad (1)$$

where C_s (the intercept) is the solubility of UDCA in the absence of HPβCD and S denotes the slope of the straight line.

The value of k' was found to be 3750 M^{-1}.

3.2. Solid-state studies

The DSC curves revealed some information on solid-state interactions of UDCA with HPβCD (Fig. 2). UDCA crystals showed the characteristic endothermic peak at 205–206°C, corresponding to drug melting. The DSC profile of HPβCD exhibited a broad endothermic peak (about 60 and 140°C) due to water loss. The appearance of two endothermic peaks corresponding to drug melting and to dehydration of HPβCD was also evident in the thermogram of the physical mixture. The UDCA melting peak disappeared in the thermogram of the solid inclusion complex.

The X-ray pattern (Fig. 3) of the physical mixture was the superimposition of UDCA and amorphous HPβCD patterns. The X-ray diffraction pattern of the inclusion complex was free of interferences, owing to the amorphous state of the product obtained by the freeze-drying process.

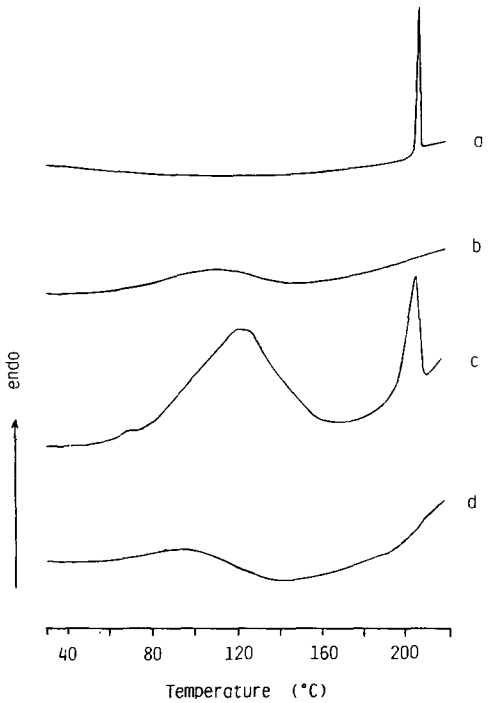

Fig. 2. DSC curves: (a) ursodeoxycholic acid; (b) 2-hydroxypropyl-β-cyclodextrin; (c) physical mixture; (d) lyophilised inclusion complex.

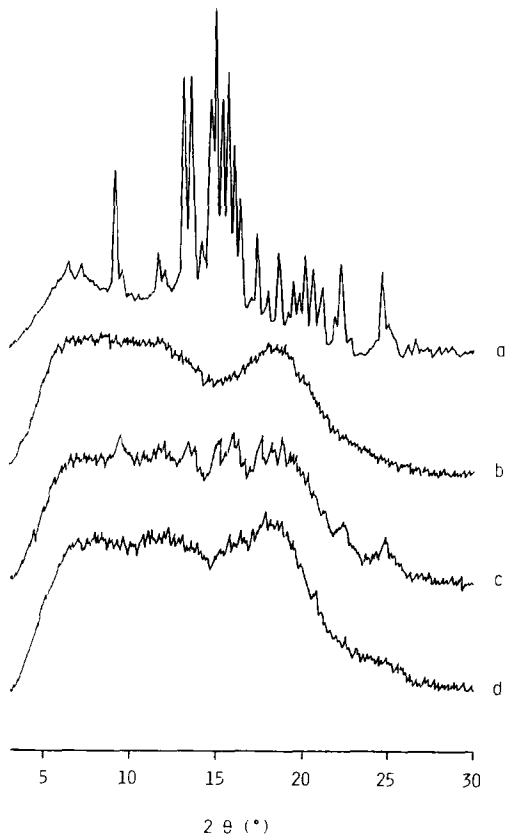

Fig. 3. X-ray diffraction patterns: (a) ursodeoxycholic acid; (b) 2-hydroxypropyl-β-cyclodextrin; (c) physical mixture; (d) lyophilised inclusion complex.

The absence of the drug melting peak in the DSC thermogram and of interferences in the X-ray diffraction pattern of the complex was attributed to the inclusion of drug within the cyclodextrin cavity (Rajagopalan et al., 1986). On the other hand, the freeze-drying process of a drug not included in cyclodextrin should lead to drug amorphisation. As the amorphisation of the drug produces the same thermal behaviour (absence of the drug melting peak) and the same flat X-ray diffraction pattern of the amorphous complex, the X-ray and DSC data cannot confirm the formation of an inclusion complex.

The IR spectroscopic technique (Fig. 4) was suitable for detecting the inclusion of UDCA within the HPβCD cavity. The HPβCD bands changed only slightly as a consequence of formation of the complex. In the spectrum of the solid

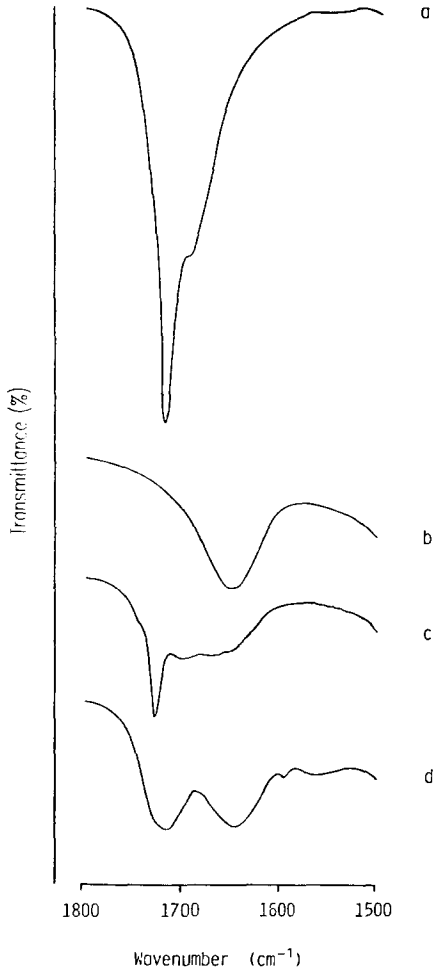

Fig. 4. Infrared absorption bands in the 1800–1500 cm^{-1} region: (a) ursodeoxycholic acid; (b) 2-hydroxypropyl-β-cyclodextrin; (c) physical mixture; (d) lyophilised inclusion complex.

inclusion complex, the $\nu(C=O)$ stretching mode of UDCA was shifted from 1717 to 1713 cm^{-1}. The shape of the C = O band was broader and less intense in the spectrum of the complex than that of the physical mixture, whereas both UDCA and the physical mixture had a similar C = O band profile.

3.3. ^{13}C-NMR studies

The classical ^{1}H-NMR approach to the study of this inclusion complex was obviated by the considerable complexity of the ^{1}H-NMR spectra of both UDCA and, especially, HPβCD.

^{13}C-NMR chemical shifts are sensitive probes of the molecular environment and can be used, alternatively, to derive information on complexation. It is well known that the transfer of the guest molecule from the free state to the cyclodextrin cavity, in solution, causes high-field (negative) shifts of the ^{13}C-NMR signals of included lead carbons and low-field (positive) shifts of the ^{13}C-NMR signals of the carbons externally close to the wider rim of the hollow cone of cyclodextrins (Inoue, 1993; Redenti et al., 1993).

The ^{13}C-NMR ^{1}H-decoupled spectrum of HPβCD presents the same complexity as its ^{1}H analogue, but that of UDCA is rather simple. Previous assignments (Waterhous et al., 1985) were checked through 2D-NMR inverse detection experiments [HMQC (Bax et al., 1983) and HMBC (Bax and Summers, 1986)] revealing two assignment inversions between C-3 and C-7 and between C-14 and C-17.

The ^{13}C-NMR data (Table 1) demonstrate a remarkable trend. The two carbons at the end of the C-17 side chain move to higher fields whereas the others move to progressively lower fields in the presence of HPβCD. C-18, C-21 and C-16 experience the highest downfield shifts. This behavior is qualitatively consistent with the intro-

Table 1
^{13}C chemical shifts (ppm) of selected carbons of ursodeoxycholic acid (UDCA) in the absence and presence of 2-hydroxypropyl-β-cyclodextrin [a] (HPβCD)

Carbon	δ UDCA	δ UDCA/HPβCD	Δδ [b]
24	178.44	178.37	−0.07
23	32.33	32.28	−0.05
22	32.68	32.77	+0.09
21	19.22	19.52	+0.30
20	36.98	37.01	+0.03
18	12.94	13.15	+0.21
17	56.88	56.80	−0.08
16	29.90	30.11	+0.21
15	28.23	20.30	+0.07
13	45.10	45.16	+0.06
12	41.88	41.99	+0.11
3	72.44	72.50	+0.06
2	31.35	31.43	+0.08
1	36.41	36.47	+0.06

[a] Δδ not reported are less than +0.04 ppm, generally about +0.02 ppm; C4 presents a negative shift of −0.02 ppm.
[b] Δδ = δ UDCA/HPβCD − δ UDCA.

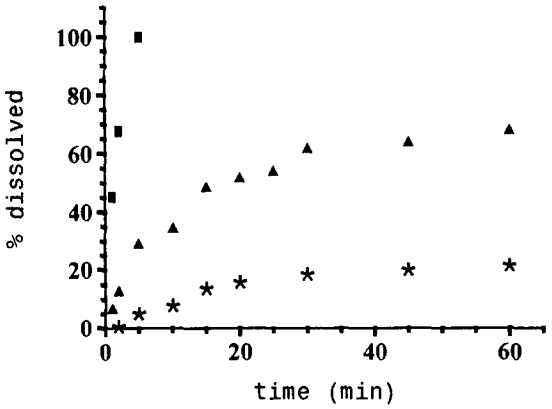

Fig. 5. Dissolution profiles in phosphate buffer (pH 7.0) at 37±0.2°C. (*) ursodeoxycholic acid; (▲) physical mixture; (■) lyophilised inclusion complex.

duction of the side chain into the HPβCD cavity. Two exceptions, represented by C-17 and C-20, are probably due to the superimposition of the high-field shift due to steric compression (Wehrli and Wirthlin, 1978) following the complexation and the downfield shift due to the complexation itself.

3.4. Dissolution studies

Fig. 5 shows the dissolution profiles of plain UDCA crystals, the physical mixture and the inclusion complex with HPβCD. Quantitative interpretation of the dissolution data according to Langenbucher (1972) shows that the value of the shape parameter '*b*' is always less than unity, indicating a steeper initial slope for the plot of dissolution processes. The dissolution rate of the complex was much greater than those of both the plain drug crystals and physical mixture, as shown by the T_d, i.e. the time interval required to dissolve 63.2% of the drug, values (> 24 h for the plain UDCA crystals; 35 min for the physical mixture; 5 min for the complex). The improvement in the dissolution characteristics can be justified through the concurrence of different factors. Inclusion of UDCA molecules into the HPβCD cavity improves wettability owing to the hydrophilicity of the exterior surface of the cyclodextrin derivative. Inclusion into the cavity of the highly soluble cyclodextrin derivative and subsequent drug amorphisation can also explain the enhanced dissolution rate of the inclusion complex.

4. Conclusion

The complexation of UDCA into the HPβCD cavity was suggested by ^{13}C-NMR and IR studies. The solid inclusion complex showed an increase in drug solubility and in drug dissolution rate. An enhanced dissolution rate offers the promise of increased UDCA bioavailability. Therefore, the above positive results motivate in vivo bioavailability studies to evaluate the feasibility of the complex in oral administration of UDCA.

Acknowledgements

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