Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **26:** 233–241, 1996.

The Interaction of Biliar Acids with 2-Hydroxypropyl-β-Cyclodextrin in Solution and in the Solid State

ADELE MUCCI and LUISA SCHENETTI
Department of Chemistry, University of Modena, via G. Campi 183, 41100 Modena, Italy

GIANFRANCO SALVIOLI and PAOLO VENTURA
Cattedra di Geriatria e Gerontologia, University of Modena, viale Vittorio Veneto 9, 41100 Modena, Italy.

MARIA ANGELA VANDELLI and FLAVIO FORNI*
Department of Pharmaceutical Sciences, University of Modena, via G. Campi 183, 41100 Modena, Italy.

(Received: 15 January 1996; in final form: 8 May 1996)

Abstract. The interaction of two biliar acids (chenodeoxycholic acid and cholic acid) with 2-hydroxypropyl-β-cyclodextrin (HPβCD) in solution and in the solid state was studied using different techniques. The formation of an inclusion complex with a 1 : 1 stoichiometry was suggested by the phase solubility studies. Both differential scanning calorimetry and X-ray diffractometry exhibited the amorphous state of the complex. The inclusion of both biliar acids into the HPβCD cavity was confirmed by the ^{13}C-NMR studies. Cholic acid showed a weaker affinity with respect to chenodeoxycholic acid probably owing to the presence of a hydroxyl group on C(12) (12α) close to the complexation site.

Key words: Biliar acid, 2-hydroxypropyl-β-cyclodextrin, interaction studies, IR, NMR, DSC, XRD.

1. Introduction

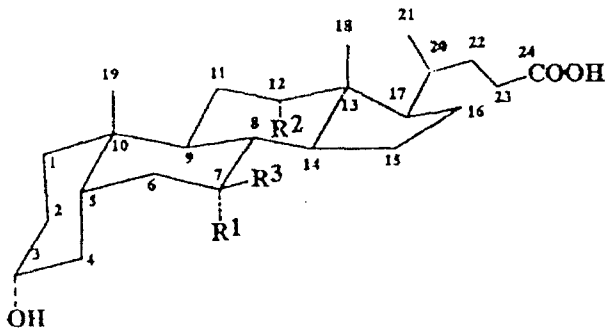
Bile acids are commonly used in the therapy of liver disease and in the dissolution of cholesterol gallstones [1, 2]. Parenteral administration of bile acids could be advantageous in patients having a severe cholestasis, but both their low water solubility and haemolytic characteristics exclude this route of administration.

The complexation of bile acids with 2-hydroxypropyl-β-cyclodextrin (HPβCD) could overcome these undesirable properties as suggested in recent studies [3, 4] thus giving a route to parenteral administration.

The complexation of ursodeoxycholic acid (UDCA) with HPβCD has been investigated in order to improve the bioavailability of this biliar acid [5]. The study of the complexation of UDCA with HPβCD indicated that a 1 : 1 molar complex was obtained. The characterisation of this complex by means of ^{13}C-NMR spectroscopy

* Author for correspondence

suggested the complex formation arose from the introduction of the side chain of UDCA into the HPβCD cavity. The ^{13}C-NMR approach appeared to be more useful in the characterisation of the inclusion complex than the more widely used ^{1}H-NMR [6] because of the relative simplicity of the $\{^{1}H\}$-decoupled ^{13}C- spectrum over the ^{1}H-NMR spectrum. To further investigate the complexation, the purpose of the present study was to investigate the inclusion complex formation of other biliar acids (chenodeoxycholic acid and cholic acid) with HPβCD in order to gain an insight into the mode of interaction in solution as well as in the solid state.



Ursodeoxycholic acid (UDCA)	$R^1 = H$;	$R^2 = H$;	$R^3 = OH$
Chenodeoxycholic acid (CDCA)	$R^1 = OH$;	$R^2 = H$;	$R^3 = H$
Cholic acid (CA)	$R^1 = OH$;	$R^2 = OH$;	$R^3 = H$

2. Experimental

2.1. Materials

Chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid; MW 392.6) (CDCA), cholic acid (3α, 7α, 12α-trihydroxy-5β-cholan-24-oic acid; MW 408.6) (CA) (Sigma, St. Louis, USA) and 2-hydroxypropyl-β-cyclodextrin (HPβCD) (average molar substitution: 0.6; average MW 1380; Aldrich, Milwaukee, USA) were used to prepare the solid inclusion complexes. All the solvents (analytical grade; Carlo Erba, Milan, Italy) and products were used as received from the manufacturers.

2.2. Solubility Studies

To determine the solubility of CA and CDCA, an excess amount of each biliar acid (BA) (250 mg) was added to 5 mL of deionized water containing different concentrations of HPβCD, ranging from 0 to 40 mM L^{-1}. The suspensions were shaken in 10 mL screw-capped vials at 30 strokes min^{-1} and equilibrated at 25 $\pm$ 1 °C for 48 h. The content of each vial was filtered through a cellulose nitrate membrane (pore size 0.45 μm; Sartorius, Göttingen, Germany). The concentration of BA was assayed in the filtered solution by an HPLC procedure [7] using a liquid

chromatograph (Beckmann; Fullerton, USA) equipped with a Lichrospher 100 RP 18 column (240 mm long, 4.6 mm i.d.; 5 μm particle size; Merck, Darmstadt, Germany). All the data are the average of three determinations.

2.3. PREPARATION OF THE PHYSICAL MIXTURES

The physical mixtures were prepared at a 1 : 1 molar ratio by simple dry mixing of equimolar amounts of either CA or CDCA with HPβCD, adopting the geometric dilution method.

2.4. PREPARATION OF LYOPHILISED INCLUSION COMPLEXES

The lyophilised inclusion complexes were prepared at a 1 : 1 molar ratio. 1.00 mM of each BA (393 mg of CA; 406 mg of CDCA) were dissolved at room temperature in 5 mL of ethyl alcohol to which 15 mL of deionized water containing 1.38 g (1.00 mM) of HPβCD were added. The solution was cooled to −18 °C for 30 min (Shell Freezer; Edwards, Crawley, United Kingdom) and then freeze-dried at 2 mbar and −40 °C for 24 h (Liovac GT2; Leybold-Heraus, Hanau, Germany).

2.5. ^{13}C-NUCLEAR MMGNETIC RESONANCE (^{13}C-NMR) STUDIES

^{13}C-NMR spectra were obtained at 300 K using a spectrometer (AMX–400 WB, Bruker) operating at 100.61 MHz, on 10^{-2} M CD_3OD solutions which, in the case of the complexes, were left to equilibrate for several days. δ values (ppm) refer to internal residual $^{13}CD_3OD$ set to 49.30 ppm. Typical parameters for ^{13}C-NMR $\{^1H\}$-decoupled spectra were: 0.7 Hz/pt resolution, 2048 scans, 5 s relaxation delay, 45° read pulse. Exponential multiplication was applied prior to Fourier transformation.

2.6. DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The thermograms were recorded on a DSC-4 differential scanning calorimeter equipped with a Thermal Analysis Data Station 3600 (Perkin-Elmer, Norwalk, USA). Indium (99.99%; Perkin–Elmer) (mp 156.6; ΔH_f28.45· J g^{-1}) was used to check the instrument. All samples (2–4 mg) were heated in sealed aluminium pans with an empty pan as reference (Perkin-Elmer) and scanned at 10 °C min^{-1} between 30 °C and 250 °C. Dry nitrogen flow (30 mL min^{-1}) was used throughout the scans.

2.7. X-RAY DIFFRACTOMETRY

The X-ray diffraction patterns were recorded using a PW 3710 powder diffractometer (Philips, Eindhoven, The Netherlands) using a voltage of 40 kV and a current of 20 mA for the generator, with Cu anode material. The wavelengths of the Ni

filtered radiation were: α_1 = 1.5406 Å and α_2 = 1.54439 Å. The diffractograms were recorded from 3° (2θ) to 40° (2θ) at an angular speed of 1° (2θ) per minute using 1–0.2–1 slits.

2.8. Infrared absorption (IR) spectroscopic studies

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

3. Results and Discussion

3.1. Phase diagram studies

The phase solubility diagram of CA and CDCA in aqueous HPβCD solutions at 25 °C are depicted in Figure 1. The solubility of both BAs increased linearly over the concentration range used in this study. According to the phase diagram classification proposed by Higuchi and Connors [8], the solubility curve could be classified as A_L-type phase behaviour. The linear relationship between the solubility and the HPβCD concentrations proved the formation of a soluble complex of first order between BA and HPβCD.

Hence, assuming that a (1 : 1) complex was formed, the apparent stability constant (k') of both inclusion complexes were calculated from the initial linear portion of the phase solubility diagram according to Higuchi and Kristiansen [9]:

$$k' = S/[C_S(1 - S)] \tag{1}$$

where C_S (the intercept) is the BA solubility in the absence of HPβCD and S is the slope of the straight line.

The k' values were found to be 2000 M^{-1} for CA and 19 000 M^{-1} for CDCA. The comparison between the k' values shows that in aqueous solution a more stable inclusion complex is formed between HPβCD and CDCA than CA.

3.2. Nuclear magnetic resonance (NMR) studies

The changes in the chemical shifts owing to the interaction between HPβCD and BA were investigated using ^{13}C-NMR spectroscopy. Although the study could be carried out in aqueous solutions either of a soluble salt or having basic pH, CD_3OD, as a common solvent for BAs, HPβCD and two complexes, was employed to compare the results with those previously obtained for UDCA [5]. The assignment of ^{13}C-NMR signals in CD_3OD has already been reported [10].

The analysis of the data was carried out on the basis of the model of Inoue [11]. According to this model, the inclusion of the guest molecule in solution from the free state into the cyclodextrin cavity causes upfield shifts of the ^{13}C-NMR signals

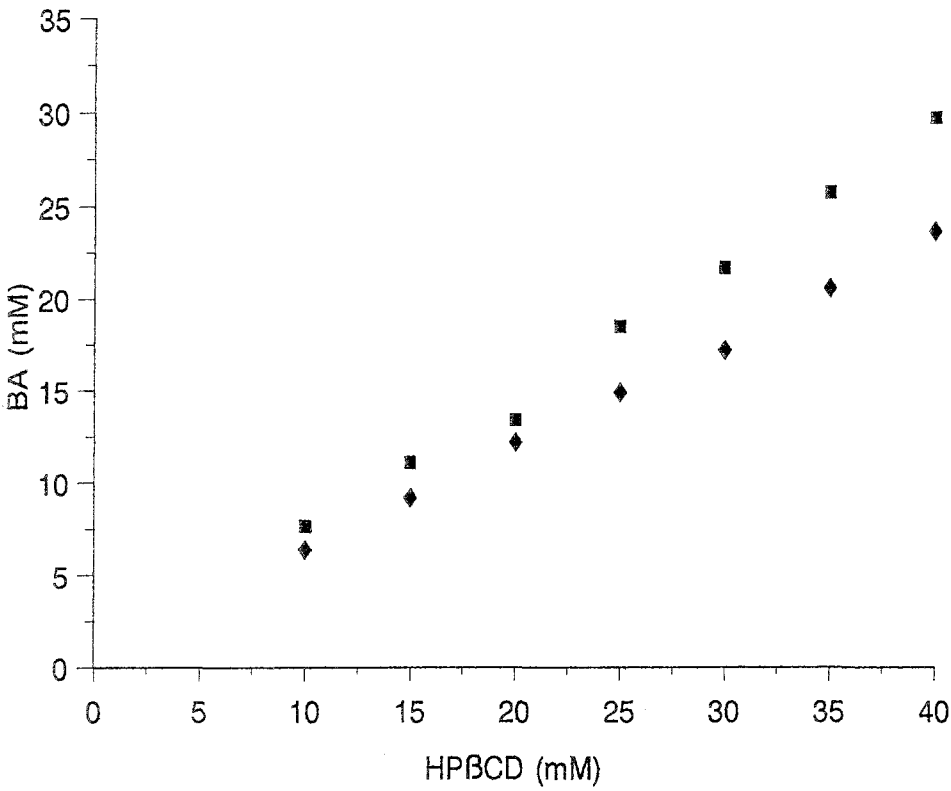


Figure 1. Phase-solubility diagrams of chenodeoxycholic acid (♦) and cholic acid (■) with 2-hydroxypropyl-β-cyclodextrin (HPβCD) in deionized water at 25 ± 1°C.

of included carbons and downfield displacements of the ^{13}C-NMR signals of the carbons externally close to the wider rim of the hollow cone of cyclodextrins [12].

On the basis of the model, the analysis of the data (Table I) is consistent with the insertion of the first carbons of the side chain of CDCA into the inner cavity of HPβCD. The two carbon atoms at the end of the side chain are shielded in the complex with respect to the free acid. With the exception of C(17), the carbons which follow are deshielded up to C(12). This exception, as well as the low deshielding on C(20), is probably due to a steric compression [13] in these two positions, following the complexation. The small $\Delta\delta$ values ($< \pm 0.05$ ppm) indicate a weak interaction of the other parts of the molecule with HPβCD. The upfield shift of the carbonyl carbon signal could also be due to changes in the hydrogen-bonding framework of the molecule, passing from the free to the complexed state [13]. In our opinion, however, the Inoue model seems to better explain the global trends of chemical shifts.

The behaviour of the CA complex is quite different form that of CDCA. All shifts caused by the complexation are smaller, even those on C(21), C(18) and

Table I. ^{13}C-NMR chemical shifts (ppm, relative to Me_4Si) of chenodeoxycholic acid (CDCA) and cholic acid (CA) free and complexed with 2-hydroxypropyl-β-cyclodextrin [1×10^{-2} M solutions in CD_3OD].

	CDCA free	CDCA complexed	$\Delta\delta$ ($\delta_{complexed} - \delta_{free}$)	CA free	CA complexed	$\Delta\delta$ ($\delta_{complexed} - \delta_{free}$)
C(1)	36.86	36.89	+0.03	36.80	36.78	−0.02
C(2)	31.66	31.69	+0.03	31.49	31.51	+0.02
C(3)	73.16	73.21	+0.05	73.19	73.18	−0.01
C(4)	40.79	40.80	+0.01	40.78	40.78	0.00
C(5)	43.49	43.50	+0.01	43.52	43.51	−0.01
C(6)	36.20	36.18	−0.02	36.15	36.12	−0.03
C(7)	69.36	69.38	+0.02	69.35	69.35	0.00
C(8)	41.08	41.13	+0.05	41.34	41.34	0.00
C(9)	34.36	34.33	−0.03	28.19	28.18	−0.01
C(10)	36.53	36.53	0.00	36.20	36.20	0.00
C(11)	22.09	22.07	−0.02	29.89	29.90	+0.01
C(12)	41.36	41.42	+0.06	74.32	74.33	+0.01
C(13)	43.98	44.06	+0.08	47.79	47.80	+0.01
C(14)	51.83	51.86	+0.03	43.30	43.31	+0.01
C(15)	24.92	24.97	+0.05	24.51	24.52	+0.01
C(16)	29.52	29.75	+0.23	28.94	28.98	+0.04
C(17)	57.65	57.51	−0.14	48.36	48.35	−0.01
C(18)	12.46	12.69	+0.23	13.26	13.34	+0.08
C(19)	23.68	23.70	+0.02	23.45	23.46	+0.01
C(20)	37.05	37.07	+0.02	37.06	37.03	−0.03
C(21)	19.09	19.50	+0.41	17.91	18.05	+0.14
C(22)	32.64	32.73	+0.09	32.63	32.68	+0.05
C(23)	32.27	32.19	−0.08	32.31	32.31	0.00
C(24)	178.44	178.26	−0.18	178.53	178.49	−0.04

C(16), suggesting a much weaker interaction in methanol-d_4 solution for CA with HPβCD.

The changes in chemical shifts of the two acids may be the reflection of a different mode of inclusion in the two complexes.

As expected from the solubility studies the results confirm that the CA molecule is more loosely bound into the HPβCD cavity than CDCA and UDCA.

3.3. SOLID STATE STUDIES

3.3.1. *Differential Scanning Calorimetry (DSC) and X-ray Diffractometry*

The information obtained by the DSC analysis and X-ray diffractometry cannot confirm the inclusion of the BA into the HPβCD cavity.

The melting peak of both BAs (CDCA: mp 166–167 °C; CA: mp 202–203 °C) disappeared in the thermogram of the solid inclusion complex. The X-ray diffraction patterns of both the inclusion complexes showed no diffraction peaks.

The absence of the drug melting peaks in the DSC thermograms and of peaks in the X-ray diffraction patterns of the complexes cannot be considered as clear evidence of the inclusion of BA molecules within the cyclodextrin cavity. In fact, the freeze-drying process of a substance produces amorphous products, which obviously shows no drug melting peak in the thermograms and flat X-ray patterns in the diffractograms. Therefore, the X-ray and DSC data cannot confirm the formation of inclusion complexes, although the inclusion of a molecule into the HPβCD cavity obviously leads to an amorphous complex.

3.3.2. *Infrared Spectroscopy*

In the spectra of both the complexes (Figure 2), no visible change of HPβCD bands was observed as a consequence of the complex formation.

In the inclusion complex, the ν (C=O) stretching mode of CDCA is shifted from 1710 to 1714 cm^{-1}. No shift is practically observed in the case of the CA complex. The shifts of the carbonyl stretching band are extremely small emphasising no remarkable changes in the force constants. The IR data, therefore, do not provide conclusive proof of the existence of a complex between BA and HPβCD.

However, the shape of the C=O bands of the BAs are broader and less intense in the spectra of both the complexes, whereas both BAs and the physical mixtures have similar C=O band profiles. The broadening of the carbonyl stretching band in the inclusion complex samples was attributed to the restriction of bending and stretching vibration within the cyclodextrin cavity [14] owing to the monomolecular dispersion of the guest.

4. Conclusions

The formation of a (1 : 1) inclusion complex of either CA or CDCA with HPβCD is suggested by phase solubility studies.

As previously reported for UDCA complexation with HPβCD (5), the ^{13}C-NMR approach, based on the Inoue model, promises to be very useful in the characterization of BA inclusion compounds. According to this model, the complex formation between either CA or CDCA and HPβCD takes place from the side chain of the BA, as hypothesised for UDCA. The weaker affinity of CA with respect to CDCA parallels that for unsubstituted β-cyclodextrin [15]. The structural difference of CA with respect to CDCA probably produces this different behaviour. In our opinion, the presence of a hydroxyl group on C(12) (12α), close to the complexation site is not favourable to the inclusion process.

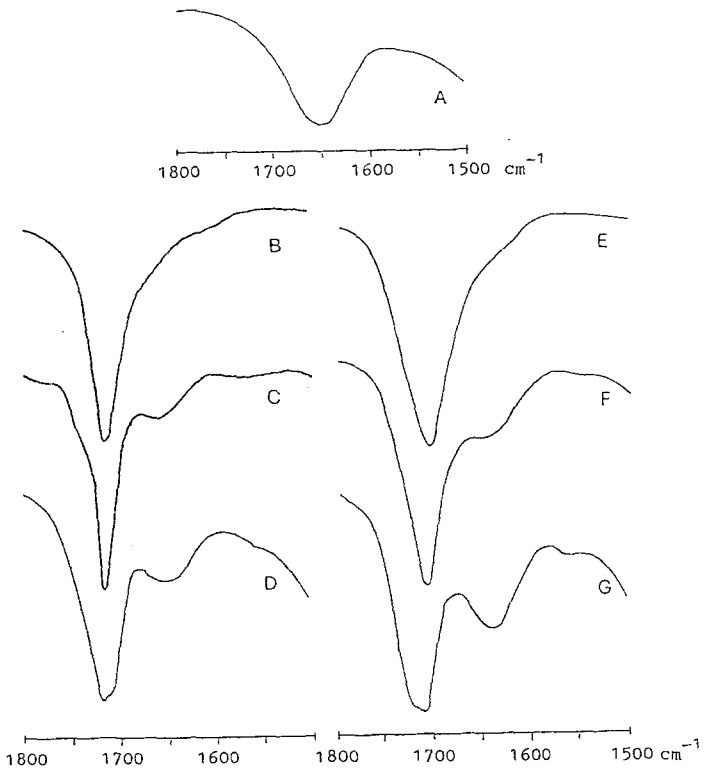


Figure 2. IR absorption bands in the 1800–1500 cm^{-1} region. (A) 2-hydroxypropyl-β-cyclodextrin (HPβCD); (B) chenodeoxycholic acid; (C) physical mixture of chenodeoxycholic acid and HPβCD; (D) lyophilised inclusion complex of chenodeoxycholic acid and HPβCD; (E) cholic acid; (F) physical mixture of cholic acid and HPβCD; (G) lyophilised inclusion complex of cholic acid and HPβCD.

Acknowledgement

This work was supported in part by a grant 'Ricerca Avanzata ex 60%' from the 'Università degli Studi di Modena'.

References

1. R. Poupon, R.E. Poupon, Y. Calmua, R. Ballet, and F. Darnis: *Lancet* **i**, 834 (1987).
2. W.H. Bachrach and A.F. Hoffmann: *Dig. Dis. Sci.* **27**, 737 (1982).
3. R. Panini, M.A. Vandelli, F. Forni, J.M. Pradelli, and G. Salvioli: *Pharmacol. Res.* **31**, 205 (1995).
4. R. Panini, M.A. Vandelli, E. Leo, G. Salvioli, and R. Cameroni: *J. Pharm. Pharmacol.* **48**, 641 (1996).
5. M.A. Vandelli, G. Salvioli, A. Mucci, R. Panini, L. Malmusi, and F. Forni: *Int. J. Pharm.* **118**, 77 (1995).
6. X. Tan and S. Lindenbaum: *Int. J. Pharm.* **74**, 127 (1991).
7. S. Scalia, P. Pazzi, and M. Guarneri: *Anal. Lett.* **22**, 915 (1989).

8. T. Higuchi and K.A. Connors: *Adv. Anal. Chem. Instrum.* **4**, 117 (1965).
9. T. Higuchi and H. Kristiansen: *J. Pharm. Sci.* **59**, 1601 (1970).
10. V.D. Waterhous, S. Barnes, and D. Muccio: *J. Lipid Res.* **26**, 1068 (1985).
11. Y. Inoue: in G.A. Webb (ed.), *Annu. Rep. NMR Spectrosc.,* Vol. 27, Academic Press (1993), pp. 59–101.
12. E. Redenti, M. Pasini, P. Ventura, A. Spisni, M. Vikmon, and J. Szejtli: *J. Incl. Phenom.* **15**, 281 (1993).
13. E. Breitmeier and W. Voelter: *Carbon–13 NMR Spectroscopy*, 3rd ed., VCH (1987), p. 115.
14. K. Uekama, T. Fujinaga, F. Hirayama, M. Otagiri, and M. Yamasaki: *Int. J. Pharm.* **10**, 1 (1982).
15. S. Comini, P. Olivier, M. Riottot, and D. Duhamel: *Clinica Chim. Acta* **228**, 181 (1994).