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Complexation of 6-Deoxy-6-(aminoethyl)amino-β-cyclodextrin with Sodium Cholate and Sodium Deoxycholate

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Abstract. In order to study its guest binding and the inclusion phenomena, 6-deoxy-6-(aminoethyl)amino-β-cyclodextrin (βCDN) was synthesised and its binding properties examined. The complexation phenomena of sodium cholate (NaC) and sodium deoxycholate (NaDC) with βCDN has been monitored by the NMR method using ^{13}C chemical shift data. The method of continuous variation "Job's method" has been used to determine the stoichiometry of these supramolecular complexes. The Job's plot confirms the 1 : 1 supramolecular complex for NaC: βCDN and the 1 : 2 supramolecular complex for NaDC: βCDN. The interaction of NaC and NaDC with βCDN has been obtained through two-dimensional Rotational Nuclear Overhauser Effect Spectroscopy (ROESY) NMR. Equilibrium constants were also obtained from ^{13}C chemical shift data (C-1, C-3 & C-4) at different pH values (7, 9, & 11).

Key words: β-cyclodextrin, 6-deoxy-6-(aminoethyl)amino-β-CD, sodium cholate, sodium deoxycholate, nuclear magnetic resonance, ROESY, Job's method.

1. Introduction

β-Cyclodextrin is a torus shaped oligosaccharide (Figure 1a) made of 7 glucopyranose units [1]. Bile salts are amphipathic compounds with distinctive detergent properties and they solubilize [2] the products of fat digestion in the form of mixed micelles. It has been recently reported [3a,b] that bile salts form 1 : 1 or 1 : 2 stoichiometric supramolecular complexes with cyclodextrin and some derivatives, depending on the number of hydroxy substituents in the steroidal group of the bile salt.

In the present paper the authors have obtained the geometry of the inclusion complexes of bile salts (NaC & NaDC) with 6-deoxy-6-(aminoethyl)amino-β-cyclodextrin (βCDN) on the basis of the shifts of ^{13}C NMR signals. Among the different experimental techniques available to study the formation of complexes [4a–g], NMR [4h] spectroscopy was preferred because previous studies [3a,b] have

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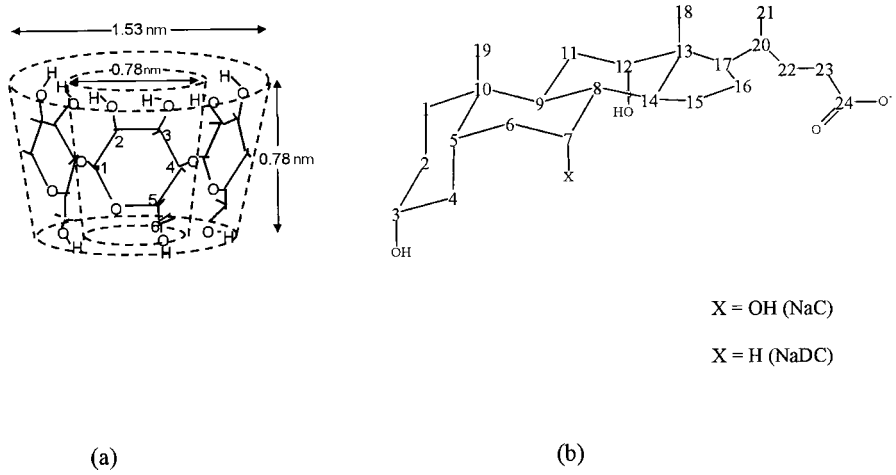


Figure 1. (a) Structure of β-CD. (b) Structure of bile salt.

clearly shown that the formation of the complex induces a change in the chemical shifts of both the βCD and the bile salt. A two-dimensional Rotational Nuclear Overhauser Effect Spectroscopy (ROESY) NMR study on the interaction between NaC and NaDC with βCDN has also been reported by the authors. It shows strong interactions between the host and guest molecules and also gives information about the structure of the complex. This data suggests that the hydrophobic steroidal ring enters into the inner cavity of the cyclodextrin derivative. The geometrical structures of the two bile salts are very similar, with the only difference being the substituent R on C-7, which is OH for NaC and H in NaDC (Figure 1b). However, their physico-chemical properties are very different. In particular, at pH values close to neutrality, NaDC forms gels, a characteristic uncommon to NaC and other bile salts [5a–c]. It should also be noted that ^{13}C-NMR chemical shifts are more sensitive to complexation than ^{1}H resonances, and that the C-1, C-3 and C-4 sites are more influenced by NaC and NaDC complexation than $\Delta\delta$ C-5 and C-6.

2. Experimental Section

2.1. MATERIALS

β-Cyclodextrin was donated by Roquette and it was purified by recrystallization from hot water and dried in a vacuum oven. NaC and NaDC (Sigma-Aldrich) were purified by dissolving in ethanol and methanol and were precipitated from ethyl acetate and acetone, respectively, and dried in a vacuum oven. Ethylenediamine obtained from Panreac was dried by refluxing in the presence of fresh KOH in an argon atmosphere just before use. Other reagents were of high quality and used without further purification.

2.2. APPARATUS

The NMR spectra were recorded using a Bruker spectrometer Model AMC 300. ^{1}H and ^{13}C NMR spectra were obtained at 300.13 MHz and 75.47 MHz, respectively. The ROESY experiments were recorded on a Bruker spectrometer Model AMX 500 at a frequency of 500.14 MHz. The deuterium oxide and DMSO-d_6 used for these studies were obtained from Sigma. For the Job's method, the inclusion complex was directly prepared in a 5 mm NMR tube with the respective buffers in D_2O. The solution of the complex was left to equilibrate overnight. NMR spectra were then obtained at 298.1 K using a thermostated Eurotherm B-VT 2000 with 0.01M D_2O-buffer solutions of bile salts and βCDN. All NMR resonances are reported in ppm.

2.3. PREPARATION OF 6-DEOXY-6-TOSYL-β-CYCLODEXTRIN (TS-β-CD)

The Ts-β-CD was prepared by a modification of the procedure of Matsui and Okimoto [6]. A solution of *p*-toluenesulfonyl chloride (3.65 g, 19.1 mmol) in 30 ml dry pyridine was added to a solution of β-CD (29.60 g, 26.1 mmol) in 300 ml dry pyridine dropwise over 15 minutes. After stirring overnight at ambient temperature in an inert atmosphere of argon, the mixture was evaporated in vacuum at 50–60 °C to dryness. The white solid was washed with 150 ml of ethyl ether, recrystallised three times from 300 ml of water (80 °C) to remove pyridine, and then dried in vacuum. ^{1}H NMR (DMSO-d_6): (a) δ 7.76 and 7.44 (d, 2H, Ar-H), (b) 4.84 and 4.77 (br, s, 4H, br, s, 3H, $CH(O)_2$), and (c) 2.34 (s, 3H, CH_3Ar).

2.4. PREPARATION OF 6-DEOXY-6-(AMINOETHYL)AMINO-β-CYCLODEXTRIN

This compound was synthesised by a modified method of Matsui et al. [7] and Nozaki et al. [8]. Ts-β-CD (1.25 g, 0.96 mmol) was dissolved in dry ethylenediamine (18 g, 299 mmol) with stirring in an argon atmosphere. The resulting solution was stirred at 40 °C for 48 hours. The progress of the reaction was monitored by TLC (silica gel 60; butanol/ethanol/water = 5 : 4 : 3 by volume). The solution was concentrated in vacuum with low heat (50 °C) to give a clear, light yellow, viscous oil which was dissolved in MeOH-water (3 : 1). The solution was added dropwise to stirred cold acetone (60 ml), producing a precipitate that was filtered to give a colourless powder. The powder was reprecipitated from acetone (3–4x) until no signs of free ethylenediamine remained as evidenced by ^{1}H-NMR in D_2O.

The colourless powder was dissolved in water and the pH of the solution was changed to 7 with a few drops of a dilute solution of hydrochloric acid. The solution was then charged into an ion-exchange CM Sephadex C-25 column and eluted with a step gradient of water and 0.2 M ammonium bicarbonate solution. The water fraction contained native β-CD and Ts-β-CD, while the ammonium bicarbonate solution contained the desired product βCDN. The yield was 55–60% with respect to the tosyl derivative.

The ^{1}H NMR of the pure βCDN indicates the following signals: (D_2O) 4.95 (s, H-1, 7H), 3.77–3.88 (m, H-6, H-3, H-5, 28H), 3.44–3.55 (m, H-2, H-4, 14H), 2.96 (s, —CH_2NH-β-CD, 2H) and, 2.78 (s, —CH_2NH_2, 2H). The ^{13}C NMR shows signals at 104.4 (C-1), 84 (C-4), 74.02, 73.4, 73.07 (C-5, C-3, C-2), 63 (C-6), 51.44 (C-6′), 48.85 (CNH—) and 41.09 (CNH_2). The DEPT 135 shows negative signals of the —CH_2 at 63 (C-6), 51.44 (C-6′), 48.85 (CNH—) and 41.09 (CNH_2).

2.5. PREPARATION OF INCLUSION COMPLEXES

Dissolution of sodium cholate and sodium deoxycholate in D_2O at 0.01 M gave solutions of pH 8.0 and 7.6, respectively. This pH will be referred to as the "natural" pH of the bile salts. In addition to natural pH, the complexation of 0.01 M bile salts and 0.01 M βCDN was measured at pH 7 (dihydrogen phosphate buffer), pH 9 (borate buffer) and pH 11 (hydrogen phosphate buffer). The appropriate buffers were prepared in D_2O; βCDN (0.01 M) and the bile salts (0.01 M) were then dissolved in these buffers. For the study of the complexation reaction as per the Job's method [9], 10 solutions with different molar ratios of βCDN and the bile salts were prepared, where the total βCDN + bile salt molar concentrations and total solution volumes were kept constant.

3. Results and Discussion

3.1. EVALUATION OF THE STABILITY CONSTANTS

The inclusion of a guest molecule inside the cavity of a cyclodextrin molecule produces a change in the electronic environment of the atoms of the cyclodextrin. Therefore, we can observe changes in the chemical shifts of the ^{13}C NMR signals between the free and complexed forms of βCDN. On the basis of ^{13}C NMR, stoichiometric considerations and ROESY studies, structural models have been proposed suggesting that NaC possesses one binding site (i.e., a 1 : 1 complex) while NaDC possesses two binding sites (i.e., a 1 : 2 complex).

3.1.1. *1 : 1 Stoichiometry Complex*

Consider the formation of the 1 : 1 complex C_{11} between the host CD (βCDN) and guest BS (NaC), where the equilibrium BS + CD $\rightleftharpoons$ C_{11} can be described by a stability constant K_{11},

$$K_{11} = \frac{[C_{11}]}{[BS] \cdot [CD]} \tag{1}$$

Under the fast exchange condition, the observed chemical shift δ_{obs} is a weighted average of the chemical shifts in the free δ_{CD} and complexed δ_{11} states,

$$\delta_{obs} = f_{CD}\delta_{CD} + f_{11}\delta_{11} \tag{2}$$

where f_{CD} and f_{11} are the mole fractions of free and complexed cyclodextrin.

Making use of $f_{CD} + f_{11} = 1$, and

$$\Delta\delta_{obs} = f_{11}\Delta\delta_C \tag{3}$$

(where $\Delta\delta_{obs} = \delta_{obs} - \delta_{CD}$ and $\Delta\delta_C = \delta_{11} - \delta_{CD}$), the chemical shift displacement of a specific nucleus of βCDN in the presence of NaC is expressed as:

$$\Delta\delta_{obs} = \frac{\Delta\delta_C}{[CD]_0} \cdot [C_{11}] \tag{4}$$

Finally, from the mass balances,

$$[BS]_0 = [BS] + [C_{11}] \text{ and } [CD]_0 = [CD] + [C_{11}] \tag{5}$$

and from Equation (1), the concentration of the complex in solution is deduced:

$$[C_{11}]^2 - ([BS]_0 + [CD_0 + 1/K_{11}]\,[C_{11}] + ([BS]_0[CD]_0) = 0. \tag{6}$$

By combining Equations (4) and (6) we get an expression for the displacement in the chemical shift of an atom of the βCDN as a function of the concentrations of bile salt and βCDN added. This expression is fitted to experimental values using a non linear least-squares computer program to obtain the stability constant (K_{11}) and the chemical shift of the complex (δ_{11} or $\Delta\delta_C$) as adjusted parameters.

A plot of $\Delta\delta_{obs}$ against [NaC] gives a sigmoid curve, but the plot of $\Delta\delta.X_{CD}$ versus X_{CD} (Figure 2a) of the hydroxy carbons (C-1, C-3 and C-4), shows a maximum at $X_{CD} = 0.5$ for all values of pH. This trend indicates the formation of 1 : 1 inclusion complex of NaC with βCDN.

3.1.2. *1 : 2 Stoichiometry Complex*

For the formation of a 1 : 2 stoichiometry complex two different hypotheses can be suggested, stepped complexation equilibria (i.e., sequential bimolecular processes) and a global complexation (intermolecular) process. The global complexation process, BS + 2 CD $\rightleftharpoons$ C_{12}, is a single step reaction having one stability constant K_{12}:

$$K_{12} = \frac{[C_{12}]}{[BS] \cdot [CD]^2} \tag{7}$$

This complexation process is more suitable in our system because if we considered two equilibrium constants as considered in stepped complexation, then the concentration of the 1 : 1 complex and the concentration of the 1 : 2 complex must be comparable. But in the present system, the maximum of the Job's Plot is a value between 0.5 and 0.67 depending on the proportion of complexes present in the given solution. Since in our studies a maximum in the Job's Plot occurs

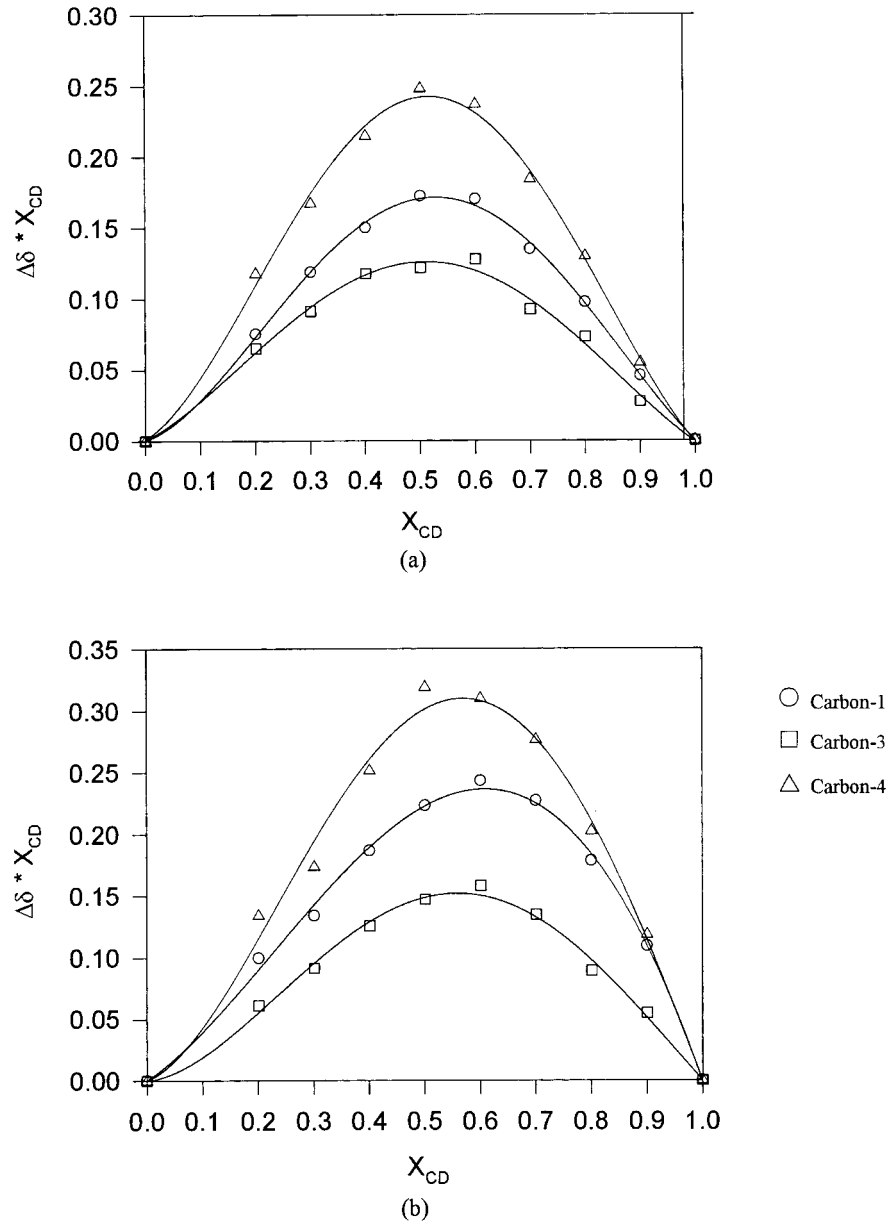


Figure 2. Job's plot of (a) NaC (b) NaDC with βCDNH at natural pH indicating formation of 1 : 1 and 1 : 2 complexes.

at 0.67 (Figure 2b) for C-1, C-3 and C-4, we conclude that the complexes are predominantly 1 : 2 with negligible contributions from the 1 : 1 complex. The form of this condition that only one complex predominates for the application of the continuous variations method has also been described by Werner [10].

Under the fast exchange condition, and considering the process expressed by the single step reaction, the chemical shift displacement observed for a cyclodextrin (βCDN) atom is now expressed as:

$$\Delta\delta_{\text{obs}} = \frac{z\Delta\delta_{\text{C}}}{[\text{CD}]_0} \cdot [\text{C}_{12}] \tag{8}$$

and finally the concentration of a 1 : 2 complex in solution is deduced by:

$$-4[\text{C}_{12}]^3 + 4(\text{CD}_0 + \text{BS}_0)\,[\text{C}_{12}]^2 - (4\text{BS}_0\text{CD}_0 + \text{CD}_0^2 + 1/\text{K}_{12})[\text{C}_{12}] + \text{BS}_0\text{CD}_0^2 = 0. \tag{9}$$

In this equation, the cubic term may be negligible if we work with appropriate initial concentrations of bile salt (NaDC) and cyclodextrin (βCDN).

Combination of Equations (8) and (9) gives us an expression for the displacement in the chemical shift of an atom of the βCDN as a function of the concentrations of NaDC and βCDN added. This expression is fitted to experimental values using a non-linear least-squares computer program to obtain the stability constant (K_{12}) and the chemical shift of the complex (δ_{12} or $\Delta\delta_{\text{C}}$) as adjusted parameters. The plot of δ_{obs} versus δ_{cal} gives a straight line having a slope of 1 for NaC and NaDC for all values of observed pH, indicating an excellent least squares analysis.

Within the same range of NaDC concentrations, the Job's plot at natural pH (Figure 2b) for C-1, C-2 and C-4 shows maxima at $X_{\text{CD}} = 0.67$ and has the same maxima for all the other values of pH, thus indicating the formation of a 1 : 2 inclusion complex of NaDC with βCDN. Based on an observed maximum at the molar ratio of 0.67, we infer that the 1:2 complex predominates (i.e., large Kz) in the continuous variations method. The fact that K_{11} for NaC is smaller (Table I) than K_{12} for NaDC (Table II) suggests a role of stronger hydrophobic interactions in the complexation of NaDC with β-CDN due to the complexation of two βCDN molecules for each molecule of NaDC.

A comparative Job's plot was obtained for C-1 at different pH values for NaC (Figure 3a) and NaDC (Figure 3b). In the case of NaC, the maximum in the Job's plot for all the pH values was at $X_{CD} = 0.5$ and for NaDC at $X_{CD} = 0.67$ indicating that pH has no effect on the ^{13}C chemical shift values. This suggests that the variation of the pH in the reaction system does not affect the complexation process (3a). The pH change in this range also does not affect the bile salt stability except for NaDC at pH 7. At this pH, NaDC can form gels and therefore at higher concentrations of [NaDC]/[βCDN] ($X_{\text{CD}} < 0.4$) the NMR spectrum presents some

Table I. Stability constant and chemical shift for the 1 : 1 NaC-βCDN interaction at four different pH values

NaC complexes		7.0	Natural	9.0	11.0
Carbon No. 1	K_{11}	$(1.7 \pm 0.4) \times 10^4$	$(1.4 \pm 0.9) \times 10^4$	$(1.3 \pm 0.4) \times 10^4$	$(0.8 \pm 0.2) \times 10^4$
	$\Delta\delta_C$	0.60 ± 0.01	0.39 ± 0.01	0.41 ± 0.00	0.38 ± 0.00
Carbon No. 3	K_{11}	$(1.8 \pm 0.6) \times 10^4$	–	–	–
	$\Delta\delta_C$	0.33 ± 0.03	0.36 ± 0.02	0.40 ± 0.01	0.34 ± 0.01
Carbon No. 4	K_{11}	$(2.6 \pm 1.2) \times 10^4$	$(1.1 \pm 0.3) \times 10^4$	$(1.2 \pm 0.7) \times 10^4$	$(0.8 \pm 0.2) \times 10^4$
	$\Delta\delta_C$	0.53 ± 0.01	0.56 ± 0.01	0.55 ± 0.01	0.61 ± 0.01

Units of K_{11} is in M^{-1} and $\Delta\delta_C$ is ppm.

Table II. Stability constant and chemical shift for the 1 : 2 NaDC-βCDN interaction at four different pH values

NaDC complexes		7.0	Natural	9.0	11.0
Carbon No. 1	K_{12}	$(5.0 \pm 0.3) \times 10^4$	$(4.5 \pm 0.6) \times 10^4$	$(4.6 \pm 1.0) \times 10^4$	$(4.7 \pm 1.1) \times 10^4$
	$\Delta\delta_C$	0.58 ± 0.08	0.71 ± 0.09	0.73 ± 0.13	0.69 ± 0.14
Carbon No. 3	K_{12}	$(4.9 \pm 0.9) \times 10^4$	$(4.7 \pm 0.5) \times 10^4$	$(4.4 \pm 1.8) \times 10^4$	$(4.9 \pm 0.4) \times 10^4$
	$\Delta\delta_C$	0.43 ± 0.12	0.41 ± 0.08	0.47 ± 0.13	0.38 ± 0.06
Carbon No. 4	K_{12}	$(4.9 \pm 0.5) \times 10^4$	$(4.7 \pm 0.5) \times 10^4$	$(4.8 \pm 1.0) \times 10^4$	$(4.9 \pm 0.3) \times 10^4$
	$\Delta\delta_C$	0.79 ± 0.13	0.84 ± 0.12	0.90 ± 0.22	0.88 ± 0.13

Units of K_{12} is in M^{-2} and $\Delta\delta_C$ is ppm.

difficulties. Even though, we observe a 1 : 2 complexation reaction. At pH above 7, NaDC forms well-behaved solutions, such that guest binding and the inclusion phenomena of the host remains relatively unaffected by the pH of the complexation reaction.

The observed chemical shifts of βCDN and bile salt carbons on complexation suggest strong interactions between the host and guest molecules. The changes in the equilibrium constant values for pure β-CD, β-CD-NH_2 and βCDN inclusion complexes with NaC & NaDC at natural pH are reported in Table III. Obviously the charged side chains in βCD have a significant effect on the binding process with the bile salts. Our previous publications (3b) on the complexation of βCD, βCDNH_2 and the present βCDN with bile salts show a significant change in the values of K_{11} and K_{12} due to the C-6 side chain of the βCD molecule.

The substituent R at C-7 in NaC is a hydroxyl group, hence one side of the molecule has a hydroxyl group and the other side a carboxyl group rendering these sides of the molecule hydrophilic; the central steroidal ring is hydrophobic in nature. The ROESY experiment provides us with detailed information (Table IV) on the proximity of βCDN and the guest protons (where *s* = strong, *m* = moderate

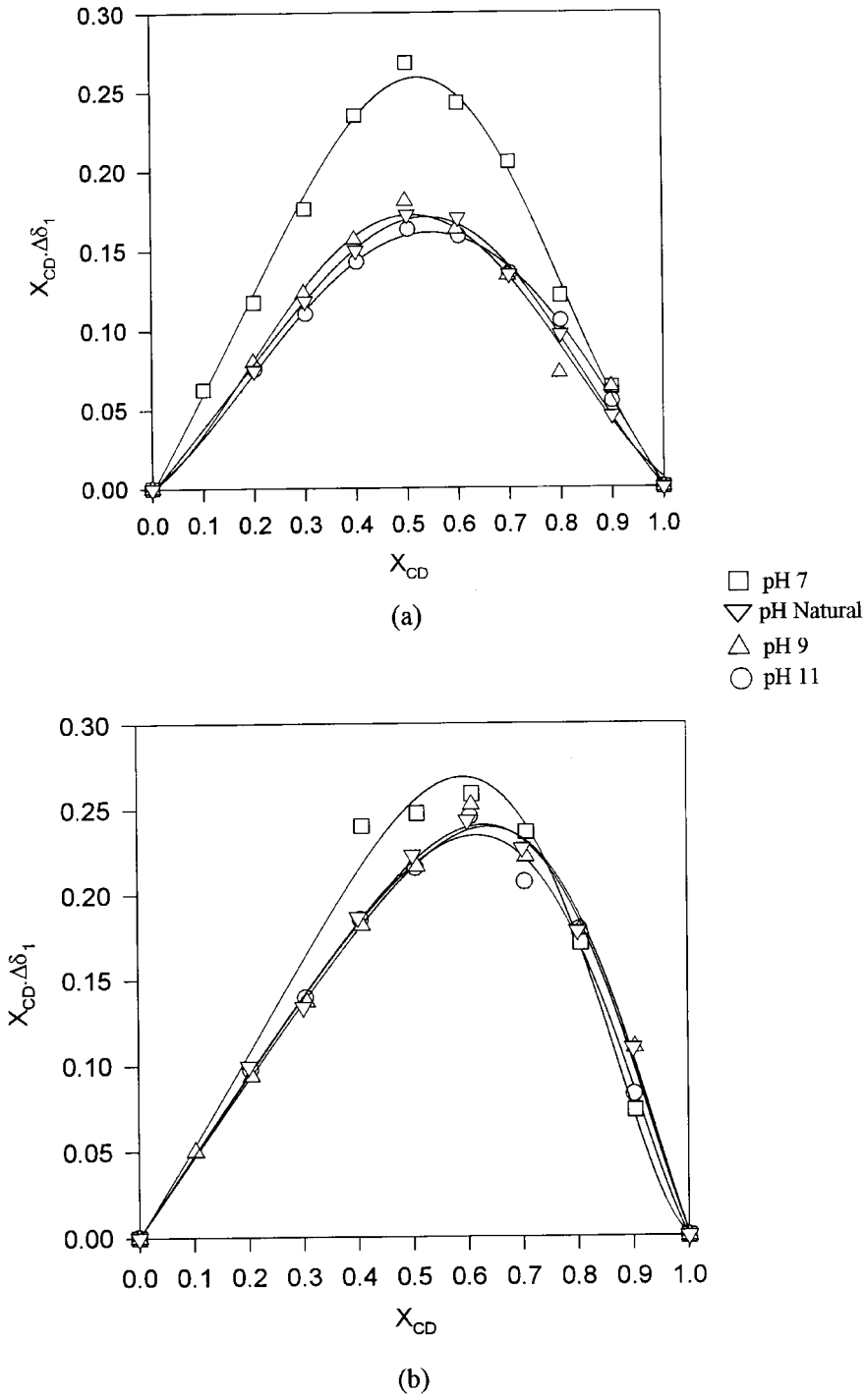


Figure 3. Job's plot of C-1 (a) the 1 : 1 inclusion complex of NaC (b) the 1 : 2 inclusion complex of NaDC with βCDN at different pH values.

Table III. Stability constant and chemical shift of pure cyclodextrin and cyclodextrin derivatives at natural pH for Carbon-1

Carbon No. 1		NaC complexes	NaDC complexes
β-CD	K	$(8.3 \pm 5) \times 10^3$	$(3.9 \pm 0.5) \times 10^4$
	$\Delta\delta_C$	0.41 ± 0.01	0.62 ± 0.01
β-CD—NH_2	K	$(11.5 \pm 1.2) \times 10^3$	$(4.9 \pm 1.2) \times 10^4$
	$\Delta\delta_C$	0.39 ± 0.02	0.53 ± 0.02
β-CDN	K	$(13.6 \pm 9) \times 10^3$	$(4.5 \pm 0.6) \times 10^4$
	$\Delta\delta_C$	0.39 ± 0.01	0.71 ± 0.09

and w = weak, interactions): (a) 23-CH_2 (s), 22-CH_2 (s), 21-CH_3 (s), 20-CH (s), 18-CH_3 (s), 17-CH (s), 16-CH_2 (m), 15-CH_2 (m), 14-CH (m), 12-CH (m), 11-CH_2 (w), 7-CH (w) showed interactions with 3-H, (b) 21-CH_3 (s), 20-CH (w), 18-CH_3 (m), 17-CH (m), 16-CH_2 (m), 15-CH_2 (w), 14-CH (w) showed interactions with 5-H, and (c) 23-CH_2 (m), 22-CH_2 (m), 21-CH_3 (m), 20-CH (w) showed interactions with 6-H. NaC therefore enters the βCDN cavity from the ring of the secondary hydroxyl groups of the β-CD (as deduced from the interactions with 3-H of βCDN with the protons of bile salt) and the hydrophobic steroidal part stays within the hydrophobic βCDN cavity (Figure 4a). While the carboxyl group on the side chain penetrates the cavity and is extended out of the cavity towards the C-6 primary hydroxyl side (as deduced from the interactions of the bile salt protons with 5-H and 6-H of the βCDN) with the —$NHCH_2CH_2NH_2$ side chain rendering a hydrophilic environment resulting in a 1 : 1 complex. Hence, an electrostatic interaction occurs between the positive charge of the protonated —$NHCH_2CH_2NH_2$ of the βCDN and the negative charge of the carboxylate group of the bile salt. As a result the equilibrium constant is higher as compared to pure cyclodextrin and comparable with cyclodextrins containing NH_2 side chains (Table III). The protonated amine groups of βCDN has a major influence on the host-guest complexation and has also been described by Bruce et al. [11]

Similarly the observed interactions (Table V) in ROESY for βCDN and NaDC protons were, (1) 23-CH_2(s), 22-CH_2 (s), 21-CH_3 (s), 20-CH (s), 18-CH_3 (s), 17-CH (s), 16-CH_2 (s), 15-CH_2 (s), 14-CH (s), 12-CH (w), 11-CH_2 (m), 8-CH (w), 5-CH (w), 4-CH_2 (m), 3-CH (m), 2-CH_2 (m), 1-CH_2 (s) interacts with 3-H, (2) 22-CH_2 (w), 21-CH_3 (s), 20-CH (w), 18-CH_3 (w), 17-CH (m), 16-CH_2 (m) interacts with 5-H and, (3) 23-CH_2 (m), 22-CH_2 (w) interact with 6-H.

The steroidal structure of NaDC has an enlarged hydrophobic region due to the presence of the H substituent at C-7. Therefore, according to the ROESY experiment, two molecules of βCDN form a stable inclusion complex with the enlarged hydrophobic region of one molecule of NaDC (Figure 4b). The main difference in the ROESY data of the NaDC inclusion complex from that of the NaC complex,

Table IV. Summary of the intermolecular ROESY cross peaks between NaC and βCDN protons*

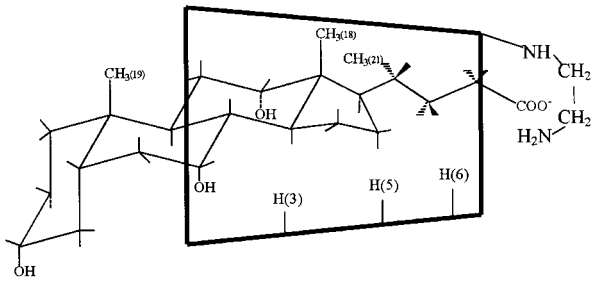
NaC hydrogens	6-deoxy-6-(aminoethyl)amino-β-cyclodextrin (βCDN) hydrogens		
	H_3	H_5	H_6
H_{23}	XXX	–	XX
H_{22}	XXX	–	XX
H_{21}	XXX	XXX	XX
H_{20}	XXX	X	X
H_{19}	–	–	–
H_{18}	XXX	XX	–
H_{17}	XXX	XX	–
H_{16}	XX	XX	–
H_{15}	XX	X	–
H_{14}	XX	X	–
H_{12}	XX	–	–
H_{11}	X	–	–
H_9	–	–	–
H_8	X	–	–
H_7	X	–	–

* The relative strength of cross peaks is indicated by '*X*, 'XX' and '*XXX*'.

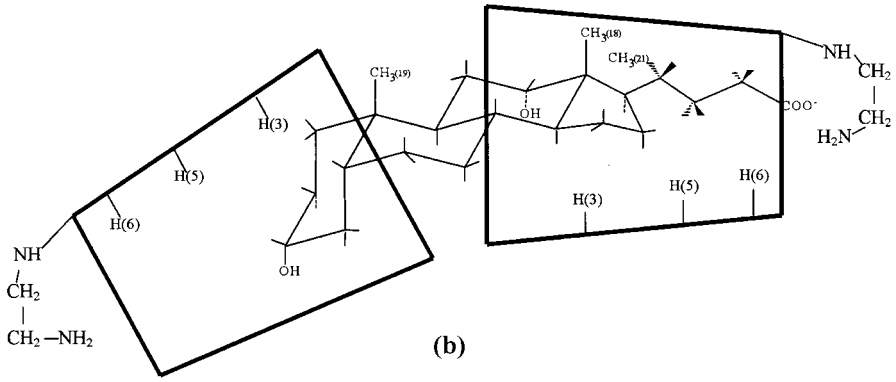
is the presence of H_1, H_2, H_3, H_4, H_5 and H_6 interactions with H-3, H-5 and H-6 of βCDN in addition to the previous interactions, confirming the formation of a 1 : 2 complex. The binding of two βCDN molecules on the same bile salt molecule reduces the stability of the second binding site. These conclusions from the ROESY experiments are in agreement with the Job's method.

4. Conclusions

A study of the ^{13}C chemical shifts as a function of concentration at natural, 7, 9 and 11 pH shows a different behaviour of complexation for NaC and NaDC with βCDN resulting in 1 : 1 and 1 : 2 inclusion complexes. However, the complexation phenomena do not depend on the pH of the solution. ^{13}C NMR chemical shifts of the host (βCDN) and guest (bile salts) molecules change on passing from the free to the complexed state. From these changes, information about the geometry, stoichiometry and stability constants of the inclusion complexes is drawn. The side chains in βCD at position C-6 have a significant effect on the complexation process with the bile salts. The ROESY experiments confirm the overlap of the NaC molecule with βCDN molecule resulting a 1 : 1 inclusion complex, while in the case of NaDC molecule, the first molecule of βCDN encapsulates the bile salt



(a)



(b)

Figure 4. Schematic representation of (a) the 1 : 1 complex of NaC with βCDN and (b) the 1 : 2 complex of NaDC with βCDN.

to a larger extent than the second molecule of βCDN, resulting a 1 : 2 inclusion complex. Hence the most important factors for the formation of a stable inclusion complex are the relative size of the βCDN and the bile salt molecules, the non-polar cavity of the βCDN, the hydrophobicity of the bile salts, and the presence of an electrostatic environment outside the toroidal cavity.

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Table V. Summary of the intermolecular ROESY cross peaks between NaDC and βCDN protons*

NaDC hydrogens	6-deoxy-6-(aminoethyl)amino-β-cyclodextrin (βCDN) hydrogens		
	H_3	H_5	H_6
H_{23}	XXX	–	XX
H_{22}	XXX	X	X
H_{21}	XXX	XXX	–
H_{20}	XXX	X	–
H_{19}	–	–	–
H_{18}	XXX	X	–
H_{17}	XXX	XX	–
H_{16}	XXX	XX	–
H_{15}	XXX	–	–
H_{14}	XXX	–	–
H_{12}	X	–	–
H_{11}	XX	–	–
H_9	-	–	–
H_8	X	–	–
H_7	–	–	
H_6	–	–	–
H_5	X	–	–
H_4	XX	–	–
H_3	XX	–	–
H_2	XX	–	–
H_1	XXX	–	–

* The relative strength of cross peaks is indicated by 'X', 'XX' and 'XXX'.

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