Journal of Inclusion Phenomena and Macrocyclic Chemistry **39:** 41–46, 2001.

Spectrometric and 2D NMR Studies on the Complexation of Chlorophenols with Cyclodextrins

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(Received: 6 October 1999; in final form: 1 May 2000)

Key words: α- and β-cyclodextrin chlorophenol complexes, 2D ^{1}H NMR TROESY, inclusion complex structures, spectrophotometry, ^{1}H NMR, equilibrium constants

Abstract

The formation and structure of inclusion complexes of α- and β-cyclodextrins with 2-chlorophenol (2CP), 3-chlorophenol (3CP), 4-chlorophenol (4CP), 2,4-dichlorophenol (24DCP), 2,6-dichlorophenol (26DCP) and 3,4-dichlorophenol (34DCP) have been studied by UV-VIS and ^{1}H NMR spectroscopy. Both cyclodextrins were found to form 1 : 1 inclusion complexes. Binding constants estimated from titration studies revealed that the stability of the complexes was highly dependent on the structure and polarity of the chlorophenol and on the cyclodextrin used. In general, weaker binding constants were observed for a given chlorophenol with α-cyclodextrin than with β-cyclodextrin. The weakest binding constants (K_b < 200 M^{-1}) were obtained for the ortho-substituted chlorophenols (2CP and 26DCP) and the largest binding constants were obtained between *para*-chlorophenols (4CP, 24DCP and 34DCP) and β-cyclodextrin. 2D-TROESY studies of chlorophenol-cyclodextrin complexes in D_2O provided insight into the structure of the complexes.

Introduction

Cyclodextrins are cyclic oligosaccharides produced by enzymatic action over starch. The most commonly used cyclodextrins contain 6, 7, or 8 units of glucose connected with α-1,4-bonds and are named α, β and γ-cyclodextrin, respectively [1]. Cyclodextrins have a toroidal shape with a hydrophobic inside and free hydroxyl groups at the two rims which render them capable of forming inclusion complexes with hydrophobic compounds in aqueous environments [1b]. Although the depth of all cyclodextrins is given by the length of one glucose unit, their diameters change in relatively large steps as the number of units in the oligosaccharide increases. The two rims of the cyclodextrins have different sizes which limit the complexation of hydrophobic compounds while influencing their directionality.

The formation of inclusion complexes is the basis for applications of cyclodextrins in several fields, including spectrophotometric analysis and chromatographic separations [2], as well as in the pharmaceutical, cosmetics, and food industries [3, 4]. The stability of the inclusion complexes depends primarily on hydrophobic interactions and on size and shape considerations [5]. Although there are many studies of cyclodextrin complexes with different classes of organic compounds [6–10], there are only a few reported studies of cyclodextrin complexes with toxic organic substances [11]. Chlorophenols are used in a variety of chemical processes as intermediates in the synthesis of dyes, herbicides, pesticides, plastics and drugs. They are present in the waste water effluents from petrochemical, coal tar, and synthetic industries. Trace levels (<1 ppm) of chlorophenols are toxic to aquatic and mammalian life and have adverse effects on the odor and taste of water. The separation and identification of chlorophenols in water is an important goal [12] and it is hoped that their complexation with suitable hosts may allow for the development of analytical techniques and remediation procedures. In this paper, we report results from complexation studies using high resolution ^{1}H NMR and UV-VIS spectroscopy to evaluate the host-guest interactions of several chlorophenols with α- and β-cyclodextrins. The aim of this work is to determine which of these chlorophenols form inclusion complexes with cyclodextrins in aqueous media, and to determine the structure of these complexes. Changes in UV-VIS absorbance and ^{1}H NMR chemical shifts caused upon complexation were quantitatively measured for each chlorophenol with α- and β-cyclodextrins to obtain the corresponding binding constants. Experimental evidence of complex formation, the average extent of penetration, and the direction of inclusion in the host were obtained by 2D-TROESY ^{1}H NMR.

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Experimental

Materials

All solvents used were of the highest purity commercially available and were used as received. All chlorophenols and methyl orange were purchased from Aldrich Chemical Co. α- and β-Cyclodextrins were generously donated by Cerestar Co. and were used without further purification.

NMR and UV measurements

All 1D-NMR spectra were acquired with a 30 degree pulse (2.8 μs on the ARX400 and 3.7 μs on the ARX500) and a repetition time of 6 s. Solutions of cyclodextrin (10^{-3} M) and chlorophenol (10^{-3}–10^{-4} M) in D_2O were placed in NMR tubes with a coaxial NMR tube containing a solution of $CDCl_3$-TMS as an external reference. 2D-TROESY (ROESY without TOCSY) experiments [13] were carried out on the ARX500 with an 11 μs 90 degree pulse in a phase sensitive mode using the States-TPPI method to introduce quadrature detection in the second dimension [14]. Clear solutions of α- or β-cyclodextrin (10^{-1}–10^{-2} M) and 4-chlorophenol (10^{-1}-10^{-2} M) in D_2O were placed in NMR tubes and were bubbled with Argon for 1 hour before 2D-TROESY measurements. UV-VIS spectra were obtained on a Hewlett Packard 8453 UV-VIS spectrophotometer. Solutions of chlorophenol (10^{-4} M) and cyclodextrin (10^{-3} M) in aqueous buffer solution (Na_2HPO_4—H_3PO_4, pH = 4.5) were used for direct spectrophotometric studies. Solutions of methyl orange (4.2×10^{-5} M), α-cyclodextrin (10^{-3} M) and chlorophenol (10^{-3}–10^{-2} M) in HCl (0.008 M) were used for competitive spectrophotometric studies. The binding constants were calculated using the Benesi–Hildebrand equation or a modified version of it [15, 16].

Results and discussion

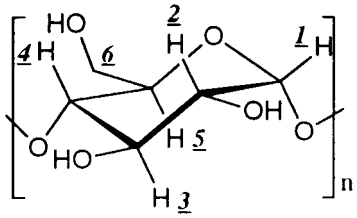
UV-VIS spectroscopy studies

The formation of inclusion complexes has been studied by a wide variety of spectroscopic methods [17–27]. Among many useful techniques, ^{1}H NMR and UV-VIS spectroscopy are among the simplest, most readily accessible, and highly informative. The evaluation of binding constants by direct spectroscopic method relies on analytical differences between the free guest and the complex [5]. Changes in the absorption intensity of 2,4-dichlorophenol at 285 nm were monitored as a function of β-cyclodextrin concentration to measure the binding constant (Table 1). Similar changes in UV-VIS spectra were observed for 26DCP and 34DCP with β-cyclodextrin but very small changes were observed for most of the chlorophenol-cyclodextrin systems. The binding constants (Table 1) for most of the chlorophenol-cyclodextrin complexes were determined by the spectrophotometric examination of the inhibitory effect of the chlorophenol on the association of the cyclodextrin with methyl orange [28].

Table 1. Binding constants for chlorophenol-cyclodextrin complexes

Host	Guest	K_b (UV-VIS, M^{-1})	K_b (^{1}H NMR, M^{-1})
α	4CP	274 ± 26[a]	331 ± 15.3[c]
α	3CP	200 ± 16[a]	324 ± 14.1[a]
α	2CP	35 ± 4[a]	[d]
α	3,4DCP	120 ± 15[a]	[d]
α	2,4DCP	210 ± 27[a]	403 ± 17[c]
α	2,6DCP	100 ± 13[a]	[d]
β	4CP	427 ± 68[a]	420 ± 16[c]
β	3CP	200 ± 47[a]	304 ± 12[c]
β	2CP	110 ± 15[a]	150 ± 9[c]
β	3,4DCP	1000 ± 60[b]	2100 ± 28[c]
β	2,4DCP	350 ± 23[a,b]	556 ± 17[c]
β	2,6DCP	50 ± 6[b]	[e]

[a]Measured by competitive spectrophotometric method in aqueous solution (0.008 M HCl).
[b]Measured by direct spectrophotometric method in aqueous buffer solution ($Na_2H_3PO_4$—H_3PO_4, pH = 4.5).
[c]Measured by ^{1}H NMR spectroscopy in D_2O solution.
[d]The $\Delta\delta$ value for H3 hydrogen was too small to calculate the binding constant.
[e]The $\Delta\delta$ value for H5 hydrogen was too small to calculate the binding constant.



Scheme 1.

1D-NMR studies

Since ^{1}H NMR spectroscopy was first introduced for the study of complex formation in aqueous solutions [29], there have been numerous studies involving aromatic compounds [7, 30–32]. The method relies on changes in chemical shifts caused by the guest and the host on each other. In the case of aromatic compounds, some of the most important spectral changes that occur upon complexation come from the diamagnetic shielding of the aromatic host on the nearby spins of the guest. In the structures α- and β-cyclodextrin, only hydrogens H3 and H5 are located inside the cavity (Scheme 1). H3 are located near the wider rim of the cyclodextrin cavity while the H5 hydrogens form a ring near the narrower rim of the methylene (H6) bearing the primary hydroxyl groups. All other hydrogens (H1, H2 and H4) are located on the exterior of the cavity.

The dynamics in the chlorophenol-cyclodextrin system are in the fast-exchange limit. Measured resonance positions are the average chemical shifts in the free and complexed cyclodextrins weighted by the fractional population in each state. Only the H3 (3.98 ppm, triplet) in α-cyclodextrin (Figure 1) showed significant up field shifts upon the addition of a given chlorophenol.

In contrast, complexation of the same series of chlorophenols with β-cyclodextrin (Figure 2) produced lar-

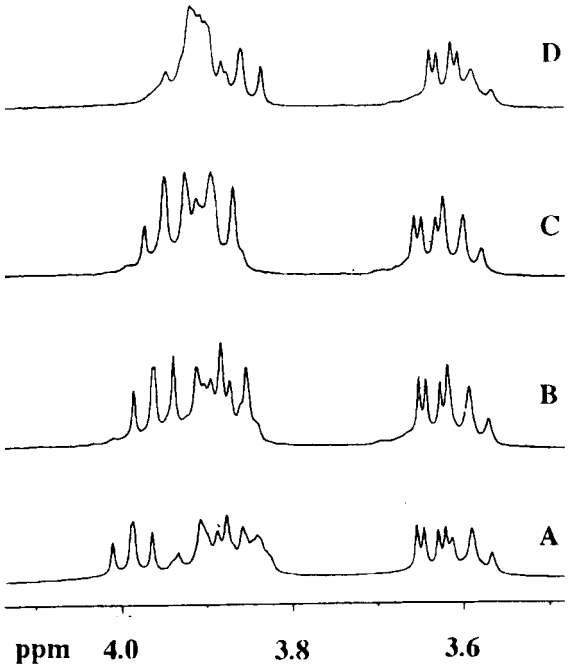


Figure 1. ^{1}H NMR (400 MHz) spectra of α-cyclodextrin and 4CP in different molar ratios: (A) 0.0, (B) 0.5, (C) 1.0 and (D) 4.0. The concentration of α-cyclodextrin is 0.001 M.

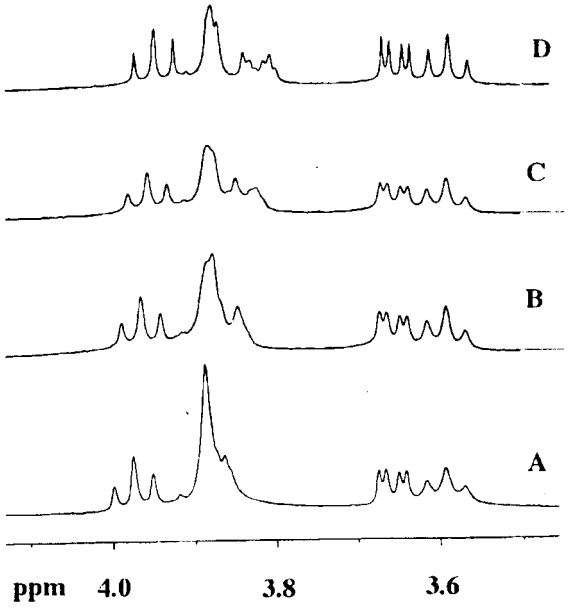


Figure 2. ^{1}H NMR (400 MHz) spectra of β-cyclodextrin and 4CP in different molar ratios: (A) 0.0, (B) 0.5, (C) 1.0 and (D) 1.5. The β-cyclodextrin concentration is constant at 0.001 M.

ger upfield shifts of both H3 (3.97 ppm, triplet) and H5 (3.87 ppm, doublet of triplets). This change indicates that the phenyl rings of the chlorophenols are more deeply inserted into the β-cyclodextrin cavity.

2D-NMR Studies

There are several NMR techniques that can give supporting evidence for specific structures in cyclodextrin complexes [33–37]. Despite challenges associated with binding dynamics, which may interfere with the development of cross relaxation interactions, the Nuclear Overhauser Effect (NOE) is one of the most widely used methods [38]. 2D-NOESY and 2D-ROESY experiments give rise to cross peaks between dipolarly coupled spins [39, 40], indicating the close proximity between atoms in the two components of the complex. Under favorable conditions, 2D NOESY and 2D ROESY experiments provide an upper limit (ca. 5 Å) on the distance between protons that produce cross peaks.

2D-NOESY and 2D-TROESY experiments were carried out with a clear solution of 4-chlorophenol (0.001 M) and β-cyclodextrin (0.001 M) in D_2O using a 200 ms mixing time on a Bruker 400 MHz ARX NMR instrument with a QNP probe. Under these conditions, no intermolecular NOEs were detected in the 2D-NOESY experiment while moderate NOEs were detected in the 2D-TROESY experiment. These results can be explained in terms of the dynamics of the complex which modulate the evolution and the sign of the NOE [41, 42]. Molecular species having motions with correlation times τ_c near the condition $\omega\tau_c = 1$, where ω is the angular Larmor frequency, have NOE effects that are near zero. Supramolecular systems will be susceptible to similar effects and will be strongly influenced by the lifetime of the complex, and by its population under equilibrium conditions. Limitations associated with restrictions given by the dynamic window associated with the Larmor frequency and the residence time of the complex may be overcome by using rotating-frame NOE techniques where cross-relaxation occurs under the smaller Larmor frequency of spin locking fields, or ROE, which gives positive NOE's for increasing values of τ_c. Therefore, 2D-TROESY [13] experiments were used to study the chlorophenol-cyclodextrin complexes. In order to improve signal-to-noise values in the case of 4-chlorophenol, higher concentrations of guest 0.02–0.04 M) and host (0.01 M) were employed and measurements were carried out on the ARX500 spectrometer with an inverse coil probe and a mixing time of 750 ms. A portion of the 2D-TROESY spectrum for the 4-chlorophenol-β-cyclodextrin complex in D_2O is shown in Figure 3. Strong cross peaks between the two sets of aromatic hydrogens at 6.80 and 7.19 ppm, and the H3 and H5 hydrogens of the β-cyclodextrin at 3.85 and 3.72 ppm, respectively, indicate that all aromatic hydrogens may be within less than 5 Å apart from the H3 and H5 cyclodextrin hydrogens.

The 2D-TROESY spectrum for the 4-chlorophenol-α-cyclodextrin complex in D_2O is shown in Figure 4. Cross peaks between the ortho (6.84 ppm) and meta (7.36 ppm) aromatic hydrogens, and the H3 hydrogens of the α-cyclodextrin at 3.78 ppm indicate their proximity. However, the H3 hydrogens of α-cyclodextrin at 3.85 ppm display cross peaks only with the meta-hydrogens of the chlorophenol at 7.36 ppm. This suggests that the ortho hydrogens in the chlorophenol are relatively far apart from the H5 hydrogens of α-cyclodextrin, in agreement with a structure having the phenol closer to the wider rim of α-cyclodextrin with the 4-chloro substituent inside the cavity and the hydroxyl group pointing to the outside (Figure 5). Similar results were recently observed by Alderfer

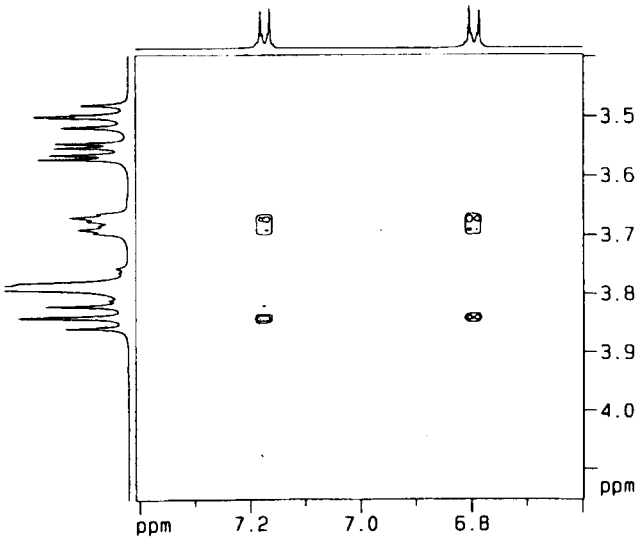


Figure 3. A section of the 500 MHz 2D-TROESY symmetrized spectrum of the 4CP-β-cyclodextrin complex in D_2O solution at ambient temperature obtained with a spin lock time of 750 ms.

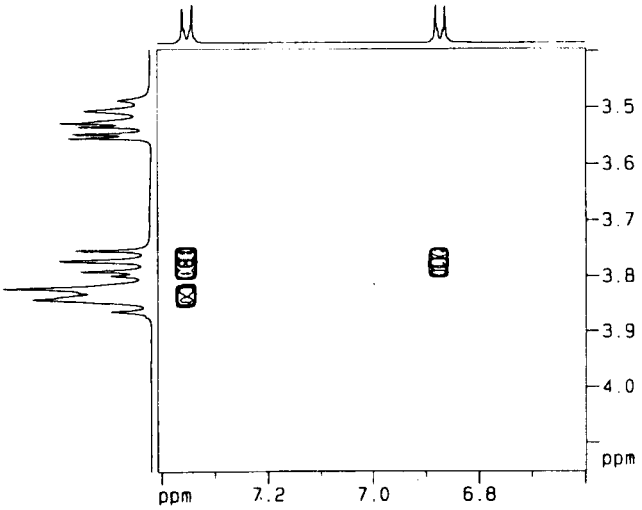


Figure 4. A portion of the 500 MHz 2D-TROESY symmetrized spectrum of the 4CP-α-cyclodextrin complex in D_2O solution at ambient temperature obtained with a spin lock time of 750 ms.

and Eliseev for the complexation of 4-fluorophenol with α-cyclodextrin [38b].

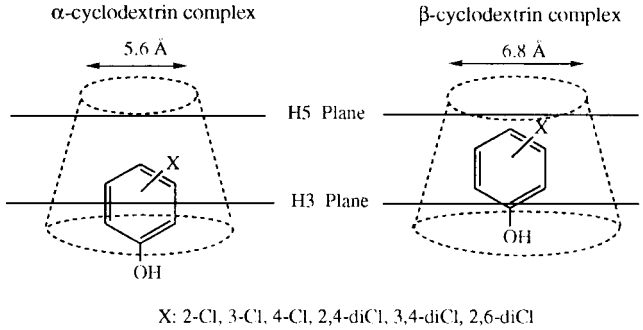


Figure 5. Structure of chlorophenol-cyclodextrin complexes in aqueous solution.

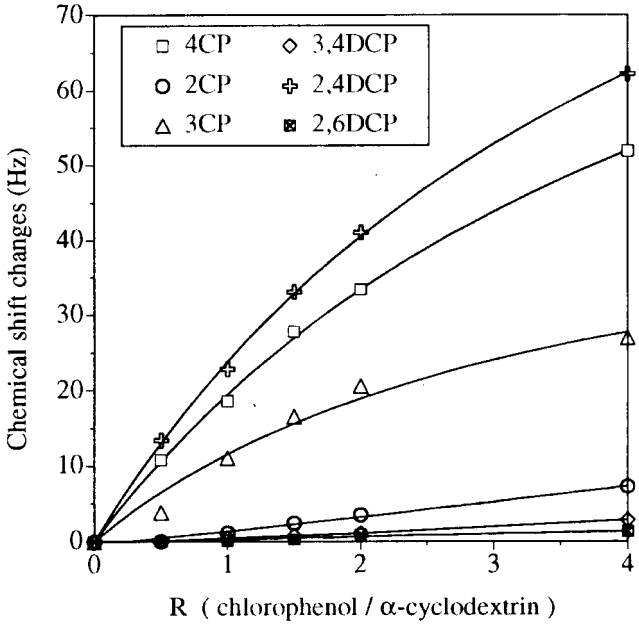


Figure 6. Plots of the chemical shift changes ($\Delta\delta$) for H3 of α-cyclodextrin as a function of R, the molar ratio of chlorophenol to α-cyclodextrin.

Binding constants and stability

All the chlorophenols analyzed in this study caused an upfield shift on the H3 signal of α-cyclodextrin. Figure 6 shows a plots of the chemical shift changes, $\Delta\delta$, for the H3 hydrogen of α-cyclodextrin versus the molar ratio R of all the chlorophenols studied. Chemical shift changes ($\Delta\delta$) of inclusion complexes showing large $\Delta\delta$ values ($\Delta\delta > 25$ Hz) gave good fits with a model involving a 1 : 1 complex [16]. Binding constants, K_b, determined from this data are included in Table 1. The binding constants determined for 4CP, 3CP and 24DCP with α-cyclodextrin were 331, 324, and 403 M^{-1}, respectively. The other three chlorophenols (2CP, 34DCP and 26DCP) gave very small $\Delta\delta$ values ($\Delta\delta < 10$ Hz) for the H3 of α-cyclodextrin indicating the formation of very weak complexes.

Five of the chlorophenols (2CP, 3CP, 4CP, 24DCP and 34DCP) caused similar upfield shifts on H3 and H5 of β-cyclodextrin. Figure 7 shows the plots of the chemical shift changes for H5 versus the molar ratio R of chlorophenol. Binding constants calculated for 2CP, 3CP, 4CP, 24DCP and 34DCP from this data were 150, 304, 420, 556 and 2100 M^{-1}, respectively. A very small chemical shift change of H5 ($\Delta\delta < 25$ Hz) observed upon addition of 26DCP to β-cyclodextrin under the same conditions indicates a very weak binding

The effect of 34DCP addition on the ^{1}H NMR spectrum of β-cyclodextrin is shown in Figure 8. Complexation of this chlorophenol with β-cyclodextrin leads to upfield chemical shift for both H3 and H5 hydrogens. In addition, there is line broadening and upfield shifting of H2 which is located on the outside of β-cyclodextrin. This observation was unique to 34DCP since H2 remains unchanged upon complexation with the other aromatic phenols analysed. We speculate that the phenolic —OH group may be hydrogen bonded to the

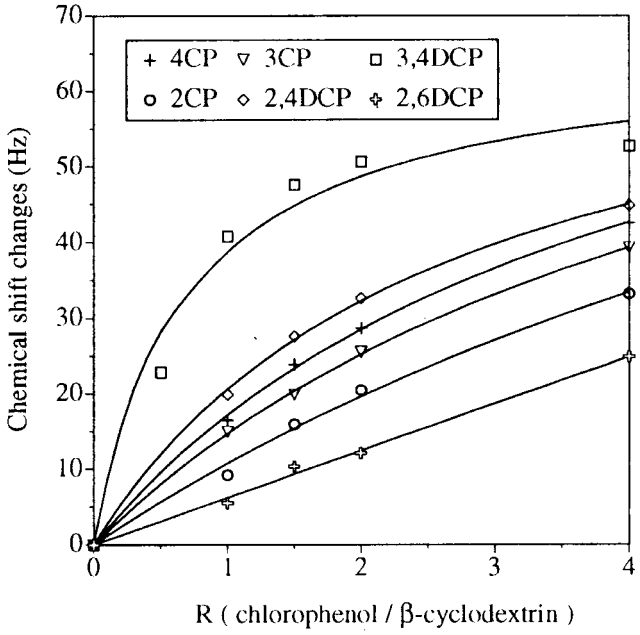


Figure 7. Plots of the chemical shift changes ($\Delta\delta$) for H5 of β-cyclodextrin as a function of R, the molar ratio of chlorophenol to β-cyclodextrin.

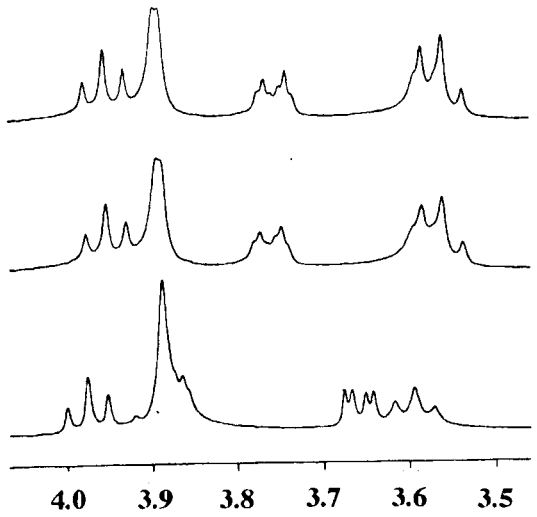


Figure 8. ^{1}H NMR (400 MHz) spectra with different molar ratios of 34DCP and β-cyclodextrin: (A) 0.0. (B) 1.5, (C) 2.0,. The concentration of β-cyclodextrin is constant at 0.001 M.

peripheral β-cyclodextrin hydroxyl group at C2, thus affecting the chemical shift of the H2 hydrogen. This hydrogen bonding interaction may also explain the higher binding constant ($K_b = 2100\ \mathrm{M}^{-1}$) observed for this complex. A similarly unusual chemical shift for the H2 hydrogen has been observed upon complexation of hydrocinnamate with β-cyclodextrin where the carbonyl group can also undergo hydrogen bonding to the hydroxyl group at C2 [43].

The K_b values in Table 1 show deviations that depend on the methods used to determine them. Although values determined by ^{1}H NMR are consistently larger than those measured by UV-VIS spectroscopy, there is good relative correlation between the K_b values calculated by either method. The larger K_b values obtained by NMR are probably due to the different media used in the experiments [5, 8]. The proposed average structures of these complexes are given in Figure 5. The position of the chlorophenol in the cyclodextrin cavity reflects a balance between steric repulsion, given by the relative sizes of the host and the guest, the hydrophobicity of the aromatic ring, and the hydrophilicity of the phenol —OH group. In general, weaker binding is observed with α-cyclodextrin. In this case, the six H5 hydrogens form an aperture with a radius of ca. 3.2 Å, which is smaller than the aperture of ca. 3.6 Å ring formed by the H3 hydrogens. In the case of β-cyclodextrin, the corresponding dimensions are formed by a ring of seven H5 hydrogens. A wider aperture allows for the phenolic compounds to penetrate more deeply into the cavity without creating severe steric interactions. It is expected that the highly hydrophilic —OH group prefers to remain exposed to the bulk of the solution. When extra chloro-substituents (2CP and 26DCP) are introduced in close proximity to the phenolic -OH group, weaker binding is observed. In contrast, larger binding is observed for the para-substituted chlorophenols (4CP, 24DCP and 34DCP) with β-cyclodextrin. This may be due not only to the better fit between the chlorophenol and the cyclodextrin cavity which allows for deeper penetration of the chloro-substituent, but also to the more favorable hydrophobic interactions. The larger binding constant measured for 34DCP may be due to hydrogen bonding between the phenolic —OH group and the external C2—OH group of the β-cyclodextrin.

Conclusions

α- and β-Cyclodextrin form inclusion complexes with several chlorophenols with modest to low equilibrium constants. Results from UV-VIS and ^{1}H NMR studies are consistent with a simple 1 : 1 stoichiometry and the stability of the complexes is dependent on the structure of the chlorophenol and the cyclodextrin used. In general, the most stable complexes are formed between β-cyclodextrin and the 4-substituted chlorophenols. This suggests that the stability of the complexes is strongly influenced by the sizes and shapes of the guest and the cavity of the host. The polarity of the host chlorophenol plays an important role on the stability of the complex but is far less important than geometric fitting. Changes in chemical shifts of hydrogens located inside the cavity (H3 and H5) and NOE effects measured by 2D-TROESY between chlorophenol and the two cyclodextrins suggest that complexation occurs through the wider rim near the secondary hydroxyl groups. Cross peaks between the ortho- and meta-hydrogens of chlorophenol and either or both H3 and H5 in the cyclodextrin, give an indication of the directionality and the extent of penetration. Strong peaks were observed between the two sets of aromatic hydrogens of 4CP and both H3 and H5 of β-cyclodextrin. In contrast, in the case of α- cyclodextrin, cross peaks were only observed between the ortho- and meta hydrogens of 4CP and the H5 of the host.

Acknowledgements

Financial support by CONACyT (Grant 485100-5-3186PA), TWAS (Grant 98-058 RG/CHE/LA) and the NSF (MAGG) are gratefully acknowledged. We thank Cerestar Company for a generous donation of cyclodextrins.

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