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The Influence of β- and γ-Cyclodextrin Cavity Size on the Association Constant with Decanoate and Octanoate Anions

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Abstract

This work evaluates the influence of the β- and γ-cyclodextrin (CD) cavity size on the association constant (K_{CDA}) with decanoate (C_{10}) and octanoate (C_8) anions. The spectral displacement technique with phenolphthalein was used to obtain the 1:1 association constant (K_{CDA}) in $NaHCO_3$/NaOH buffer pH 10.5 at 25 °C. The K_{CDA} value obtained were 2.6 ($\pm$0.2) $\times$ 10^3, 2.5 ($\pm$0.5) $\times$ 10^2, for βCD–C_{10} and γCD–C_{10} inclusion complexes, and 5.1 ($\pm$0.2) $\times$ 10^2 and 4.7 ($\pm$0.2) $\times$ 10^1 for βCD–C_8 and γCD–C_8 inclusion complexes, respectively. The K_{CDA} values of either acid with βCD is approximately 10 times higher than for the same acid with γCD, where as for the same cyclodextrin, the K_{CDA} value is 5 times higher for the C_{10} association than for the C_8. The data demonstrate that the cyclodextrin cavity size exerts a greater influence on the association constant than the chain length of the acid for these compounds. ^{1}H NMR studies show that fatty acid protonation has a distinct effect on the chemical shift of CD protons.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides containing 6 to 12 glucose units linked by α-1,4-glucosidic bonds. They have the shape of a hollow truncated cone, resulting in a hydrophobic cavity. The more common cyclodextrins are α-, β- and γ-CD with 6, 7 and 8 glucose units, respectively, each having a slightly larger cavity size [1].

The formation of inclusion complexes between small organic molecules and cyclodextrins has proven to be an excellent method for studying the nature of noncovalent binding forces in solution [2]. The principal factors involved in binding are believed to be van der Waals and hydrophobic interactions, although hydrogen bonding and steric effects may also play a role [3].

A number of experimental techniques have been employed in the determination of the affinity between cyclodextrins and guest molecules. These include fluorescence spectroscopy [4], potentiometric titration, conductance [5, 6], NMR titration [7, 8] and UV-visible spectroscopy [9–11].

A spectral technique, using phenolphthalein (PH) as competitive chromophoric binder, can be used when the guest does not absorb in the visible region. This method is based on the displacement of the competitive agent upon complexation of the desired guest to the CD cavity, and allows a determination of the association constant [12]. This method has been used to investigate the association constant between βCD and a homologous series of fluorocarbon and hydrocarbon anionic surfactants [10] and the influence of tetrahydrofuran on the association between βCD-phenolphthalein [2].

Another technique applicable to the study of inclusion complexes in CD is ^{1}H NMR spectroscopy. The chemical shifts (δ) of both the interior protons of the CD (H_3 and H_5) and the guest protons can be analyzed to provide information about the inclusion mode and binding affinity between CD and guest [8].

Little studied is the influence of the cyclodextrin size on the association of different fatty acids, which may be of importance in optimizing hydrophobic interactions within the inclusion complex. Therefore, in this work the association constants of two cyclodextrins, βCD and γCD, with octanoate (C_8) and decanoate (C_{10}) anions were determined by using phenolphthalein as a competitive agent. ^{1}H NMR spectra of βCD-C_{10} and C_8, γCD-C_{10} and C_8 systems were also obtained in order to measure the variation of chemical shift of cyclodextrin protons in the presence of fatty acids at different pHs.

Experimental

Reagents and solutions

Decanoic acid 99% and octanoic acid 99% were purchased from Fluka. D_2O, KOD and phenolphthalein were purchased from Aldrich. $NaHCO_3$ and NaOH were obtained from Nuclear. Cerestar (USA) donated β-CD and γ-CD and both

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cyclodextrins were re-crystallized in aqueous medium. The water content was determined from thermal analysis.

A stock solution of phenolphthalein (PH) was prepared in aqueous $NaHCO_3$/NaOH buffer, with 2% ethanol (v/v) at pH 10.5. This solution was prepared every three days of continued use. It has been demonstrated that ethanol concentrations of up to 5 % (v/v) in the stock solutions of phenolphthalein does not cause suppression of absorbency or a competitive binding in the cavity of cyclodextrin [10].

βCD–PH and γCD-PH association constant determination. Several experimental β-CD solutions with 0.2×10^{-4} mol L^{-1} to 2.0×10^{-4} mol L^{-1} were prepared from a 7.5×10^{-4} mol L^{-1} stock solution of βCD. An aliquot of the stock solution of PH was added, and the solution filled with $NaHCO_3$/NaOH buffer to give a final concentration of 4×10^{-5} mol L^{-1}.

The same experimental procedure were used for the γCD-PH system, but with concentrations of 4.5 to 11×10^{-5} mol L^{-1} of γCD, and 4×10^{-5} mol L^{-1} of PH in each solution. All solutions were allowed to equilibrate for 12 hours before measurements.

βCD–C_{10}, γCD-C_{10}, βCD-C_8 and γCD-C_8 association constant determination. After equilibration, different amounts of fatty acid stock solutions (C_8 or C_{10}) were added to the CD–PH solution. For the βCD experiments, the concentrations of each component were [PH] = 4×10^{-5} mol L^{-1}, [βCD] = 1×10^{-3} mol L^{-1} and the concentration range for C_{10} was 4×10^{-4} to 8×10^{-3} mol L^{-1} and for C_8 was 2.5×10^{-3} to 3×10^{-2} mol L^{-1}. The concentrations in the γCD experiments were [PH] = 4×10^{-5} mol L^{-1}, [γCD] = 2×10^{-3} mol L^{-1} and the concentration range for C_{10} was 4×10^{-4} to 1×10^{-3} mol L^{-1} and the concentration range for C_8 was 5×10^{-3} to 7×10^{-2} mol L^{-1}.

Instrumentation and measurement

For the competitive determinations, absorbance measurements were taken at 550 nm, while in a thermostated bath at 25.0 °C. Each experiment was repeated at least three times and the value for the constant is the average of the measurements. The absorption spectra were recorded using a Hitachi U-3000 UV-vis spectrophotometer. All measurements were carried out using standard 1 cm quartz cells.

For the ^{1}H NMR characterization, the inclusion complexes were analyzed in D_2O with TMS as internal reference at concentrations of 0.05 M for both the host and guest. The solutions were prepared in pD 4 and pD $\geq$ 10, with addition of KOD. The ^{1}H NMR spectra were recorded with a Bruker AC-200F, 200 MHz spectrometer.

Table 1. Association constants for β- and γ-cyclodextrin with phenolphthalein

System	K_{CD-PH} = [CD–PH]/[CD][PH]		
	This work[a]	Reference [11][b]	Reference [10][c]
βCD–PH	$(1.21 \pm 0.16) \times 10^4$	$(3.1 \pm 0.3) \times 10^4$	$(2.5 \pm 0.3) \times 10^4$
γCD–PH	$(2.02 \pm 0.14) \times 10^3$	–	–

[a] at 25 °C, in a buffer pH 10.5 ($NaHCO_3$/NaOH), 2% of ethanol, and [PH] = 4×10^{-5} mol L^{-1}.
[b] Determined by potentiometric and spectrophotometric methods, the solvent was 35% (v/v) ethanol/water.
[c] Determined by spectrophotometric method in solutions having 0.04% ethanol, assuming no absorption due to CD–PH species.

Results and discussion

Determination of βCD-PH and γCD-PH association constants

The equilibrium between CD and PH is described by Equation 1, where CD is β- or γCD and PH represents phenolphthalein. The absorbing species are free PH and complexed PH (CD-PH). The association constants for the PH complex with β- and γCD were obtained by using the linear equation of Hildebrand and Benesi [13] (Equation 2), where PH_t is the total phenolphthalein concentration, CD_t is the total cyclodextrin concentration, ΔA is the difference between the absorbency of free and complexed phenolphthalein and $\Delta\varepsilon$ is the difference between the molar absorbtivity of free and complexed phenolphthalein. The experimental data shows a linear relationship over a range of concentrations. The values obtained for $K_{\beta CD-PH}$ are compared in Table 1 with values reported in the literature.

$$CD + PH \rightleftharpoons CD\text{–}PH \quad K_{CD-PH} = \frac{[CD\text{–}PH]}{[CD][PH]} \tag{1}$$

$$\frac{PH_t}{\Delta A} = \frac{1}{\Delta\varepsilon K_{CD-PH}} \frac{1}{CD_t} + \frac{1}{\Delta\varepsilon} \tag{2}$$

Determination of βCD-C_{10} and C_8, γCD-C_{10} and C_8 association constant

The equilibrium between CD and C_{10}, and that for CD-C_8 are described by Equation (3), where A is the guest C_{10} or C_8:

$$CD + A \rightleftharpoons CDA \;\; K_{CDA} = \frac{[CDA]}{[CD][A]} \tag{3}$$

Connors *et al.* developed a linear equation to determine the constant for cyclodextrin-guest association [12]. By considering the guest mass balance ($[A]_t = [A] + [CDA]$), the cyclodextrin and phenolphthalein mass balance, and incorporating the term Q = [PH]/[CD-PH], Equation (4) is obtained.

$$CD_t - \frac{1}{Q+1} - \frac{PH_t}{Q+1} = \frac{A_t K_{CDA}}{QK_{CD-PH} + K_{CDA}} \tag{4}$$

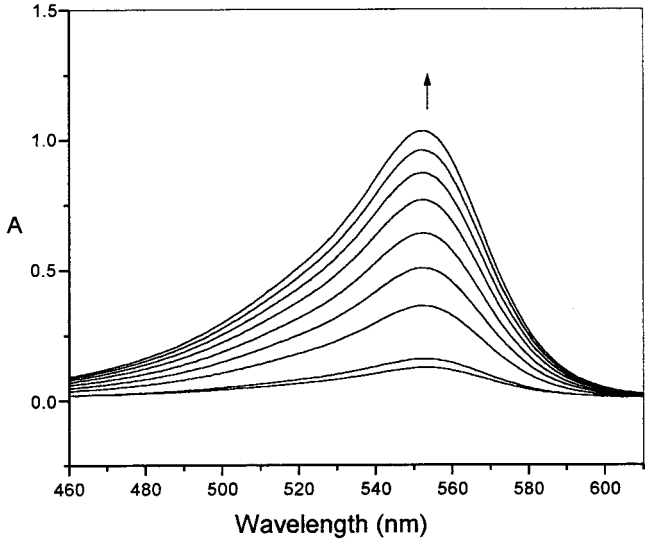


Figure 1. Absorbance increase for the βCD–C_{10} system due to increasing concentrations of C_{10}. Conditions: [PH] = 4.0×10^{-5} mol L^{-1}, [βCD] = 1.0×10^{-3} mol L^{-1}, C_{10}: from 0.40×10^{-4} mol L^{-1} to 8.0×10^{-1} mol L^{-1}, pH 10.5 buffer ($NaHCO_3$/NaOH) with 2% ethanol.

Denoting the left term in Equation (4) as P, and rearranging yields a linear dependence, Equation (5).

$$\frac{A_t}{P} = \frac{K_{CD-PH}}{K_{CDA}}Q + 1 \tag{5}$$

Substituting the phenolphthalein mass balance in the expression Q = [PH]/[CD–PH], Equation (6) is obtained. When the Q and P values are known, it is possible to determine K_{CDA}.

$$Q = \frac{PH_t}{\Delta A/\Delta\varepsilon} - 1. \tag{6}$$

The $\Delta\varepsilon$ value for the βCD–PH system is 48.300 cm^{-1} L mol^{-1} and for the γCD-PH system is 44.820 cm^{-1} L mol^{-1}. Formation of 2 : 1 CD per acid inclusion species were not considered, as it has been reported that only alkyl chains with more than 16 carbon atoms can form this species in solution [10].

Figure 1 shows the increase in absorbance at 550 nm as C_{10} acid is added to the βCD/phenolphthalein system. The increase is due to competitive complexation of C_{10} acid by the βCD cavity, which displaces PH from the cavity to the solution. Similar behavior is observed for the βCD–C_8, γCD- C_8 and γCD- C_{10} systems.

Figures 2 and 3 show the linear correlation of the results for the βCD-C_{10} and γCD-C_8 systems, where the visible spectra data were treated with the aid of Equation (9). The βCD–C_8 (not shown) and γCD-C_{10} (not shown) systems present similar behavior. The computed association constants are reported in Table 2.

^1H NMR characterization

^{1}H NMR is an important technique in the studies of inclusion complexes because the chemical shift of the cyclodextrin and guest protons can be related to the strength

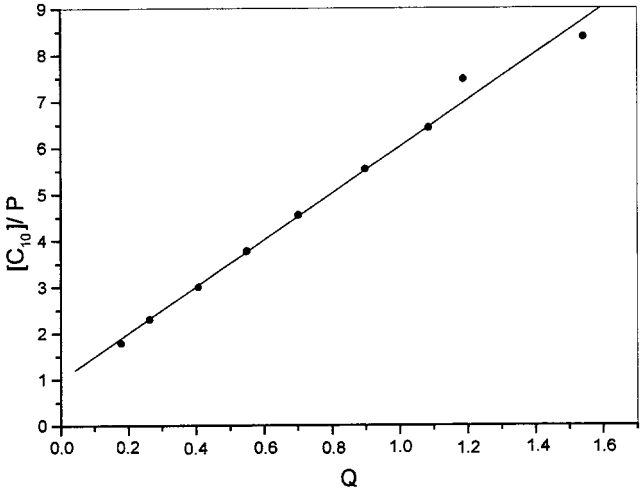


Figure 2. Linear plot from absorbance data in Figure 1 for the βCD–C_{10} system, obtained by the use of Equation (9) (fit correlation coefficient = 0.9985).

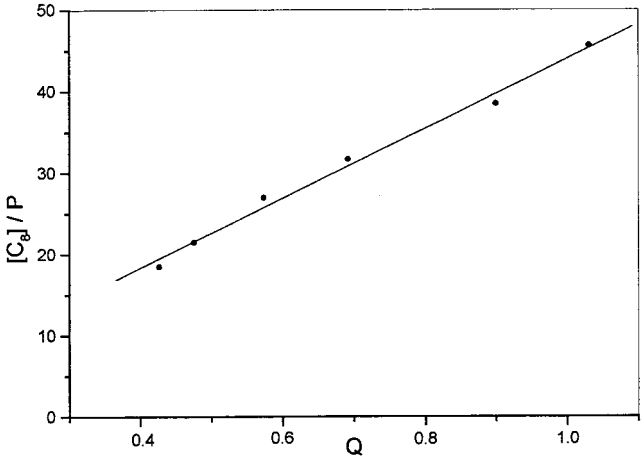


Figure 3. Linear plot from absorbance data for the γCD–C_8 system, obtained by the use of Equation (9) (fit correlation coefficient = 0.9956). Conditions: pH 10.5 buffer ($NaHCO_3$/NaOH), 2% ethanol, [PH] = 4.0×10^{-5} mol L^{-1}, and [γ-CD] = 2.0×10^{-3} mol L^{-1}.

of cyclodextrin-guest interaction [7]. The chemical shifts of the interior proton resonances, H_3 and H_5 of cyclodextrin (Figure 4), may be used to demonstrate the strength of interaction of the host within the cavity of cyclodextrins. The H_3 and H_5 proton resonances shift to high field in the presence of guests, due to the shielding of these protons from solvent, and thus are a measure of the interaction with the guest [7].

Figure 4 shows the βCD ^{1}H NMR spectra and Figure 5 shows a typical βCD–C_8 spectra at pD ca. 10 with host and guest concentrations of 50 mM. At pD above 10, the H_3 and H5 peaks shift only 0.06 ppm for both protons in the βCD–C_8 inclusion complex (Figure 5) reflecting the hydrophobic host/guest interaction at this pH. Under identical conditions at pD above 10, the βCD–C_{10} inclusion complex displays larger shifts than βCD–C_8, of 0.06 and 0.11 ppm for the H_3 and H_5 peaks, implying a tighter complexation. At pD 4 the fatty acids are protonated and neutral, thus resulting in larger shifts, 0.08 ppm for the H_3 and 0.11 ppm for the H_5 in the βCD–C_8 inclusion complex, due to stronger hydrophobic interaction.

Table 2. Association constants (K_{CDA}) for βCD–C_{10}, γCD–C_{10}, βCD–C_8, and γCD–C_8 systems

System	K_{CDA} = [CDA]/[CD][A]			
	This work[a]	Reference [8][b]	Reference [9][c]	Reference [10][d]
βCD–C_{10}	$(2.6 \pm 0.2) \times 10^3$	$(8.0 \pm 0.02) \times 10^3$	$(3.8 \pm 0.1) \times 10^3$	$(5.1 \pm 0.6) \times 10^3$
γCD–C_{10}	$(2.5 \pm 0.5) \times 10^2$	–	–	–
βCD–C_8	$(5.1 \pm 0.2) \times 10^2$	$(7.0 \pm 2.9) \times 10^2$	$(4.8 \pm 0.5) \times 10^2$	$(6.6 \pm 0.8) \times 10^2$
γCD–C_8	$(4.7 \pm 0.2) \times 10^1$	–	–	–

[a] At 25 °C; buffer pH 10.5 ($NaHCO_3$/NaOH) and 2% of ethanol. [PH] = 4×10^{-5} mol L^{-1}, [βCD]= 1×10^{-3} mol L^{-1}, [γCD] = 2×10^{-3} mol L^{-1}.
[b] Determined by ^{1}H NMR shifts of the fatty acid CH_3 resonance.
[c] Determined by the same method at 21 °C, [PH] = 1×10^{-4} M.
[d] Determined by spectrophotometric competitive method in solutions having 0.04% of ethanol, but assuming no absorption due to CD–PH species.

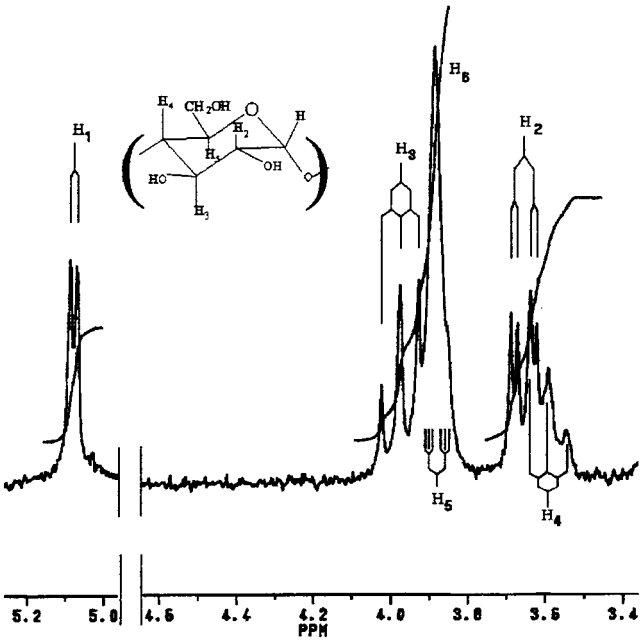


Figure 4. β-CD ^{1}H NMR spectrum in D_2O.

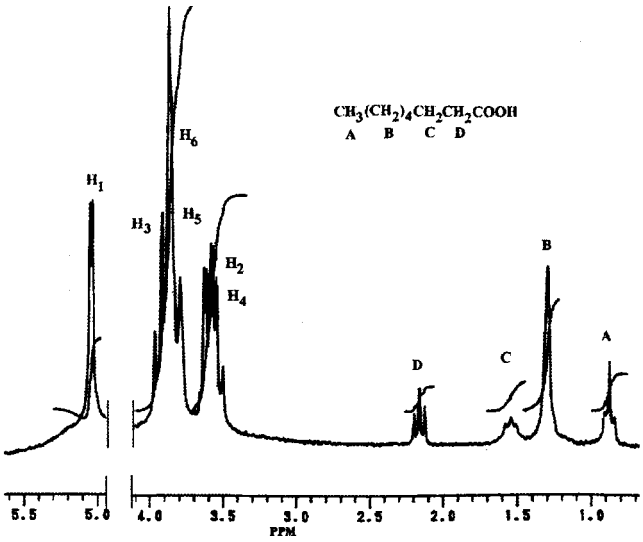
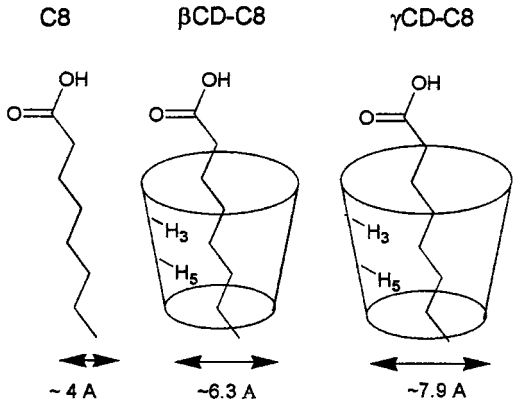


Figure 5. βCD-C_8 ^{1}H NMR spectrum in D_2O, pD 10.



Scheme 1.

Comparison of the data

Comparing the K_{CDA} values in Table 2, for the binding of different acid chains, it can be observed that the K_{CDA} value of the C_{10} inclusion complexes for both β and γCD is ca. 5 times stronger than that for the C_8 inclusion complexes. The increase in the hydrocarbon chain length increases the number of possible conformers within the cavity of cyclodextrin, reflected in the observed larger association constants. These results are in agreement with others reported for substrates with general formula $CH_3(CH_2)_nX$, where X = CH_3, COOH, COO^-, OH^-, SO_3^- [10, 16].

However, comparing the binding ability of different CDs with the same guest, it is observed that the K_{CDA} values of the βCD inclusion complexes are both ca. 10 times higher than that for the γCD complexes ($K_{\beta CD-C_{10}}/K_{\gamma CD-C_{10}} \simeq K_{\beta CD-C_8}/K_{\gamma CD-C_8} \simeq 10$). Thus the formation of CD-fatty acid complexes is most dependent on the cyclodextrin cavity size. The cavity diameter of βCD is ~6.3 Å, which is little larger than that of a methyl group (~4 Å), whereas the cavity diameter of γCD is ~7.9 Å, almost twice that of a methyl group, Scheme 1 [14, 15]. These results imply that the appropriate cavity size maximizes the interactions between the hydrocarbon chain of the fatty acids and the CD.

There are significant differences between the K_{CDA} values for the βCD-C_{10} and βCD-C_8 systems obtained in this work and in literature values (Table 2), likely due to different solvent composition and temperatures employed. Different stock phenolphthalein concentrations may cause a variation in the K_{CDA} value, as demonstrated by Georgiou *et al.* [9].

Likewise, in the ^{1}H NMR technique, there is a dependence of the K_{CDA} values on which proton resonances are used for the calculation. But the association constants determined in this work vary by substrate in a similar manner to those reported in the literature, and therefore the relative effects found to be due to host cavity size and guest chain length should hold true despite systematic differences in the observed association constants.

Conclusion

In summary, the spectral competitive technique was used to determine the association constants for inclusion of fatty acids into βCD and γCD, and to our knowledge, the first reports of such constants for the γCD systems. The data demonstrate that βCD is superior to γCD for encapsulating fatty acids, and that the cavity size difference plays a greater role than the fatty acid tail length in the stability of the inclusion compounds studied in this work. The ^{1}H NMR spectra demonstrate the importance of the hydrophobicity in the inclusion complexes, protonated fatty acids cause a larger chemical shift, and by implication a tighter binding, than when they are deprotonated.

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