Complex Formation Between α-Cyclodextrin and 4-Substituted Phenols Studied by Potentiometric and Competitive Spectrophotometric Methods

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Received February 11, 1982, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706.* Accepted for publication October 8, 1982.

Abstract □ Stability constants for complex formation between α-cyclodextrin and the conjugate acid and base forms of nine phenols were measured in aqueous solution at 25°. The potentiometric method, in which the apparent acid dissociation constant of the phenol is measured as a function of cyclodextrin concentration, was supplemented by a modified version of a competitive spectrophotometric methyl orange method. For all phenols, the 1:1 stability constant for the conjugate base form (K_{11b}) was larger than K_{11a} for the conjugate acid form. Finite K_{12b} values were found for phenols whose 4-substituents could tolerate a positive charge by electron delocalization. Complex stability, as measured by K_{11a} and K_{11b}, increases with electron density and polarizability at the 4-substituent. It is concluded that the 4-substituent is the sole or predominant site of binding for both the conjugate acid and base forms of the phenols. The general result that K_{11b} is greater than K_{11a} for any phenol is accounted for by relative delocalization of charge in the anion and neutral species.

Keyphrases □ α-Cyclodextrin—complex formation with 4-substituted phenols, stability constants, potentiometric and competitive spectrophotometric methods □ 4-Substituted phenols—complex formation with α-cyclodextrin, stability constants, potentiometric and competitive spectrophotometric methods □ Competitive spectrophotometry—with methyl orange, α-cyclodextrin–4-substituted phenol complexation, stability constants, comparison with potentiometric methods

In an earlier paper (1), the potentiometric method for studying cyclodextrin complexes was applied to a series of 4-substituted benzoic acids. This method has now been used to investigate the complexing of α-cyclodextrin with 4-substituted phenols.

THEORETICAL

Potentiometric Method—The theory of the method was described earlier (1); only a summary of the points pertinent to the present application is given here. The symbolism is identical with that used in the prior treatment (1).

The procedure is to measure the apparent dissociation constant (pK_a') of the phenol (substrate) at total concentration S_t in the presence of a total concentration L_t of α-cyclodextrin (the ligand), L_t being varied from zero up to nearly its solubility limit. The quantity $\Delta pK_a'$ is defined by:

$$\Delta pK_a' = pK_a' - pK_a \qquad \text{(Eq. 1)}$$

where pK_a is the dissociation constant when $L_t = 0$. The theory of the method (1), for a system that may contain 1:1 (SL) and 1:2 (SL_2) complexes of both the conjugate acid and base forms of the substrate, shows that $\Delta pK_a'$ is related to the complex stability constants by:

$$\Delta pK_a' = \log C = \log\left(\frac{1 + K_{11a}[L] + K_{11a}K_{12a}[L]^2}{1 + K_{11b}[L] + K_{11b}K_{12b}[L]^2}\right) \qquad \text{(Eq. 2)}$$

where K_{11a}, K_{12a}, K_{11b}, and K_{12b} are stepwise complex stability constants, the numerical subscripts indicating stoichiometry and the letters the conjugate acid–base form, and [L] is the equilibrium (unbound) concentration of ligand. For carboxylic acids it appears to be a general result that $\Delta pK_a'$ is a positive quantity (1–3); hence, C is greater than unity. For phenols, however, $\Delta pK_a'$ is negative, so C is smaller than one, and it becomes more convenient to work with its reciprocal C'. The basic relationship is then:

$$C' = \frac{1 + K_{11b}[L] + K_{11b}K_{12b}[L]^2}{1 + K_{11a}[L] + K_{11a}K_{12a}[L]^2} \qquad \text{(Eq. 3)}$$

All of the systems here can be described as special cases of Eq. 3. The particular case to which a system belongs is diagnosed by plotting C' against L_t; then, an appropriate linearized plotting form is applied to extract stability constant estimates. Since $L_t \neq [L]$, the free concentration [L] is calculated, and iterations are made until the stability constant estimates converge. The four special cases of Eq. 3, the linear plotting forms, and the equations for the calculation of [L] are:

Case I:

$$C' = \frac{1 + K_{11b}[L]}{1 + K_{11a}[L]} \qquad \text{(Eq. 4)}$$

$$\frac{C' - 1}{[L]} = K_{11b} - C'K_{11a} \qquad \text{(Eq. 5)}$$

$$[L] = L_t - \frac{S_t}{X + 1} \qquad \text{(Eq. 6)}$$

where:

$$X = \frac{(C' + 1)(R' - C')}{(C' - 1)(R' + C')} \qquad \text{(Eq. 7)}$$

and $R' = K_{11b}/K_{11a}$.

Case II:

$$C' = 1 + K_{11b}[L] \qquad \text{(Eq. 8)}$$

$$[L] = L_t - \frac{(C' - 1)}{2C'} S_t \qquad \text{(Eq. 9)}$$

Case III:

$$C' = 1 + K_{11b}[L] + K_{11b}K_{12b}[L]^2 \qquad \text{(Eq. 10)}$$

$$\frac{C' - 1}{[L]} = K_{11b} + K_{11b}K_{12b}[L] \qquad \text{(Eq. 11)}$$

$$[L] = \frac{2C'(L_t - S_t) + 2S_t}{2C' - S_tK_{11b}} \qquad \text{(Eq. 12)}$$

Case IV:

$$C' = \frac{1 + K_{11b}[L] + K_{11b}K_{12b}[L]^2}{1 + K_{11a}[L]} \qquad \text{(Eq. 13)}$$

$$\frac{C' - 1}{[L]} + C'K_{11a} = K_{11b} + K_{11b}K_{12b}[L] \qquad \text{(Eq. 14)}$$

$$K_{11a}[L]^2 + \left[1 + S_tK_{11a}\left(\frac{3}{2} - \frac{R'}{2C'}\right) - L_tK_{11a}\right][L] - \left[L_t - \frac{S_t(C' - 1)}{C'}\right] = 0 \qquad \text{(Eq. 15)}$$

To make the case IV linear plot according to Eq. 14, K_{11a} must be known. For case IV systems, K_{11a} was determined by a competitive spectrophotometric method as described in the following section.

Competitive Indicator Method—In 1953 Broser (4) showed that methyl orange and α-cyclodextrin form a complex in acidic solution, the complexed form of the indicator absorbing light much less intensely than the free form. If a third solute, capable of forming a cyclodextrin complex, is added to the solution, some of the complexed indicator will be competitively displaced, with a corresponding increase in the absorption intensity. This is the basis of the competitive methyl orange spectrophotometric method, which was used by Lautsch *et al.* (5) to study adrenalin–α-cyclodextrin complex formation. Casu and Ravà applied this method to measure K_{11a} values for many substituted benzoic acid substrates (6). The stability constants reported by these workers were, however, not in good agreement with those found potentiometrically in this laboratory (1). For this reason, the methyl orange method has been reexamined and modified as follows.

0022-3549/83/1100-1333$01.00/0

Let S represent the substrate of interest, L the cyclodextrin, and I the indicator. The competitive complexation equilibrium is shown in Scheme I:

$$IL + S \rightleftarrows SL + I$$

Scheme I

Mass balance equations on the substrate, ligand, and indicator are:

$$S_t = [S] + [SL] \quad \text{(Eq. 16)}$$

$$L_t = [L] + [SL] + [IL] \quad \text{(Eq. 17)}$$

$$I_t = [I] + [IL] \quad \text{(Eq. 18)}$$

(It is, in general, not permissible to neglect the [IL] term in Eq. 17.) Stability constants for the complexes SL and IL are defined, where 1:1 stoichiometry is assumed, as:

$$K_{11S} = \frac{[SL]}{[S][L]} \quad \text{(Eq. 20)}$$

$$K_{11I} = \frac{[IL]}{[I][L]} \quad \text{(Eq. 21)}$$

In the systems of present interest, where the substrate is the conjugate acid form of a phenol, K_{11S} is equivalent to K_{11a}.

Eqs. 16–21 are combined to give:

$$L_t = [L] + \frac{K_{11S}[L]S_t}{1 + K_{11S}[L]} + \frac{K_{11I}[L]I_t}{1 + K_{11I}[L]} \quad \text{(Eq. 22)}$$

Defining the indicator ratio $Q = [I]/[IL]$ allows Eq. 21 to be written as $K_{11I} = 1/Q[L]$, which, substituted into Eq. 22, gives:

$$L_t = \frac{1}{QK_{11I}} + \frac{S_tK_{11S}}{QK_{11I} + K_{11S}} + \frac{I_t}{Q + 1} \quad \text{(Eq. 23)}$$

The quantity P is defined as:

$$P = L_t - \frac{1}{QK_{11I}} - \frac{I_t}{Q + 1} \quad \text{(Eq. 24)}$$

Therefore, Eq. 23 may be written:

$$P = \frac{S_tK_{11S}}{QK_{11I} + K_{11S}}$$

or

$$\frac{S_t}{P} = \frac{K_{11I}}{K_{11S}}Q + 1 \quad \text{(Eq. 25)}$$

The indicator ratio Q can be measured by means of:

$$Q = \frac{\epsilon - \epsilon_{IL}}{\epsilon_I - \epsilon} \quad \text{(Eq. 26)}$$

where ϵ_I and ϵ_{IL} are the molar absorptivities of free and complexed indicator, respectively, and ϵ is the apparent molar absorptivity in any solvent containing both forms. If the total indicator concentration is constant in all solutions, the absorptivities can be replaced by absorbances.

K_{11I} can be obtained by independent measurements on solutions of indicator and ligand by the conventional spectrophotometric method. Eq. 25 therefore provides a graphical approach to determining K_{11S} by plotting S_t/P against Q, where P is obtained using Eq. 24.

The possible formation of a substrate–indicator complex (SI) has been analyzed (7) for its effect on the determination of K_{11S}. It was found that such an effect can be minimized if S_t and [I] are small; [I] is small when Q is small, when I_t is small, or when L_t is large. Another complicating factor is self-association of the indicator. DeVylder and DeKeukeleire (8) observed self-association of methyl orange in pH 2.2 aqueous solutions at indicator concentrations $>10^{-4}$ M. In the present work I_t was 1.67×10^{-5} M; hence, self-association could be neglected.

According to Eq. 25, the best estimate of K_{11S} should be possible if K_{11I} is approximately equal to K_{11S}, for then the slope will be approximately one. The method may therefore be useful for a wide range of substrates if indicators are identified with appropriate K_{11I} values.

EXPERIMENTAL

Materials—α-Cyclodextrin[1] was dried at 95° for 48 hr. Methyl orange[2] was recrystallized from water, then washed with ethanol followed by ether (9); its molar absorptivity in 0.08 M HCl at 508 nm was 4.82×10^4. Solid phenols[3] were recrystallized until their melting points agreed with literature values. 4-Methoxyphenol was distilled under reduced pressure; bp 111°/2 mm Hg, mp 55°. Water was redistilled from alkaline permanganate.

Potentiometric Studies—A stock solution of the phenol was prepared such that its final diluted total concentration (S_t) would be 0.003–0.004 M, and the phenol was half-neutralized with 0.10 M NaOH. The solution was brought to the mark with 0.10 M NaCl.

α-Cyclodextrin was weighed into 5-ml volumetric flasks in amounts that covered its full solubility range. Portions (4.0 ml) of the phenol stock solution were pipetted into each flask, and the solutions were brought to volume with 0.10 M NaCl. They were equilibrated at 25.0; to ensure adequate mixing, a 10-mm stirring bar was added and was driven by a submerged water-driven magnetic stirrer. The solution was transferred to a 5-ml test tube, and the combination pH electrode was lowered into the solution, which was protected from contact with the atmosphere. The pH was measured[4], and pK_a' was calculated using:

$$pK_a' = pH - \log \frac{b + [H^+] - [OH^-]}{S_t - (b + [H^+] - [OH^-])} \quad \text{(Eq. 27)}$$

where b is the added concentration of sodium hydroxide; in these studies $b = S_t/2$. Reproducibility on duplicate solutions was 0.003 pH unit or better. All studies were at 25.0° and ionic strength 0.10 M.

Competitive Methyl Orange Spectrophotometric Method—Portions (4.0 ml) of a stock solution containing 4.185×10^{-5} M methyl orange in 0.20 M HCl were pipetted into 10.0-ml volumetric flasks containing 2.0 ml of 0.01 M α-cyclodextrin aqueous solution. Substrate (phenol) solutions at variable concentrations such that the final substrate concentrations (S_t) were from 2.0×10^{-3} M to 1.38×10^{-2} M were added to the flasks, and the solutions were brought to volume. The reference solution was prepared with the same concentration of α-cyclodextrin in 0.08 M HCl. The final solutions were equilibrated at 25.0°, and absorbances were read[5] at 508 nm in 1-cm cells. For calculating Q by Eq. 26, the molar absorptivities were measured in 0.08 M HCl at 508 nm, giving $\epsilon_I = 4.82 \times 10^4$ and $\epsilon_{IL} = 8.72 \times 10^2$. The stability constant K_{11I} for the 1:1 complex of methyl orange with α-cyclodextrin at 25.0° in 0.08 M HCl was determined by the conventional Benesi–Hildebrand spectrophotometric method (10); K_{11I} was found to be 672.9 M^{-1} (standard deviation 5.0 M^{-1}).

RESULTS

Nine phenols were studied by the potentiometric method. 4-Cyanophenol and 4-nitrophenol could be described as case I systems; Fig. 1 shows the plot of C' against L_t for the 4-cyanophenol–α-cyclodextrin system, and Fig. 2 is the corresponding plot according to Eq. 5. Phenol, 4-methylphenol, and 4-fluorophenol were case II systems, as indicated for 4-methylphenol in Fig. 3. The C' *versus* L_t plot for 4-methoxyphenol (Fig. 4) shows that it is a case III system; Fig. 5 is the linearized plot according to Eq. 11 for this system. 4-Iodophenol, 4-bromophenol, and 4-chlorophenol showed case IV behavior, as seen in Fig. 6 for 4-chlorophenol.

For case IV systems, an independent study is necessary to determine K_{11a} for use in Eq. 14. The UV absorption spectra of these compounds were not significantly altered by the presence of α-cyclodextrin, so simple UV spectrophotometry did not provide a method for measuring K_{11a}. The competitive methyl orange spectrophotometric method was therefore used. Since the potentiometric data showed that these were case IV systems, evidently $K_{12a} = 0$, and only 1:1 stoichiometry need be considered for the conjugate acid forms of the substrates. The studies were made at constant L_t and varying S_t. Figure 7 is the plot according to Eq. 25 for the 4-chlorophenol–methyl orange–α-cyclodextrin system. Figure 8 shows the linear plot of Eq. 14 for 4-chlorophenol, in which the K_{11a} value determined from Fig. 7 has been used to construct the ordinate values.

The validity of the assignments of case I systems was tested by treating them according to case IV equation (Eq. 14), which should give a slope of zero for a case I system. Similarly, case II assignments were tested by plotting according to the case III equation. The stability constants are listed in Table I. The K_{12b} values for the methoxy, iodo, bromo, and chloro compounds are clearly significant; these are the case III and IV systems. The K_{12b} values evaluated for the case I and II systems are not significantly different from zero, except for phenol. However, the corre-

[1] Sigma Chemical Co. (Lot 29C-0425).
[2] Eastman Organic Chemicals.
[3] Aldrich Chemical Co. These compounds were purified by A. B. Wong.
[4] Orion 701A pH meter equipped with a Sargent-Welch S30072-15 electrode.
[5] On Cary Model 14 or Perkin-Elmer Model 559 spectrophotometers.

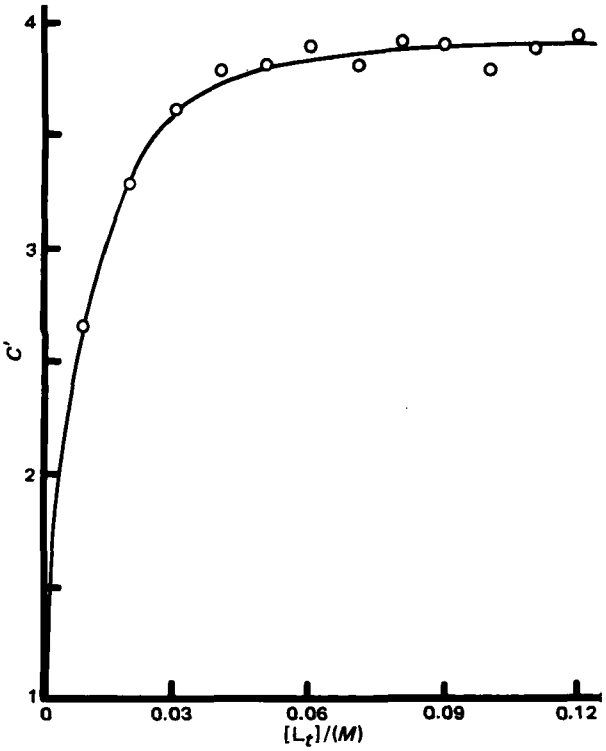


Figure 1—*Plot of C′ against L_t for 4-cyanophenol–α-cyclodextrin, a case I system.*

lation coefficient was 0.998 for the case II linear plot, whereas it was 0.553 for treatment as a case III system; hence, phenol is better described as a case II system, with K_{12b} equal to zero.

DISCUSSION

Competitive Indicator Method—To test the competitive methyl orange spectrophotometric method, it was applied to the determination of K_{11a} for 4-nitrophenol, which can be measured by several techniques. Table II lists stability constants for the 4-nitrophenol–α-cyclodextrin

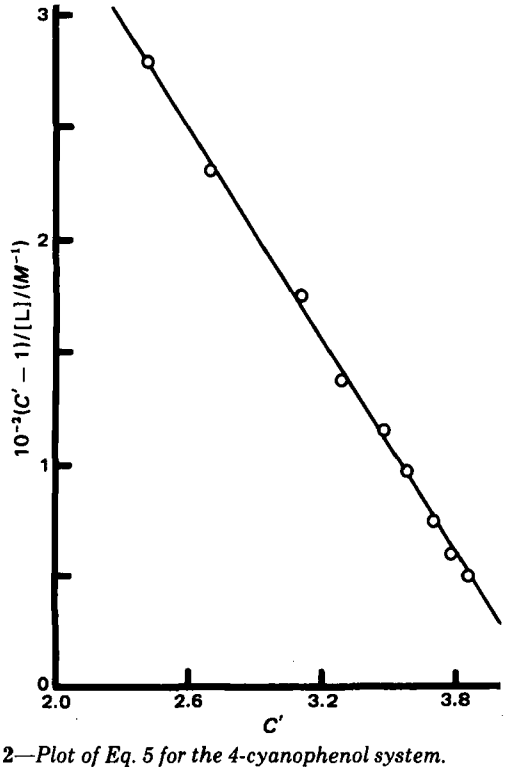


Figure 2—*Plot of Eq. 5 for the 4-cyanophenol system.*

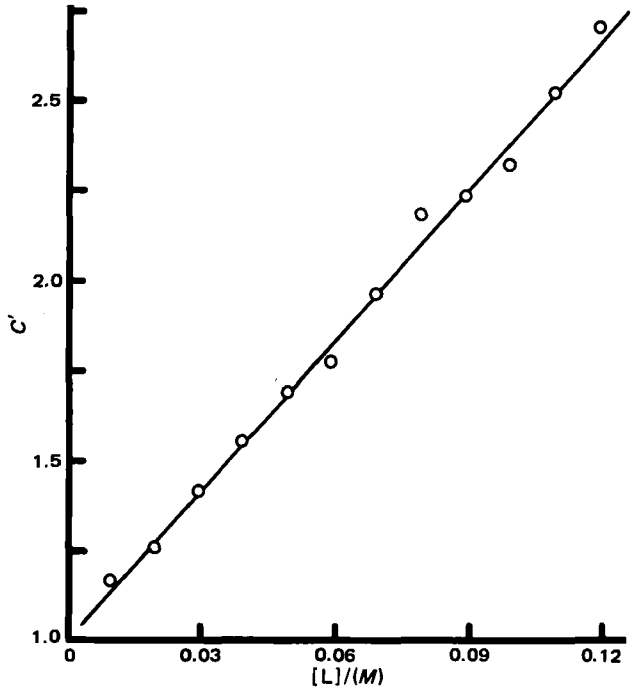


Figure 3—*Plot of Eq. 8 for the 4-methylphenol system.*

system determined in this work and as reported by other investigators. It is clear that the methyl orange method gives a result consistent with other methods. The methyl orange method was also applied to the benzoic acid–α-cyclodextrin system, yielding K_{11a} = 810 M^{-1}. This is significantly lower than the value 1050 M^{-1} reported by Casu and Ravà (6) for this system studied by their version of the competitive methyl orange method (which neglects the [IL] term in Eq. 17), but is consistent with recent values determined by other methods (1).

The method as described, using methyl orange as the indicator, is applicable only in an acidic solution, but with other indicators it may be useful under other conditions. The plot according to Eq. 25 should yield an intercept of unity, and this was verified in the present applications. When K_{11I} is approximately equal to K_{11S}, competition to displace the

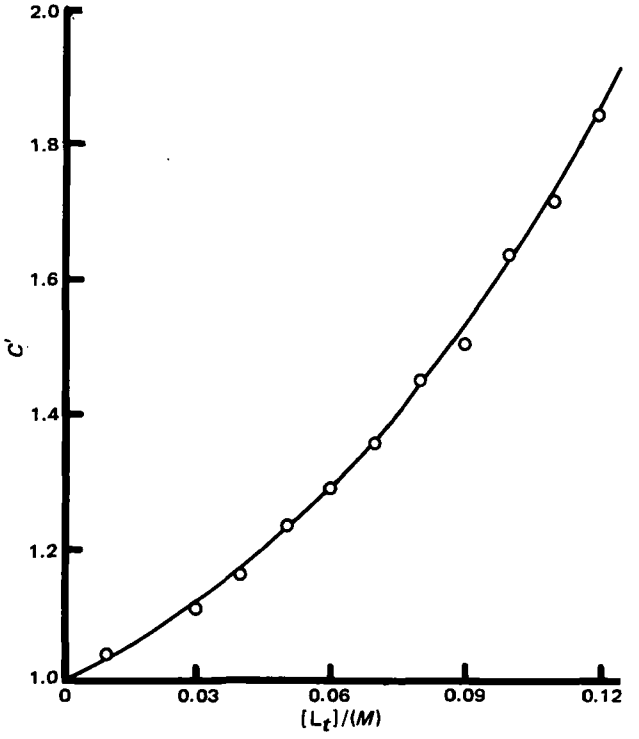


Figure 4—*Plot of C′ against L_t for 4-methoxyphenol, a case III system.*

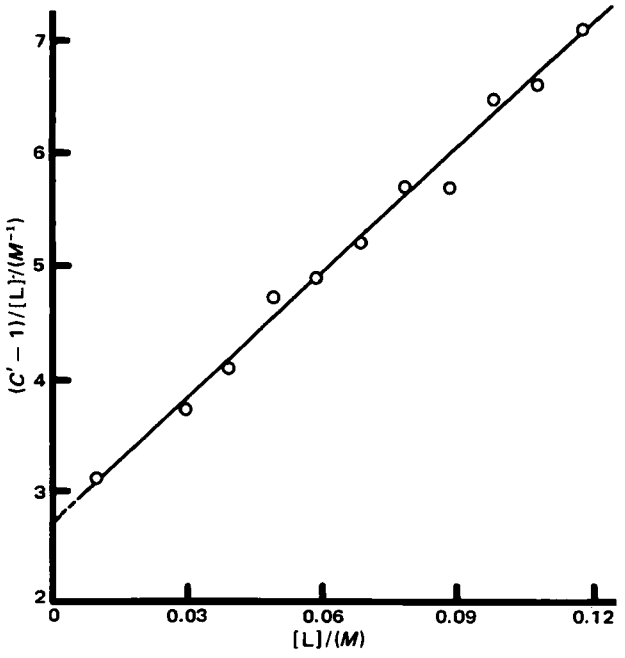


Figure 5—*Plot of Eq. 11 for the 4-methoxyphenol system.*

indicator requires that L_t and S_t be of comparable magnitude, although L_t can be much larger than I_t. If K_{11S} is much larger than K_{11I}, a smaller concentration of substrate would suffice to displace the indicator from its complex. Equation 25 can accommodate changes in both S_t and L_t in an experiment, but it was found convenient to hold L_t constant and to vary S_t.

Binding Sites and Complex Stability—These phenolic compounds can be viewed as substrates having two possible binding sites, namely the hydroxyl group and the 4-substituted moiety, that can interact with the cavity of the α-cyclodextrin. A model for such a system was described earlier (1, 18). For a two-binding site substrate that forms only 1:1 and 1:2 complexes, the experimental stability constants K_{11} and K_{12} are related to the binding site stability constants by:

$$K_{11} = K_X + K_Y \qquad \text{(Eq. 28)}$$

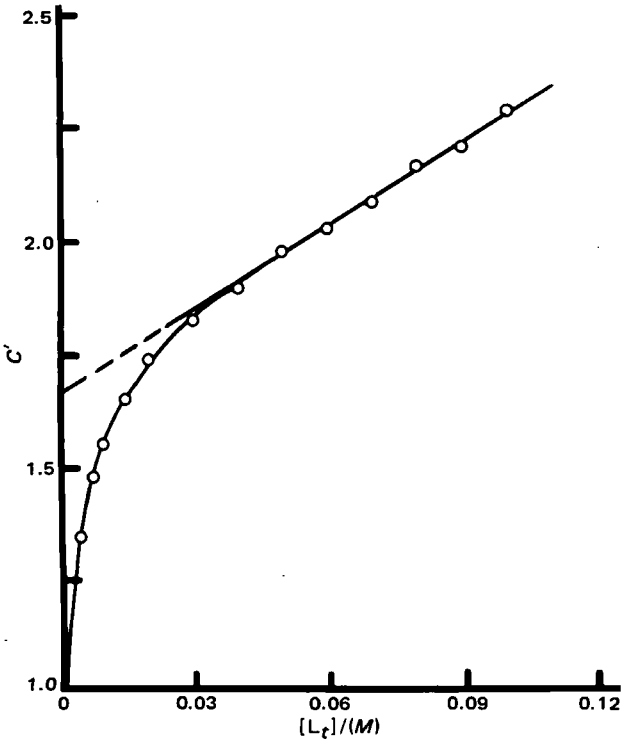


Figure 6—*Plot of C′ against L_t for 4-chlorophenol, a case IV system.*

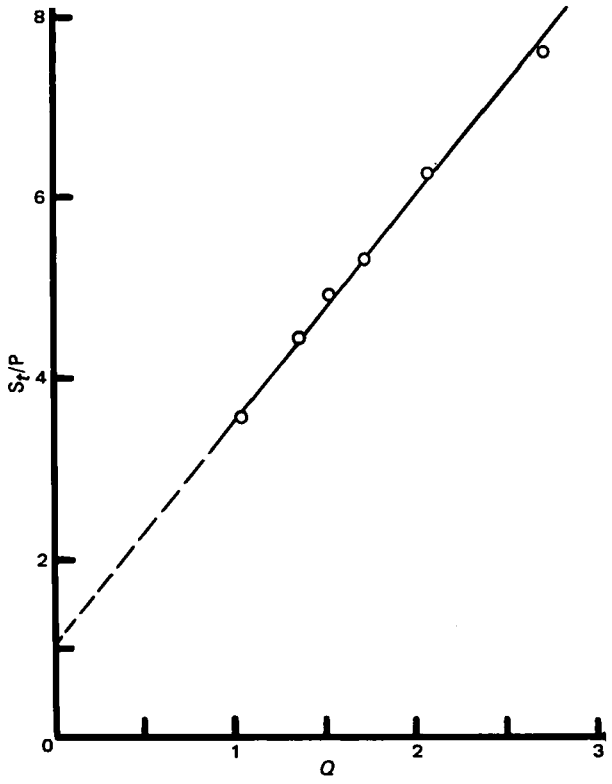


Figure 7—*Plot of Eq. 25 for the 4-chlorophenol–methyl orange–α-cyclodextrin system.*

$$K_{12} = \frac{aK_XK_Y}{K_{11}} \qquad \text{(Eq. 29)}$$

In these relationships K_X and K_Y are 1:1 stability constants for binding to sites X and Y, and a is a parameter that measures interaction between the two sites in a 1:2 complex. Such a two-site substrate is therefore capable of forming two isomeric 1:1 complexes and one 1:2 complex. This model leads to interpretations in terms of binding sites. The derivation

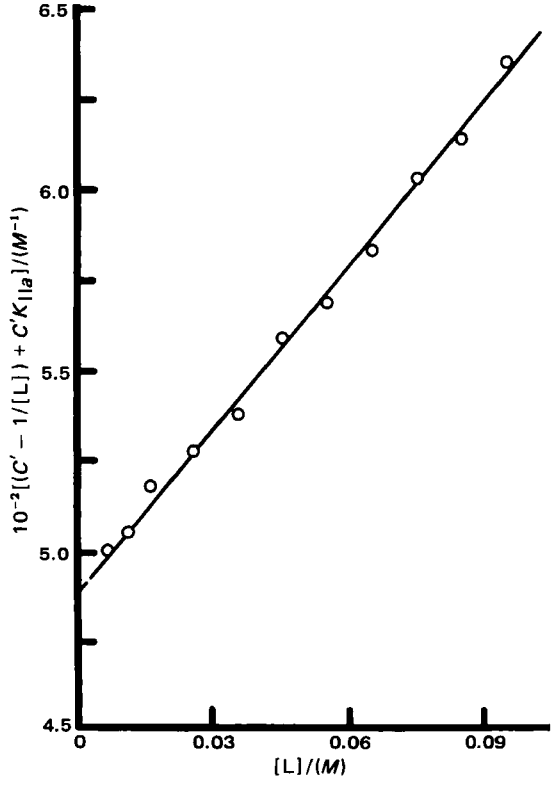


Figure 8—*Plot of Eq. 14 for the 4-chlorophenol system.*

Table I—Stability Constants for α-Cyclodextrin Complexes of 4-Substituted Phenols at 25° [a]

4-Substituent	K_{11b}, M^{-1}	K_{12b}, M^{-1}	K_{11a}, M^{-1}
OCH_3	2.7 (0.10)	13.3 (0.68)	0
CH_3	13.9 (0.34)	−0.08 (0.36)	0
H	10.9 (0.19)	0.46 (0.21)	0
COO^- [b]	—	—	16.6 (0.23)
F	15.6 (0.70)	−1.00 (0.95)	0
I	3955 (26.3)	2.4 (0.09)	2316 (65.5)
Cl	487.9 (1.6)	3.1 (0.06)	272 (9.9)
Br	1221 (7.4)	4.7 (0.09)	704 (31.8)
COOH [b]	—	—	1130 (7.7)
CN	662 (9.0)	0.09 (0.08)	158.3 (2.7)
NO_2	2408 (87.6)	−0.14 (0.16)	245 (10.2)

[a] Standard deviations in parentheses. [b] Taken from Ref. 1.

of Eq. 29 is given elsewhere (1), and the significance of a has also been discussed previously (1).

Whether a cyclodextrin interacts with a binding site is largely dependent on the size and shape of the site; but if the site can enter the cyclodextrin cavity to form an inclusion complex, the strength of the complex will be controlled mainly by the electron density, the polarizability, and the polarity of the binding site. Increases in site electron density and polarizability will tend to increase complex stability, whereas high polarity will decrease complex stability (in a polar solvent). In a series of closely related substrates, the trend of complex stabilities with these factors may therefore give information about the binding sites. In this way Hammett plots of K_{11a} and K_{11b} for 4-substituted benzoic acids (1) led to the conclusion that K_{11a} describes mainly binding to the carboxylic acid site, whereas K_{11b} describes binding solely at the 4-substituent site.

This approach will be used to discuss the data in Table I. It is postulated that complex stability in a series is primarily determined by site electron density, modified by polarizability and polarity. The substrates in Table I are arranged in order of increasing Hammett substituent constant σ for the 4-substituent; σ is a measure of the electron-attracting ability of the substituent. For both K_{11a} and K_{11b} the same rough trend is seen, namely an increase in complex stability with increasing electron density at the 4-substituent. These data therefore suggest that the primary binding site, for both the conjugate acid and the conjugate base forms of the substrates, is the 4-substituent end of the molecule.

The correlations of K_{11a} and K_{11b} with σ are obviously not closely followed, the halogens I and Br having complex-strengthening effects not accounted for by their σ values. But these substituents have high polarizabilities, which will tend to increase complex stability; K_{11a} and K_{11b} can be well correlated by:

$$\log K_{11a} = -0.96 + 0.12R_D - 0.10\mu \qquad \text{(Eq. 30)}$$

$$\log K_{11b} = -1.41 + 5.33\sigma + 0.15R_D - 0.81\mu \qquad \text{(Eq. 31)}$$

where R_D is the molar refraction of $X—C_6H_5$ and μ is the dipole moment of $X—C_6H_4—OH$[6]. These correlations have no theoretical significance, but they suggest that this interpretation is reasonable.

If K_{11b} and K_{11a} describe binding at the 4-substituent, then K_{12b} and K_{12a} describe binding at the hydroxyl site, because the 1:2 complex is formed by adding a second cyclodextrin to the 1:1 complex. But K_{12a} is zero for all systems, leading to the conclusion that no significant binding occurs at the phenolic hydroxyl site; this conclusion applies also to those phenolate substrates for which K_{12b} is zero. There exist, however, some finite K_{12b} values, and it is notable that in each of these substrates (the methoxy, iodo, chloro, and bromo compounds) the 4-substituent can tolerate a positive charge by electron release through resonance delocalization; this may sufficiently counter the electron release of the phenolate site to allow significant binding at that site.

K_{11a} for the phenol $^-OOC—C_6H_4—OH$ is evidently the same quantity as K_{11b} for the benzoic acid anion $HO—C_6H_4—COO^-$. When this was discussed as a benzoate (1), it was concluded that the carboxylate site binding was negligible, leaving binding at the phenolic site to account for the observed binding. On the other hand, it has been concluded here that binding at the hydroxyl site in phenols is probably negligible. This inconsistency suggests that it may not always be permissible to make a common assignment of binding mode to all of the members of a series. In this case, the negative charge on the carboxylate may oppose the usual electron release by the hydroxyl group, leaving the hydroxyl site susceptible to binding.

[6] The correlation coefficients are 0.99 for both equations. The standard deviations are 0.06 in log K_{11a} and 0.13 in log K_{11b}.

Table II—Comparison of Stability Constants for the 4-Nitrophenol–α-Cyclodextrin System [a]

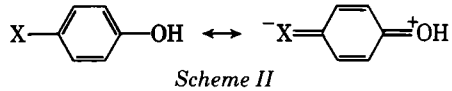
Method	K_{11b}, M^{-1}	K_{11a}, M^{-1}	Reference
Spectrophotometry	2230	290	11
Thermometry	—	126	12
Potentiometry	2200	200	2
Spectrophotometry	2512	190	13
Polarography	2439	—	14
Potentiometry	2143	211	15
Spectrophotometry	2720	250	16
Potentiometry	2382	270	17
Potentiometry	2408	245	This work
Spectrophotometry [b]	—	249	This work
Competitive spectrophotometry [c]	—	249	This work

[a] At 25°; ionic strengths differ. [b] Studied at 317 nm. [c] Methyl orange method described herein.

Effect of Phenol Ionization on Complex Stability—It is a general result that K_{11b} is greater than K_{11a} for the cyclodextrin complexes of phenols (Table I); that is, the anionic form of the substrate "partitions" into the relatively nonpolar cyclodextrin cavity more favorably than does the un-ionized form. This unexpected behavior (which is the reverse of the situation seen with carboxylic acid substrates) has been discussed for the particular case of 4-nitrophenol by several authors. Dunn and Bernhard (19) accounted for the greater stability of the 4-nitrophenoxide–α-cyclodextrin complex in terms of a stronger dispersion force field between the included nitrophenoxide and the interior of the cavity. These authors assumed that the nitro end of the substrate was inserted into the cavity. Bergeron and Channing (20) have shown that this is in fact the geometry of the complex. Benzoic acid, on the other hand, complexes with the carboxylic acid end of the molecule penetrating the cavity (1, 15, 21). Bergeron *et al.* (21) have called attention to this difference in substrate–ligand orientation and have related it to the corresponding difference in relative stabilities of the neutral and charged complexes. Their argument is that, if induced dipole–dipole interaction is primarily responsible for binding, the nitrophenolate will be more strongly bound than is the neutral phenol.

It is not necessary to specify the types of forces responsible for binding to account generally for the result that $K_{11b} > K_{11a}$. This result is surprising only if an ion is regarded as a point charge. But a phenolate ion is decidedly not a point charge on the molecular scale. The concept described in the preceding section, namely that complex stability is determined by *binding site* electron density, polarizability, and polarity, will be applied to the phenol and phenolate complexes with cyclodextrin.

Neutral phenols are highly dipolar species, especially those with electron-withdrawing 4-substituents, as revealed by their dipole moments, which can be accounted for by electron delocalization as shown in Scheme II.



Scheme II

Electron density is increased at the 4-substituent and is decreased at the hydroxyl group; moreover the polarizability of the hydroxyl group is decreased. Thus, complexing with the cyclodextrin cavity should occur at the 4-substituent site rather than at the hydroxyl site, and this is consistent with the trend of K_{11a} values as discussed above.

On ionization of the hydroxyl group, delocalization disperses the excess electronic charge as in Scheme III.

Scheme III

The electron density at the 4-substituent is greater in the anion than in the neutral form, hence the complex stability at this binding site is greater in the anion. As shown above, K_{11a} represents solely binding at the 4-substituent, and K_{11b} represents predominantly 4-substituent binding. (Those substrates with finite K_{12b} values include, in K_{11b}, a small contribution from binding at the hydroxyl site, according to Eq. 28.) Therefore, K_{11b} will be larger than K_{11a}. Moreover, the increased charge density on the oxygen in the phenolate species actually may lead to some binding at this site, which in the current context is actually less polar than the hydroxyl group in the neutral substrate. Localized negative charge

(as in carboxylic acid anions) may be destabilizing, positive charge (as in protonated amines) will always be destabilizing, but delocalized negative charge (as in phenol anions) may be stabilizing.

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ACKNOWLEDGMENTS

This study was supported in part by a grant from The Upjohn Company. Preliminary studies by Dr. Albert B. Wong are gratefully acknowledged.

Improvement of the Oral Bioavailability of Digitalis Glycosides by Cyclodextrin Complexation

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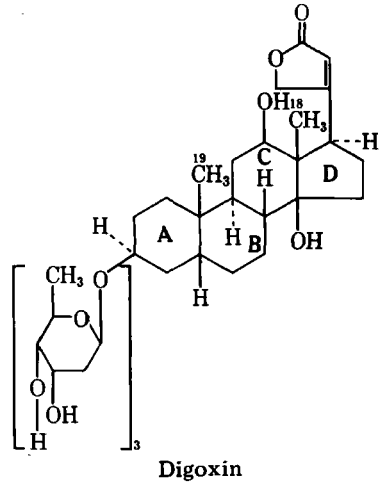
Abstract □ Inclusion complexes of the digitalis glycosides digitoxin, digoxin, and methyl digoxin with three cyclodextrins (α-, β-, γ-homologues) in water and in the solid state were studied by a solubility method, IR and ^{1}H-NMR spectroscopy, and X-ray diffractometry. Solid complexes (in a molar ratio of 1:4) of the digitalis glycosides with γ-cyclodextrin were prepared and their *in vivo* absorption examined. The rapidly dissolving form of the γ-cyclodextrin complex significantly increased plasma levels of digoxin (~5.4-fold) after oral administration to dogs.

Keyphrases □ Bioavailability—oral, digoxin, digitoxin, methyl digoxin, complexation with cyclodextrins □ Digoxin—complexation with cyclodextrins, oral bioavailability, digitoxin, methyl digoxin □ Cyclodextrins—complexation with digitalis glycosides, oral bioavailability, digoxin, digitoxin, methyl digoxin

The bioavailability of the digitalis glycosides from commercial tablets varies significantly (1–3). The main cause of this variability appears to be related to such factors as low water solubility (4–6) and chemical instability in acidic media (7–9). Cyclodextrins have been used extensively to improve various physicochemical properties of drug molecules (10–12) by forming inclusion complexes in which the drug molecules are included in the relatively hydrophobic cavity of the cyclodextrins (13).

The present study describes the inclusion complexes of the digitalis glycosides digitoxin, digoxin, and methyl digoxin with the three cyclodextrins (α-, β-, γ-homologues). Complex formation in water and in the solid state was studied by a solubility method, IR and ^{1}H-NMR spectroscopy, and X-ray diffractometry. Plasma levels of digoxin were determined after the oral administration of the digoxin–γ-cyclodextrin complex to dogs.



Digoxin

0022-3549/83/1100-1338$01.00/0