

Potentiometric Study of Molecular Complexes of Weak Acids and Bases Applied to Complexes of α -Cyclodextrin with *para*-Substituted Benzoic Acids

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Abstract □ The theory of the potentiometric method for studying complexes of ionizable substrates was developed, and graphical techniques are described for obtaining stability constant estimations from the data. The method described is for a system in which the conjugate acid and base forms of the substrate (S) are capable of forming 1:1 (SL) and 1:2 (SL₂) complexes with the ligand (L). It was applied to complexes of α -cyclodextrin (cyclohexaamylose) with 10 *para*-substituted benzoic acid derivatives. Letting K_{11a} and K_{12a} be stability constants for the conjugate acid forms of the substrates, and K_{11b} , K_{12b} for the conjugate base forms, it was found that K_{12b} is zero for all substrates, K_{12a} is zero for seven of the substrates, and $K_{11a} > K_{11b}$ in every case. Hammett plots yielded ρ_{11a} and ρ_{11b} values of -0.31 and 0.77 , respectively, which was interpreted to mean that K_{11a} mainly represents binding at the carboxylic acid site, and K_{11b} describes binding at the site of the *para*-substituent. This model of the complexing suggests that K_{12a} represents binding at the *para*-substituent, and therefore K_{12a} should vary roughly with substituent as K_{11b} does; this trend was observed.

Keyphrases □ Complexation—potentiometric study, α -cyclodextrins with *para*-substituted benzoic acids □ Stability constants— α -cyclodextrins with *para*-substituted benzoic acids, potentiometric studies □ α -Cyclodextrins—complexes with *para*-substituted benzoic acids, potentiometric study

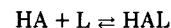
If the conjugate acid and base forms of a weak acid–base substrate form cyclodextrin (also called cycloamylose) complexes of different strengths, then addition of cyclodextrin to a solution of the substrate will result in a pH change. Cramer *et al.* (1) reported a change in the apparent dissociation constant of *p*-nitrophenol in the presence of α -cyclodextrin (cyclohexaamylose). A method was described previously based on the measurement of apparent dissociation constants as a function of cyclodextrin concentration in order to extract complex stability constants for both the conjugate acid and base forms of the substrate, assuming 1:1 stoichiometry (2). Miyaji *et al.* (3) developed a similar technique. A potentiometric method for 1:1 and 1:2 stoichiometric systems was also described (4, 5).

Evidence is accumulating (5–7) that in α -cyclodextrin systems both 1:1 and 1:2 complexes must be considered when measuring complex stability constants¹. The potentiometric method offers a rapid and convenient experimental approach to this problem for ionizable substrates. The present report describes the theory of the method, and examines new graphical techniques for evaluating the stability constants. These techniques are applied to the study of complexing between α -cyclodextrin and a series of *p*-substituted benzoic acids.

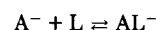
THEORY

Let L represent cyclodextrin and HA a neutral weak acid substrate. For 1:1 and 1:2 stoichiometry, there are four complexation equilibria:

¹ The weak acid–base is the substrate (S), α -cyclodextrin is the ligand (L), and stoichiometric relationships are expressed in the form SL and SL₂, these complexes having 1:1 and 1:2 stoichiometry, respectively.



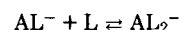
Scheme I



Scheme II



Scheme III



Scheme IV

These equilibria have corresponding stability constants:

$$K_{11a} = \frac{[HAL]}{[HA][L]} \quad (\text{Eq. 1})$$

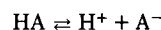
$$K_{11b} = \frac{[AL^-]}{[A^-][L]} \quad (\text{Eq. 2})$$

$$K_{12a} = \frac{[HAL_2]}{[HAL][L]} \quad (\text{Eq. 3})$$

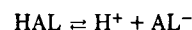
$$K_{12b} = \frac{[AL_2^-]}{[AL^-][L]} \quad (\text{Eq. 4})$$

where brackets signify molar concentrations, and activity coefficients are assumed to be constant. Thus, the stability constants may be interpreted as thermodynamic quantities with the experimental solvent as the reference state.

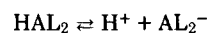
There are three acid–base equilibria in this system:



Scheme V



Scheme VI



Scheme VII

They have the acid–base dissociation constants:

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad (\text{Eq. 5})$$

$$K_{a11} = \frac{[H^+][AL^-]}{[HAL]} \quad (\text{Eq. 6})$$

$$K_{a12} = \frac{[H^+][AL_2^-]}{[HAL_2]} \quad (\text{Eq. 7})$$

From Eqs. 1–7 these relationships are found:

$$K_{11a}K_{a11} = K_aK_{11b} \quad (\text{Eq. 8})$$

$$K_{12a}K_{a12} = K_{a11}K_{12b} \quad (\text{Eq. 9})$$

Letting S_t and L_t be the total molar concentrations of substrate and ligand, respectively, the mass-balance equations are:

$$S_t = [HA] + [A^-] + [HAL] + [AL^-] + [HAL_2] + [AL_2^-] \quad (\text{Eq. 10})$$

$$L_t = [L] + [HAL] + [AL^-] + 2[HAL_2] + 2[AL_2^-] \quad (\text{Eq. 11})$$

For later convenience the quantities A, B, M, and, N are defined:

$$A = 1 + K_{11a}[L] + K_{11a}K_{12a}[L]^2 \quad (\text{Eq. 12})$$

$$B = 1 + K_{11b}[L] + K_{11b}K_{12b}[L]^2 \quad (\text{Eq. 13})$$

$$M = K_{11a}[L] + 2K_{11a}K_{12a}[L]^2 \quad (\text{Eq. 14})$$

$$N = K_{11b}[L] + 2K_{11b}K_{12b}[L]^2 \quad (\text{Eq. 15})$$

Algebraic combination leads to:

$$S_t = [\text{HA}] \left[\frac{A[\text{H}^+] + BK_a}{[\text{H}^+]} \right] \quad (\text{Eq. 16})$$

$$L_t = [\text{L}] + [\text{HA}] \left[\frac{M[\text{H}^+] + NK_a}{[\text{H}^+]} \right] \quad (\text{Eq. 17})$$

Eliminating $[\text{HA}]$ from Eqs. 16 and 17 gives a general relationship between $[\text{L}]$ and L_t :

$$L_t = [\text{L}] + S_t \left[\frac{M[\text{H}^+] + NK_a}{A[\text{H}^+] + BK_a} \right] \quad (\text{Eq. 18})$$

The electroneutrality equation for this system is:

$$[\text{Na}^+] + [\text{H}^+] = [\text{OH}^-] + [\text{A}^-] + [\text{AL}^-] + [\text{AL}_2^-] \quad (\text{Eq. 19})$$

where it is supposed that sodium is the counterion to the substrate. Combining Eq. 19 with the preceding expressions gives:

$$[\text{Na}^+] + [\text{H}^+] - [\text{OH}^-] = \frac{S_t BK_a}{A[\text{H}^+] + BK_a} \quad (\text{Eq. 20})$$

The quantity $[\text{Na}^+]/S_t$ is the analytical fraction of substrate in the conjugate base form. Equation 20 is the general equation relating hydrogen-ion concentration to free ligand concentration $[\text{L}]$. By means of Eqs. 18 and 20, $[\text{H}^+]$ is related to total ligand concentration L_t . In these equations, $[\text{Na}^+]$, S_t , and L_t are independent variables; $[\text{H}^+]$ and $[\text{L}]$ are dependent variables.

Equation 20 can be cast in a more useful form. If $[\text{Na}^+] \gg ([\text{H}^+] - [\text{OH}^-])$, then Eq. 20 becomes:

$$[\text{Na}^+] = \frac{S_t BK_a}{A[\text{H}^+] + BK_a} \quad (\text{Eq. 21})$$

For a solution in which L_t is 0, it follows that $[\text{L}] = 0$, $A = 1$, $B = 1$, and Eq. 21 becomes:

$$[\text{Na}^+]_0 = \frac{S_t K_a}{[\text{H}^+]_0 + K_a} \quad (\text{Eq. 22})$$

A series of measurements of $[\text{H}^+]$ as a function of L_t is made at constant S_t and $[\text{Na}^+]$; hence $[\text{Na}^+] = [\text{Na}^+]_0$, and these equations give:

$$\frac{[\text{H}^+]_0}{[\text{H}^+]} = \frac{A}{B} = C \quad (\text{Eq. 23})$$

Defining a quantity $\Delta\text{pH} = \text{pH} - \text{pH}_0$:

$$\Delta\text{pH} = \log C \quad (\text{Eq. 24})$$

Equation 24 describes the change in the solution pH as a function of free ligand concentration for fixed $[\text{Na}^+]/S_t$. If $[\text{Na}^+]/S_t = 1/2$, then the pH can be interpreted as pK'_a (apparent dissociation constant), and Eq. 24 becomes $\Delta\text{pK}'_a = \log C$, or:

$$\Delta\text{pK}'_a = \log \left[\frac{1 + K_{11a}[\text{L}] + K_{11a}K_{12a}[\text{L}]^2}{1 + K_{11b}[\text{L}] + K_{11b}K_{12b}[\text{L}]^2} \right] \quad (\text{Eq. 25})$$

which is the equation used previously (5) to interpret the cinnamic acid- α -cyclodextrin system. This derivation shows the level of approximation involved in interpreting ΔpH as $\Delta\text{pK}'_a$.

More generally, the exact Eq. 20 must be used. Defining the operational dissociation constant K'_a by Eq. 26:

$$K'_a = \frac{[\text{H}^+]([\text{Na}^+] + [\text{H}^+] - [\text{OH}^-])}{S_t - ([\text{Na}^+] + [\text{H}^+] - [\text{OH}^-])} \quad (\text{Eq. 26})$$

Substitution into Eq. 26 from Eq. 20 leads to $K'_a/K_a = A/B = C$, or $\Delta\text{pK}'_a = \log C$. That is, Eq. 25 is general, provided the dissociation constants are evaluated by Eq. 26, which takes into account solvent dissociation. An important feature of this equation is that C is a ratio of polynomials. If $\Delta\text{pK}'_a = 0$ at all L_t , then $K_{11a} = K_{11b}$ and $K_{12a} = K_{12b}$.

On chemical grounds, and also by means of Eq. 18, it can be seen that the limits of $[\text{L}]$ are $[\text{L}] = L_t$ and $[\text{L}] = L_t - 2S_t$. In the potentiometric method, S_t must be appreciable in order to provide buffer capacity; hence it is seldom permissible to set $[\text{L}] = L_t$, as may often be done in other experimental techniques (such as spectroscopy).

One approach to obtaining the stability constants is to treat them as adjustable parameters, and to curve-fit the data using Eqs. 18 and 25. Although it is possible to achieve good fit to the experimental points (2, 8), it was observed that an apparently infinite number of satisfactory solutions can be obtained in this way (8). Therefore, the curve-fitting approach was abandoned, and graphical techniques leading to unique solutions have been developed.

General Evaluation of Stability Constants—The experimental data consist of $\Delta\text{pK}'_a$ values measured as a function of L_t , at constant S_t and reaction conditions. Since $\Delta\text{pK}'_a = \log C$, the quantity C is available. This is related to the stability constants by:

$$C = \frac{1 + K_{11a}[\text{L}] + K_{11a}K_{12a}[\text{L}]^2}{1 + K_{11b}[\text{L}] + K_{11b}K_{12b}[\text{L}]^2} \quad (\text{Eq. 27})$$

The problem is to evaluate the stability constants from these data. Considerable experience with such systems has shown that it is seldom necessary to invoke all four constants, and some important special cases of Eq. 27 suffice to account for most of these systems. These cases are treated in the following section.

To find the stability constants, Eq. 18 must be solved for $[\text{L}]$, but this requires the stability constants. The following approach is effective. In this work, the ratio $[\text{Na}^+]/S_t = 1/2$, so $[\text{H}^+] = K_a$, to a level of accuracy acceptable for the present purpose. Since $C = K_a/K'_a$, this gives $[\text{H}^+] = K_a/C$. Substitution into Eq. 18 gives:

$$L_t = [\text{L}] + S_t \left[\frac{M}{2A} + \frac{N}{2B} \right] \quad (\text{Eq. 28})$$

The value of $[\text{L}]$ is estimated by combining Eq. 28 with the appropriate special case of Eq. 27. In this development the quantity C is incorporated into the expression for $[\text{L}]$ to the greatest extent possible. This has two advantages: first, since the stability constants are needed in the calculation, their introduction in the form of experimental C values ensures that the correct values are being used; and second, the solution of the equation for $[\text{L}]$ is usually simplified, since much of the algebraic complexity is possessed by the numerical C values. The particular equations for $[\text{L}]$ will be given when the special cases are discussed.

The general approach is to make a plot of C versus L_t . From the shape of this curve, the system is tentatively assigned to one of the special cases. The free ligand concentration $[\text{L}]$ is calculated, and stability constants are evaluated from the appropriate linear plot. Iterations are carried out until the stability constants are essentially unchanged.

The following treatment considers systems in which C is greater than unity. If C is less than unity, it is convenient to work with its reciprocal, $C' = 1/C$. Then all of the equations are applicable with the subscripts a and b interchanged.

Special Cases—Case I: $K_{12a} = 0$, $K_{12b} = 0$ —Equation 27 becomes:

$$C = \frac{1 + K_{11a}[\text{L}]}{1 + K_{11b}[\text{L}]} \quad (\text{Eq. 29})$$

A plot of C versus $[\text{L}]$ or L_t will approach a limiting value at high ligand concentration. Diagnosis of Case I behavior is tentative because Eq. 27 also results in this type of curve.

The ligand concentration is calculated with:

$$[\text{L}] = L_t - \frac{S_t}{X + 1} \quad (\text{Eq. 30})$$

where:

$$X = \frac{(C + 1)(R - C)}{(C - 1)(R + C)} \quad (\text{Eq. 31})$$

and $R = K_{11a}/K_{11b}$. The parameter R is estimated by extrapolating a plot of $\log C$ versus $1/L_t$ to $1/L_t = 0$, since C approaches R as L_t approaches infinity. A plot is then made according to:

$$\frac{C - 1}{[\text{L}]} = K_{11a} - CK_{11b} \quad (\text{Eq. 32})$$

which is obtained from Eq. 29. The constants K_{11a} and K_{11b} are obtained from this plot, which will be linear for a Case I system. The calculated R is compared with the initial estimate; if they differ significantly, the process is repeated.

Case II: $K_{11b} = 0$, $K_{12a} = 0$, $K_{12b} = 0$ —From Eq. 27:

$$C = 1 + K_{11a}[\text{L}] \quad (\text{Eq. 33})$$

where C is a linear function of ligand concentration. Of course, any system will approach linearity at sufficiently low ligand concentration, so L_t must be made large enough to determine if curvature exists. The free ligand concentration is obtained with Eq. 34.

$$[\text{L}] = L_t - \frac{(C - 1)S_t}{2C} \quad (\text{Eq. 34})$$

Case III: $K_{11b} = 0$, $K_{12b} = 0$ —Equation 27 becomes:

$$C = 1 + K_{11a}[\text{L}] + K_{11a}K_{12a}[\text{L}]^2 \quad (\text{Eq. 35})$$

Table I—Stability Constants for α -Cyclodextrin Complexes of *para*-Substituted Benzoic Acids at 25°

Number	σ^a	X ^b	K_{11a}/M^{-1} $\pm SD$	K_{11b}/M^{-1} $\pm SD$	K_{12a}/M^{-1} $\pm SD$
1	-0.84	NHCH ₃	1301 (15.9)	6.1 (0.26)	-0.010 (0.14)
2	-0.66	NH ₂	1341 (11.1)	9.0 (0.22)	-0.024 (0.10)
3	-0.37	OH	1130 (7.7)	16.6 (0.23)	-0.048 (0.063)
4	-0.27	OCH ₃	884 (5.1)	3.5 (0.10)	0.0064 (0.067)
5	-0.17	CH ₃	1091 (7.4)	6.6 (0.14)	0.0026 (0.071)
6	0.00	H	722 (9.7)	11.2 (0.35)	-0.0047 (0.13)
7	0.06	F	504 (4.4)	14.2 (0.26)	0.020 (0.081)
8	0.50	CH ₃ CO	899 (45.0)	60.3 (11.7)	28.8 (1.63)
9	0.66	CN	471 (7.5)	79.2 (4.6)	25.0 (0.47)
10	0.78	NO ₂	350 (16.2)	81.0 (9.3)	20.2 (1.14)

^a Hammett substituent constant. ^b X in X—C₆H₄—COOH.

The plot of *C* versus *L_t* will reveal curvature that is concave upward; [*L*] is calculated with:

$$[L] = \frac{2C(L_t - S_t) + 2S_t}{2C - S_t K_{11a}} \quad (\text{Eq. 36})$$

The linear form of Eq. 35 is:

$$\frac{C-1}{[L]} = K_{11a} + K_{11a}K_{12a}[L] \quad (\text{Eq. 37})$$

Case IV: $K_{12b} = 0$ —Equation 27 becomes:

$$C = \frac{1 + K_{11a}[L] + K_{11a}K_{12a}[L]^2}{1 + K_{11b}[L]} \quad (\text{Eq. 38})$$

The plot of *C* versus *L_t* will approach a linear segment of positive slope at high *L_t*. The equation of this line is:

$$C = R + RK_{12a}[L] \quad (\text{Eq. 39})$$

where $R = K_{11a}/K_{11b}$. This gives part of the desired information. The concentration [*L*] is given by:

$$K_{11b}[L]^2 + \left[1 + S_t K_{11b} \left(\frac{3}{2} - \frac{R}{2C}\right) - L_t K_{11b}\right][L] - \left[L_t - \frac{S_t(C-1)}{C}\right] = 0 \quad (\text{Eq. 40})$$

Several methods have been devised to extract the stability constants. It is a general result from Eq. 27 that:

$$\frac{dC}{d[L]} = \frac{BM - AN}{B^2[L]} \quad (\text{Eq. 41})$$

Thus the slope at [*L*] = 0 is equal to $K_{11a} - K_{11b}$. This result, together with the value of *R* from Eq. 39, gives the stability constants. Another method constructs, on the *C* versus [*L*] plot, lines such that the quantity [*L*]_X is found as the value of [*L*] when *C* = *R*. Then it is easily shown that:

$$K_{11b} = \frac{R-1}{RK_{12a}[L]_X^2} \quad (\text{Eq. 42})$$

However, both of these approaches rely on data at very low [*L*], and it is in this region that the relative error in [*L*] is the greatest. A better procedure is to measure K_{11b} by a different, independent, experimental method, such as spectroscopy. This will usually be straightforward, the Case IV diagnosis having shown that $K_{12b} = 0$. The linear plot according to Eq. 43 is made:

$$\frac{C-1}{[L]} + CK_{11b} = K_{11a} + K_{11a}K_{12a}[L] \quad (\text{Eq. 43})$$

This plot is also useful for confirming Case I systems, which should yield a slope equal to zero.

EXPERIMENTAL

Materials— α -Cyclodextrin² was dried at 95° for 48 hr. A sample of α -cyclodextrin recrystallized from water and dried under reduced pressure over phosphorus pentoxide for 12 hr (9) gave identical results. The benzoic acids³ were recrystallized until their melting points agreed with literature values. Water was redistilled from alkaline permanganate.

Procedures—Potentiometric Studies—A stock solution of the substrate was prepared such that its final diluted concentration would be 0.003–0.004 *M*, and the substrate was half-neutralized with 0.10 *M* NaOH. It was filled to the mark with 0.10 *M* NaCl.

α -Cyclodextrin was weighed into 5-ml volumetric flasks in amounts to cover its full solubility range. Portions of the substrate stock solution (4.0-ml) 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 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 measured⁴ and p*K*' was calculated according to Eq. 26. Reproducibility on duplicate solutions was 0.003 pH units or better. All studies were at 25.0° and ionic strength was 0.10 *M*.

Substrates with ionizable *para*-substituents (compounds 1–3 in Table I) have p*K*' values separated sufficiently (10–12) that the measurement of p*K*' for the carboxylic acid group leaves the *para*-substituent in its nonionized state.

Variation of *S_t* by a factor of 2 resulted in no significant variation in the stability constants.

Spectrophotometric Studies—For Case IV systems, K_{11b} was measured spectrophotometrically⁵. The substrate was dissolved in pH 9.18 tromethamine buffer, ionic strength 0.10 *M*; *S_t* was 5.6–8.4 × 10⁻⁵ *M*. The data were analyzed by the double-reciprocal linear form with a weighted least-squares treatment (13). The wavelengths of observation were: for CH₃CO—C₆H₄COOH, 253 nm; NC—C₆H₄COOH, 231 and 237 nm; and O₂N—C₆H₄COOH, 265 and 315 nm. Values of K_{11b} were not wavelength dependent.

RESULTS

This potentiometric method was applied to 10 benzoic acid derivatives. All of these compounds gave positive $\Delta pK_a'$ values and hence *C* values greater than unity, apparently a general result for carboxylic acids (2). These systems could be described as Case I or Case IV systems⁶.

Figure 1 shows the plot of *C* versus *L_t* for *p*-hydroxybenzoic acid, a typical Case I system, and Fig. 2 is the corresponding linear plot according to Eq. 32. Figure 3 shows *p*-cyanobenzoic acid, a Case IV system, and Fig. 4 is the linear plot according to Eq. 43. Usually two iterations sufficed to yield final estimates of the stability constants.

Table I shows the stability constants evaluated in this way. For all of these systems, K_{12b} is zero by definition, since they can be adequately described by Cases I or IV. For Case I systems, K_{12a} is zero. However, this assignment can be tested by treating a Case I system as if it were Case IV, making the plot according to Eq. 43. The values of K_{12a} in Table I were evaluated in this way. For compounds 1–7, K_{12a} is clearly not significantly different from zero, whereas substrates 8–10 possess very significant K_{12a} values. For Case I systems, the relative standard deviation of K_{11a} ranges from 0.6–1.3%, and of K_{11b} from 1.4–4.3%. The precision of Case IV systems is poorer, as seems reasonable, since these are three-parameter systems.

DISCUSSION

The Potentiometric Method—For complexing systems in which the

⁴ Orion 701A pH meter equipped with a Sargent-Welch S30072-15 electrode.

⁵ Perkin-Elmer model 559 spectrophotometer.

⁶ Phenols and amines give negative $\Delta pK_a'$ values. Some amines have been found to be Case II and Case III systems.

² Lot 29C-0425, Sigma Chemical Co.

³ Aldrich Chemical Co. and Eastman Organic Chemicals.

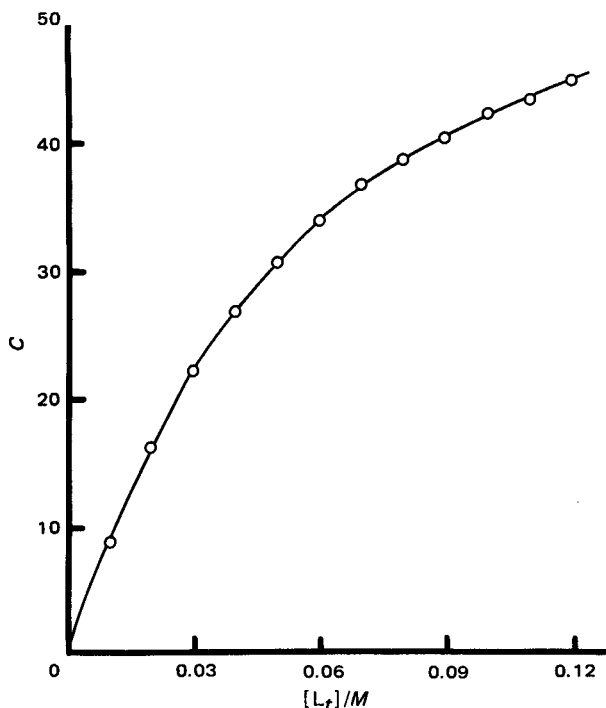


Figure 1—Plot of C versus L_t for the *p*-hydroxybenzoic acid- α -cyclodextrin system.

full four-parameter equation (Eq. 27) is not required, this potentiometric method with graphical treatment of the data is simple, rapid, and effective. Because of the linear graphing forms, the usual least-squares statistical treatment is appropriate. The error in the abscissa is much smaller than that in the ordinate, as is required by the conventional treatment. The great sensitivity of the quantity $(C - 1)/[L]$ at low $[L]$ values made it necessary to reject some data points at very low $[L]$ in the plots according to Eqs. 32 and 43. At least 10 points were used in every plot.

The assumption that activity coefficients are constant as L_t changes is fundamental to the interpretation of potentiometric data in terms of

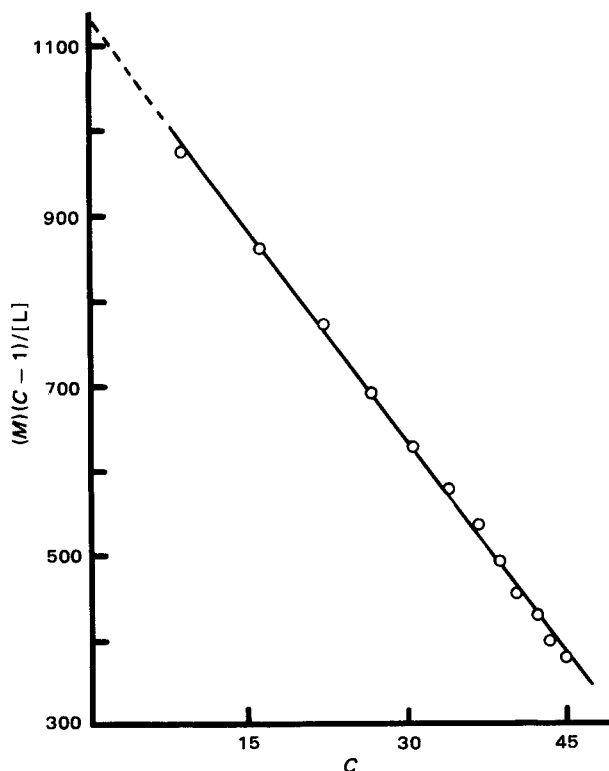


Figure 2—Plot of Eq. 32 for the *p*-hydroxybenzoic acid- α -cyclodextrin system.

Table II—Comparison of Stability Constants for the Benzoic Acid- α -Cyclodextrin System ^a

Method	K_{11a}	K_{11b}	Ref.
Competitive spectrophotometry	1050	—	14
Kinetics	—	12.3	15
Thermometry	1000	—	16
Potentiometry	1400	38	2
PMR	233; 800	9.8	17
Potentiometry	751	10.5	4
Solubility	500	—	18
UV spectrophotometry	660	—	18
Competitive spectrophotometry	—	13	19
Potentiometry	722	11.2	This work

^a At 25°; ionic strengths differ.

1:1 and 1:2 complex formation. It is conceivable that the observation of finite 1:2 stability constants represents a measurement of changing activity coefficients rather than actual 1:2 complexation. Gelb *et al.* (4) described evidence that ionic activity coefficients are not changed as a consequence of changes in cyclodextrin concentration. That K_{12a} for several acids was found to be negligible (Table I) suggests further that activity coefficient changes are not responsible for the observed 1:2 stability constants. Nevertheless, the role of cyclodextrin in modifying the properties of the solvent cannot be overlooked as a possible complicating factor in such studies.

Several studies of the benzoic acid- α -cyclodextrin system were described earlier and are listed chronologically in Table II. The earliest potentiometric result (2) was obtained by curve-fitting, and is now rejected. It is evident that the method proposed in the present paper is capable of generating stability constants consistent with those obtained by other techniques and in other laboratories.

An advantage of potentiometry is that it yields information about the complexing of both the conjugate acid and base forms of the substrate. A limitation is that it can serve as an independent method only when the system includes two (or fewer) complex equilibria, *i.e.*, when the equation

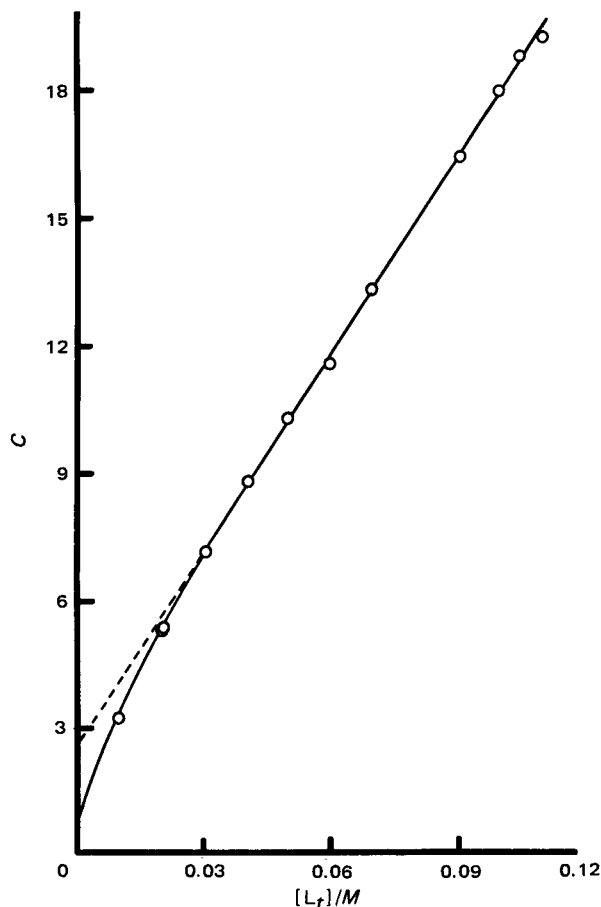


Figure 3—Plot of C versus L_t for the 4-cyanobenzoic acid- α -cyclodextrin system.

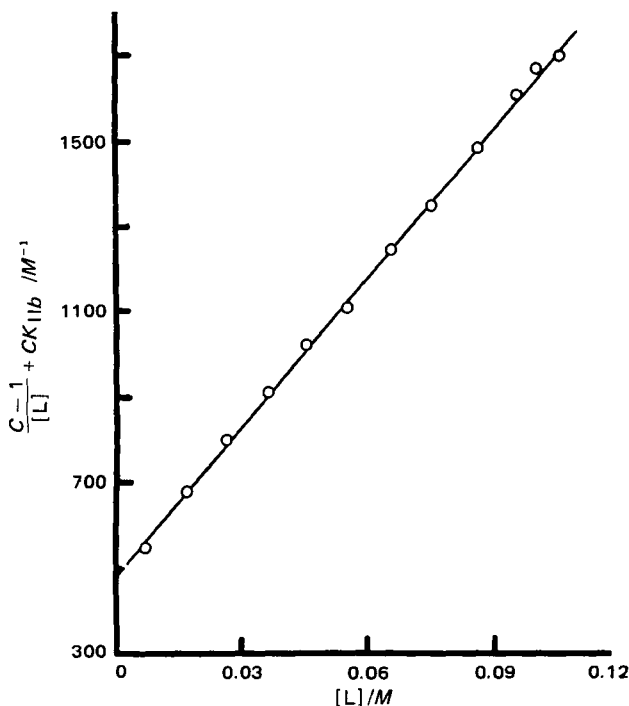


Figure 4—Plot of Eq. 43 for the 4-cyanobenzoic acid- α -cyclodextrin system.

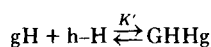
has two parameters. For the three-parameter Case IV system, it was judged necessary to make use of a separate experimental determination of one of the parameters. This combination of several techniques is essential if the equation has four parameters, as in the cinnamic acid system (5).

Interpretation of Stability Constants—An earlier report (20) introduced a model of cyclodextrin complex formation helpful in interpreting the present data. The substrate (guest) is symbolized g-G, the lower and upper case letters signifying two binding sites; the cyclodextrin (host) is represented h-H because the two rims of the cavity are chemically different. It was shown that it is possible for four 1:1, four 2:1, and four 1:2 complexes to be present in a system. However, if there are no 2:1 complexes present, it is a reasonable inference that only one end (e.g., H) of the host can be entered. It follows that there can be only two 1:1 complexes (gH and GH) and one 1:2 complex. The 1:2 complex is formed by the addition of a host molecule to either of the 1:1 complexes.

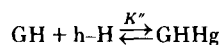
The observed K_{11} value for the system is the sum of the stability constants for the isomeric 1:1 complexes, hence:

$$K_{11} = K_{gH} + K_{GH} \quad (\text{Eq. 44})$$

The 1:2 complex can be formed *via* the gH or the GH route:



Scheme VIII



Scheme IX

The observed 1:2 stability constant is given by (20):

$$K_{12} = \frac{K'K''}{K' + K''} \quad (\text{Eq. 45})$$

It is also seen that:

$$\frac{K'}{K''} = \frac{K_{GH}}{K_{gH}} \quad (\text{Eq. 46})$$

Writing $K' = aK_{GH}$, with Eq. 46, it follows that $K'' = aK_{gH}$. Combination with Eq. 45 gives:

$$K_{12} = \frac{aK_{gH}K_{GH}}{K_{gH} + K_{GH}} \quad (\text{Eq. 47})$$

The factor a is a measure of the extent of interaction between the two sites in a 1:2 complex. If the two binding sites are independent, $a = 1$ and

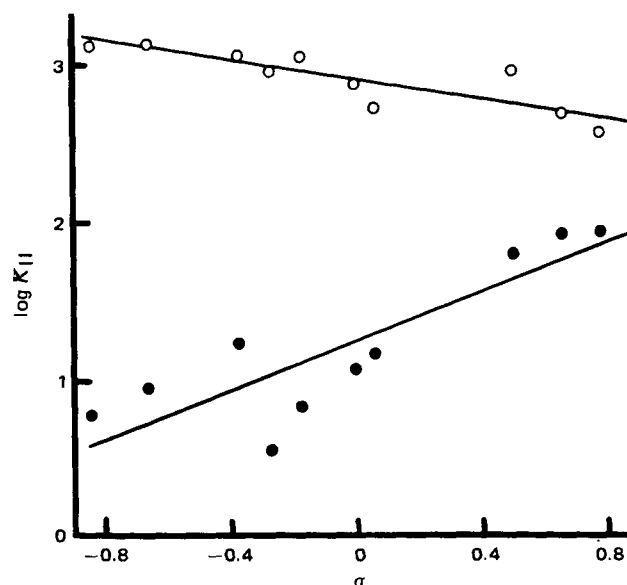


Figure 5—Hammett plots of K_{11a} (O) and K_{11b} (●) data from Table I.

the isomeric stability constants K_{gH} and K_{GH} can be evaluated with Eqs. 44 and 47. For the *para*-substituted benzoic acids it seems improbable that the sites are independent because of their proximity (the two sites being the X-end and the COOH-end of the molecule). Nevertheless, the model is useful in describing the nature of K_{11} and K_{12} , since it shows that K_{12} is actually a constant for the formation of a 1:1 complex from an already formed 1:1 complex. The model leads to interpretations in terms of binding sites.

Figure 5 is a Hammett plot of the K_{11a} and K_{11b} data in Table I. The obvious result is that these quantities vary with substituent constant in opposite ways. The slopes (ρ values) are -0.31 (0.07 SD) for K_{11a} and $+0.77$ (0.17 SD) for K_{11b} . Casu and Ravà (14) studied K_{11a} for some of these acids; their constants are significantly different from those in Table I, but the general behavior is the same, namely that ρ is negative. Electron-withdrawing X groups decrease complex stability in the conjugate acid series.

This behavior is explicable if K_{11a} primarily represents binding at the COOH-end of the substrate molecule. The binding interaction will be increased by an increase in electron density at the binding site, and will be associated with a negative ρ value. This hypothesis is supported by PMR data on benzoic acid (17), which indicate that the carboxyl group enters the cavity.

The value of K_{11b} is always smaller than K_{11a} for the same substrate. This also is consistent with the view that K_{11a} represents mainly COOH-site binding. Upon ionization of this group, its affinity for the cavity will be greatly decreased because of the high polarity of COO^- and the nonpolarity (relative to the solvent) of the cavity. Therefore K_{11b} represents binding at the remaining site, namely X. Thus the more electron-withdrawing the X group, the larger is K_{11b} . Since K_{11b} describes X-site binding, the correlation with σ for X is not expected to be as precise as when the substituent is distant from the reaction site.

If K_{11a} describes COOH-site binding (mainly), then K_{12a} must represent mainly binding at the X-site. Therefore, this model predicts that K_{12a} should respond to X-substituent effects the same way that K_{11b} does, since they describe roughly the same process. Table I shows that this is indeed the case, in a general way; both K_{11b} and K_{12a} tend to increase together, and to tend in the opposite sense to K_{11a} along the σ scale.

The Hammett plot suggests that if a substituent with sufficiently large σ were available, K_{11b} would become larger than K_{11a} . This might not result in $\Delta pK_a'$ becoming negative, however, because K_{12a} will also increase.

These correlations clearly show trends rather than highly precise relationships, and factors other than electronic density must play a role. For example, K_{11b} for substrates 1–3 seems anomalously high. These are the only X groups capable of functioning as hydrogen-bond donors, and this capability may increase complex stability. The trend in K_{12a} for compounds 8–10 appears to oppose that for K_{11b} , and this may be the result of some additional factor, which could be size, shape, polarity, or polarizability. In fact, K_{12a} for these substrates seems to be a resultant

of the electronic density effect measured by σ , and the X-site polarity as measured by group dipole moments. These moments are, for X = COCH₃, 3.00 D; CN, 4.39; and NO₂, 4.21 (21). An increase in site polarity will tend to decrease the site binding constant (20), and both K_{11b} and K_{12a} may reflect this effect superimposed on the electron density effect.

This model of complexing suggests that K_{12} values are especially useful, leading to the question: Why is K_{12} ever equal to zero (for a two-site substrate)? According to Eq. 47, K_{12} can be zero only if $a = 0$ or if one of the site binding constants (K_{gH} or K_{GH}) is zero. This leads to the following argument. If the given site assignments are correct, K_{12b} represents binding at the COO⁻ site (since K_{11b} mainly describes X-site binding), and it was found that $K_{12b} = 0$ for all substrates. The existence of finite K_{12a} values (substrates 8–10) means that a is finite for these acid substrates, and suggests that it will also be finite in the corresponding base substrates. It follows that K_{12b} is zero as a consequence of the site binding constant for COO⁻ being zero. Letting K_{gHb} represent this quantity, and K_{GHb} the site binding constant for the X-site in the conjugate base series, it follows that:

$$K_{11b} = K_{gHb} + K_{GHb} = K_{GHb} \quad (\text{Eq. 48})$$

i.e., K_{11b} can be identified solely with the binding at site X.

Extension to the acid series requires the assumption that K_{GHb} for binding to X in the base series is identical with K_{GHa} for binding to X in the acid series. If approximately so, then $K_{11a} = K_{gHa} + K_{GHa} = K_{gHa} + K_{GHb} = K_{gHa} + K_{11b}$, and the site binding constant K_{gHa} for the COOH site is given approximately by $K_{11a} - K_{11b}$.

With these estimates of K_{gHa} ($K_{11a} - K_{11b}$) and K_{GHa} (K_{11b}), Eq. 47 leads to estimates of a for these substrates, since:

$$K_{12a} = \frac{aK_{gHa}K_{GHa}}{K_{gHa} + K_{GHa}} = \frac{aK_{11b}(K_{11a} - K_{11b})}{K_{11a}} \quad (\text{Eq. 49})$$

For compound number 8, $a = 0.51$; number 9, $a = 0.38$; and number 10, $a = 0.33$. Because of the several approximations, these calculations are unlikely to be accurate, but they are reasonable in magnitude. The interpretation of complex stability data in terms of this model and Eqs. 44 and 47 seems to be a potentially useful means for describing, understanding, and perhaps predicting complex formation behavior.

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Reversed-Phase Ion-Pair Chromatography of Tetracycline, Tetracycline Analogs, and Their Potential Impurities

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Abstract □ Methods are presented for the separation of tetracycline, tetracycline analogs, and their potential impurities by reversed-phase ion-pair chromatography. The mobile phase consisted of a phosphate buffer with tripropylamine or *N,N*-dimethyloctylamine as counterions and acetonitrile as the organic modifier. The chromatographic properties of the tetracyclines were significantly improved by addition of the tertiary amines. The best result was obtained with *N,N*-dimethyloctylamine as the counterion, which gave good separation efficiency and peak symmetry for most of the substances studied. Addition of the tertiary amines to the mobile phase also significantly affected the capacity factors of the tetracyclines. Stability studies of the tetracyclines showed a fast degradation

of chlortetracycline to isochlortetracycline and of lymecycline to tetracycline in phosphate buffer solutions of different pH. The purity of pharmaceutical preparations of tetracyclines was also investigated.

Keyphrases □ Tetracycline—potential impurities of the drug and its analogs, reversed-phase ion-pair chromatography □ High-performance liquid chromatography—tetracycline, tetracycline analogs, and their potential impurities □ Counterions—*N,N*-dimethyloctylamine, tripropylamine, in reversed-phase ion-pair chromatography of tetracycline, tetracycline analogs, and their possible impurities

The most important impurities in tetracycline and tetracycline analogs are the epimerized, dehydrated, and epimerized dehydrated forms of the tetracyclines. Thus, quatrimecin (epitetracycline), anhydrotetracycline, and epianhydrotetracycline are the most frequently found impurities in tetracycline. Epianhydrotetracycline has been reported to be toxic in humans, but the exact safety

level in pharmaceutical preparations is not known. Permitted concentrations of epianhydrotetracycline and other impurities are fixed by the European Pharmacopoeia.

BACKGROUND

Various chromatographic techniques, such as TLC (1, 2) and paper chromatography (3), have been used to analyze tetracycline, but they are