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Improved Competitive Indicator Methods for the Study of α-Cyclodextrin Complexes

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Abstract □ The competitive indicator method for studying molecular complexes is extended to systems forming 1:1 (SL) and 1:2 (SL_2) complexes of substrate (S) and ligand (L). A modification is described for slightly soluble substrates, in which the presence of solid substrate establishes a constant concentration of uncomplexed substrate. These methods are applied to complexes of α-cyclodextrin with some aromatic substrates, with methyl orange as the indicator in acid solution; nitrazine yellow is introduced as an indicator for these studies in basic solution.

Keyphrases □ α-Cyclodextrin—competitive indicator methods, methyl orange, nitrazine yellow □ Competitive indicator methods—α-cyclodextrin, methyl orange, nitrazine yellow complexes

The cyclodextrins are naturally occurring cyclic oligosaccharides possessing a central cavity into which smaller molecules may "partition," forming noncovalently bonded inclusion complexes. The physical and chemical properties of the included molecule (the substrate or guest) may be altered by complexation with the cyclodextrin (the ligand or host). This phenomenon has attracted much recent attention, in part because of its potential applications in drug dosage forms (1–3). Among the properties of a drug that can be affected by cyclodextrin complexation are its solubility, dissolution rate, chemical stability, and bioavailability.

Systematic studies in this laboratory have been designed to provide information on the stoichiometry and thermodynamic stability of cyclodextrin complexes, with the goal of understanding structure-stability relationships and thus developing predictive capability. It has been necessary, in these studies, to make use of several experimental techniques for the measurement of complex stability constants and in some instances to develop new methods. One technique that has been found useful is the so-called "competitive indicator" method. In this technique, an equilibrium is established between the ligand (cyclodextrin) and an indicator whose absorption spectrum differs significantly in its complexed and uncomplexed forms. This equilibrium is then perturbed by the addition of a substrate, which competes with the indicator for the ligand. By measuring the spectral change produced by this perturbation, the stability constant for the substrate–ligand complex can be deduced. The methyl orange–α-cyclodextrin equilibrium was exploited in this way by Broser and co-workers (4) to study the adrenalin–α-cyclodextrin complex and by Casu and Ravà (5) to study a series of substituted benzoic acids. The method was recently improved in this laboratory and was applied to the study of the α-cyclodextrin complexes of some phenols (6). All of these applications have made use of methyl orange as the indicator in acidic solution and have been limited to the study of 1:1 complexes between substrate and ligand.

Our most recent work has required three new capabilities of the competitive indicator method: (*a*) that it be applicable to systems containing both 1:1 (SL) and 1:2 (SL_2) complexes, where S and L represent substrate and ligand respectively; (*b*) that it be applicable to slightly soluble substrates; (*c*) that it be applicable in alkaline medium. The present paper describes extensions to the theory and practice of the method that permit its application in these circumstances.

THEORETICAL SECTION

Standard Competitive Indicator Method—Attention is restricted to systems containing a 1:1 complex IL of indicator I with ligand plus the 1:1 (SL) and 1:2 (SL_2) complexes of substrate with ligand. The average number of ligand molecules bound per substrate molecule ($\bar{n}$) is:

$$\bar{n} = \frac{\Sigma(\text{L bound to S})}{\Sigma(\text{all S})} \qquad \text{(Eq. 1)}$$

The mass balance equations for S and L are:

$$S_t = [S] + [SL] + [SL_2] \qquad \text{(Eq. 2)}$$

$$L_t = [L] + [SL] + 2[SL_2] + [IL] \qquad \text{(Eq. 3)}$$

where S_t and L_t are the (known) total concentrations of substrate and ligand. Further define the quantity P as Σ (L bound to S) can be defined by:

$$P = [SL] + 2[SL_2] = L_t - [L] - [IL] \qquad \text{(Eq. 4)}$$

Combining Eqs. 1, 3, and 4:

$$\bar{n} = \frac{P}{S_t} \qquad \text{(Eq. 5)}$$

The equilibrium constants in this system are given by:

$$K_{11} = \frac{[SL]}{[S][L]} \qquad \text{(Eq. 6)}$$

$$K_{12} = \frac{[SL_2]}{[SL][L]} \qquad \text{(Eq. 7)}$$

$$K_I = \frac{[IL]}{[I][L]} \qquad \text{(Eq. 8)}$$

Combining Eqs. 5, 6, and 7 yields:

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$$\bar{n} = \frac{K_{11}[L] + 2K_{11}K_{12}[L]^2}{1 + K_{11}[L] + K_{11}K_{12}[L]^2} \quad \text{(Eq. 9)}$$

Equation 9 is the classical expression for the Bjerrum formation function, which occurs in the study of metal ion coordination complexes. It can be rearranged into the linear plotting form (7, 8):

$$\frac{\bar{n}}{(1-\bar{n})[L]} = K_{11} + K_{11}K_{12}[L]\frac{(2-\bar{n})}{(1-\bar{n})} \quad \text{(Eq. 10)}$$

To use Eq. 10, the quantities $\bar{n}$ and [L] are needed. These are obtained by spectrophotometric measurements at a wavelength at which the indicator absorbs light, but neither the substrate nor the ligand absorbs.

The ratio [I]/[IL] is labeled Q; this quantity is evidently inversely proportional to the free ligand concentration:

$$Q = \frac{[I]}{[IL]} = \frac{1}{K_I[L]} \quad \text{(Eq. 11)}$$

Combination of a mass balance on the indicator with Eqs. 4 and 8 gives:

$$P = L_t - \frac{1}{QK_I} - \frac{I_t}{Q+1} \quad \text{(Eq. 12)}$$

where I_t is the total indicator concentration. Moreover, Q is experimentally measurable by use of Eq. 13, where ϵ_I and ϵ_{IL} are the molar absorptivities of the indicator in the uncomplexed and complexed forms, respectively, and ϵ is the apparent molar absorptivity in a solution containing both forms:

$$Q = \frac{\epsilon - \epsilon_{IL}}{\epsilon_I - \epsilon} \quad \text{(Eq. 13)}$$

Thus, $\bar{n}$ can be experimentally measured. These symbols were introduced to achieve consistency with the earlier development (6); Eq. 10 becomes:

$$\frac{PQK_I}{S_t - P} = K_{11} + \frac{K_{11}K_{12}}{K_I} \cdot \frac{1}{Q} \cdot \frac{(2S_t - P)}{(S_t - P)} \quad \text{(Eq. 14)}$$

P and Q can be measured, S_t is known, and K_I can be determined in a separate experiment, so the stability constants K_{11} and K_{12} can be obtained from the slope and intercept of a plot according to Eq. 14. If $K_{12} = 0$, the slope of this line will be zero, and a simpler plotting form, Eq. 15, results, as was shown earlier (6).

$$\frac{S_t}{P} = 1 + \frac{K_I}{K_{11}}Q \quad \text{(Eq. 15)}$$

Solubility: Competitive Indicator Method— For substrates of low solubility, it is difficult to establish accurate values of S_t for use in the aforementioned competitive indicator method, and the following useful modification has been devised. The experiment is carried out by varying total ligand concentration, L_t, but by maintaining the free substrate concentration [S] constant at its saturation solubility S_o; this is accomplished by establishing the equilibria in the presence of solid substrate. Then Eq. 4 can be written:

$$P = K_{11}S_o[L] + 2K_{11}K_{12}S_o[L]^2 \quad \text{(Eq. 16)}$$

Substituting from Eq. 11 for [L] and rearranging gives:

$$\frac{PQK_I}{S_o} = K_{11} + \frac{2K_{11}K_{12}}{K_IQ} \quad \text{(Eq. 17)}$$

Thus, a plot of PQK_I/S_o against $1/Q$ should be linear.

This combined solubility-competitive indicator method can be extended to include systems in which a 1:1 substrate-indicator complex SI is formed, provided the stability constant, K_{SI}, can be independently measured and a wavelength can be selected such that the molar absorptivities of SI and I are equal. The analysis (9) shows that Eq. 17 remains applicable but Q and P must be evaluated by Eqs. 18 and 19, which may be compared with Eqs. 11 and 12.

$$Q = \frac{\epsilon - \epsilon_{IL}}{(\epsilon_I - \epsilon)(1 + K_{SI}S_o)} \quad \text{(Eq. 18)}$$

$$P = L_t - \frac{1}{K_IQ} - \frac{I_t}{1 + Q + QK_{SI}S_o} \quad \text{(Eq. 19)}$$

EXPERIMENTAL SECTION

Materials—α-Cyclodextrin[1] was dried for 3 h at 105°C. The methyl orange indicator was purified by the method of Lin and Connors (6). Nitrazine yellow indicator[2] (C.I. 14890) was recrystallized from water to a constant molar absorptivity of 4.045×10^4 at λ_{max} 588 nm; it was dried for 3 h at 105°C before use. Substrates were from commercial sources and were recrystallized before use (9). Buffer components were of reagent grade. Spectrophotometric[3] and pH[4] measurements were made at 25.0°C.

Procedures—*Measurement of* Q—Throughout a study, the total indicator concentration, I_t, is held constant; hence, absorptivities in Eq. 11 can be replaced by absorbances:

$$Q = \frac{A - A_{IL}}{A_I - A} \quad \text{(Eq. 20)}$$

A_I is readily measured in a solution of the indicator in the absence of ligand. A_{IL} is obtained as the product $\epsilon_{IL}I_t$; ϵ_{IL} is found as follows. In a solution containing only indicator and ligand, the mass balance on ligand is:

$$L_t = [L] + [IL] \quad \text{(Eq. 21)}$$

This, combined with Eq. 8 and the mass balance on indicator, gives:

$$[I] = \frac{I_t}{1 + K_I[L]} \quad \text{(Eq. 22)}$$

An iterative process is used. First, the assumption is made that $[L] = L_t$, and Eq. 22 is solved for [I] (making use of an independently measured value of K_I). Then, [IL] is found from the mass balance $I_t = [I] + [IL]$, and this is used in Eq. 21 to produce a refined estimate of [L]. This process is repeated until no change in [L] is noted in the fourth decimal place. The absorbance of the solution (path length = 1 cm) is $A = \epsilon_I[I] + \epsilon_{IL}[IL]$, hence:

$$\epsilon_{IL} = \frac{A - \epsilon_I[I]}{[IL]} \quad \text{(Eq. 23)}$$

This provides the information necessary to evaluate Q with Eq. 20.

Standard Method—Methyl orange studies were conducted in 0.10 M HCl, whereas nitrazine yellow studies were carried out in 0.05 M Tris buffer at pH 9.2 with the ionic strength adjusted to 0.10 M with NaCl. Portions of stock solutions of indicator, substrate, and ligand were added to 10-mL volumetric flasks to produce a series of solutions having constant indicator concentration ($2\text{-}4 \times 10^{-5}$ M) with substrate and ligand concentration varying as required by optimization considerations (to be described). In some instances, the substrate and ligand were weighed directly into the flasks. After equilibration of the solutions at 25.0°C, the absorbances were measured at the wavelength of maximal spectral shift.

Solubility Method—Indicator solutions containing varied ligand concentrations were prepared in 10-mL volumetric flasks. Each of these was transferred to a 15.5-mL vial containing solid substrate (in excess of its total solubility). After the vials were sealed, they were rotated end-over-end at 32 rpm in a 25.0°C water bath for 24 h. They were then placed upright in the 25°C bath for time sufficient for the solid substrate to settle. The clear solution was aspirated and its absorbance was measured at 25°C.

Data Treatment—Equations 14, 15, and 17 are linear, and the slopes and intercepts of these plots were obtained by conventional linear least-squares analysis. The variances of the stability constant estimates were then found by a propagation of errors treatment (9). Although in each of these equations the variable playing the role of x, the "independent" variable, has measurable uncertainty associated with it, the y variable possesses greater uncertainty; hence, the usual linear regression treatment is suitable.

If treatment according to Eq. 14 showed that the slope was not significantly different from zero, then the data were analyzed by means of Eq. 15.

Optimization of Conditions—For a system containing only a 1:1 (SL) complex, the quantity $\bar{n} = P/S_t$ can range from 0 to 1, whereas for a system capable of forming both SL and SL_2, $\bar{n}$ can range from 0 to 2. Thus, $\bar{n}$ is a useful indication of the extent of the binding isotherm that has been examined. In a 1:1 system, $\bar{n}$ is equal to f_{11}, the fraction of substrate present as the 1:1 complex, but in a 1:1 + 1:2 system, $\bar{n} = f_{11} + 2f_{12}$.

From Eq. 14, it is clear that the quantity $(S_t - P)$ control the reliability of the plotting variables. Combining Eqs. 2 and 4 gives:

$$S_t - P = [S] - [SL_2] \quad \text{(Eq. 24)}$$

Thus, $(S_t - P)$ may be positive, zero, or negative, whereas $2S_t - P$ must be positive, as must the other variables and parameters in Eq. 14. Therefore, data points plotted according to Eq. 14 must lie in either the first or third quadrants, with the point at $S_t = P$ being indeterminate. Points in the first and third quadrants correspond to the conditions $\bar{n} < 1$ and $\bar{n} > 1$, respectively. In the present studies, all systems appeared in the first quadrant.

The experimental data consist of absorbance values, which are bounded

[1] Sigma Chemical Co.
[2] Aldrich Chemical Co.
[3] Cary Varian Model 2200 or Perkin-Elmer Model 559 spectrophotometer.
[4] Orion Model 701A pH meter with Corning semi-micro combination electrode (catalog number 476050).

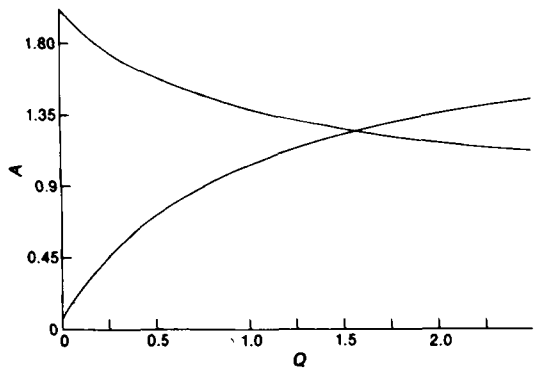


Figure 1—*Relationship between absorbance, A, and corresponding Q value (Eq. 20) for hypothetical indicator–α-cyclodextrin systems. Methyl orange (positive slope), $I_t = 4.17 \times 10^{-5}$ M; nitrazine yellow (negative slope), $I_t = 3.92 \times 10^{-5}$ M.*

by the limits A_I and A_{IL}. With Eq. 20 these are converted to Q values, which may range from 0 to ∞. The relationship between absorbance values, A, and corresponding Q values is shown in Fig. 1 for typical solutions of methyl orange and nitrazine yellow. At large Q values, the slope is so small that a small absorbance error results in a large error in Q. At low Q values, a small absorbance error results in a small absolute but large relative error in Q. These effects can be quantified by differentiating Eq. 20 to find dQ/dA. In the present work it was decided, for systems containing both 1:1 and 1:2 equilibria, to exclude data points having Q values outside the range 0.3–2.5.

According to Eq. 11, Q is uniquely related to free ligand concentration (for a given indicator). Thus, the limits placed on Q establish corresponding limits on [L]. To extend these limits in order to examine a greater range of the binding isotherm, it would be necessary to have available a series of indicators with different K_I values.

For a given indicator and substrate, each pair of plotting variables in Eq. 14 is associated with a unique Q ([L]), which may be realized by innumerable combinations of I_t, S_t, and L_t. It is necessary to limit these quantities to values that will give acceptable uncertainties in P and in $S_t - P$. This is done as follows:

1. Choose I_t such that A_I or A_{IL} (whichever is larger) ≈ 2.
2. Select a series of Q values in the 0.3–2.5 range and for each Q calculate the corresponding [L] with Eq. 11.
3. With preliminary estimates of K_{11} and K_{12}, calculate $\bar{n}$ values for each [L] value, using Eq. 9. Reject any systems giving $\bar{n}$ values ~ >0.7.
4. For each acceptable [L] value, choose L_t such that $L_t \geq 2[L]$. Calculate P with Eq. 12.
5. For each set of conditions, calculate S_t from $S_t = P/\bar{n}$.

Often it will be satisfactory to work with a fixed S_t and simply calculate the desired L_t levels from Eq. 25, which is obtained from Eqs. 5 and 12:

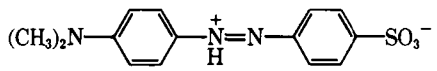
$$L_t = \bar{n}S_t + [L] + \frac{I_t}{Q+1} \qquad \text{(Eq. 25)}$$

S_t should be chosen such that $L_t \geq 2[L]$.

This procedure will result in data points in the first quadrant when plotted according to Eq. 14. All Q values will be in the desired range and the errors in P and $S_t - P$ will be acceptable. If the required S_t values exceed the solubility of the substrate, the solubility-competitive indicator method should be used.

RESULTS AND DISCUSSION

Indicator Properties.—A requirement of these competitive indicator methods is that the indicator must form only a 1:1 complex with the ligand. This imposes limits on acceptable indicator structures. However, indicator selection is greatly facilitated by adopting a point of view provided by a recently proposed binding site model of cyclodextrin complex formation (10, 11). To ensure only 1:1 complex formation, it is sufficient that the indicator possess only one potential binding site capable of entering the cyclodextrin cavity. For α-cyclodextrin, there is considerable evidence that uncharged substituents on an aromatic (benzene) ring, either *meta* or *para* to another group, can act as binding sites, but that charged groups have little affinity for the cavity. By inspection of indicator structures, it is therefore possible to identify likely candidates, and in this way nitrazine yellow was selected. Methyl orange has long been used (in strongly acid medium) (4). The structures of methyl orange (I) and nitrazine yellow (II) are shown:



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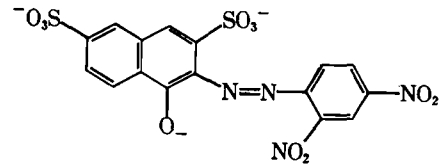

II

A second requirement is that the indicator must exhibit a significant spectral change upon complexation with α-cyclodextrin. In Table I, the properties of these indicators pertinent to these studies are gathered. The K_I values were determined by a Benesi-Hildebrand treatment; the result for methyl orange is in excellent agreement with that reported by Lin and Connors (6). Several pieces of evidence are consistent with the assumption that nitrazine yellow forms only a 1:1 complex with α-cyclodextrin: (*a*) the K_I value is independent of wavelength; (*b*) there is a single isosbestic point (at 590 nm); (*c*) graphical treatment of a matrix of spectrophotometric data (12, 13) provides evidence

Table I—Properties of Indicators in Aqueous α-Cyclodextrin Solutions [a]

Indicator	pH	Analytical Wavelength, nm	Molar Absorptivities: Free (ϵ_I)	Molar Absorptivities: Complexed (ϵ_{IL})	Equilibrium Constant K_I, M^{-1} [b]
Methyl orange	1.0	508	47,980	1,465	664 (4.7)
Nitrazine yellow	9.2	634.5	19,500	50,980	74.3 (0.9)

[a] At 25°C, ionic strength 0.10 M. [b] *SD* in parentheses.

Table II—Stability Constants Measured with Methyl Orange and Nitrazine Yellow Indicators [a]

Substrate	Competitive Indicator Method [b]	K_{11}, M^{-1}	K_{12}, M^{-1}
	Methyl Orange Indicator		
1,4-Dimethoxybenzene	Standard	55.3 (22)	208 (90)
1,4-Dimethoxybenzene	Solubility	75.4 (2.6)	221 (8.6)
4-Nitrobenzoic acid	Solubility	320 (12)	26.9 (6.5)
4-Cyanobenzoic acid	Standard	485 (11)	57 (4.7)
	Nitrazine Yellow Indicator		
Benzoate ion		12.8 (0.2)	
3-Nitrobenzoate ion		124.5 (9.1)	
4-Nitrobenzoate ion		60.0 (3.0)	

[a] At 25°C and ionic strength 0.10 M. *SD* are in parentheses. [b] Methods as described in text.

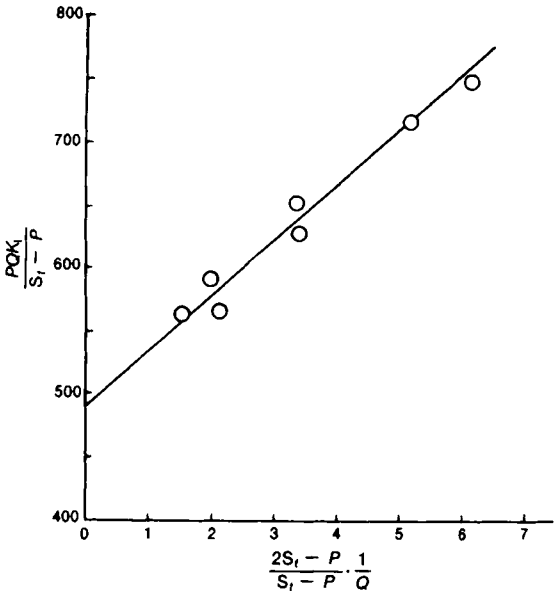

Figure 2—*Plot of Eq. 14 for the methyl orange- 4-cyanobenzoic acid-α-cyclodextrin system. $I_t = 3.0 \times 10^{-5}$ M; S_t varies from 2.3 to 6.3 × 10^{-3} M; L_t varies from 2.5 to 6.6 × 10^{-3} M.*

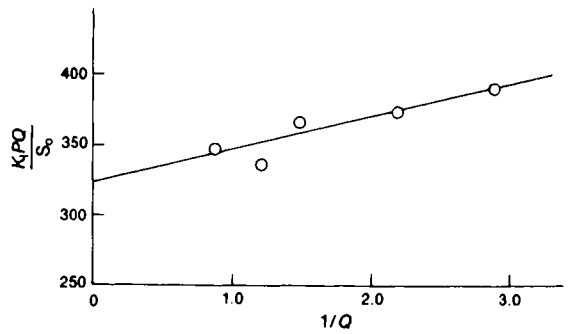

Figure 3—*Plot of Eq. 17 for the methyl orange-4-nitrobenzoic acid-α-cyclodextrin system. $I_t = 4.8 \times 10^{-5}$ M; $S_0 = 9.68 \times 10^{-4}$ M; L_t varies from 1.8 to 6.0 × 10^{-3} M.*

for the existence of only two indicator species in solution, presumably I and IL.

Stability Constant Results—These new modifications, consisting of the standard competitive indicator method for 1:1 + 1:2 systems, the solubility-competitive indicator method, and the introduction of nitrazine yellow as an indicator in basic media, were applied to the study of some substrates of interest in our systematic investigation of α-cyclodextrin complexation. The reference methods were UV spectrophotometry, potentiometry (11), and solubility (14, 15). Methyl orange was the indicator in studies (at pH 1.0) of 1,4-dimethoxybenzene, 4-nitrobenzoic acid, and 4-cyanobenzoic acid; nitrazine yellow (at pH 9.2) was employed to study the anions of benzoic, 3-nitrobenzoic, and 4-nitrobenzoic acids.

Table II lists the results. In basic medium, the carboxylate anions do not form 1:2 complexes, so the nitrazine yellow data were processed with Eq. 15. The systems studied with methyl orange in acidic medium showed both 1:1 and 1:2 complex formation, so Eqs. 14 and 17 were used. Figure 2 is a plot of Eq. 14, the standard competitive indicator method and Fig. 3 shows a plot of Eq. 17, the solubility-competitive indicator method. Table III compares the stability constants obtained by these competitive indicator methods with values obtained by other techniques. It is apparent that the competitive indicator approach is measuring the same quantity as the other methods. It must be pointed out that some of these systems, especially those in acidic media, are difficult to study, in part because of their limited solubilities. The 1,4-dimethoxybenzene system is especially interesting. The initial result on this system was obtained by the classical solubility method (14, 15), and it yielded the extremely unusual observation that $K_{12} > K_{11}$ (with rather large uncertainties in the stability constants). Application of the two new competitive indicator methods described in this paper gave results that were entirely consistent; this type of independent confirmation is useful. The only discordant result in Table III is K_{12} for 4-cyanobenzoic acid by the standard competitive indicator method. This is thought to be a consequence of the particular combination of the indicator and the substrate, possibly caused by the formation of a substrate-indicator complex. The low solubility of 4-nitrobenzoic acid did not permit a reliable application of the standard method, but preliminary results indicated behavior similar to that observed with 4-cyanobenzoic acid. Spectroscopic observations of more soluble benzoic acids in the presence of methyl orange showed small shifts in the spectrum of the indicator suggestive of the formation of complexes. It therefore seems possible that SI complexes may lead to systematic errors in methyl orange-substituted benzoic acid systems. The solubility competitive indicator method appears to be less susceptible to these errors.

CONCLUSIONS

These results have usefully extended the competitive indicator method to systems containing both 1:1 and 1:2 complexes. It may be noted that the theoretical basis is general, although the applications reported here were to complexes of α-cyclodextrin. Nitrazine yellow has been found effective for studies in alkaline solution.

The competitive indicator method will seldom be viewed as the "method of choice" when other techniques are available, for the following reasons. The addition of another complex former, namely the indicator, complicates the equilibrium system. In this system, the formation of the SL complex generates a perturbation whose observation provides information about K_{11}. The presence of complex SL_2 produces a further perturbation that gives an estimate of K_{12}. Thus, the information about K_{12} is a consequence of a perturbation

Table III—Comparison of Stability Constants by Various Methods

Substrate	Method	K_{11}, M^{-1}	K_{12}, M^{-1}	Ref.
1,4-Dimethoxybenzene	Std. comp. ind.[a]	55.3	208	This work
	Sol. comp. ind.[b]	75.4	221	This work
	Solubility	55.8	193	9
4-Nitrobenzoic acid	Sol. comp. ind.	320	26.9	This work
	Solubility	277	29.6	9
	Potentiometric	350	20.2	11
	Potentiometric	293	25.9	9
	Potentiometric	331	25.1	9
4-Cyanobenzoic acid	Std. comp. ind.	485	57	This work
	Potentiometric	471	25.0	11
	Potentiometric	428	24.1	9
	Potentiometric	441	26.2	9
Benzoate	Std. comp. ind.	12.8	—	This work
	Potentiometric	11.2	—	11
	Potentiometric	10.5	—	16
3-Nitrobenzoate	Std. comp. ind.	124.5	—	This work
	Potentiometric	94.2	—	9
	Potentiometric	126		16
4-Nitrobenzoate	Std. comp. ind.	60.0	—	This work
	Spectrophotometry	75.7	—	9
	Spectrophotometry	81.0	—	11

[a] Standard comparative indicator. [b] Solubility comparative indicator.

of a perturbation. Despite this limitation, the competitive indicator method has one notable feature that will often make it a valuable resource, namely, it allows observations to be made of a substrate whose own properties do not provide quantitative measures of complex formation; the indicator spectral change is a surrogate property.

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Metabolism of Meperidine in Several Animal Species

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Abstract □ Four new meperidine metabolites were identified by GC–MS in the urine of rats, guinea pigs, rabbits, cats, and dogs. In addition to known meperidine metabolites, 4-ethoxycarbonyl-4-phenyl-1,2,3,4-tetrapyridine (dehydronormeperidine; IV, the *N*-hydroxydehydro derivative of normeperidine (X), the dihydroxy derivative of meperidine (XII), and the dihydroxy derivative of normeperidine (XIII) were identified. The possible role of the *N*-hydroxy derivative of normeperidine (IX) in the pharmacological interaction of meperidine (I) with MAO inhibitors, seen selectively in the rabbit (and humans), is discussed. Following the administration of the *p*-hydroxy derivative of meperidine (VII), the major metabolite was conjugated VII. Trace amounts of the *p*-hydroxy derivative of normeperidine (VIII), the methoxy hydroxy derivative of meperidine (XI), XII, and XIII also were detected as metabolites of VII. The degree of *N*-demethylation of VII, both *in vitro* and *in vivo*, was small.

Keyphrases □ Meperidine—TLC, GC–MS, identification of metabolites, interaction with monoamine oxidase inhibitors □ Monoamine oxidase inhibitors—metabolites of meperidine, TLC, GC–MS

Meperidine is extensively metabolized by humans and animals. The major metabolites of meperidine identified in the urine of rats, guinea pigs, dogs, and humans are normeperidine and (1-methyl-4-phenyl-4-piperidine carboxylic) and (4-phenyl-4-piperidine carboxylic) acids (1–12), while the minor metabolites are the *p*-hydroxy derivative of meperidine (5), the *N*-oxide of meperidine (5, 6), the methoxy hydroxy derivative of meperidine (7), and the *N*-hydroxy derivative of normeperidine (7). In the urinary excretion studies of meperidine and its metabolites [normeperidine, and (1-methyl-4-phenyl-4-piperidine carboxylic) and (4-phenyl-4-piperidine carboxylic) acid], the amount of each excreted by the rabbit was found to be low (16–53% of the administered dose) when compared with other species (13). Of this total, meperidine and normeperidine accounted for only ~0.4 and 4–8.5%, respectively, of the administered dose. The remainder of the dose excreted represented unidentified metabolites. Severe interaction between monoamine oxidase inhibitors (MAOI) and meperidine have been observed in rabbits and humans, but seldom observed in other species (14–16). The purpose of this study was to identify new metabolites in rabbits and other species which might contribute to the severe interaction of meperidine and MAOI observed in the rabbit. The metabolism of the *p*-hydroxy derivative of meperidine (VII) was also investigated in a preliminary study in the rat in an attempt to determine if VII is metabolized *in vivo* to VIII.

EXPERIMENTAL SECTION

Drugs—Meperidine hydrochloride[1], normeperidine hydrochloride[1], and the *p*-hydroxy derivative of meperidine[2] (5) were obtained commercially. The *N*-oxide of meperidine was synthesized as follows. Meperidine hydrochloride (200 mg) was dissolved in 2 mL of water, basified with ammonium hydroxide, and extracted with benzene. After removal of the solvent under reduced pressure, the residue was dissolved in 1 mL of absolute ethanol, and 1 mL of 30% H_2O_2 was added. The solution was heated in a water bath at 70°C for 2 h. At that time, TLC showed no starting material. The solution was transferred to a dish and evaporated to near dryness on a steam bath. The residue was dissolved in isopropyl alcohol and transferred to a 15-mL centrifuge tube. The solvent was removed under a stream of nitrogen, to give a white liquid. Ether was added to the residue to give a white precipitate. The mixture was refrigerated and centrifuged, and the liquid phase was transferred to another centrifuge tube. By repeating this treatment, two products were obtained: ether-soluble material and a white precipitate. The solid material was recrystallized from absolute ethanol to yield 18 mg of white crystals, mp 173–175°C. The solid material showed a single spot on TLC (R_f 0.37 and

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