

Multiple Association Equilibria in the Self-Association of Methylene Blue and Other Dyes¹

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Abstract: The self-association of methylene blue has been shown to involve multiple stepwise association to form dimers, trimers, tetramers, and higher multimers. The "isoextraction" method employed gives values of the equilibrium dye monomer concentration, b , as a function of the total equivalent concentration B . The use of such $b(B)$ data to obtain quantitative information about stepwise association constants is investigated. Direct methods are usually not sufficiently precise. The dimerization constant, K_2 , however, has been obtained with high precision without recourse to any model of self-association. The stepwise trimerization constant, K_3 , in spite of the expected higher charge repulsion, is found to be greater than K_2 . To investigate the nature of the variation of the higher stepwise association constants, several one- and two-parameter models of stepwise self-association have been examined. The best model is one in which the higher stepwise association constants, K_4 , K_5 , etc., decrease in a mildly varying sequence, in accord with the electrostatic effects expected in dye association, based on calculated charge repulsions and activity coefficients. Some literature data on the solubility of an anionic dye, Orange II, have been analyzed and shown to be described well by the association model derived for methylene blue, suggesting that the model, or close relatives thereof, may be of general validity for the self-association of ionic dyes.

In the previous paper⁴ we have derived the qualitative features of a model of the self-association of methylene blue (MB) in aqueous solutions. The "isoextraction" method employed allows the determination of the equilibrium monomer concentration, b , as a function of the total equivalent concentration of MB, B . The present paper is concerned with the problems involved in the quantitative estimation of self-association constants from the $b(B)$ data and the applicability of various quantitative models of stepwise self-association to these data. Some solubility data of the anionic dye Orange II in NaCl and Na₂SO₄ from the literature⁵ have also been analyzed.

The symbols β_2 , β_3 , K_2 , K_3 , K_A , and $K_{A(0)}$ are used as defined in the previous paper.⁴

Direct Determination of K_2

To obtain reliable enthalpy and entropy data for the association of MB, it is necessary to isolate at least one K value with some precision, and to study its temperature dependence.

The $b(B)$ data can, in principle, be analyzed to obtain β_2 , β_3 , etc., by a reiterative procedure.⁶ Thus, considering that

$$B = b + 2\beta_2 b^2 + 3\beta_3 b^3 + \dots \quad (1)$$

a function of F_2 can be obtained, where

$$F_2 = \frac{B - b}{b^2} = 2\beta_2 + 3\beta_3 b + \dots \quad (2)$$

(1) Based in part on the doctoral dissertation of A. K. Ghosh, Calcutta University, 1963; presented in part at the 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966.

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(4) P. Mukerjee and A. K. Ghosh, *J. Amer. Chem. Soc.*, **92**, 6403 (1970).

(5) A. B. Meggy, *Discuss. Faraday Soc.*, **16**, 149 (1954).

(6) F. J. C. Rossotti and H. Rossotti, *J. Phys. Chem.*, **65**, 926, 930 (1961).

The value of F_2 at $b = 0$ is $2\beta_2$. Similar manipulation with $(F_2 - 2\beta_2)/b$ should yield β_3 , and so on.

Unfortunately, because of the multiple equilibria involved, this method gives imprecise information. Figure 1 shows a plot of F_2 vs. b for MB at 25.1° in the lower concentration range. The curve is nonlinear and the intercept depends strongly on the data at the lowest concentrations, where the amount of association is the least. The approximate value of K_2 from this plot is $(2.1 \pm 0.3) \times 10^3$ l./mol.

A considerably more reliable estimate of K_2 can be obtained from our data⁴ because of the fortunate circumstance that the K_A vs. B plots are linear at low concentrations over a substantial range (Figure 2). As the K_2 value thus derived is important for thermodynamic analysis,⁷ we discuss briefly its reliability, which depends on how linear is the variation of K_A with B in the range of Figure 2. To investigate this, the following regression analyses were performed. If K_A is represented by the polynomial

$$K_A = K_{A(0)} + PB + QB^2 + \dots \quad (3)$$

where P , Q , etc., are constants, the ratio $P/K_{A(0)} = K' = 2K_2$, as discussed later. On omitting the QB^2 and higher terms, the linear equation fitted the unweighted data to within 1.4%, with a correlation coefficient of 0.997, the standard error in P being 1.6%. To determine the possible uncertainty in K' , the second-degree polynomial was fitted to the unweighted data and both the linear and second-degree equations were fitted to the data weighted in an approximate fashion, after corrections for activity coefficients. This last correction was considered because, although our experiments were designed to keep the ionic strength, μ , low and nearly constant, small variations remained, the range being 0.0011–0.0016. The mean activity coefficients, f_{\pm} , of the extracting species, MBClO_4 , were estimated

(7) P. Mukerjee and A. K. Ghosh, *J. Amer. Chem. Soc.*, **92**, 6419 (1970).

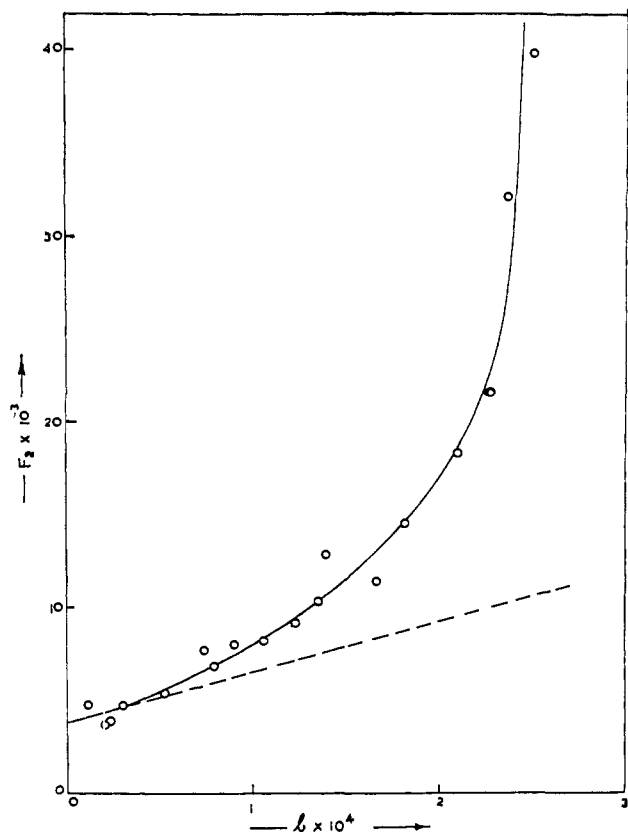


Figure 1. Variation of F_{\pm} (l./equiv) with the monomer concentration b (equiv/l.).

from NaCl data at the same μ and K_A was multiplied by f_{\pm}^2 . The variation in μ should not affect the values of K_2 and K_3 by more than about 1 and 2%, respectively.

The results are given in Table I. The value of Q was very small in all cases, and was both positive and negative, its standard error being several times the value itself. It seems, therefore, that linearity is a good

Table I. Polynomial Representation of K_A - B Data of MB at 25.1°

| Treatment of data | Eq | $K_{A(0)} \times 10^3$, (equiv/l.) ² | $P \times 10^3$, equiv/l. | $K' \times 10^{-3}$, l./equiv |
|---|-----------|--|----------------------------|--------------------------------|
| Unweighted, uncorrected | Linear | 3.41 | 1.540 | 4.52 |
| | Quadratic | 3.42 | 1.509 | 4.41 |
| Weighted | Linear | 3.41 | 1.536 | 4.50 |
| | Quadratic | 3.41 | 1.538 | 4.51 |
| Unweighted, corrected for activity coefficients | Linear | 3.16 | 1.416 | 4.48 |
| | Quadratic | 3.16 | 1.421 | 4.50 |

^a B is in the range 7.64×10^{-6} – 3.88×10^{-4} equiv/l.

approximation. The f_{\pm} corrections reduce the value of $K_{A(0)}$ appreciably but have very little effect on K' , except to reduce the standard errors somewhat. The overall standard error in K' is estimated to about 2%.

As $K_A/K_{A(0)} = B/b$,⁴ we can reorganize eq 3 to

$$K_A/K_{A(0)} = B/b = 1 + K'B + K''B^2 + \dots \quad (4)$$

where $K'' = Q/K_{A(0)}$. From eq 4 it can be derived that

$$B = b + K'b^2 + (K'^2 + K'')b^3 + \text{higher terms} \quad (5)$$

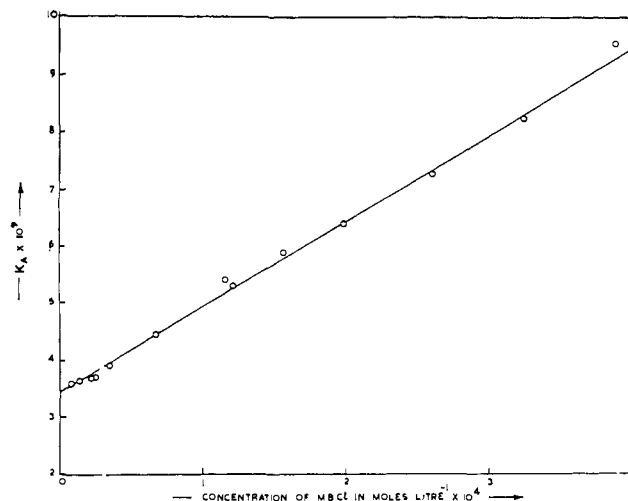


Figure 2. Linear variation of K_A [(equiv/l.)²] with B (equiv/l.) at low concentrations and 25.1°.

Comparison with eq 1 shows that $\beta_2 = K_2 = K'/2$, and $\beta_3 = (K'^2 + K'')/3$. K_2 can thus be determined to the same precision as K' . Thus, although eq 4 and 5 are quite general, the precision of K_2 is materially improved because of the linearity of Figure 2.

The value of K_2 at 25.1° is 2.24×10^3 l./mol, with an estimated standard error of about 2%. Previous spectral measurements have led to somewhat discordant values, 3.6×10^3 l./mol at 27°,⁸ 5.9×10^3 at 25°,⁹ and 2.0×10^3 at 30°.¹⁰

Estimate of K_3 . In the range of Figure 2, the regression analyses suggest that K'' is negligible with respect to K'^2 so that $K_3 = \beta_3/K_2$ has a value of $4/3 K_2$ or 2.99×10^3 l./mol. Taking the data at higher concentrations into account, it seems that the value of K_3 is somewhat uncertain, but it exceeds K_2 by a factor of 1.3–2, in spite of the extra charge repulsion associated with K_3 (see later). Thus, a substantial attractive interaction between second-nearest neighbors is indicated in dye association, a fact that should have important bearing on the cooperativeness of "stacking" interactions.⁷ Braswell's spectral study also led to a K_3/K_2 ratio of 1.5.¹⁰

Stepwise Association Models

The polynomial analysis of the data becomes rapidly unreliable for higher association constants. To investigate the nature of the self-association of higher multimers further, we have, therefore, attempted following the suggestions of Rosotti and Rosotti⁶ to analyze all of the data⁴ in terms of several models of stepwise association, in which some predetermined explicit relation between the successive equilibrium constants is invoked. The physical implications of the models and their relative ability to fit all of the data provide further insight into the nature of the stepwise equilibria. Interesting conclusions can be derived even when a model does not fit very well. The number of independent parameters is restricted to one or two. The symbol K is used for all models as a generalized parameter whose relation to actual K_2, K_3, \dots values is determined by the assumptions of the model.

(8) E. Rabinowitch and L. F. Epstein, *J. Amer. Chem. Soc.*, **63**, 69 (1941).

(9) K. Bergmann and C. T. O'Konski, *J. Phys. Chem.*, **67**, 2169 (1963).

(10) E. Braswell, *ibid.*, **72**, 2477 (1968).

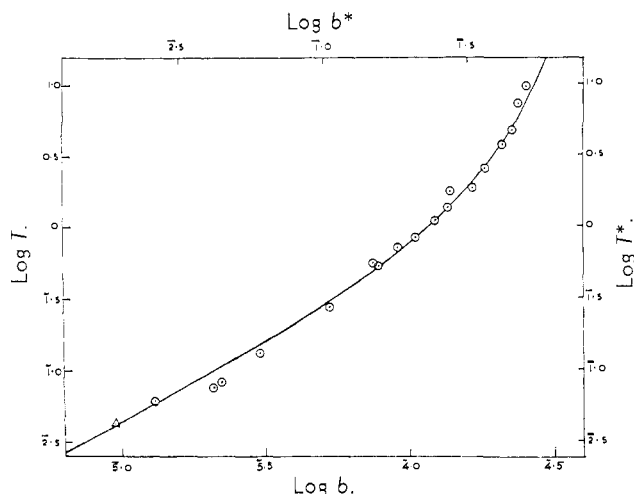


Figure 3. Description of the self-association behavior of MB over the entire concentration range at 25.1° by the two-parameter model IV.

One-Parameter Models. Model X. This model⁶ uses the simplest assumption that all K 's are the same, *i.e.*, $K_2 = K_3 = K_q = K_\infty = K$, and leads to the relation

$$\left(\frac{b}{B}\right)^{1/2} = 1 - Kb \quad (6)$$

The equation fits the data moderately well. Model X is vastly superior to any one-parameter model using one multimer, *e.g.*, the dimer. The value of K obtained is 2.5×10^3 l./mol, which is higher than K_2 obtained directly, indicating that at least one K_q is higher than K_2 . This agrees with the previous deduction that $K_3 > K_2$. The moderate success with this model suggests strongly that only mild variations in K_q with q will be permitted.

Model Y. This model was devised to accord with the linear behavior of K_A vs. B (Figure 2). It assumes that K_q increases slowly with q

$$K_q = K(q - 1)/q \quad (7)$$

so that $K_2 = K/2$, $K_3 = 2K/3$, $K_q \rightarrow K$ as $q \rightarrow \infty$. On summing the series, the predicted relation is

$$B/b = 1 + KB \quad (8)$$

as observed at low concentrations. This model, obviously, yields the same K_2 value as derived directly but probably gives a low K_3 . As the experimental K_A - B curve falls off at higher concentrations,⁴ the increasing sequence of K_q with q is not maintained although $K_3 > K_2$.

Two-Parameter Models. The two-parameter models below⁶ consider K_2 as an independent parameter, the higher K_q 's being related to the second parameter K .

Model I. Higher K_q 's are identical.

$$K_3 = K_4 = K_q = K \quad (9)$$

Model II. Higher K_q 's increase in a mild sequence.

$$K_q = K(q - 2)/(q - 1) \quad (q \geq 3) \quad (10)$$

Thus, $K_3 = K/2$, $K_4 = 2K/3$, $K_q \rightarrow K$ as $q \rightarrow \infty$.

Model III. Stepwise association becomes progressively more difficult. Higher K_q 's decrease relatively rapidly with q .

$$K_q = K/(q - 1) \quad (q \geq 3) \quad (11)$$

Thus, $K_3 = K/2$, $K_4 = K/3$, $K_q \rightarrow 0$ as $q \rightarrow \infty$.

Model IV. Higher K_q 's decrease mildly with q .

$$K_q = K(q - 1)/(q - 2) \quad (q \geq 3) \quad (12)$$

so that $K_3 = 2K$, $K_4 = 3/2K$, $K_q \rightarrow K$ as $q \rightarrow \infty$.

To treat the experimental results, two normalized, dimensionless variables are defined.

$$T^* = TK/K_2 \quad (13)$$

$$b^* = Kb \quad (14)$$

where

$$T = (B - b)/b \quad (15)$$

Unique normalized curves of $\log T^*$ against $\log b^*$ can be calculated from each model according to the following equations⁶

$$\text{model I} \quad T^* = \frac{b^*(2 - b^*)}{(1 - b^*)^2} \quad \text{for } b^* < 1 \quad (16)$$

$$\text{model II} \quad T^* = \frac{b^*}{1 - b^*} - \ln(1 - b^*) \quad \text{for } b^* < 1 \quad (17)$$

$$\text{model III} \quad T^* = e^{b^*}(1 + b^*) - 1 \quad (18)$$

$$\text{model IV} \quad T^* = 2b^*/(1 - b^*)^3 \quad \text{for } b^* < 1 \quad (19)$$

To determine if a model fits the data, $\log T$ against $\log b$ curves from experimental data are plotted on transparent graph papers, using the same scales as the $\log T^*$ against $\log b^*$ curves. If the experimental curve can be superimposed on any model curve by suitable displacements along the coordinate axes, the model fits. From the positions of best fit, K_2 and K are determined using eq 13 and 14. Tolerable displacements of the experimental curve over the normalized curve give rough limits of error.

When our data at 25.1, 34.9, and 10.0°⁷ were considered together, it was found that, although none of the models fitted exactly, models I and II were distinctly less satisfactory than III or IV. Of the latter two, IV appeared to be slightly better. Table II collects some

Table II. Stepwise Association Constants (l./mol $\times 10^{-3}$) for MB at 25.1° from Various Models of Association

| | Direct determin ^a | Model | | |
|------------|---------------------------------|----------------|------------------|-----------------|
| | | X ^b | III ^c | IV ^c |
| K_2 | 2.26 | 2.5 | 2.16 | 2.09 |
| K_3 | | 2.5 | 4.35 | 3.99 |
| K_4 | | 2.5 | 2.90 | 3.00 |
| K_∞ | | 2.5 | 0 | 2.00 |

^a From the initial linear curve of K_A against B (Figure 2), without activity corrections. ^b One parameter. ^c Two parameters.

K_q values obtained from various models. The best values of K_2 from models III and IV agree reasonably well with that directly determined, but not exactly. Both models gave higher K_3 values than K_2 , in agreement with previous deductions. The description of the data at 25.1° by model IV is shown in Figure 3.

Electrostatic Interactions

The models examined above involve conveniently summable infinite series but are not the only ones possible. Although models III and IV showing decreasing K_q at higher q values are in qualitative agreement with the increasing charge repulsion expected, it is of some interest to examine briefly if these interactions are accounted for in a reasonably quantitative manner in the models.

Charge Repulsion. The qualitative effect of charge repulsion is to reduce the association constants. We make the simplifying assumptions that in a multimer of MB the distance, r , separating any two dye ions in contact is the same, that the energy of repulsion between any two dye ions follows a Coulomb's law type of behavior, *i.e.*, it is inversely proportional to the distance separating the dye ions, and that the energies of repulsion are additive. It follows, therefore, that if the stepwise association constants corrected for charge repulsions are given by K_2^* , K_3^* , K_q^* , etc., and if K_2^*/K_2 is given by the factor x , so that $\log x$ is proportional to the free energy of repulsion and, therefore, to $1/r$, then

$$\log K_q^* - \log K_q = (\log x) \sum_{i=1}^{q-1} 1/q \quad \text{for } q \geq 2 \quad (20)$$

A rough estimate of x , a measure of the charge repulsion in the dimer of MB, can be obtained from our study of the mutual association of MB with quinoline and quinolinium ion.¹¹ On the introduction of the charge, the mutual association constant decreases by a factor of 5. This factor is probably an overestimate for x above, considering that the introduction of the charge should reduce the attractive hydrophobic interactions between MB and quinoline somewhat, and that the charge on the quinolinium ion is localized, whereas that on MB must be quite diffuse because of resonance. For two point charges separated by 3.5 Å, which is characteristic of aromatic ring systems in face-to-face contact, $x = 7.4$, using Coulomb's law and the dielectric constant of water.

To compare with models III and IV, we assume further that $K_3^* = K_4^* = K_q^*$, *i.e.*, there are no attractive interactions between dye ions when they are separated by two or more dye ions. Thus, $\log K_q - \log K_3$ when $q > 3$ should be given by charge effects alone. Figure 4 compares this difference calculated from models III and IV, and that calculated assuming values of 2, 3, and 5 for x , using eq 20. Model III seems to be in better accord than model IV, and is compatible with a reasonable value of 3 for x .

Activity Coefficient Effects. The charge repulsions above apply only at infinite dilution. At a finite ionic strength, they are countered to some extent by the ion atmosphere. This last effect can be estimated from activity coefficients, and because of our low ionic strengths, the Debye-Hückel limiting law should have moderate validity. At 25° for a $q:1$ valence-type electrolyte, we have¹²

$$-\log f_{\pm(q:1)} = 0.51q\sqrt{\mu} \quad (21)$$

(11) P. Mukerjee and A. K. Ghosh, unpublished results; A. K. Ghosh, Ph.D. Dissertation, Calcutta University, 1963.

(12) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," 2nd ed, Butterworths, London, 1959.

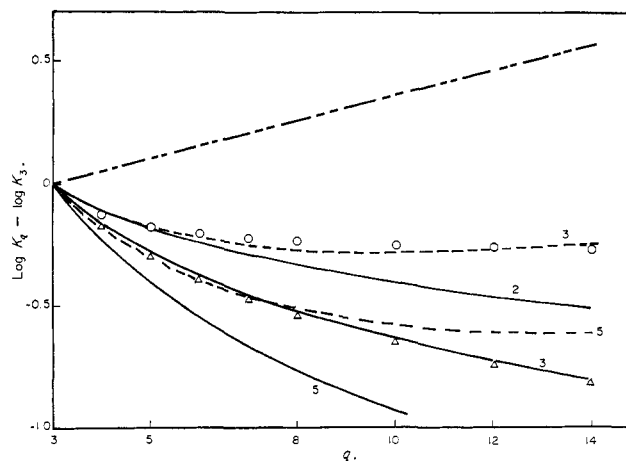


Figure 4. Effect of electrostatic interactions on stepwise association constants. Calculated effects of charge repulsion alone, — (for $x = 2, 3$, and 5); activity coefficients alone, - - - -; combination of charge repulsion and activity coefficients, - - - - ($x = 3, 5$); self-association model III, Δ ; model IV, \circ .

If $K_{q,t}$ is the true thermodynamic constant, it follows that

$$K_{q,t} = \frac{[b_q]}{[b_{q-1}]b f_{\pm(q-1:1)} f_{\pm(1:1)}^{q-1}} \quad (22)$$

Using eq 21 we have

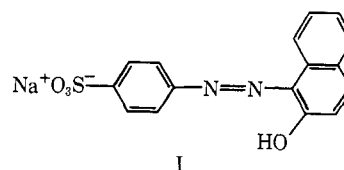
$$\log K_q = \log K_{q,t} + 1.02(q-1)\sqrt{\mu} \quad (23)$$

The activity coefficients in eq 22 cancel to a great extent, but not exactly, as suggested recently.¹⁰ Figure 4 shows the effect of activity coefficients on $\log K_q - \log K_3$, calculated from eq 23, using a mean value of 0.05 for $\sqrt{\mu}$. The actual values of $\sqrt{\mu}$ fall in the range of 0.035–0.065.

The activity coefficient effects increase the value of K_q as q increases. The combination of the activity coefficient effects and a value of 3 for x in eq 20 comes quite close to model IV. Thus models III and IV are not unreasonable with respect to the electrostatic interactions involved in dye association and, at moderate μ , model IV, with a mildly decreasing sequence of K_q values, is probably preferable to model III, in which $K_q \rightarrow 0$ as $q \rightarrow \infty$. At high μ , even model IV may overestimate the charge interactions. For uncharged species, in particular, model I with $K > K_2$ is likely to be more satisfactory in all "stacking"-type interactions.

Self-Association of Orange II from Solubility Data

It is of considerable interest to examine how broadly the general conclusions derived above apply to other systems. Unfortunately, there are very few studies in the literature in which equilibrium methods have been used. The solubility data of the dye Orange II (I)⁶ in NaCl and Na₂SO₄ solutions appear to be moderately



suitable, particularly because a different experimental method is involved. The data in NaCl are given in Table III.

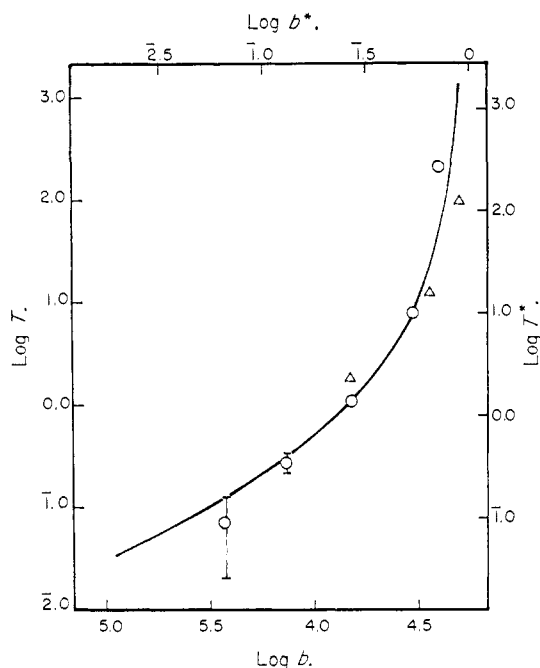


Figure 5. Description of the self-association behavior of Orange II by the two-parameter model IV. The vertical line heights correspond to an error of $\pm 5\%$ in the estimated S_0 ; O, NaCl solutions; Δ , Na_2SO_4 solutions.

To analyze the data, we have assumed activity coefficients to remain constant. This is less drastic an approximation than may appear at first sight. The data used for quantitative calculations have ionic strengths in the range 0.2–2 mol/l. In this range, f_{\pm} of the supporting electrolyte, NaCl, varies by only about 12%.¹² Since, by Harned's rule,¹² the activity coefficients of the dye species must be modified to a great extent by those of NaCl, their variations are also reduced considerably. As over this range in concentration, the apparent solubility product changes by a factor of about 100, mainly because of self-association, the changes in activity coefficients can produce only a minor effect.¹³

The apparent solubility product, S_A , is given by $S_A = B[\text{Na}^+]$. On extrapolating $S_A(B)$ data to $B = 0$, we obtain the true solubility product, $S_0 = b[\text{Na}^+]$. The ratio S_A/S_0 gives the value of B/b at any concentration B . Table III shows that extremely large values of B/b are attained at low salt and high dye concentrations. Previously suggested explanations in terms of

(13) A. K. Ghosh and P. Mukerjee, *J. Amer. Chem. Soc.*, **92**, 6413 (1970).

Table III. Solubility Data of Orange II in NaCl at 25°, $S_0 = 7.5 \times 10^{-6}$ (equiv/l.)²

| [NaCl], M | B , ^a equiv/l. | S_A , ^b = $B[\text{Na}^+]$ | B/b | b , ^c M |
|--------------|--------------------------------|---|-------|-----------------------|
| 2.0 | 4.01×10^{-5} | 8.02×10^{-5} | 1.07 | 3.75×10^{-5} |
| 1.0 | 9.58×10^{-5} | 9.58×10^{-5} | 1.28 | 7.50×10^{-5} |
| 0.5 | 3.20×10^{-4} | 1.60×10^{-4} | 2.13 | 1.50×10^{-4} |
| 0.25 | 2.70×10^{-3} | 6.82×10^{-4} | 9.1 | 2.96×10^{-4} |
| 0.10 | 5.00×10^{-2} | 7.50×10^{-3} | 126 | 3.97×10^{-4} |
| 0.05 | 1.65×10^{-1} | 3.55×10^{-2} | 473 | 3.49×10^{-4} |
| 0.02 | 2.13×10^{-1} | 4.96×10^{-2} | 661 | 3.22×10^{-4} |
| 0.00 | 2.90×10^{-1} | 8.41×10^{-2} | 1120 | 2.59×10^{-4} |

^a Solubility of Orange II. ^b Apparent solubility product. ^c Concentration of monomers.

variations in activity coefficients⁵ and tetramerization of Orange II¹⁴ appear to be unlikely.^{4,14}

For applying our self-association models, we note that when B/b values are extremely high, large multimers must form extensively. As μ is also high, association of counterions may not always be negligible, particularly because the charge in Orange II is localized. If, as a result, the concentration of counterions is depleted substantially the calculated S_A values will be too high, leading to low estimates of b . This seems to be the case at the three lowest NaCl concentrations in Table III, for which the estimated b values decrease somewhat with B . At the higher NaCl concentrations even substantial counterion binding should cause little error in S_A .

If we disregard the three highest solubility data on this basis, the remaining data, including three measurements in Na_2SO_4 solutions at 0.5, 0.2, and 0.1 N,⁵ are well represented by model IV, as shown in Figure 5. Considering that the data include B/b values well over 100, *i.e.*, a monomer fraction less than 0.01, this is a fairly stringent test of the model. The similarity with the MB system extends even to the fact that the estimated K_3 , 3.5×10^3 l./mol, is higher than K_2 , 1.4×10^3 l./mol. We conclude, therefore, that the broad features derived for the self-association of MB are probably characteristic of dye systems generally. These features are presumably due to the fact that dyes are basically flat aromatic molecules, which associate in the form of stacks, the important interactions being relatively nonspecific van der Waals' forces, hydrophobic interactions, and electrical repulsions.^{7,11}

Acknowledgment. A. K. G. is grateful to the Council of Scientific and Industrial Research, New Delhi, India, for financial support.

(14) G. A. Gilbert, *Discuss. Faraday Soc.*, **16**, 234 (1954).