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Studies of the Aggregation of Dyes. The Scope of Application of the Maximum-slope Method

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The maximum-slope method, previously suggested by one of the authors has proved to be more advantageously applied to the determination of the aggregation constant (K_n) and the degree of aggregation (n) in a dye solution than is Zanker's method. Changes in the spectra of the aqueous or ethanolic solutions of Rhodamine 6G, 3,6-diaminoacridine and thionine were studied as their concentration increased by means of both the maximum-slope method and Zanker's method. The aggregation characteristics obtained by these two methods were then compared with each other and discussed.

Recently the aggregation of molecules in solutions has been drawing much attention from men in various fields because it has a wide application.

Spectroscopic measurement has been employed in determining the aggregation constant and the degree of aggregation because of its accuracy and its simplicity.

It is, however, rather difficult to make a reliable estimation of the aggregation parameters, even for a simple equilibrium system between a monomer and a polymer of one definite degree of aggregation, by means of the graphic analysis of the spectroscopic data. The reason for this is that there are more unknown parameters than the number of given equations.

A way to overcome this difficulty has already been developed. Zanker derived the following equation (Eq. (1))[1]:

$$\log C_0(\varepsilon_1-\varepsilon) = n\log C_0(\varepsilon-\varepsilon_n/n) + \log K_n n(\varepsilon_1-\varepsilon_n/n)^{1-n} \qquad (1)$$

were ε is the apparent absorptivity at a difinite wave length, where ε_1 and ε_n are the absorptivities of the monomer and the aggregate respectively, at the same wavelength, and where C_0 is the total concentration. He assumed suitable values for ε_1 and ε_n/n, and plotted $\log C_0(\varepsilon_1-\varepsilon)$ against $\log C_0(\varepsilon-\varepsilon_n/n)$. The degree of aggregation, n, was given by the slope of this plotted straight line, and the equilibrium constant (K_n) was calculated from the intercept.

This method has been recognized as the simplest and most convenient, bus it cannot be advantageously applied to the cases where the value of either ε_1 or ε_n/n has not been determined accurately.

Another graphic method has been developed by one of the present authors (M.H.). The method, which is called "the maximum-slope method"[2], estimates the ε_1 value by extrapolating the ε-$\log C_0$ curve to an infinite dilution, while the maximum slope (m_0) is determined by the tangent of the curve. The values of n, ε_n/n, and K_n are selected in such a way that they give the best agreement with the experimental curve.

In this paper, the maximum-slope method will be compared with Zanker's method and the advantages and applicability of the former will be discussed.

Results and Discussion

1. Analysis of the Computed Aggregation System. By taking suitable values for ε_1, ε_n/n, and $\log K_n$, the apparent absorptivities were computed over a range of concentrations between 10^{-6} and 10^{-3} mol/l, and the ε-$\log C_0$ curve was analyzed graphically by both the maximum-slope method and Zanker's method. A single equilibrium system between the monomer and the dimer was postulated for the sake of simplicity. The apparent absorptivities (ε) of this system were calculated by means of following equation:

$$\varepsilon = \varepsilon_2/2 + \frac{(\varepsilon_1-\varepsilon_2/2)}{4K_2}\left(-1+\sqrt{1+8K_2C_0}\right) \qquad (2)$$

Assuming ε_1 and $\varepsilon_2/2$ to be 1×10^5 and 0.0 respectively, the apparent absorptivities were computed for $\log K_2=2,3,4$ and 5. Curve (1) in Fig. 1 suggests that the dimerization of $\log K_2=5$ commences even in a very dilute range (below 10^{-5} mol/l); consequently, ε_1 cannot be accurately determined. If

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[1] V. Zanker, *Z. Phys. Chem.*, **200**, 250 (1952).

[2] a) M. Hida, A. Yabe, H. Murayama and M. Hayashi, This Bulletin, **41**, 1776 (1968). b) Equations (4) and (6) in previous paper (Ref. 2a) should be read $f=K_nC_1{}^nC^{-1}$ and $\alpha=(nK_n)^{1/(n-1)}(\varepsilon_1-\bar{\varepsilon}_n)^{-1}$, respectively.

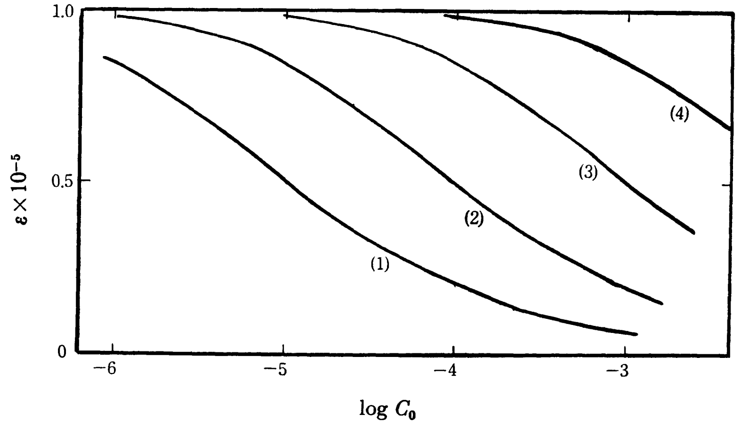

Fig. 1. Computed absorptivity *vs.* log C_0.
(1); log K_2=5.0, (2); log K_2=4.0, (3); log K_2=3.0, (4); log K_2=2.0

ε_1 is estimated by extrapolation at 0.95×10^5, $\varepsilon_2/2$ and $\log K_2$ will be found to be -0.06×10^5 and 4.81 respectively by the use of the maximum slope method. These smaller values may be caused by the underestimation of the value of ε_1. If the errors made in estimating ε_1 and in drawing the maximum slope (m_0) are designated as $\delta\varepsilon_1$ and δm_0, the errors in the values of ε_n/n and $\log K_n$ obtained by the maximum-slope method are given by Eqs. (3) and (4):

$$\delta(\varepsilon_n/n) = \delta\varepsilon_1 - (\varepsilon_1-\varepsilon_n/n)_t(\delta m_0/m_0) \tag{3}$$

$$\delta(\log K_n) = (n-1)\delta\varepsilon_1/m_0 + n\log(\overline{1-r/r\sqrt{n}}) + (n-1)\log(1+\delta m_0/m_0) \tag{4}$$

where $r=(m/m_0)/(1+\sqrt{n})$ and where the subscript *t* denotes the true value.

By substituting the numerical values listed below for $\delta\varepsilon_1$, δm_0, *r*, and m_0 in Eqs. (3) and (4), $\delta(\varepsilon_2/2)$ and $\delta(\log K_2)$ are obtained as -0.06×10^5 and -0.114 respectively.

TABLE 1. COMPARISON OF THE TRUE AND OBTAINED VALUES OF THE AGGREGATION PARAMETERS

No.	True values			Values obtained by: The maximum slope method				Zanker's method			
	$\varepsilon_1\times10^{-5}$	$\bar{\varepsilon}_2\times10^{-5}$	log K_2	$\varepsilon_1\times10^{-5}$	$\bar{\varepsilon}_2\times10^{-5}$	log K_2	*n*	$\varepsilon_1\times10^{-5}$	$\bar{\varepsilon}_2\times10^{-5}$	log K_2	*n*
1	1.0	0.0	5.0	0.95	−0.06	4.81	2	0.95	0	4.92	2
2	1.0	0.0	4.0	1.0	0.0	4.00	2	analysis is impossible			
3	1.0	0.0	3.0	1.0	0.0	3.00	2				
4	1.0	0.0	2.0	1.0	0.20	2.18	2				

$$\delta\varepsilon_1 = -0.05\times10^5,$$
$$\delta m_0/m_0 = -0.01,$$
$$r = (1-0.01)/(1+\sqrt{2}) = 0.411,$$
$$m_0 = 0.396\times10^5$$

Therefore, the values of $\varepsilon_2/2$ and $\log K_2$ obtained by the maximum-slope method would be -0.06×10^5 and 4.886 (*cf.* Table 1).

The maximum-slope method can derive correct values of $\varepsilon_2/2$ and $\log K_2$ for the dimerization of $\log K_2=4$ and 3, because the values of ε_1 and the maximum slope can be determined accurately. On the other hand, Curve (4) ($\log K_2=2$) in Fig. 1 shows that the amount of the aggregate is not sufficient to determine the accurate maximum slope. If the slope at $\log C_0=-3.5$, which is eight-tenths of the maximum value, is taken as the maximum slope, we obtain:

$$\delta m_0/m_0 = -0.20.$$

Consequently, $r=(1-0.20)/(1+\sqrt{2})=0.331$. The absorptivity of the monomer (ε_1) can be determined precisely by means of curve (4); that is:

$$\delta\varepsilon_1=0.$$

By substituting the numerical values for these quantities in Eqs. (3) and (4), the deviations of the values of $\varepsilon_2/2$ and $\log K_2$ were found to be 0.2×10^5 and 0.213 respectively. Therefore, the values of $\varepsilon_2/2$ and $\log K_2$ obtained by the maximum-slope method are 0.2×10^5 and 2.21 respectively. These

TABLE 2. AGGREGATION PARAMETERS OF RHODAMINE 6G IN AQUEOUS SOLUTION

Method	Without KCl				With KCl			
	n	$\varepsilon_1\times10^{-4}$	$\varepsilon_2\times10^{-4}$	$\log K_2$	n	$\varepsilon_1\times10^{-4}$	$\varepsilon_2\times10^{-4}$	$\log K_2$
Max. slope	2	8.50	1.50	3.41	2	8.55	0.67	3.57
Zanker	2	8.50	0.39	3.35	2	8.55	0.0	3.51

values agree well with the values shown in Table 1. Table 1 may suggest that the maximum-slope method can more reasonably be applied, if the maximum slope and either ε_1 or $\varepsilon_2/2$ are determined, than Zanker's method, which is confined within narrower limits of $\log K_2$, because the values of both ε_1 and $\varepsilon_2/2$ should be determined.

2. Aggregation of Rhodamine 6G in an Aqueous Solution. The changes in the spectrum of the solution with the dye concentration were analyzed by both methods. The results are compared in Table 2.

Baranova and Levshin reported that Rhodamine 6G aggregates in the dimerization stage at concentrations below 2×10^{-3} mol/l, but higher aggregates are formed at higher concentrations.[3] The plotting of ε against $\log C_0$ (Fig. 2) also shows that this dye appears to exist in an equilibrium between the monomer and the dimer in the concentration range below $10^{-3.5}$ mol/l, but, since there was some discrepancy between the measured value and the calculated curve ($n=2$) in the concentration range above about $10^{-3.5}$ mol/l, Rhodamine 6G is likely to exist in equilibria between the monomer and higher aggregates ($n\geq3$) in a higher concentration range.

Table 2 also shows no influence of the added potassium chloride (0.2 mol/l) on the aggregation, while Glowacki and his co-workers observed some effect of added salts (>0.24 mol/l) on the aggregation and interpreted the effect on the basis of the structural change in the aqueous solution and the salt effect. Therefore, it seems that the formation of the ion pair does play little part in the aggregation of Rhodamine 6G in a solution which has no added salt.

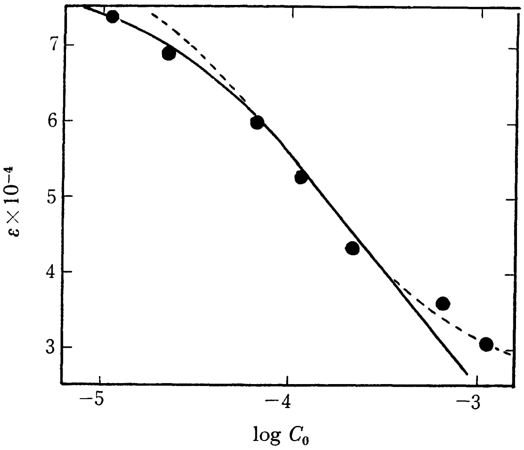

Fig. 2. Apparent absorptivity *vs.* log C_0 of rhodamine 6G in aqueous solution.
● observed
— calculated ($n=2$)
--- calculated ($n=3$)

Figure 3 shows the spectra of the monomer and dimer, computed from those of Rhodamine 6G at different concentrations by means of the least-squares method. The spectrum of the monomer has two absorption bands in the visible region; their maximum wavelengths are 525 and about 490 mμ, while the absorption band of the dimer is at 490 mμ. This hypsochromic shift may be interpreted in terms of the weak coupling theory suggested by DeVoe.[4]

According to the weak coupling theory, the dimer absorptivity of the band M at the wave number σ is expressed by Eq. (5):

$$\varepsilon_{\rm M}(\sigma)=2\varepsilon^0_{\rm M}(\sigma)[1-2(F_{\rm MM}(\sigma)\alpha_{\rm M}(\sigma)+F_{\rm MN}(\sigma)\alpha_{\rm N}(\sigma))] \quad (5)$$

where $\varepsilon_{\rm M}(\sigma)$ and $\varepsilon^0_{\rm M}(\sigma)$ denote the monomer and dimer absorptivities respectively, where $\alpha_{\rm M}$ is the partial group polarizability of the band M of the

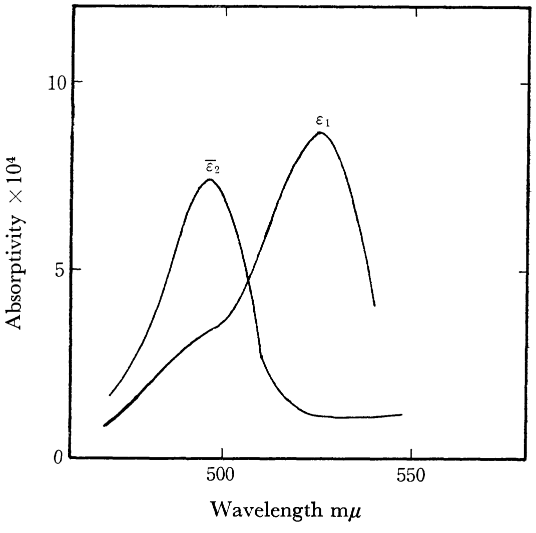

Fig. 3. Calculated absorption spectra of monomer and dimer of Rhodamine 6G in aqueous solution.

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3) E. G. Baranova and V. L. Levshin, *Optika i Spektroskopia*, **10**, 182 (1961).

4) H. DeVoe, *J. Chem. Phys.*, **37**, 1534 (1962).

monomer, and where F_{MN} is a group interaction factor between the monomer bands M and N. The value of F_{MN} in (erg D^{-2}) can be calculated by means of Eq. (6):

$$F_{\mathrm{MN}} = (10^{-36}/2)\sum_{i}\sum_{j\neq i}(R_{ij})^{-3} \times[\mathbf{e}_{i\mathrm{M}}\mathbf{e}_{j\mathrm{N}}-3(\mathbf{e}_{i\mathrm{M}}\cdot\mathbf{e}_{ij})(\mathbf{e}_{j\mathrm{N}}\cdot\mathbf{e}_{ij})]\times(\mathbf{e}_{i\mathrm{M}}\cdot\mathbf{e}_{j\mathrm{N}}) \quad (6)$$

where R_{ij} is the distance (cm) between the molecules i and j, where $\mathbf{e}_{i\mathrm{M}}$ and $\mathbf{e}_{j\mathrm{N}}$ are the unit vectors of the transition moments, and where $\mathbf{e}_{ij}$ is a unit vector along the line connecting the molecules.

The partial group polarizability of the band M (α_{M}) is given by:

$$\alpha_{\mathrm{M}}(\sigma) = 0.923\times10^{14}\int\frac{\varepsilon^{0}_{\mathrm{M}}(\mathrm{S})}{S^{2}-\sigma^{2}}\mathrm{d}s$$

The value of $\alpha_{\mathrm{M}}(\sigma)$ is positive for wave numbers shorter than σ_0, where a nearly maximum absorption is obtained,5) and negative for wave numbers larger than σ_0.

If the transition moments of the two monomer bands (A and B) are perpendicular to each other, and if the molecular plane is perpendicular to a common axis,6) the dimer spectrum may be predicted to be as follows: (1) When neighbouring moments, $\mu_{1\mathrm{A}}$ and $\mu_{2\mathrm{A}}$ ($\mu_{1\mathrm{B}}$ and $\mu_{2\mathrm{B}}$), are parallel to each other in the dimer,

$$F_{\mathrm{AA}}>0,\ F_{\mathrm{BB}}>0,\ \text{and}\ F_{\mathrm{AB}}=0;$$

namely, there is self-interaction between the monomer bands, A and B, but no mutual interaction between them.7) In this case, both monomer bands (A and B) can be predicted to shift hypsochromically and hyperchromically. (2) On the other hand, when the monomer bands, A and B, interact with each other without self-interaction, that is, when $F_{\mathrm{AB}}>0$ and $F_{\mathrm{AA}}=F_{\mathrm{BB}}=0$ (this is possible if the neighbouring moments, $\mu_{1\mathrm{A}}$ and $\mu_{2\mathrm{A}}$ ($\mu_{1\mathrm{B}}$ and $\mu_{2\mathrm{B}}$), are perpendicular to each other,8)) the absorptivity of the band with the longer λ_{max} may decrease, while that with the shorter λ_{max} may increase.

Thus, the dimer spectra observed may be understood in terms of the weak coupling mechanism, in which monomer bands interact with one another

TABLE 3. AGGREGATION PARAMETERS OF 3,6-DIAMINOACRIDINE IN AQUEOUS AND ETHANOLIC SOLUTIONS

Solvent	n	$\varepsilon_1\times10^{-4}$	$\bar{\varepsilon}n\times10^{-4}$	$\log K_2$	Δ*	Method
H_2O	2	4.20	2.15	2.95	0.0139	Max. Slope
	3	4.11	2.88	7.04	0.0086	Min. Dev.
	2	4.17	2.60	3.37	—	Zanker
EtOH	2	5.20	0.0	3.52	—	Max. Slope
	2	5.20	0.0	3.74	—	Zanker

* $\Delta = \frac{1}{N}\sum|\varepsilon_{obs} - \varepsilon_{calc}|/\varepsilon_{obs}$

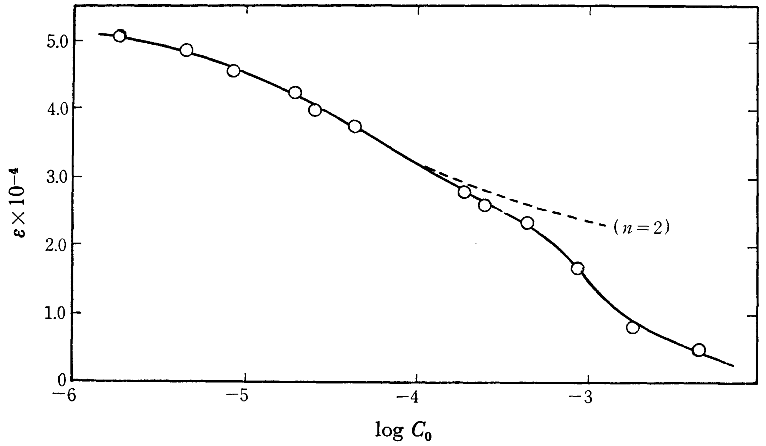

Fig. 4. Apparent absorptivity *vs.* log C_0 of 3,6-diaminoacridine in ethanolic solution.

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5) The partial group polarizability is not always zero at the wave number of maximum absorption. When the monomer absorptivity ($\varepsilon_M{}^0(\sigma)$) is symmetrical about the wave number of maximum absorption, the wave number, where α_M is zero, is very near to that of the maximum absorption.

6) The spectra of dimer with another geometry will be discussed elsewhere.

7) Self-interaction means vibronic interaction of monomer band with the other wave number regions of the same band.

8) Therefore, μ_{1A} and μ_{2B} (μ_{1B} and μ_{2A}) are parallel to one another.

with or without self-interaction. However, much more work has to be done in this connection.

3. 3,6-Diaminoacridine in Aqueous and Ethanolic Solutions. Since the change in the spectrum of an aqueous solution of 3,6-diaminoacridine with the increase in the concentration was not very large, reliable results could not be obtained. Assuming that $\varepsilon_n/n=0$, Mataga determined the degree of aggregation and $\log K_n$ as 2 and 3.43 respectively by means of Zanker's method.[9]

The maximum-slope method suggested that the monomer-dimer system agrees better with the experimental data than any other system, as is shown in Table 3. If the aggregation parameters are corrected by the minimum deviation method and have a minimized mean relative deviation,[10] the monomer-trimer system seems to agree better with the experimental data. However, the two mean relative deviations (Δ) calculated by taking $n=2$ and 3 are sufficiently smaller than the experimental error (about 2%). Thus, we were not able to conclude which model was preferable.

Figure 4 shows that 3,6-diaminoacridine aggregates in two steps in an ethanolic solution. In the concentration range below 10^{-4} mol/l, 3,6-diaminoacridine seems to exist in a monomer-dimer equilibrium system, but higher aggregations proceed above 10^{-4} mol/l as:

$$nD_2 \rightleftarrows D_{2n}.$$

A specific value of n was not determined in the experiment because of a lack of sufficient reliable experimental data.

The aggregation parameters obtained by different methods are compared in Table 3.

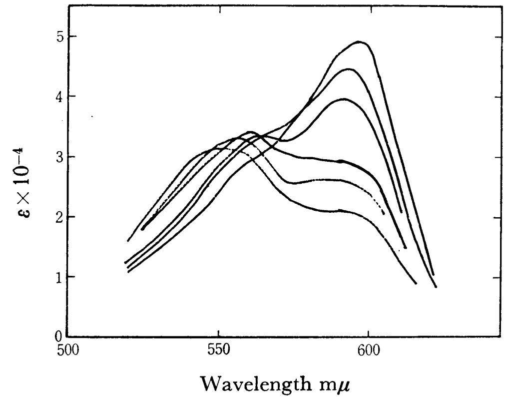

Fig. 5. Spectral change of thionine with increase of concentration.

4. Thionine (T^+Cl^-) in Aqueous Solution. Since no isosbestic point is observed, as is shown in Fig. 5, it seems that there are more than three species of thionine in its aqueous solution. The following species may be considered:

(i) several aggregates with different degrees of aggregation: $(T^+)_n$,
(ii) acid-base reaction products: TH^{2+} or TOH,
(iii) their aggregate(s), and
(iv) the ion pair and its aggregate(s): TCl and $(TCl)_n$.

In order to examine whether or not acid-base reaction products take part in the aggregation of thionine, it is necessary to determine the pK_a and pK_b values of thionine. The apparent absorptivity (ε) of the system of the protonation reaction (Eq. (7)) is given by Eq. (8):

$$T^+ + nH^+ \rightleftarrows (TH_n)^{(n+1)+} \tag{7}$$

$$\varepsilon = (\varepsilon_T[T^+]+\varepsilon_{TH_n}[TH_n])/([T^+]+[TH_n]) \tag{8}$$

By combining Eq. (8) with the law of mass action (Eq. (9)), Eq. (10) is obtained:

$$K = [TH_n]/[T^+][H^+]^n \tag{9}$$

$$\log(\varepsilon - \varepsilon_{TH_n}) - \log(\varepsilon_T - \varepsilon) = n\text{pH} + \text{p}K_b \tag{10}$$

If the values of both ε_T and ε_{THn} are determined, the slope and the intercept of the straight line made by plotting $\log(\varepsilon - \varepsilon_{THn})/(\varepsilon_T - \varepsilon)$ against the pH give the degree of protonation and the pK_b of thionine.[11] On the other hand, when either ε_{THn} or ε_T cannot be determined, the values of n and pK_b are obtained by a procedure similar to the maximum-slope method, because the maximum slope (m_0) of the tangent of the ε-pH curve is given by Eq. (11):

$$m_0 = \left(\frac{d\varepsilon}{d\text{pH}}\right)_{\max} = \frac{n}{4}(\varepsilon_T - \varepsilon_{TH_n}) \tag{11}$$

The n and pK_a values of the hydroxylation reaction can be similarly determined by analyzing the chages in the spectrum of the alkaline solution. The pK_a and pK_b values of thionine, as determined by the method described above, are shown in Table 4.

TABLE 4. THE VLAUES OF pK_a AND pK_b OF THIONINE

Wave-length (mμ)	$\varepsilon_T\times10^{-4}$	$\varepsilon_{TH_n}\times10^{-4}$	n	pK_b
597	4.90	1.56	1	0.72
670	0.0	4.00	1	0.86
		mean	1	0.79
Wave-length (mμ)	$\varepsilon_{T^+}\times10^{-4}$	$\varepsilon_{TOH}\times10^{-4}$	n	pK_a
597	4.90	1.00	1	8.8

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9) N. Mataga, This Bulletin, **30**, 375 (1957).

10) Adjustment of the parameters by trial and error method is repeated until the minimum value of deviation is attained and the final values of the aggregation parameters are regarded as the best ones (Ref. 2). But it can be shown that the final values obtained somewhat depend on definition of deviation. This subject will be discussed further elsewhere.

11) In very high acid concentration H_0-function should be used in site of pH.

TABLE 5. AGGREGATION PARAMETERS OF THIONININE IN AQUEOUS SOLUTION

n	$\varepsilon_1\times10^{-4}$	$\bar{\varepsilon}_2\times10^{-4}$	$\bar{\varepsilon}_3\times10^{-4}$	$\log K_2$	$\log K_3$	$\varDelta$	Method
2	5.25	0.0	—	2.90	—	0.0237	max. slope
3	5.25	—	1.39	—	6.52	0.0511	max. slope
2+3	5.03	2.38	0.93	2.90	6.52	0.0049	min. dev.

These results indicate that the amounts of TOH and TH^{2+} are negligibly small in the pH range between 1 and 8. Hence, the existence of acid-base reaction products and thir aggregates can be disregarded.

In addition, the ion pair and its aggregate(s), TCl and $(TCl)_n$, can also be neglected because no change was observed in the spectrum upon the addition of potassium chloride in the concentration range between 2×10^{-3} and 2 mol/*l*. Therefore, the absence of an isosbestic point in the change in the thionine spectrum implies that several aggregates are equilibrated; the aggregation system may, then, be in a multistage equilibrium. Although the maximum-slope method cannot be advantageously employed for the analysis of the system which includes several species with different degrees of aggregation, the change in absorptivity at 597 mμ (wavelength where the maximum absorption of the monomer is observed) was analyzed by this method, assuming that the aggregation system could be approximated by a single equilibrium system. The results (shown in Table 5) indicate that thionine may aggregate in the dimer and the trimer in an aqueous solution.

In order to minimize the mean relative deviation, the molar absorptivities of the monomer and the aggregates and the formation constants of the aggregates were adjusted by the minimum deviation method. These corrected values are also given in Table 5. Thus, thionine seems to aggregate in the monomer-dimer-trimer system. The wavelengths of the maximum absorptions of the spectra of the monomer, the dimer, and the trimer, obtained by means of least-squares calculations, are shown in Table 6. A hypsochromic shift in the spectrum of thionine was observed just as in the case of Rhodamine 6G.

TABLE 6. COMPARISON OF THE WAVELENGTHS OF MAXIMUM ABSORPTION OF THE MONOMER, DIMER AND TRIMER OF THIONINE*

	$\lambda_{\max}$ (mμ)/$\varepsilon_{\max}\times10^{-4}$	
Monomer	595/5.1,	~560/2.9**
Dimer	~590/3.8,**	557/6.6
Trimer	550/3.5	

* By taking $\log K_2=2.90$ and $\log K_3=6.52$, the spectra were computed by means of the least-squares method.

** shoulder.

Experimental

The prepared dye solutions were left at 25°C until no further spectral changes were observed. This took about 12 hr in the cases of Rhodamine 6G and 3,6-diaminoacridine, but 24 hr for the thionine solution.

Commercial Rhodamine 6G was purified by repeated recrystallizations from water and was then dried at 110°C in a vacuum. Pure 3,6-diaminoacridine was obtained by treating purified proflavine sulfate with aqueous ammonia. The purification of proflavine sulfate was carried out by repeated recrystallizations from an aqueous alcoholic solution. Commercial thionine was also purified by repeated recrystallizations from an aqueous ethanolic solution. The purities of the dyes were confirmed by means of paper chromatography, elementary analysis, and a study of their absorption spectra.