the writer's opinion, Buerger's proposed procedure is much less promising than that described here.

Acknowledgment.—The writer is pleased to acknowledge the advice and coöperation of various others in these laboratories, especially Dr. S. E. Sheppard, Dr. F. Perrin, and Mr. C. N. Nelson.

Summary

The optical method, proposed in 1929 by Bragg, for making Fourier syntheses for use in crystal structure analysis has been modified to make it more accurate and much faster and to eliminate the over-all background density in the resulting photograph.

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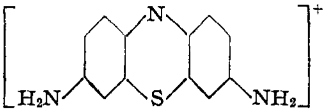
Polymerization of Dyestuffs in Solution. Thionine and Methylene Blue[1]

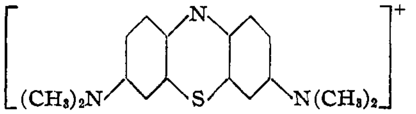
BY EUGENE RABINOWITCH AND LEO F. EPSTEIN

I. Introduction

In studying the photochemical[2a] and photoelectric[2b] properties of thiazine dyes, certain concentration effects were observed which led us to inquire into the molecular state of these dyes in solution. An investigation has been made of the spectra of thionine (I) and methylene blue (II) (Color Index, Nos. 920 and 922) under different conditions.



(I) Thio$^+$



(II) MB$^+$

(I) is the purple form in which thionine occurs in the *p*H range 2–10; (II) is the blue form of methylene blue, stable in the *p*H range 2–12. Each formula stands for two resonating structures, with one or the other of the two benzene rings in the para-quinonoid state.

We used commercial dyestuffs, the "Certified Stain" Thionine of the National Aniline and Chemical Company, and Merck Reagent Methylene Blue, the chlorides of the ions (I) and (II). The materials were dried for thirty minutes at 105°[3] and it was assumed that the products were the anhydrous salts of molecular weights 264 and 320, respectively. Experiments were also made with recrystallized material dissolved in conductivity water to check on possible effects of impurities in the dyes or in the solvent. Our stock solutions were almost saturated, containing 2.5×10^{-3} mole per liter in water and 2.0×10^{-3} mole per liter in ethanol. In soft glass vessels, thionine and methylene blue are adsorbed on the walls and gradually decomposed. The decomposition is slowed down (but not entirely prevented) by acid; in Pyrex bottles at *p*H 3.5, the solutions are quite stable.

II. Concentration Effect in Aqueous Solutions

Figures 1 and 2 show the extinction curves of Thio$^+$ and MB$^+$ in water at concentrations from 2.5×10^{-7} to 2.5×10^{-3} mole/liter for thionine, and 2.0×10^{-6} to 2.0×10^{-3} *M* for methylene blue, obtained on the Hardy recording spectrophotometer,[4,5] using calibrated cells 10, 1, 0.1 and 0.01 cm. thick.[6] The curves indicate the spectra to be made up by the superposition of two bands: one (M-band) with a maximum at 597 mμ for Thio$^+$, and 656.5 mμ for MB$^+$ is more prominent in dilute solutions; the second, with a maximum at 557 mμ for Thio$^+$ and 600 mμ for MB$^+$, is stronger in concentrated solutions (D-band).

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(1) Presented at the Symposium on Structure of Molecules and Aggregates of Molecules, Division of Physical and Inorganic Chemistry, Columbia University, New York, N. Y., December 30, 1940, to January 1, 1941.

(2) (a) E. Rabinowitch, *J. Chem. Phys.*, **8**, 551 (1940); (b) **8**, 560 (1940).

(3) *Cf.* W. M. Clark, H. D. Gibbs and B. Cohen, *U. S. Public Health Repts.*, **40**, 1131 (1935).

(4) A. C. Hardy, *J. Optical Soc. Am.*, **25**, 305 (1935).

(5) Our thanks are due to Dr. S. Q. Duntley of the M. I. T. Color Measurement Laboratory, for his coöperation in these experiments.

(6) *Cf.* L. F. Epstein, F. Karush and E. Rabinowitch, "8th Conference on Spectroscopy," Massachusetts Institute of Technology, 1940; *J. Opt. Soc. Am.*, January (1941).

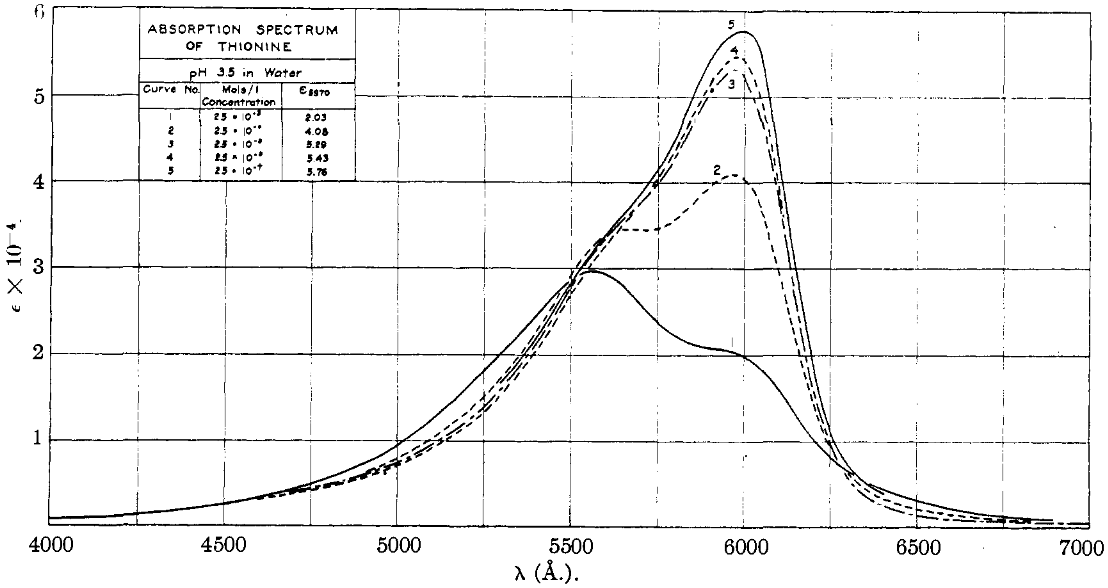

Fig. 1.—Molar extinction of thionine, $\epsilon = \log_{10}(I_0/I)/dC$. I_0 and I are the intensities of the incident and the transmitted beams, respectively, d is the cell thickness in cm., C is the concentration in moles per liter.

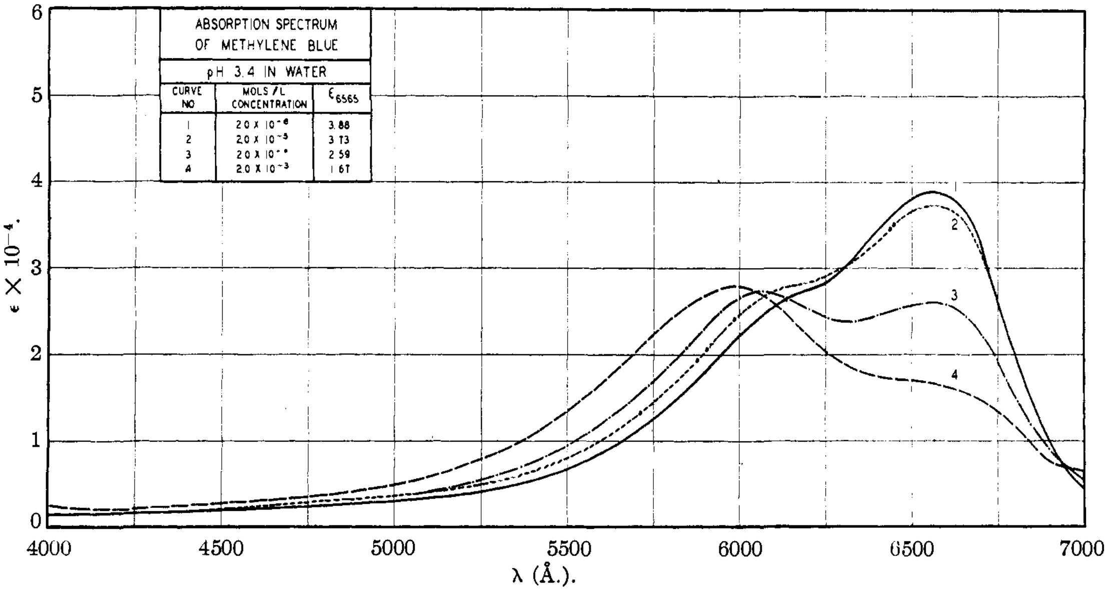

Fig. 2.—Molar extinction of methylene blue.

The M-band of thionine has a hump in the region of 557 mμ, whose coincidence with the D-band may be purely accidental. Thionine and methylene blue do not obey Beer's law, even in concentrations as low as 10^{-7} mole/liter.[7] Deviations from Beer's law are usually indicative of association, as in the familiar case of the $2NO_2 \rightleftarrows N_2O_4$ equilibrium. Two kinds of association are possible for our dyes: polymerization, *i. e.*, association of the dyestuff cations, and the association of cations with anions. Figure 3 shows the absorption spectrum of thionine in a solution molar in potassium chloride, which contains four hundred times as many Cl^- ions as the most concentrated solution in Fig. 1. Both the M- and the D-bands are weakened, either by the formation of undissociated Thio Cl molecules, or by an electrostatic interaction of $Thio^+$ and Cl^- ions in pairs of the kind considered by Bjerrum.[8] The relative in-

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[7] *Cf.* (a) S. Granick, L. Michaelis and M. P. Schubert, This Journal, **62**, 1802 (1940); (b) L. Michaelis, M. P. Schubert and S. Granick, *ibid.*, **62**, 204 (1940).

[8] N. Bjerrum, *Kgl. Danske Vidensk. Selskab Biol. Medd.*, **7**, 2 (1926).

tensity of the two bands, however, is not affected by Cl^- ions; the effects shown in Figs. 1 and 2 thus must be due to the polymerization of the cations. We ascribe the M-band to the monomeric ions T^+ (Thio$^+$ or MB$^+$), and the D-bands to the dimeric ions T_2^{++}. The equilibrium between T^+ and T_2^{++} must satisfy the relation

$$K = [T^+]^2/[T_2^{++}] \qquad (1)$$

where K is the dissociation constant of the dimer. The observed extinction coefficient ϵ is

$$\epsilon = \epsilon_M x + {}^1/_2\epsilon_D(1 - x) \qquad (2)$$

where ϵ_M and ϵ_D are the molar extinctions of the monomer and of the dimer, respectively, and x is the fraction of the dye in the monomeric form. K and x are related by the equation

$$K = 2Cx^2/(1 - x) \qquad (3)$$

where C is the total concentration of the dye, $C = [T^+] + 2[T^{++}]$·ϵ_M and ϵ_D can be obtained by extrapolating the experimental values of ϵ to $C = 0$ and ∞, respectively. We may then calculate the x's from Eq. (2), and K from Eq. (3). The following mean values of K were obtained in this way:

Dye	$K \times 10^4$ (t = 26.7°)
Thionine	10.0
Methylene blue	2.8

Tables I and II give a comparison of the observed extinction coefficients with those calculated from these values of K. They show that the dimerization hypothesis explains the effect of concentration within the limits of experimental error. Some experiments were also made on thionine–methylene blue mixtures indicating that mixed double ions Thio MB^{++} are formed in this case.

TABLE I

THIONINE (f = 0.58)

		$\epsilon \times 10^4$					
		597 mμ (M-band)			557 mμ (D-band)		
C	x	Obsd.	Calcd.	Δ	Obsd.	Calcd.	Δ
0	1.000	..	5.58	..	..	3.23	..
2.5×10^{-7}	1.000	5.76	5.58	+0.18	3.25	3.23	+0.02
2.5×10^{-6}	0.995	5.43	5.56	− .13	3.10	3.22	− .12
2.5×10^{-5}	.955	5.29	5.33	− .04	3.19	3.22	− .03
2.5×10^{-4}	.732	4.08	4.09	− .01	3.27	3.13	+ .14
2.5×10^{-3}	.359	2.03	2.02	+ .01	2.96	2.98	− .12
∞	0	..	0.104	..	..	2.93	..

TABLE II

METHYLENE BLUE (f = 0.45)

		$\epsilon \times 10^4$					
		656.5 mμ (M-band)			600 mμ (D-band)		
C	x	Obsd.	Calcd.	Δ	Obsd.	Calcd.	Δ
0	1.000	..	4.00	..	..	2.07	..
2.0×10^{-6}	0.986	3.88	3.93	−0.05	1.81	2.08	−0.27
2.0×10^{-5}	.887	3.73	3.64	+ .09	2.46	2.19	+ .27
2.0×10^{-4}	.557	2.59	2.64	− .05	2.66	2.55	+ .11
2.0×10^{-3}	.232	1.67	1.66	+ .01	2.80	2.89	− .19
∞	0	..	1.02	..	..	3.14	..

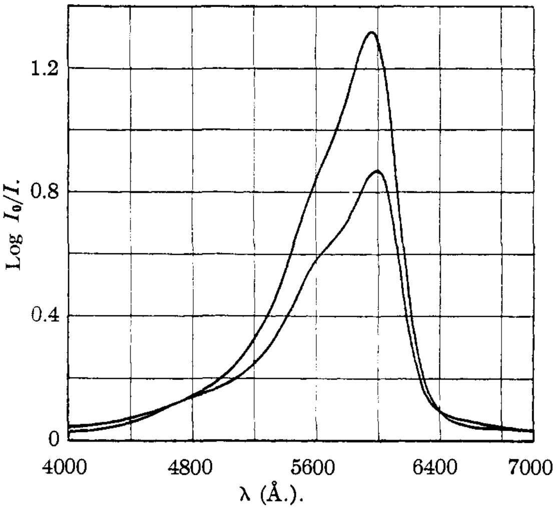

Fig. 3.—Absorption spectrum of thionine (2.5×10^{-5} M) in pure water (upper curve) and in a molal potassium chloride solution.

III. Temperature Effect and the Heat of Dimerization

Increase in temperature has an influence on the absorption spectrum similar to that of dilution (Fig. 4). Assuming that ϵ_M and ϵ_D are independent of temperature over the temperature range

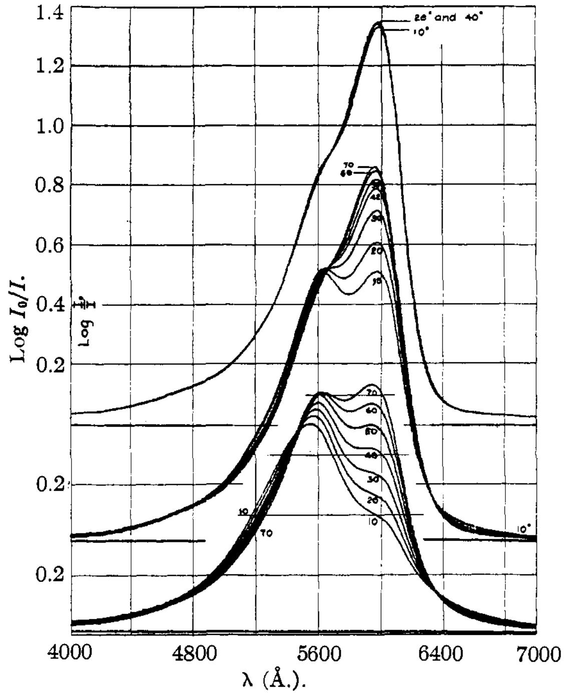

Fig. 4.—Effect of temperature on the absorption of thionine for three different concentrations (reading from top to bottom 2.5×10^{-5}, $\times 10^{-4}$, and $\times 10^{-3}$ m./l.); temp. in °C.

covered (10–70°), K can be calculated as a function of temperature. The results for thionine (Table III) can be represented by the equation

$$\log_{10} K = 1.9886 - (1492/T) \qquad (4)$$

as shown by Fig. 5. Table III also gives the *free energy of dimerization* ΔF, calculated by the relation $\Delta F = -RT \ln K_{\text{calcd.}}$, the *heat of dimerization* $\Delta H = RT^2 (\text{d} \ln K/\text{d}T)$, and the *entropy of dimerization* $\Delta S = (\Delta H - \Delta F)/T$. The free energy of dimerization of methylene blue at 26.7° is 4.9 kcal./mole.

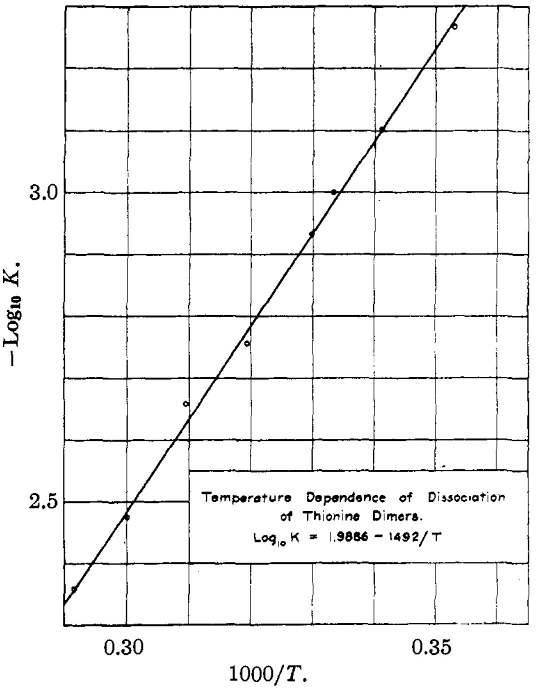

Fig. 5.—Temperature coefficient of the dissociation of thionine dimers.

TABLE III

THERMODYNAMIC PROPERTIES OF THIONINE SOLUTIONS

t, °C.	$\text{Log}_{10} K$ Obsd.	$\text{Log}_{10} K$ Calcd. (Eq. 4)	Δ	ΔF (kcal./mole)
10	−3.268	−3.280	+0.012	4.248
20	−3.101	−3.101	.000	4.158
26.7	−3.000	−2.986	− .014	4.096
30	−2.933	−2.932	− .001	4.066
40	−2.756	−2.775	+ .019	3.976
50	−2.658	−2.628	− .030	3.884
60	−2.474	−2.489	+ .015	3.793
70	−2.359	−2.359	.000	3.703

ΔH = 6.82 kcal./mole
ΔS = 9.1 cal./mole-degree

The D-bands of thionine and methylene blue lie about 40 mμ and 56 mμ on the short wave side of the M-bands ($\nu_D - \nu_M$ = 1150 cm.$^{-1}$ and 1450 cm.$^{-1}$). Figure 6 shows the probable shape of the potential energy curves of two Thio$^+$-ions.

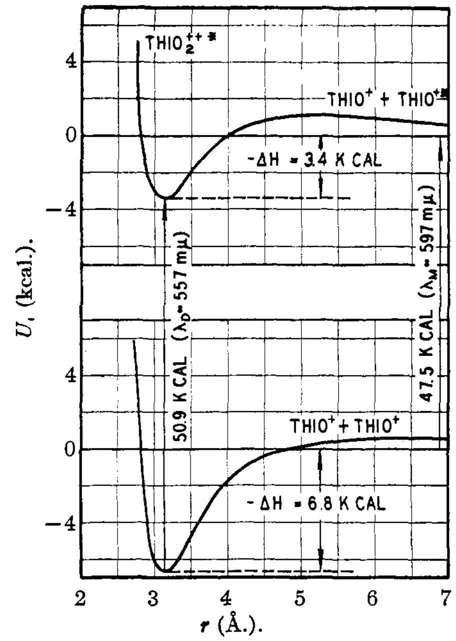

Fig. 6.—Potential energy curves of normal and excited thionine molecules in water.

The lower curve corresponds to the interaction of two normal ions, the upper to that of a normal ion and an excited ion. The relation $\lambda_D < \lambda_M$ indicates that the dimerization energy ΔH^* (asterisks referring to the excited state) is smaller than ΔH. This conclusion may be affected (on the Franck–Condon principle) by a difference between the equilibrium distances r_0 and r_0^* (assumed to be equal in Fig. 6). It is, however, improbable that this effect could reverse the relation $\Delta H^* < \Delta H$. ΔH^* is positive, *i. e.*, the excited dimer does not dissociate spontaneously into Thio$^+$ and Thio^{+*}.

It is noteworthy that the absorption of thionine in the monomeric form is about twice as strong as in the dimeric form; and that the binding energy of one excited and one normal Thio$^+$-ion is about half that of two normal ions.

IV. Electrochemical Properties of Dyestuff Solutions

Thionine and methylene blue form reversible oxidation–reduction systems. Dimerization should cause a change in the normal potential E_0'. Michaelis and co-workers[7a] stated that the titration curves of thionine reveal no polymerization effects. However, their data show a downward trend of E_0' with concentration (from

-0.0768 v. at 2.0×10^{-5} M to -0.0796 at 2.0×10^{-4} M). A similar trend was observed by Clark and his associates[3] in methylene blue solutions. Figure 7 shows the values of E_0' plotted against C, together with the values of

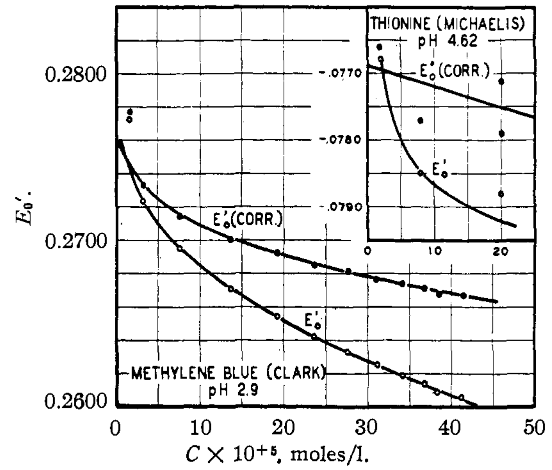

Fig. 7.—Oxidation-reduction potentials of thionine and methylene blue.

E_0'(cor.) calculated by allowing for dimerization to the extent determined from optical data. The corrected values show a much smaller trend, although the latter is not entirely eliminated.

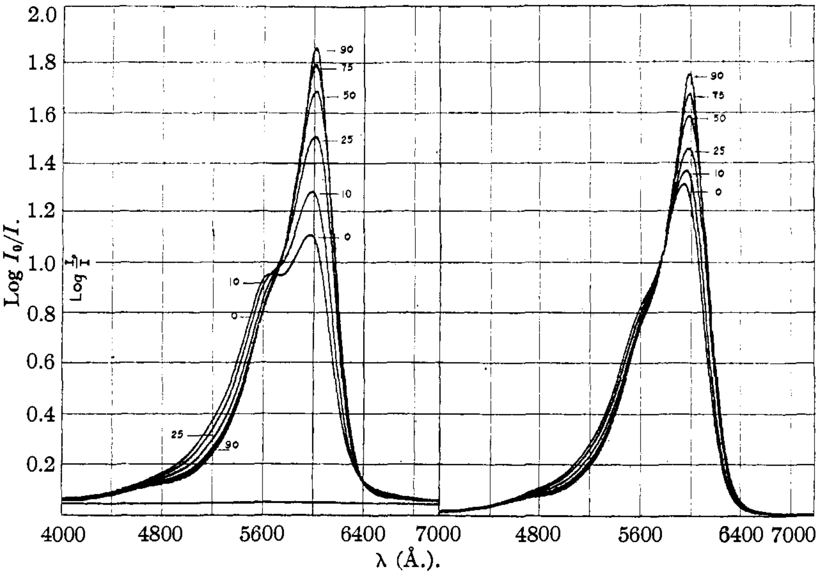

Fig. 8.—Absorption spectrum of thionine in ethanol–water mixtures at 2.5×10^{-4} (left) and 2.5×10^{-5} mole per liter. The figures are the percentages of ethanol by volume in the solvent.

Thus the e. m. f. data are not incompatible with the dimerization hypothesis, but indicate an even stronger tendency toward the formation of double molecules than the absorption spectra would seem to require.

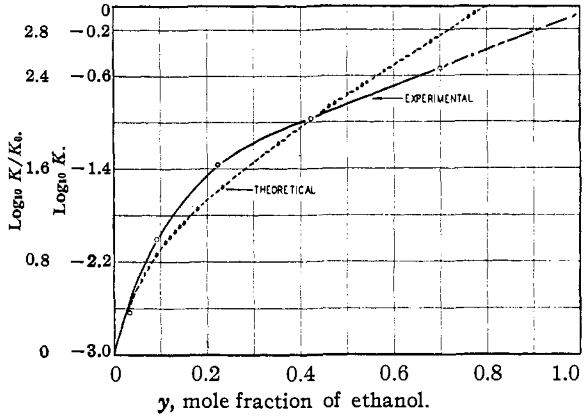

Fig. 9.—Dissociation constant of dimeric thionine in ethanol–water mixtures. The "theoretical" curve is calculated from equation (8) using the constants r_0 = 3.12 Å., $[1/D(y) - 1/D_0] = 0.0288$ (*cf.* 25) and the mean value from all the points for $1/K_A$, 3.94.

Measurements of the electrical conductivity[9] also give evidence of polymerization of methylene blue. (Robinson treated the phenomenon as a micelle formation; but from figures calculated by Robinson and Garrett[10] it appears that the average number of ions in a "micelle" is two or less.)

V. Alcoholic Solutions

Figure 8 shows the extinction curves of thionine solutions in ethanol–water mixtures. In pure alcohol, the extinction is independent of concentration from 2×10^{-7} to 2×10^{-3} mole/liter. The spectrum is similar to that of dilute aqueous solutions, although the band is sharper and the maximum is shifted from 597 to 603 mμ (in agreement with Kundt's rule).

Figure 9 gives the log of the dissociation constant (t = 26.7°) relative to the constant in water K_0 as a function of the molar composition of the solvent. The addition of a few per cent. of alcohol causes a sharp drop in the free energy of dimerization,

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[9] C. Robinson, *Trans. Faraday Soc.*, **31**, 245 (1935).

[10] C. Robinson and H. E. Garrett, *ibid.*, **35**, 771 (1939).

followed by an approximately linear decrease. Extrapolation to 100% ethyl alcohol gives log $K = 0$, which explains the practical absence of dimerization in pure ethanol.

Figure 10 shows that the increase in the intensity of the M-band with temperature, as observed in water and in dilute alcohol, is replaced in more strongly alcoholic mixtures by a reverse effect. Similar phenomena have previously been observed in acetone–water solutions of certain dyes.[11]

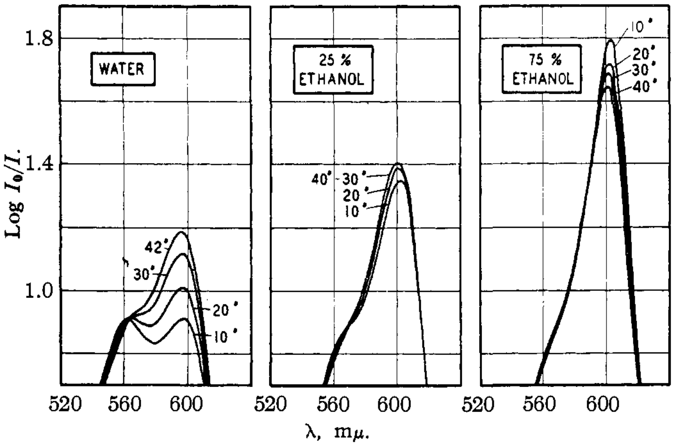

Fig. 10.—Effect of temperature on the absorption spectrum of thionine in ethanol–water mixtures.

VI. Fluorescence

Thionine fluoresces with red light. The yield of fluorescence should be different for monomeric and dimeric ions. Figure 11 shows a simple arrangement used for the measurement of the fluorescence. Filters F_1 ($CuSO_4$), F_2 (didymium glass) and F_3 (Wratten No. 61) isolate the 546 mμ mercury line; the filter F_4 (Corning No. 243) transmits the red fluorescence, but absorbs the exciting green line. We used solutions 2.5×10^{-3}, $\times 10^{-4}$ and $\times 10^{-5}$ *M* in cells 0.01, 0.1 and 1 cm. thick, respectively. According to Fig. 1, the green line is absorbed almost equally by monomeric and dimeric thionine ions. Thus its absorption is the same in all three cells, and the intensity of fluorescence is a direct measure of the relative fluorescence yield φ.

TABLE IV

FLUORESCENCE AND DIMERIZATION OF THIONINE

Concentration (mole/liter)	x (Table I)	$\varphi(H_2O)$ (in relative units)	$\varphi(C_2H_5OH)$ (in relative units)	$(\varphi/x)_{H_2O}$
2.5×10^{-3}	0.359	2.7	20	7.5
2.5×10^{-4}	.732	6.8	19	9.3
2.5×10^{-5}	.955	10.0	20	10.5

The decline in the yield of fluorescence with increased concentration ("self-quenching") closely parallels the decline in x, the fraction of the ions in the monomeric form, thus indicating that these ions alone are capable of fluorescence (Table IV). This is confirmed by the constancy of the fluorescence yield in alcohol.[11a]

At the highest concentration studied in Table IV, the quenching is more effective than can be accounted for by dimerization alone. This points to an additional quenching by collisions. However, if we assume that collisions between monomeric ions result in dimerization, the two kinds of quenching become almost identical: fluorescence does not occur if the dyestuff molecules are in the dimeric form at the moment of excitation, or if they form a dimer during the excitation period. The fluorescence yield of organic molecules is usually limited by an internal conversion of electronic excitation energy into vibrational energy. The tendency toward this internal dissipation of energy apparently is increased by the formation of the dimer.

The energy dissipation by dimeric molecules may account for the effect of concentration on the quantum yield of the thionine–ferrous iron reaction[2a] and for the consequent decrease in the sensitivity of the thionine–ferrous iron photogalvanic cell.[2b]

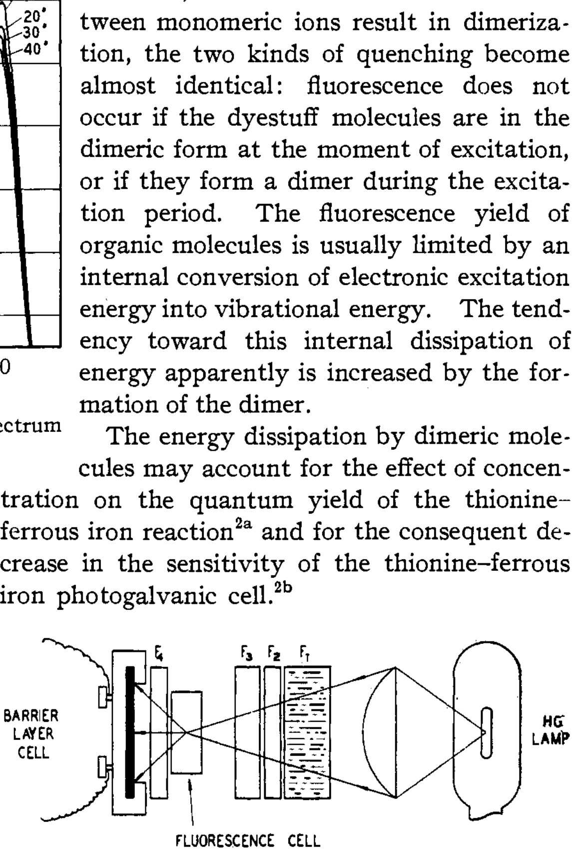

Fig. 11.—Apparatus for measurements of fluorescence

VII. Comparison with Other Dyestuffs

Holmes,[12] in an investigation of the absorption spectra of over three hundred dyes, found that

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(11) (a) V. L. Lewshin, *Acta Physicochim. U. R. S. S.*, **1**, 685 (1935); (b) **4**, 221 (1936).

(11a) Self-quenching was ascribed to polymerization in the fundamental papers on this phenomenon due to B. Walter, *Wied. Ann. Physik*, **34**, 316 (1888); **36**, 502, 518 (1889), as well as in some more recent papers on the subject, for example, by V. L. Lewshin, *Z. Physik*, **43**, 230 (1927), and F. C. Ghosh and S. B. Sengupta, *Z. physik. Chem.*, **B41**, 117 (1938), who, however, thought more of the formation of colloidal micelles than of simple dimerization. Mostly, however, self-quenching was interpreted as an effect due to collisions or to a somewhat mysterious "mutual induction" between distant dyestuff molecules.

(12) (a) W. C. Holmes, *Ind. Eng. Chem.*, **16**, 35 (1924); (b) *Am. Dyestuff Rptr.*, **16**, 429 (1927); (c) THIS JOURNAL, **50**, 1923 (1928).

practically all of them fail to follow Beer's law, and that the typical concentration effect is a weakening of the main absorption band and the appearance of a new band. The explanation suggested by him for this phenomenon is inadequate, and his results should be considered as indicating a universal tendency of organic dyes to polymerize. The D-bands usually lie 10–500 Å. on the violet side of the M-bands; for a few dyes, the relation is reversed. According to paragraph III, this indicates differences in the relative magnitudes of the dimerization energies ΔH and ΔH^*.

For a few dyes more complete information is available in papers by Speas,[13] Körtum,[14] Lewshin,[11] and Scheibe.[15] In the spectra of cyanine dyes, Scheibe found not only bands of the dimers, but also others, attributed by him to trimers and higher polymers. Lewshin calculated the dimerization constant of Rhodamine 6G Extra. Reduced to the units used in this paper his value is 1.1×10^{-4}. The free energy of dimerization of Rhodamine 6G Extra is thus 5.3 kcal./mole compared with 4.1 for Thionine and 4.9 for Methylene Blue (26.7°).

Measurements of Robinson and his co-workers[9,10,16] and Valkó[17] on diffusion coefficients, osmotic pressures and transport numbers of a large number of dyestuff ions give further evidence of the polymerization hypothesis, indicating the formation of trimers and higher polymers as well as dimers, in accordance with Scheibe's experiments.

VIII. Discussion

Is the polymerization of thionine, methylene blue, and similar molecules fundamentally associated with their dyestuff character? Many colorless organic molecules also form dimers, whose heats of dissociation are often even larger than those of the dimeric dyestuffs. We need not consider polymerizations due to homopolar valencies, *e. g.*, aldol condensations, which require high temperatures and catalysts. The reversible dimerizations of formic acid[18] ($-\Delta H = 14.1$ kcal.) and acetic acid[19] ($-\Delta H = 16.4$ kcal.) and similar compounds resemble the dimerization of dyestuffs more closely. They are usually attributed to hydrogen bonds.[20] The following facts indicate, however, that the association of dyestuff ions must be explained in a different way:

(a) Dyestuffs are dimerized more strongly in aqueous solution than in organic solvents, whereas carboxylic acid dimers exist in the vapor and in non-polar solvents, but are dissociated in water.

(b) In contrast to the behavior of the dimerizing carboxylic acids, the polymerization of dyestuffs does not stop at the dimeric stage, but often proceeds to trimers and higher polymers.

(c) Colorless leucodyes dimerize, if at all, to a smaller extent than the corresponding colored forms.

These facts agree with the postulate that the polymerization of dyes is due to additive forces of the van der Waals type.[20a] It may seem that these forces are too weak to account for dimerization energies of the order of 7 kcal. However, according to London's theory,[21] the mutual potential energy of two identical molecules possessing a single long-wave electronic absorption band λ_0 (a description which fits most dyestuffs) is to a first approximation proportional to $f^2\lambda_0^3$, where f is a measure of the excitation probability (so-called "number of absorption electrons" or "oscillator strength"). Since large values of f and λ_0 are exactly the properties which make a compound strongly colored, the attractive forces between dyestuff molecules must be considerably larger than those between similar colorless molecules (*e. g.*, the corresponding leucodyes). These forces are additive and thus can account for the formation of higher polymers as well as dimers.

The (virtual) dipole–dipole attraction term of London,[21] in the case of dyestuffs, should be supplemented by dipole–quadrupole and quadrupole–quadrupole terms, as calculated by Margenau.[22] Since we are dealing with two ions of the same sign, we must subtract the coulombic electrostatic repulsion energy Z^2e^2/Dr from the London–Margenau attraction energy. Finally, the usual repulsion energy between neutral molecules must also be taken into account; we do this by assuming a repulsive potential of the Lennard-Jones

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(13) W. E. Speas, *Phys. Rev.*, **31**, 569 (1928).

(14) G. Körtum, *Z. physik. Chem.*, **B30**, 317 (1935); **B33**, 1 (1936); **B34**, 255 (1936); **B31**, 137 (1935); **B33**, 243 (1936).

(15) (a) G. Scheibe, *Kolloid-Z.*, **82**, 1 (1938); (b) G. Scheibe and L. Kandler, *Naturwissenschaften*, **26**, 412 (1938); (c) G. Scheibe, A. Schöntag, J. Kopske, and K. Menle, *Z. Wiss. Phot.*, **38**, 1 (1939); (d) G. Scheibe, A. Schöntag, and E. Katheder, *Naturwissenschaften*, **27**, 499 (1939); *cf.* also H. Ecker, *Kolloid.-Z.*, **92**, 35 (1940).

(16) C. Robinson and J. W. Selby, *Trans. Faraday Soc.*, **35**, 780 (1939).

(17) Valkó, *ibid.*, **31**, 230 (1935).

(18) A. S. Coolidge, THIS JOURNAL, **50**, 2166 (1928).

(19) F. H. Macdougall, *ibid.*, **58**, 2585 (1936).

(20) *Cf.* L. Pauling, "Nature of the Chemical Bond." 2d ed., Cornell University Press, Ithaca, N. Y., 1940, pp. 306–307.

(20a) Similar assumptions were made by Körtum[14] and Scheibe.[15]

(21) F. London, *Z. physik. Chem.*, **B11**, 222 (1930).

(22) H. Margenau, *Rev. Modern Physics*, **11**, 1 (1939).

type,[23] X/r^n, with $n = 12$. We obtain in this way the following expression for the mutual potential energy of two dyestuff ions

$$U = -\frac{3N}{4m^2}\left(\frac{h}{c^2}\right)\left(\frac{e}{2\pi}\right)^4\frac{f^2\lambda_0{}^3}{r^6}\left[1 + 5\left(\frac{h}{4\pi^2cm}\right)\frac{f\lambda_0}{r^2} + \frac{105}{8}\left(\frac{h}{4\pi^2cm}\right)^2\left(\frac{f\lambda_0}{r^2}\right)^2\right] + \frac{NZ^2e^2}{Dr} + \frac{X}{r^{12}} \qquad (5)$$

$$= -1.0742 \times 10^{-7}\frac{f^2\lambda_0{}^3}{r^6} - 3.2884 \times 10^{-10}\frac{f^3\lambda_0{}^4}{r^8} - 5.2850 \times 10^{-13}\frac{f^4\lambda_0{}^5}{r^{10}} + \frac{329.6}{Dr} + \frac{X}{r^{12}}$$

when U is in kcal./mole and λ_0 and r are in Å. In this equation λ_0 is approximately equal to the wave length of the band maximum, and f can be determined by integration of the extinction curves:[24]

$$f = \frac{1000\ mc^2 \ln 10}{N\pi e^2}\int_{-\infty}^{\infty} \epsilon\, d\left(\frac{1}{\lambda}\right) \qquad (6)$$

$$= 4.314 \times 10^{-9}\int_{-\infty}^{\infty} \epsilon\, d\left(\frac{1}{\lambda}\right)$$

This integration gives the following results

Dye	f
Thionine	0.58
Methylene Blue	0.45
Rhodamine 6G Extra[11]	0.54

Using for D, the dielectric constant[25] of water at 26.7°, 77.93, we calculate X for thionine by assuming that the minimum in the potential energy curve is equal to the experimental heat of dissociation of $\text{Thio}_2{}^{++}$ (−6.82 kcal.). (A small error is introduced here by using the heat of dimerization at room temperature instead of the value at absolute zero.) In this way, we obtained the complete potential energy curve for two thionine ions in water (Fig. 12). Further assuming X to be independent of the solvent, and using Wyman's value[25] 24.01 for the dielectric constant, we can calculate the corresponding curve for ethanol.

An interesting feature of these curves is the occurrence of a maximum (at about 6.75 Å. in water and 5.65 Å. in ethanol). This potential barrier (due to the electrostatic repulsion) indicates the existence of an *activation energy of dimerization.* The height of this potential barrier is of the order of magnitude of RT at room temperature and therefore does not preclude the possibility of forming a dimer at the first "encounter,"[26,27] as was assumed above in the explanation of the self-quenching of fluorescence. However, potential barriers of this type may account for the changes in the absorption spectra of certain dyes, which become stable only several hours after the dilution of the concentrated stock solutions.[28]

The equilibrium distance in Fig. 12, $r_0 = 3.12$ Å., is not an unreasonable value for the distance of closest approach of two ions of the type I, if for example we imagine the two ring systems to lie flat on top of one another, like stacked coins.

Figure 12 indicates, for thionine in ethanol, a dimerization energy of −3.8 kcal., as against −6.8 in water. This difference is due entirely to the increase in repulsive energy, which is proportional to $1/D$. Since in ethanol–water mixtures $1/D$ is nearly linear in the mole fraction of alcohol y, U (at any fixed distance r) should also be a linear function of y. However, according to Fig. 9, $-\Delta F$ ($=RT \log K$), the free energy of dimerization, changes rapidly upon the addition of the first small quantities of alcohol, and only becomes linear in y at higher concentrations. Thus ethanol has a stronger influence on the stability of the dimer than can be accounted for by the change in the dielectric constant in equation (5) alone. We may assume that the Thio^+ ions have an affinity for ethanol molecules, and that their association with the alcohol tends to prevent dimerization. (Similarly, the formation of hydrogen bonds between RCOOH and water prevents the dimerization of carboxylic acids in aqueous solution.)[28a] If we postulate that the competition between the A-molecules (A = alcohol) and the Thio^+ ions is described by the simple equations:

$$\text{Thio}^+ + \text{Thio}^+ \longrightarrow \text{Thio}_2{}^{++} \text{ (Dissociation constant } K_\text{D})$$
$$\text{Thio}^+ + \text{A} \longrightarrow \text{Thio A}^+ \text{ (Dissociation constant } K_\text{A})$$

we can express K, the experimental dissociation constant of $\text{Thio}_2{}^{++}$, in terms of the two equilibrium constants

$$K = K_\text{D}\left(1 + \frac{\eta}{K_\text{A}}\right)^2 \qquad (7)$$

where η is the activity of alcohol. Log K_D is linear in y, because of the previously mentioned linearity of $1/D$ with y. The complete expression for log K is

$$\ln\frac{K(y)}{K_0} = 2\ln\left[1 + \frac{\eta}{K_\text{A}}\right] + \frac{Ne^2}{RTr_0}\left[\frac{1}{D(y)} - \frac{1}{D_0}\right] \qquad (8)$$

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[23] Lennard-Jones, *Proc. Roy. Soc.* (London), **A56**, 463 (1924).

[24] (a) R. Ladenburg, *Verhandl. deut. Physik. Ges.*, **16**, 775 (1914); *Z. Physik*, **4**, 351 (1921); (b) R. Ladenburg and F. Reiche, *Naturwissenschaften*, **11**, 584 (1923); **14**, 1208 (1926).

[25] J. Wyman, THIS JOURNAL, **53**, 3293 (1931).

[26] E. Rabinowitch, *Trans. Faraday Soc.*, **33**, 1225 (1937).

[27] R. H. Fowler and N. B. Slater, *ibid.*, **34**, 81 (1938).

[28] Private communication from the M. I. T. Color Measurement Laboratory.

[28a] This effect is another example of the "Sorting of Mixed Solvents by Ions" discussed by Scatchard, *J. Chem. Physics*, January, 1941.

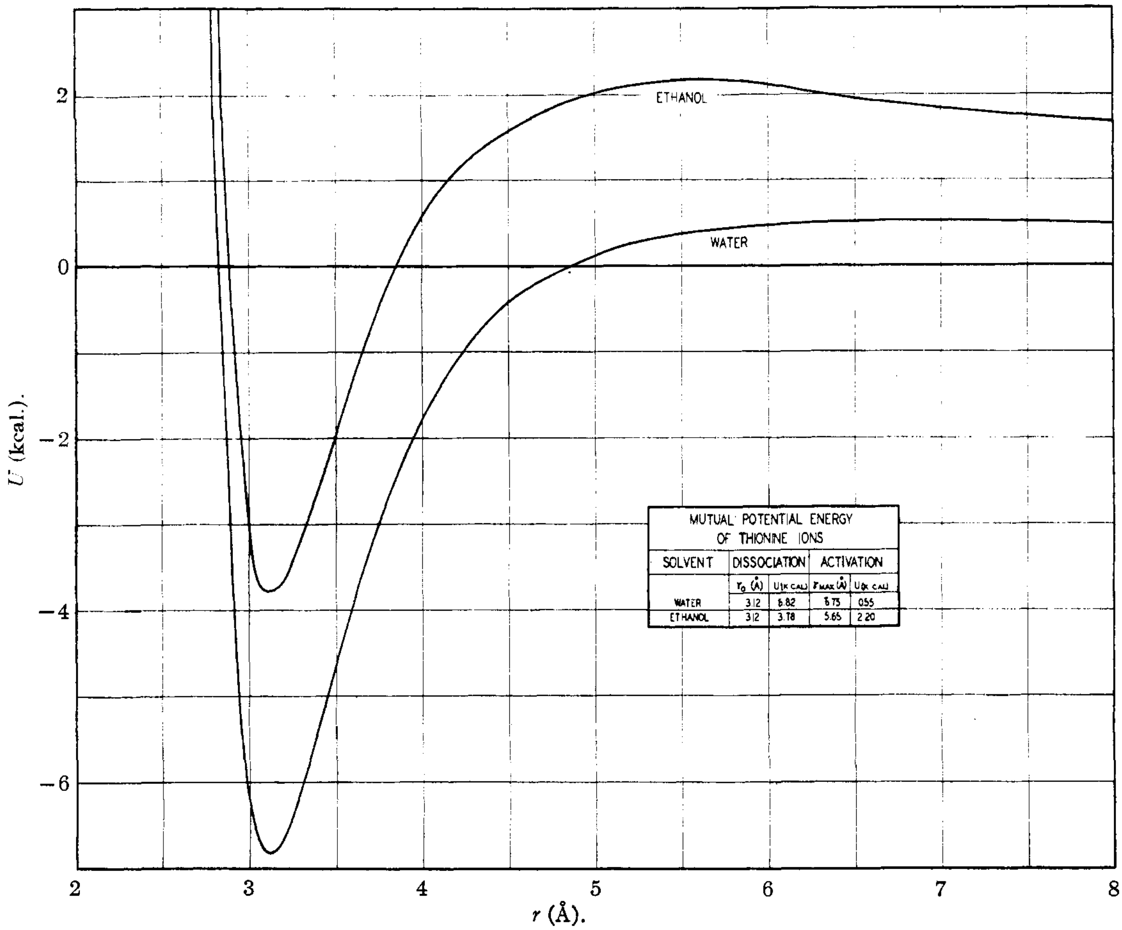

Fig. 12.—Potential energy curves of thionine.

η was determined as a function of y from the vapor pressure data at 20°.[29] Introducing the value of r_0 determined from Fig. 12 (3.12 Å.) we obtain the dotted curve in Fig. 9. Its general shape agrees with the experimental data, but the deviations appear to be outside the limits of experimental error. They may be due to several causes: first, the effective distance between the *charges* in a dimeric ion Thio_2^{++} may be different from the effective distance between the virtual *dipoles* responsible for the London attraction forces. (In general, the assumption of spherical symmetry for assymetric molecules of the kind considered here is a very crude first approximation.) Secondly, the use of the macroscopic dielectric constants at small distances of separation of the two ions is also only approximate.[30] Uncertainties about the effective value of D at short distances should not seriously affect the shape of Fig. 12, but they may be of considerable importance in the equation containing the difference between the $1/D$'s. Thirdly, the representation of the interaction of Thio^+ and A by a simple binary compound Thio A^+ is certainly very rough. Finally, in the derivation of equation (5), we did not consider changes in the energy of hydration caused by dimerization. In other words, we have assumed that dimerization does not interfere with the haloes of solvent molecules clustering around the charges on the ions.

The extrapolation of log K to pure ethanol in Fig. 9 gives log $K = 0$, and thus $\Delta F = 0$. The entropy of dimerization in alcohol must be somewhat smaller than in water, where $T\Delta S = 2.2$ kcal. Consequently the heat of dimerization in alcohol must be greater than −2.2 kcal./mole, that is, considerably larger than the value −3.8 shown in Fig. 12. This is a consequence of the stabilization of the monomeric ions by association with alcohol molecules, which was not taken into

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[29] "International Critical Tables," Vol. III, p. 250.

[30] *Cf.* E. Hückel, *Physik. Z.*, **26**, 93 (1925); P. Debye and L. Pauling, THIS JOURNAL, **47**, 2129 (1925).

account in this figure. However, even according to this estimate, ΔH has the same sign in pure alcohol as in water. It is therefore impossible to explain the reversal of the temperature effect in Fig. 10 by assuming that $Thio_2^{++}$ becomes endothermal in strongly alcoholic solutions. The "negative" temperature effect in alcohol cannot therefore be due to the dimerization equilibrium; we may attribute it instead to the dissociation of the $ThioA^+$ complexes, or it may be simply the effect of increased rotation and collision frequency, which often causes a lowering and broadening of absorption bands with increasing temperature.

Summary

1. Extinction curves of thionine and methylene blue were determined at different concentrations and temperatures, in water, ethanol and ethanol–water mixtures.

2. In water, deviations from Beer's law appear even at the lowest concentrations ($<10^{-6}$ mole/liter) and can be explained quantitatively by the formation of dimeric ions $Thio_2^{++}$ and MB_2^{++}. Dissociation constants and the heats and entropies of formation of the dimers are calculated.

3. Dimerization does not occur in pure ethanol, and it is reduced considerably in alcohol–water mixtures.

4. Dimeric ions do not fluoresce; self-quenching of fluorescence at the higher concentrations of the dyestuff is due to dimerization. No self-quenching occurs in alcohol.

5. Other properties of dyestuff solutions (oxidation–reduction potentials, conductivity, osmotic pressure, etc.) are in accord with the dimerization hypothesis.

6. Dimerization (and higher polymerization) is a universal property of organic dyestuffs, fundamentally associated with the property of color. It can be interpreted quantitatively on the basis of London's theory of intermolecular forces.

7. Potential energy curves are constructed for the interaction of two $Thio^+$ ions in water and in ethanol. Because of the coulombic repulsion between ions of the same sign, dimerization is found to require an activation energy.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, TECHNICAL COLLEGE, SYDNEY]

Chelation in Metallic Triazene Salts

BY F. P. DWYER

Recently it was shown that if the silver salt of the *aci* form of 4-nitrodiazoaminobenzene was allowed to revert to the triazene form, and then methylated, instead of the expected N-methyl derivative with the methyl group adjacent to the nitro group, a mixture of equal amounts of N-methyl-4-nitrodiazoaminobenzene and N-methyl-4′-nitrodiazoaminobenzene resulted.[1] This suggested that the silver salt of the triazene form exhibited dynamic isomerism or, more probably, could be best formulated as an internal complex in which the triazene group acted as a chelate group.

In order to obtain more adequate support for the latter view, a number of other metallic complexes with diazoaminobenzene and 4,4′-dimethyldiazoaminobenzene have been examined. The complexes showed the ready solubility in organic media that is characteristic of covalent compounds. The molecular weights in most instances were normal, and from coördination experiments with ethylenediamine and pyridine the chelating function of the triazene group seems definitely indicated.

Experimental

Since most preparations of diazoamino compounds contain traces of diazoaminoazo compounds that have led to much confusion in the chemistry of diazoamino compounds, the diazoaminobenzene and 4,4′-dimethyldiazoaminobenzene were specially purified. The pure lemon-yellow compounds melted at 100 and 119°, respectively.[2]

Palladium Complexes with Diazoaminobenzene.—A cold solution of sodium chloropalladite, 35 ml. containing 1.06 g. of palladium, mixed with 15 ml. of sodium acetate solution (3 *N*) and added rapidly to an ice-cold solution of diazoaminobenzene, 3.94 g. in methanol, 100 ml., gave a voluminous precipitate of **tris-diazoaminobenzenepalladium** (I). After washing with 80% methanol the substance was dissolved in ice cold acetone, filtered as rapidly as possible, and immediately precipitated with ice water. The resulting unstable brown powder was extremely soluble in all organic solvents and even petroleum ether to

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[1] F. P. Dwyer, *J. Australian Chem. Inst.*, **6**, 362 (1939).

[2] F. P. Dwyer, *J. Soc. Chem. Ind.*, **56**, 70 (1937).