Thermodynamic Aspects of the Self-Association and Hydrophobic Bonding of Methylene Blue. A Model System for Stacking Interactions[1]

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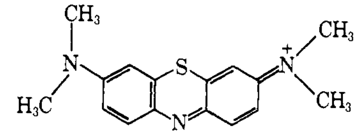
Abstract: The self-association of flat aromatic or heteroaromatic dye ions in aqueous solutions provides a good model system for "stacking" interactions. The self-association behavior of the cationic dye methylene blue has been studied by the "isoextraction" method at 34.9 and 10.0°; the results are qualitatively very similar to those at 25.1°. From the values of the dimerization constant K_2 at three temperatures, the entropy and enthalpy of dimerization have been derived. The "unitary" entropy of dimerization is positive, suggesting that water-structure contributions are appreciable in the stability of the dimers. The enthalpy of dimerization is negative and substantial in magnitude. Possible contributory factors are discussed. The stepwise trimerization constant K_3 is found to be greater than K_2 at all temperatures, in spite of stronger charge repulsion. There is thus an appreciable second-nearest neighbor attractive interaction in the stacking process, contributing to its cooperativity. The "extra" stability of the trimer reflected in the ratio K_3/K_2 appears to be due to a favorable positive entropy change overcoming a positive enthalpy change. This is indicative of a long-rang econtribution of water-structure effects in promoting association of organic molecules in aqueous solutions.

The face-to-face association of flat organic molecules, aromatic or heteroaromatic, produces one of the simplest types of structural organization in many aqueous and biological systems, and is often described as a "stacking"-type interaction. Such interactions are probably greatly responsible for the stability of double-stranded DNA molecules and the photosynthetic apparatus, and they are involved in a variety of association phenomena in aqueous media and at various interfaces. The self-association of flat dye molecules in aqueous media, of considerable interest by itself, provides an excellent model system for stacking interactions and the important role of water as a solvent medium.[4-8]

The present paper deals with the thermodynamic aspects of the self-association of methylene blue (MB^+-Cl^-), a cationic dye (I), studied by the "isoextraction" method.[5-7] The enthalpy and entropy changes associated with dimerization are reported and their probable interpretations discussed. The relation of the stepwise trimerization constant K_3 to the dimerization constant K_2 is also investigated.



I, one of four resonance forms for MB^+

The symbols b, B, K_A, $K_{A(0)}$, and K_q are used as defined previously.[5] The apparatus and chemicals have been used as described.[5]

Results

K_A values, obtained by the isoextraction method as described earlier[5] at various values of the equivalent dye concentration, B, at 34.9 and 10.0°, are reported in Tables I and II. The data can be used to calculate

Table I. Isoextraction Data at 34.9° [a]

B, equiv/l.	$K_A \times 10^9$, (equiv/l.)^2	B, equiv/l.	$K_A \times 10^9$, (equiv/l.)^2
9.86×10^{-6}	6.12	1.19×10^{-4}	8.38
1.20×10^{-5}	6.13	1.27×10^{-4}	8.48
1.63×10^{-5}	6.30	2.60×10^{-4}	11.0
1.64×10^{-5}	6.12	4.05×10^{-4}	13.9
1.65×10^{-5}	6.39	5.24×10^{-4}	16.8
3.71×10^{-5}	6.76	7.00×10^{-4}	19.2
3.82×10^{-5}	6.75	8.72×10^{-4}	22.8
4.10×10^{-5}	6.68	9.52×10^{-4}	24.1
5.95×10^{-5}	7.06	1.054×10^{-3}	25.0
6.95×10^{-5}	7.24	1.37×10^{-3}	29.6
7.24×10^{-5}	7.29	1.40×10^{-3}	30.5
8.08×10^{-5}	7.52	1.72×10^{-3}	34.0
1.15×10^{-4}	8.08	3.18×10^{-3}	49.0

[a] $K_{A(0)} = 5.96 \times 10^{-9}$ (equiv/l.)^2.

Table II. Isoextraction Data at 10.0° [a]

B, equiv/l.	$K_A \times 10^9$, (equiv/l.)^2	B, equiv/l.	$K_A \times 10^9$, (equiv/l.)^2
8.72×10^{-6}	1.34_5	1.945×10^{-4}	3.14
1.049×10^{-5}	1.44_4	2.734×10^{-4}	3.72
2.92×10^{-5}	1.58_5	3.20×10^{-4}	4.20
6.20×10^{-5}	1.93_2	4.80×10^{-4}	5.52
1.087×10^{-4}	2.38	5.59×10^{-4}	5.92
1.114×10^{-4}	2.45	9.47×10^{-4}	8.43
1.522×10^{-4}	2.99	1.02×10^{-3}	9.18

[a] $K_{A(0)} = 1.31_0 \times 10^{-9}$ (equiv/l.)^2.

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(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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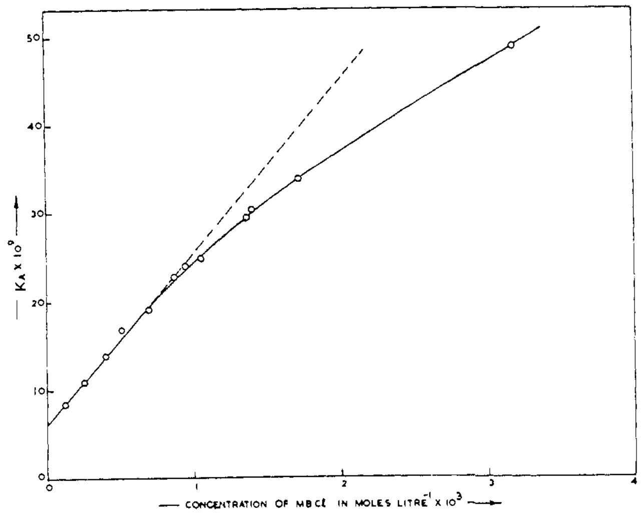

Figure 1. Variation of K_A [(equiv/l.)^2] with MBCl concentration (B) at 34.9°. Not all data in dilute solutions are shown.

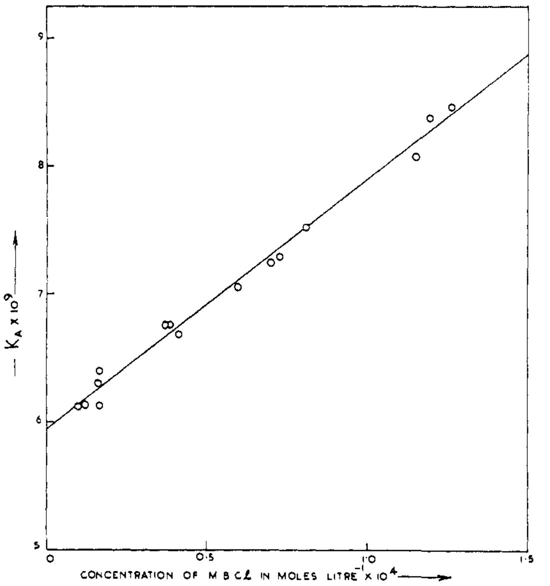

Figure 2. Variation of K_A [(equiv/l.)^2] with MBCl concentration (B) at 34.9° in dilute solutions. The linear variation extends up to an MBCl concentration of 8.72×10^{-4} equiv/l. K_2 was evaluated from the best straight line representing the data from 9.86×10^{-6} to 8.72×10^{-4} equiv/l.

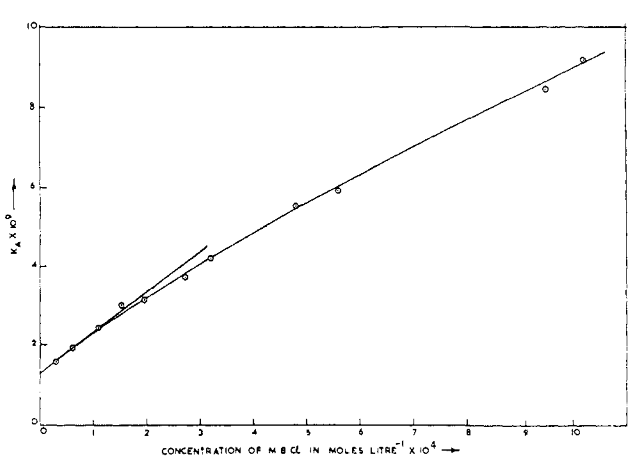

Figure 3. Variation of K_A [(equiv/l.)^2] with MBCl concentration (B) at 10.0°. Not all data in dilute solutions are shown.

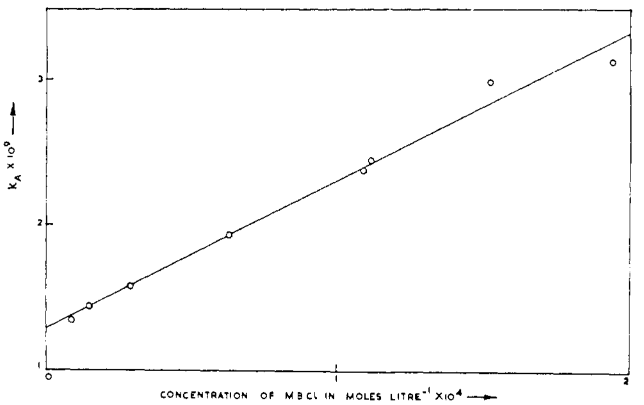

Figure 4. Linear variation of [K_A (equiv/l.)^2] with MBCl concentration in dilute solutions at 10.0°.

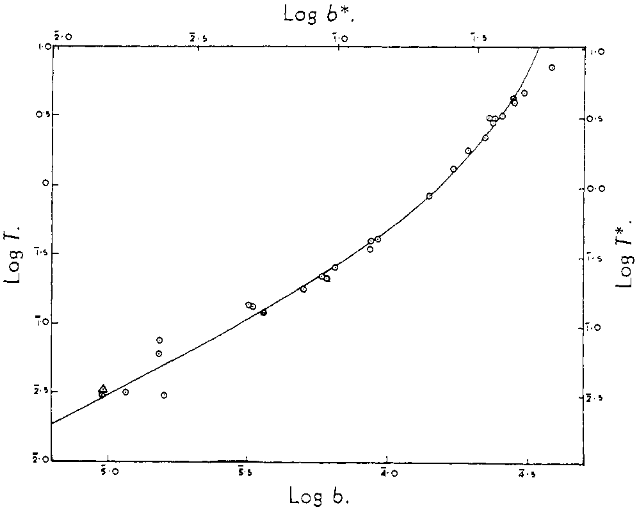

Figure 5. Description of the self-association behavior of MB over the entire concentration range at 34.9° by the two-parameter model IV,[6] with $K_2 = 1.48 \times 10^3$ l./mol and $K_3 = 3.01 \times 10^3$ l./mol.

the equilibrium monomer concentration, b, from the relation $K_A/K_{A(0)} = B/b$.[5] Figures 1–4 show that the nature of the variation of K_A with B at these two temperatures is very similar to that at 25.1°.[5,6] In particular, the K_A–B plots are accurately linear over a substantial range at low concentrations (Figures 2 and 4). From the slopes of these lines and the extrapolated $K_{A(0)}$, determined by linear regression analysis, K_2 can be determined with fair precision (~2% standard error) without taking recourse to any model of self-association.[6] K_3 is estimated to be 1.3–2.5 times K_2 at all temperatures. Over the complete range of concentrations, the two-parameter self-association model IV,[6] in which the higher K_q values decrease in a mild sequence, gives a fairly good representation of the data as shown in Figures 5 and 6. Model IV was previously found suitable for MB^+ at low[6] and high[5] ionic strengths, as also for an anionic dye, Orange II.[6]

Figure 7 shows a plot of log K_2 *vs.* $1/T$, where T is the absolute temperature. The variation is linear. The changes in standard free energy, ΔG, the enthalpy, ΔH, and the entropy, ΔS, associated with the dimerization of MB^+ were calculated from the following equations.

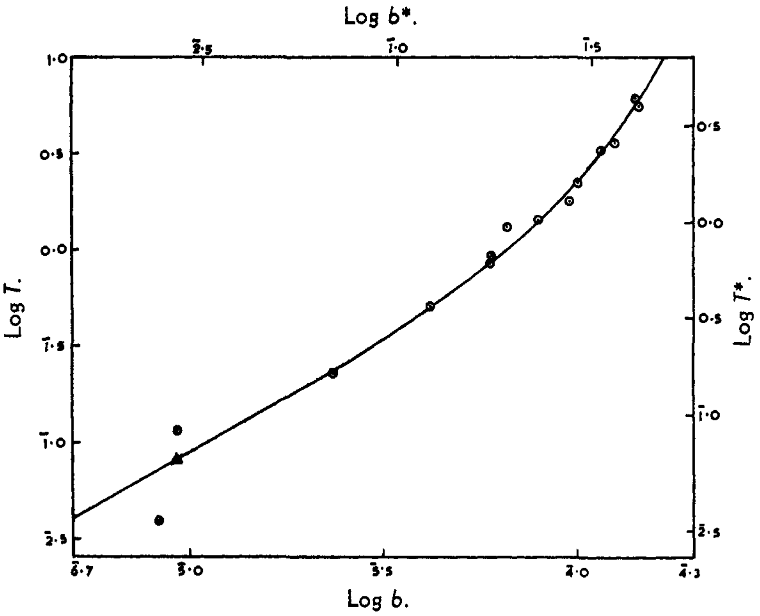

Figure 6. Description of the self-association behavior of MB over the entire concentration range at 10.0° by the two-parameter model IV,[6] with K_2 = 3.98 × 10^3 l./mol and K_3 = 5.76 × 10^3 l./mol.

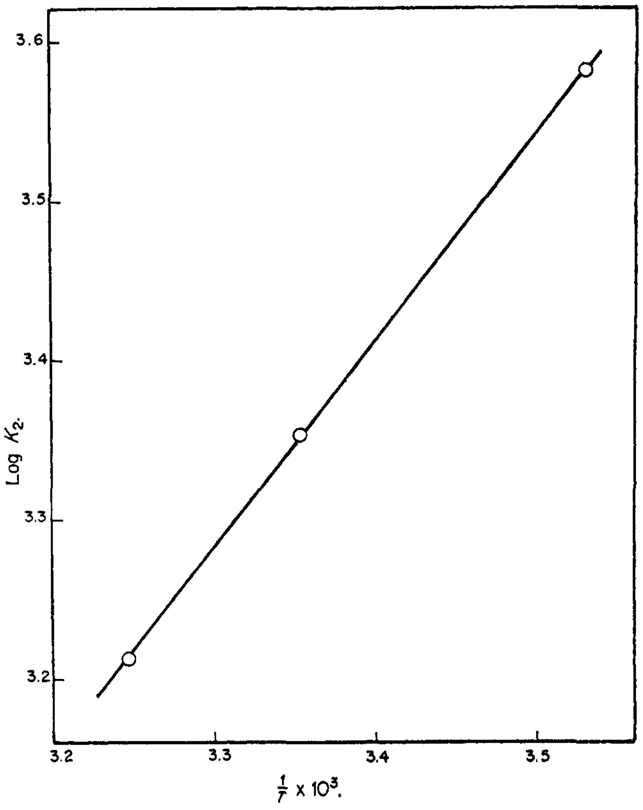

Figure 7. Linear variation of log K_2 with $1/T$, K_2 obtained directly from the initial slopes of K_A–B plots.[6]

$$\Delta G = -RT \ln K_2 \tag{1}$$

$$\Delta H = RT^2 \frac{\mathrm{d} \ln K_2}{\mathrm{d}T} \tag{2}$$

$$\Delta G = \Delta H - T\Delta S \tag{3}$$

The K_2 values (Table III) used for obtaining ΔH were derived from the K_A–B curves in the linear range without correcting for small changes in activity coefficients. The corrections, found to have a small effect on K_2 at 25.1°,[6] have equally small effects at 10.0 and 34.9°.

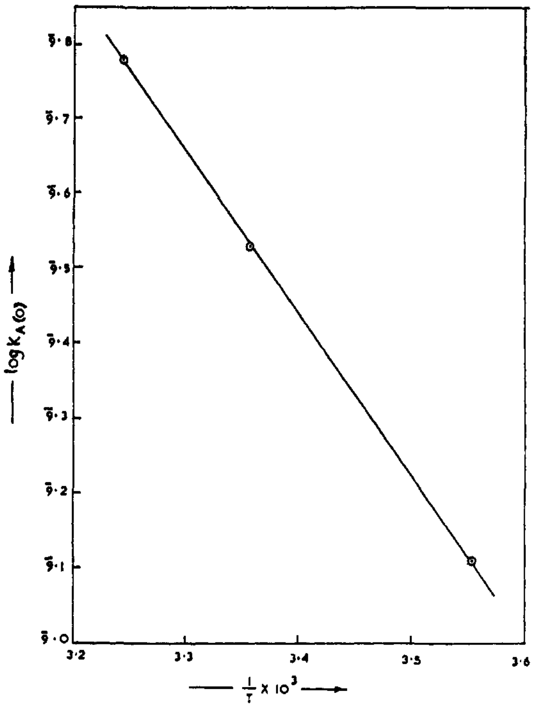

Figure 8. Linear variation of log $K_{A(0)}$ with $1/T$.

The thermodynamic quantities associated with dimerization of MB^+ are given in Table III. Figure 8 shows a plot of log $K_{A(0)}$ against $1/T$. Its linearity provides additional support for the reliability of the isoextraction method, as $K_{A(0)}$ is a quantitative measure of the extractivity of monomeric $MBClO_4$ into chloroform.

Table III. Thermodynamic Quantities Related to the Dimerization of Methylene Blue

Temp, °C	$K_2 \times 10^{-3}$, l./mol	ΔG^a	ΔH^a	ΔS^b	$\Delta S_u{}^b$	$\Delta G_u{}^a$
10.0	3.83					
25.1	2.26	−4.58	−5.9	−4.5	3.5	−6.96
34.9	1.63					

[a] In kcal/mol. [b] In eu.

Discussion

ΔS and Water-Structure Effects. In the formation of a dimer from two monomers, the number of molecules decreases. As a result, an entropy of mixing is associated with ΔS,[9,10] which must be corrected for to obtain the "unitary" entropy change ΔS_u, the quantity characteristic of the interactions associated with dimerization. ΔS_u (Table III) can be calculated by expressing K_2 in mole-fraction units. It is about 8 eu more positive than the ΔS calculated from K_2 expressed in l./mol units.

The formation of a dimer from two monomers must involve some loss of rotational and translational entropy. An additional entropy change arises from the charge repulsion in the dimer. This electrical contribution to ΔS_u, ΔS_{el}, can be evaluated roughly in the following manner. From the interactions of quinoline and quinolinium ion with MB$^+$,[11] the factor x by which charge repulsion reduces K_2 has been estimated to be somewhat lower than 5.[6] The electrical contribution to ΔG, ΔG_{el}, can be written as

$$\Delta G_{el} = RT \ln x = \frac{Ne^2}{\epsilon_{eff} r_{av}} \tag{4}$$

assuming a Coulomb's law type of repulsion, where N is Avogadro's number, e is the electronic charge, ϵ_{eff} is the "effective" dielectric constant, and r_{av} is the average value of the separation of the charges which are delocalized because of resonance. If r_{av}, *i.e.*, the dimer geometry, is assumed to be independent of temperature, we obtain

$$\Delta S_{el} = -\frac{d\Delta G_{el}}{dT} = \frac{Ne^2}{r_{av}} \frac{1}{\epsilon_{eff}{}^2} \frac{d\epsilon_{eff}}{dT} = \Delta G_{el} \frac{d \ln \epsilon_{eff}}{dT} \tag{5}$$

The value of d ln ϵ/dT for water is -4.6×10^{-3}/°C at 25°.[12] d ln ϵ_{eff}/dT may differ in magnitude somewhat but is unlikely to differ in sign. As ΔG_{el} is positive and has a value of 600–900 cal/mol, ΔS_{el} is negative and has a value of several entropy units.

Thus the small positive ΔS_u of dimerization is a result of the cancellation of several negative contributions with a substantial positive contribution to ΔS_u. The most likely candidate for the source of this positive entropy change is the Frank and Evans type of "icebergs" or structured regions of water around nonpolar solutes.[13] On the formation of the dimer, some of the icebergs around the monomers "melt." The value of ΔS_u thus provides strong evidence of the importance of water-structure effects in promoting stacking-type association of flat organic molecules. Previous work on the effect of dissolved urea on the self-association of MB, its activity coefficient, and the metachromatic interactions of MB$^+$ with negatively charged polyelectrolytes led to a similar conclusion.[4]

The Enthalpy of Dimerization. The substantial negative value of ΔH (Table III) clearly indicates that water-structure effects are only partly responsible for the self-association of MB$^+$. The ΔH value can arise from a combination of several types of interactions, particularly dispersive interactions of the London type and hydrogen bonding. The highly polarizable aromatic systems are likely to show stronger dispersive interactions with each other in the dimer than with the solvent water in the monomeric form, leading to a net attractive contribution to dimerization. The hydrogen bonding contribution to the stability of the MB$^+$ dimer is likely to be an indirect one, as MB$^+$ itself has no hydrogen-bond donor. However, if the dye monomer causes some hydrogen-bond breaking among water molecules, and if on association of two monomers some of the displaced water molecules can form hydrogen bonds with each other, a substantial negative enthalpy contribution may result. Quantitative calculations are difficult. Because of the mobility of the π-electron systems, their dispersive interactions at short distances are likely to be much more complicated than those between isolated atoms.[14] Moreover, additional factors, such as the relative stabilization of the dye monomer by hydrogen bonding to the π-electron system itself[15] as well as the interactions of the monomer dipoles and higher multipoles with the solvent and each other, may be significant.

As a combination of many of the interactions mentioned above is responsible for the interfacial tension of aromatic organic liquids against water, a quantity readily measurable by bulk techniques, it is of some interest to examine the applicability of the interfacial free energy change as a measure of the tendency of monomers to associate in the same sense as it is a measure of the tendency of oil drops to coalesce in water. If the molar volume of MB$^+$ is estimated and the area per face is calculated assuming the typical thickness of 3.5 Å of aromatic molecules, the free energy change calculated on the loss of the interfacial area corresponding to two such faces on dimerization is −13.4 kcal/mol, assuming the interfacial free energy to be that of benzene against water, 35 ergs/cm^2. This value must be compared with ΔG_u corrected for ΔG_{el}, *i.e.*, ~ -8 kcal/mol. The agreement might materially improve if the true area of contact in the dimer was considered, since the two monomeric faces may make an angle with each other in the dimer and may be oriented in an antiparallel fashion, so that the dimethylamino groups of the monomers may not be in contact.[16] The agreement in order of magnitude is of some interest in view of the well-known parallelity of interfacial free energies and hydrophobicity. For

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many organic liquids, for example, solubilities in water are roughly related to interfacial tensions.[17,18] It is also well known that the tendency of organic molecules to associate with each other in such processes as micelle formation[19] and dye association[4,20] decreases rapidly as organic solvents are added to water and its surface tension reduced.

That the interfacial free energy considerations are only of qualitative validity for molecular interactions between organic solutes is clearly indicated by the positive ΔS_u value. The contribution from interfacial free energies is expected to be negative.[18,21] However, it may be significant that the ΔH is substantially negative and does correspond qualitatively with the large enthalpy component of the interfacial free energy.[18,21] For small aliphatic chains, even this qualitative correspondence is not observed. The negative unitary free energies of transfer of small hydrocarbons from aqueous to nonaqueous media are associated with positive enthalpy changes.[10] On the other hand, as the chain length increases, the incremental change in the transfer free energies on adding a CH_2 group, $\Delta\Delta G$, is composed in major part of a negative $\Delta\Delta H$ contribution, although $\Delta\Delta S$ still continues to be positive.[22-24] Aromatic systems with several fused rings and long-chain aliphatic systems may thus form the suspected transition region between small molecules, whose hydrophobicity is controlled mainly by water-structure effects and oil drops, whose hydrophobicity is known to arise from interfacial free energies.[25]

Sinanoğlu and coworkers[26] have recently adopted considerations somewhat similar to the classical interfacial free energy considerations[25,27-29] mentioned above in their study of "solvophobicity." The interactions of a molecule with the solvent are broken up into roughly two parts, those involved in the production of a cavity in which the solute molecule is to be placed, and the interactions of the solute molecule with the liquid when it is placed in the cavity. The free energy needed to produce a cavity is assumed to be mainly due to surface free energy. As the surface free energy of water, 72.7 ergs/cm^2 at 20°, is associated with a surface energy of considerably higher magnitude, $\sim$120 ergs/cm^2,[21] *i.e.*, the surface entropy is positive, this picture is unlikely to lead to the positive ΔS_u found for the dimerization of MB^+. For aliphatic molecules also, the difficulties with this picture remain about the same as those faced by the classical interfacial free energy arguments. The pronounced effect of dissolved urea in reducing hydrophobic interactions in both aliphatic micellar systems[30,31] and the self-association of MB^+, as well as the more extensive associations involved in the so-called metachromatic-type interactions of MB^+ with anionic polyelectrolytes,[4] is another experimental fact to be considered. Urea *increases* the surface tension of water, yet it acts, qualitatively, as an organic liquid such as ethanol.[4] It is difficult to understand the action of urea without involving water-structure considerations, although the possibility that urea increases the van der Waals interactions of the monomeric MB^+ with the medium,[32] thus stabilizing it, must also be considered.

Finally, the fact that the absorption spectrum of the dimer is considerably different from that of the monomers[16,17] is indicative of some interactions of the electron donor–acceptor or charge-transfer type. However, whether such interactions contribute much to the stability of the dimer in the ground state is debatable, particularly in view of the questions raised recently about the magnitude of such stabilization for the so-called charge-transfer complexes themselves.[33] The mobility of the charge in MB^+, and any associated extra polarizability of its π-electron system, is also another factor which probably makes only a minor contribution to the ground-state stability of the dimer. It has been shown[5] that the self-association behavior of the anionic dye Orange II closely parallels that of MB^+. Orange II is roughly of the same size as MB^+, but its charge is localized in a $-SO_3^-$ group. Dyes, particularly azo dyes, with localized charges show little spectral change on self-association when compared with dyes with mobile charges.[34] In our study[11] of the mutual association of MB^+ with several heteroaromatic compounds of different sizes such as pyridine, quinoline, quinolinium ion, and acridinium ion, a rough correlation has been found between the free energy of mutual association with the size of the ring, suggesting that somewhat nonspecific polarization–dispersion forces and water-structure effects are mainly responsible for the association of flat molecules in aqueous media.

The Relation of K_3 to K_2. Cooperativity of Stacking Interactions. In spite of the expected additional charge repulsion in stepwise trimerization, we have found that K_3 is higher than K_2 in several systems.[5,6,8] For MB in dilute solutions the ratio K_3/K_2 is in the range of 1.3–2.5. As the extra charge repulsion should reduce K_3/K_2 by roughly the square root of the estimated factor 3–5 by which K_2 is lowered,[6] the ratio K_3/K_2, corrected for charge effects, should be in the range 2.2–4.0. Thus the "unitary" free energy of stepwise trimerization, when corrected for charge effects, is about 700 cal/mol or 9% more negative than the corresponding value for dimerization at 25°.

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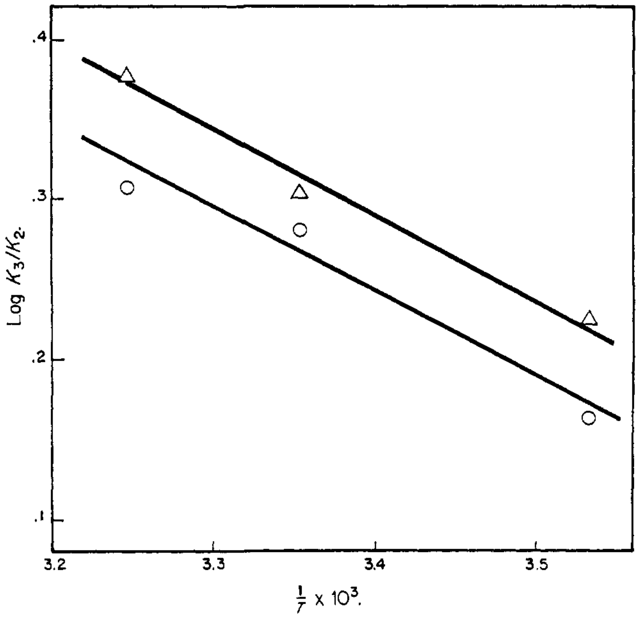

Figure 9. Variation of log K_3/K_2 with $1/T$. K_3/K_2 ratios are obtained from self-association models III,[6] Δ, and IV,[6] ○.

The indication of a substantial attractive second-nearest neighbor contribution in stacking interactions is clearly of considerable interest as regards the "cooperativity" of stacking interactions, it being easier to add a monomer to a dimer than to a monomer. This factor should be important for the stability of many biologically important systems, such as double-stranded DNA molecules.

It is difficult to estimate how well the principle of pairwise additivity is obeyed by the variety of interactions discussed. It seems reasonable, nevertheless, to assume that long-range interactions make an important contribution to the extra stability of the trimer.

Dispersive interactions, because of their approximate additivity, can lead to substantial long-range interactions and appear to be the most obvious source of the extra stability of the trimer. That other factors are of equal or greater importance is suggested by Figure 9, in which log K_3/K_2 is plotted against $1/T$. The K_3/K_2 ratios were derived from two self-association models, III and IV,[6] found to be the most satisfactory in describing the self-association behavior of MB^+.[6] Although the two models give somewhat different magnitudes of the K_3/K_2 ratio, the temperature dependences are similar: K_3/K_2 *increases* at higher temperatures. Figure 9 suggests that at 25° the free energy difference corresponding to the *extra* stability of the trimers, defined as $\Delta G_{ex} = -RT \ln K_3/K_2$, has a value of about -400 cal/mol, whereas the corresponding ΔH_{ex} is about 2500 cal/mol and ΔS_{ex} is about 10 eu. The numerical values of ΔG_{ex}, ΔH_{ex}, and ΔS_{ex} are probably not very reliable because of the shortcomings of the assumed models of self-association. However, for ΔH_{ex} and ΔS_{ex}, only relative comparisons of K_3/K_2 ratios are involved, and it will require large errors in the relative values of K_3/K_2 for ΔH_{ex} and ΔS_{ex} to be wrong in sign. Considering that the extra charge repulsion associated with K_3 must make a negative contribution to ΔS_{ex}, as discussed earlier, it seems that the extra stability of the trimer is not due to a favorable enthalpy contribution, as would be expected from dispersive interactions, but to a favorable entropy contribution, which overcomes an unfavorable enthalpy contribution.

The ΔH_{ex} and ΔS_{ex} quantities strongly suggest that the cooperativity in stacking interactions arises primarily from water-structure effects. As the formation of structured regions in water around nonpolar solutes, the icebergs, probably involves several layers of water molecules, an appreciable long-range contribution of water-structure effects in conferring stability on multimers larger than dimers appears to be a reasonable possibility. Detailed mechanisms, however, can only be speculative in view of the little that is known with certainty about the structure of water and its perturbation by nonpolar solutes.

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