

CHAPTER - IV

Studies on Aggregation of Dyes

A. Introduction:

The changes of the absorption spectra of certain basic dyes on increasing the concentration of an aqueous solution of the dye has been known for over 50 years (1). A blue shift of the major absorption band is usually found. Certain concentration effect is also observed in studying the photochemical and photoelectrical properties of the thiazine dyes, which leads one to enquire into the molecular state of these dyes in solution (2).

Various physical descriptions of this phenomenon have been proposed to account for the behaviour of the dye spectrum, But the superiority of any one is yet to be demonstrated. In this chapter attempts have been made to understand the nature of dye-dye aggregation, the effect of increase in chain length on aggregation and the probable structure of dye aggregates. The knowledge about the state of the dyes in solution is necessary for satisfactory explanation of data on dye-poly-nucleotides and dye-DNA interactions incorporated in the thesis.

The unperturbed absorption of dyes is directly related to the refractive index of the solvent (3). It has become customary to refer to the unperturbed transitions obtained in

highly diluted aqueous or alcoholic solutions as (for molecular absorption) and to call H-bands (for hypochromism) those blue shifted transitions, including D-bands (for dimers) that frequently appear when the concentration of the dye is increased or when its temperature or other environmental factors are altered. At wave lengths longer than the M-bands the appearance of a new maximum may also be caused for such concentration or environmental changes. This new band is called a J-band after Jelly (4), if it is narrow, intense and exhibits resonance fluorescence. The author together with Scheibe (5) first described its properties. It has been shown by Holst (6), Rabinowitch and Epstein (7) and others (8-12) that the deviations from Beer's law with appearance of H or J bands in aqueous solution could be related to the formation of dimers of the dye. Aggregation may also lead to an important change in emission spectra of dye in that the quantum yield of phosphorescence ($T \rightarrow S$ emission) may be enhanced at the expense of fluorescence ($S' \rightarrow S$ emission). Several instances of the enhancement of phosphorescence under conditions favouring aggregation (high concentration, absorption of high polymers) have been recorded.

Nature of Dimers:

The dimerization of many organic acids and similar compounds is attributed to hydrogen bonds. But the association

of dye stuff must be explained in a different way for the following reasons:

(a) Dye stuffs dimerize more strongly in aqueous solution than in organic solvents, whereas carboxylic acid dimers exist in the vapour and in non-polar solvents, but are dissociated in water.

(b) In contrast to the behaviour of the dimerizing carboxylic acids, the polymerization of dye stuffs does not stop at the dimeric state, but often proceeds to trimers and higher polymers.

(c) Colourless leuco dyes dimerize to a smaller extent than the corresponding coloured forms.

The facts agree with the postulate that the polymerization of dyes is due to additive forces of van der Waal's type. 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 (13), the mutual potential energy of two identical molecules possessing a single long-wave electronic absorption band λ_0 (a description which fits most dye stuffs) is to a first approximation proportional to $f^2\lambda^2$, where f is a measure of the excitation probability (called number of absorption electrons or oscillator strength). Since large values of f and λ are exactly the properties which

make a compound strongly coloured, the attractive forces between dye stuff molecules must be considerably larger than those between similar coloured molecules (e.g. the corresponding leuco dyes). These factors are additive and thus account for the formation of high polymers as well as dimers.

The (virtual) dipole-dipole attraction of London in the case of dye stuffs should be supplemented by dipole-quadrupole and quadrupole-quadrupole terms. Since two ions are of the same kind, one must subtract the coulombic electrostatic repulsion energy $z^2 e^2 / Dr$ from the London-Margenau attraction energy (14). Finally, the usual repulsion energy between neutral molecule must also be taken into account. This can be done by assuming a repulsive potential of the Lennard-Jones (15) type with $n = 12$. We obtain in this way the following expression for the mutual potential energy of two dye stuff ions:

$$\begin{aligned}
 U &= - \frac{3N}{4m^2} \left(\frac{h}{c^2} \right) \left(\frac{e}{2\pi} \right)^4 \frac{f^2 \lambda^3}{r^6} \left[1 + 5 \left(\frac{h}{4\pi^2 cm} \right) \frac{f \lambda_0}{r^2} + \right. \\
 &\quad \left. \frac{105}{8} \left(\frac{h}{4\pi^2 cm} \right)^2 \left(\frac{f \lambda}{r^2} \right)^2 \right] + \frac{Nz^2 e^2}{Dr} + \frac{X}{r^{12}} \\
 &= - 1.0742 \times 10^{-7} \frac{f^2 \lambda_0^3}{r^6} - 3.2884 \times 10^{-10} \frac{f^3 \lambda_0^4}{r^8} \\
 &\quad - 5.285 \times 10^{-3} \frac{f^4 \lambda_0^5}{r^{10}} + \frac{329.6}{Dr} + \frac{X}{r^{12}}
 \end{aligned}$$

when U is in Kcal./mole and A_0 and r are in \AA . In this equation λ_0 is approximately equal to the wave length of the band maximum of f can be determined by integration of the extinction curves.

$$f = \frac{1000 mc^2 \ln 10}{N \pi e^2} \int_{-\infty}^{+\infty} \epsilon d\left(\frac{1}{\lambda}\right)$$
$$= 4.314 \times 10^{-9} \int_{-\infty}^{+\infty} \epsilon d\left(\frac{1}{\lambda}\right)$$

Quantum Theoretical Aspects of Dimeric Spectra:

As Forster (16,17) has pointed out, the electronic degeneracy that arises in the solid state of a dimer that has absorbed one photon causes a splitting of the excited electronic level into two. Quantitative development of the theory (18,19,20) in terms of the interaction between the transition dipoles of the resonating dimeric structures, in which one or the other of the linked monomers carries the excitation energy shows that if the dimer is composed of two identical molecules with their chromophores aligned parallel, the optical transition from the ground state to the higher of the split levels is intense, whereas that to the lower level is in the limit of exact parallelism of identical

chromophores in a structure possessing a centre of symmetry, optically forbidden. The excited level associated with the strong transition arises from an interaction between transition moments in the same phase, and the other with an interaction of the moments in the opposite phases. Deviations from parallelism because of vibrational motion of the chromophores with respect to each other or an unsymmetrical structure of the dimer will allow the transition to the lower excited state to occur, but less intensely than to the upper state. The dimeric spectrum associated with a parallel arrangement of the co-linked flat monomer is expected, therefore, to consist of an intense band at the shorter wave length and a feebler band at longer wave lengths. This is, in fact, observed for the dimers of the thiocyanine, xanthine and rhodamine classes of dyes.

One can, therefore, conclude in general that the dimeric structure of dyes consists of two monomers held together with their planes, or at least their chromophores essentially parallel to each other, with some deviations from parallelism because of thermal vibrations or structural asymmetry.

An important consequence of the sandwich dimer model and its split excited state levels is the expectation of an

intense blue shifted transition relative to the $0 \rightarrow 0'$ maximum of the monomer as well as a weak red shifted band associated with the forbidden transition of the low energy. The energy difference between the monomer maximum and that of the blue shifted dimer should be roughly proportional to the dyes extinction co-efficient or more precisely to its transition dipole moment.

B. Results and Discussions:

It has been shown that the absorption spectra of aqueous dye solutions could be interpreted quantitatively on the assumption of the monomer-dimer equilibrium (21). Let us consider the equilibrium



We may define the dimer association constant

$$K = \frac{C_m^2}{C_d} \quad \text{IV - 2}$$

where C_m and C_d are the molar concentration of the monomer and dimer respectively. Neglecting high polymers in the concentration range studied, the total molar concentration of the dye as monomer is

$$C = C_m + 2 C_d \quad \text{IV - 3}$$

If α is the fraction of dye present as monomer, then

$$\alpha = \frac{C_m}{C}, \text{ and it follows that}$$
$$K = \frac{2C^2}{(1-\alpha)} \quad \text{IV - 4}$$

If A is the absorbancy of the solution at a total dye concentration C and l is the path length, we may define as effective molar absorbancy index

$$a = \frac{A}{l \cdot C} \quad \text{IV - 5}$$

Assuming that Beer's law holds for each component, it follows that

$$a = \alpha a_m + (1 - \alpha) \frac{a_d}{2} \quad \text{IV - 6}$$

A check of the validity of equation IV-6 was obtained by plotting the molar absorbancy indices for constant wave length vs. α for MB, assuming certain values (Fig. 2-b) of K. In the neighbourhood of $K = 2.0 \times 10^{-4}$ for MB, straight lines were obtained in the region $5000 \text{ \AA} < \lambda < 7000 \text{ \AA}$. By extrapolating the straight lines to $\alpha = 0$, the absorption spectrum of the dimer was obtained at different wave lengths. Similarly for TET (Fig. 3-b) straight lines were obtained in

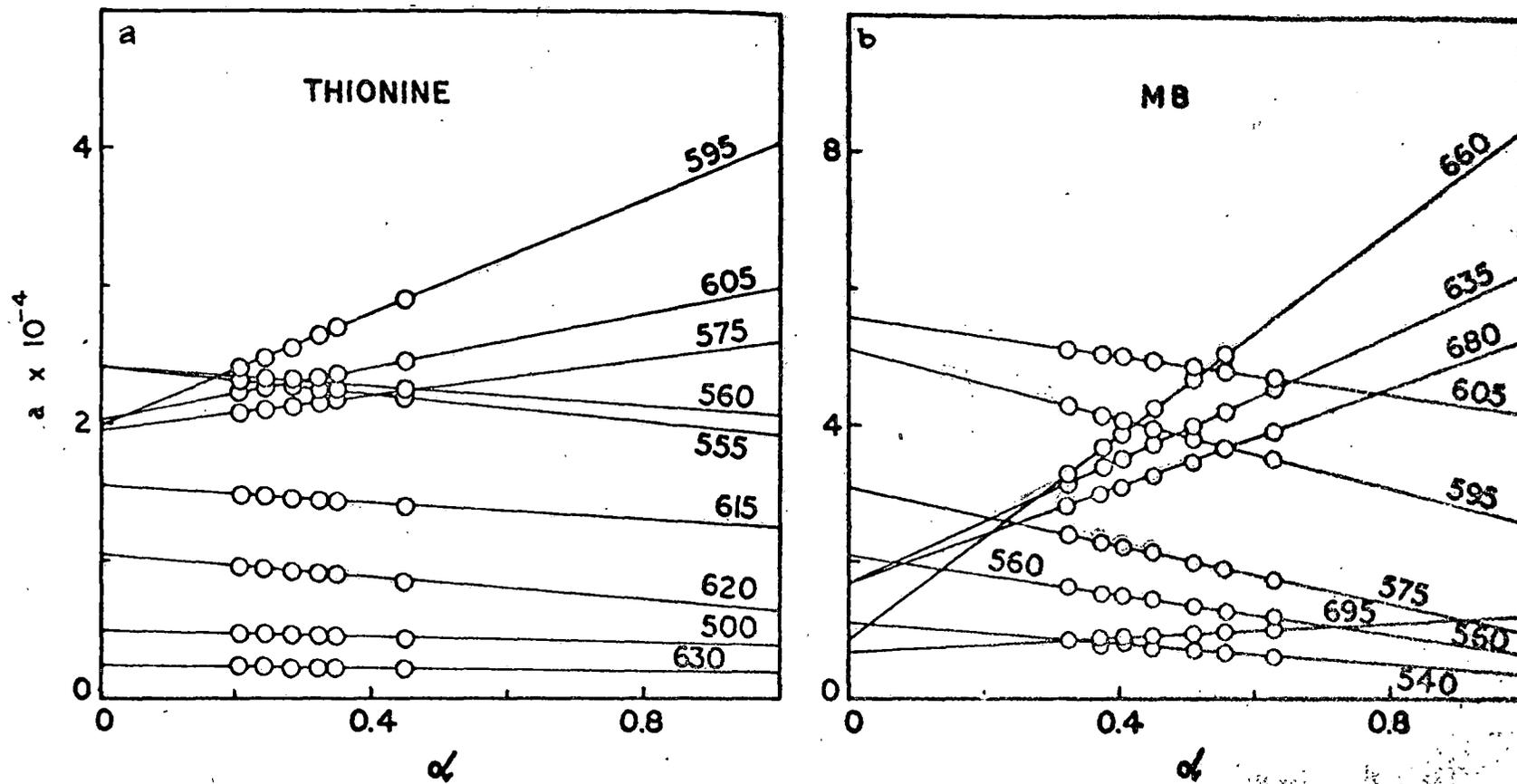


FIG. 2. STRAIGHT LINE PLOT OF THE EFFECTIVE MOLAR ABSORBANCY INDEX OF AQUEOUS (a) THIONINE AND (b) MB SOLUTIONS AT VARIOUS WAVE LENGTHS Vs. MONOMER FRACTION,

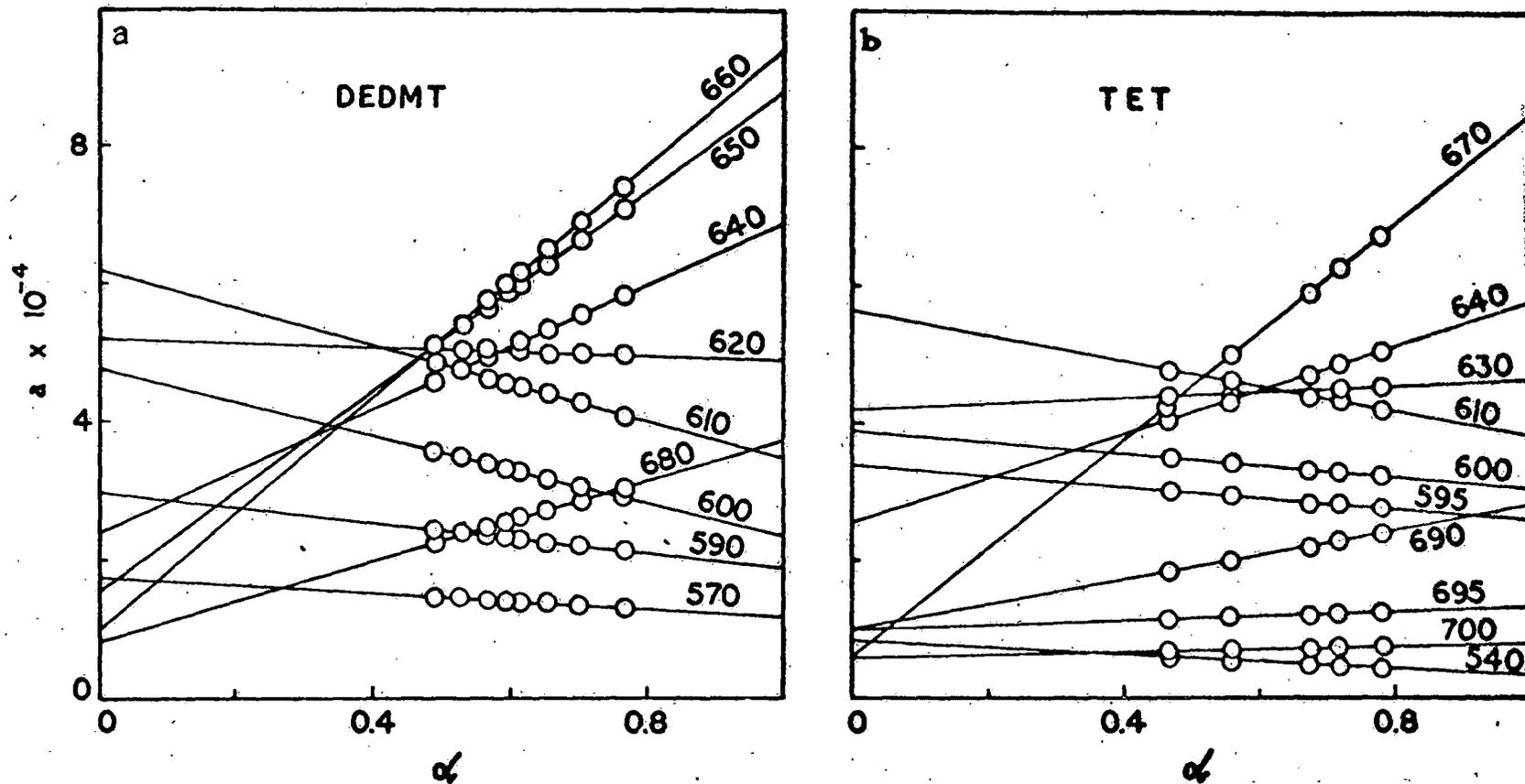


FIG. 3. STRAIGHT LINE PLOT OF THE EFFECTIVE MOLAR ABSORBANCY INDEX OF AQUEOUS (a) DEDMT AND (b) TET SOLUTIONS AT VARIOUS WAVE LENGTHS Vs. MONOMER FRACTION,

the region $5200 \text{ \AA} < \lambda < 7200 \text{ \AA}$ for $K = 4.5 \times 10^{-4}$; for DEDMT straight lines were obtained in the region $5100 \text{ \AA} < \lambda < 7200 \text{ \AA}$ for $K = 2.2 \times 10^{-4}$ (Fig. 3-a) and for Thionine straight lines were obtained in the region $4500 \text{ \AA} < \lambda < 6300 \text{ \AA}$ for $K = 0.5 \times 10^{-4}$ (Fig. 2-a). Trial and error fitting involving the straight line plot was not sensitive enough to distinguish between several equilibrium constants in the neighbourhood of $K = 2.00 \times 10^{-4}$ mole/l for MB; 4.7×10^{-4} mole/l for TBT; 2.2×10^{-4} mole/l for DEDMT and 0.6×10^{-4} mole/l for Thionine. It was possible to find an accurate value for the dimer dissociation constant from the condition that the dimer should show neither a shoulder nor a hollow in the region of monomer maximum. Thus, the accurate values calculated on this basis are tabulated below.

Table - 3

Dye	Dissociation Constant
Thionine	0.5×10^{-4} mole/l.
Methylene Blue	1.7×10^{-4} mole/l.
Diethyl dimethyl Thionine	2.2×10^{-4} mole/l.
Tetraethyl Thionine	4.5×10^{-4} mole/l.

Over the easily measured absorption range, the long wave length absorption peak decreases and the intermediate wave length peak increases in such a manner as to form an isobestic point indicating that primarily there are present only two absorbing species. Figures 4(a), 4(b), 4(c) and 4(d) show the spectra of pure Thionine, MB, DEDMT and TET monomers and dimers respectively. All the four monomer spectra of dyes show their respective shoulders at approximate wave lengths 555, 610, 613 and 615 nm. The appearance of these shoulders has been explained by fluorescence observation as a vibrational component of a single electronic band. The dimer spectra of all the four dyes differ widely from those of monomers because of the delocalised charge cloud interaction between two monomer dye molecules. In each case two peaks are obtained in dimer spectra, one called d_1 and the other d_2 . These two, an intense blue shifted transition relative to $0 \rightarrow 0'$ maximum of the monomer (d_1) as well as a weak red shifted band associated with the forbidden transition (d_2) are expected of the sandwich dimer model.

The transition moment lengths 'q' of the dye monomer and dimer were determined from areas under the absorption curves [Figs. 5(a, b, c and d)] using equation

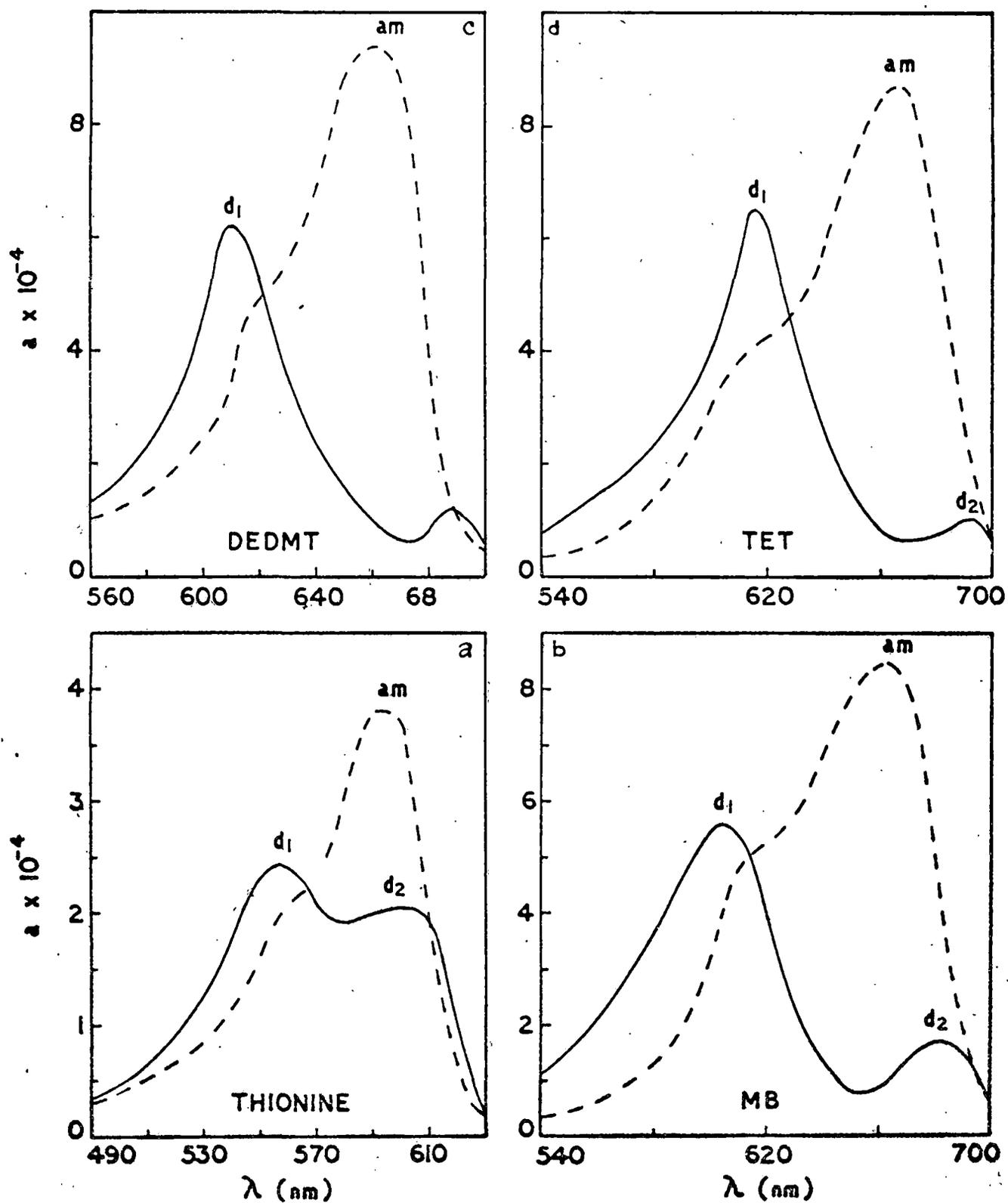


FIG. 4. MOLAR ABSORBANCY INDICES OF PURE MONOMER (a_m) AND DIMER (a_d) OF AQUEOUS DYE SOLUTIONS. (a) THIONINE ; (b) MB ; (c) DEDMT ; (d) TET SOLUTION.

$$q^2 = -1.09 \times 10^{-19} \int a \, d \log \lambda \quad \text{IV - 7}$$

The values of q 's calculated for each monomer in its aqueous solution and those of its dimers are listed in table below:

Table - 4
Transition moment length in Å

Dye	Monomer (q_m)	Dimer (q_{d_1})	Dimer (q_{d_2})
Thionine	1.48	1.24	0.75
MB	2.14	1.69	0.76
DEDMT	2.15	1.68	0.47
TET	2.13	1.27	0.36

Oscillator strengths for each monomer and dimer were obtained by integrating over the absorption band of the plot of a vs. $\bar{\nu}$ (Figs. 6a, 6b, 6c and 6d), employing the relation

$$f = 4.32 \times 10^{-9} R \int a \, d \bar{\nu} \quad \text{IV - 8}$$

where $\bar{\nu}$ is the wave number and R is an integral field function given by

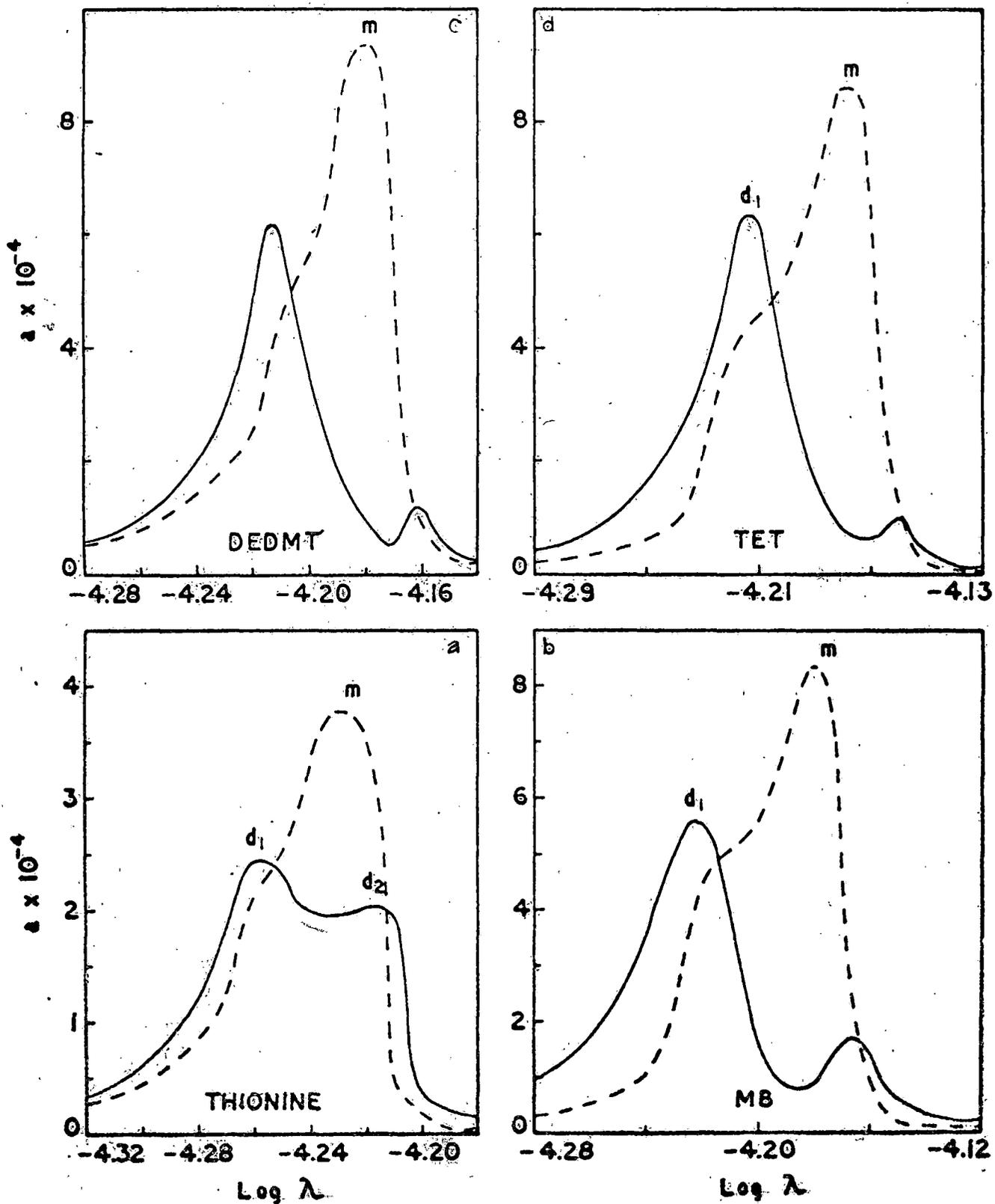


FIG. 5. MOLAR ABSORBANCY INDICES OF PURE MONOMER (a_m) AND DIMER (a_d) OF AQUEOUS DYE SOLUTIONS. (a) THIONINE ; (b) MB ; (c) DEDMT AND (d) TET SOLUTION.

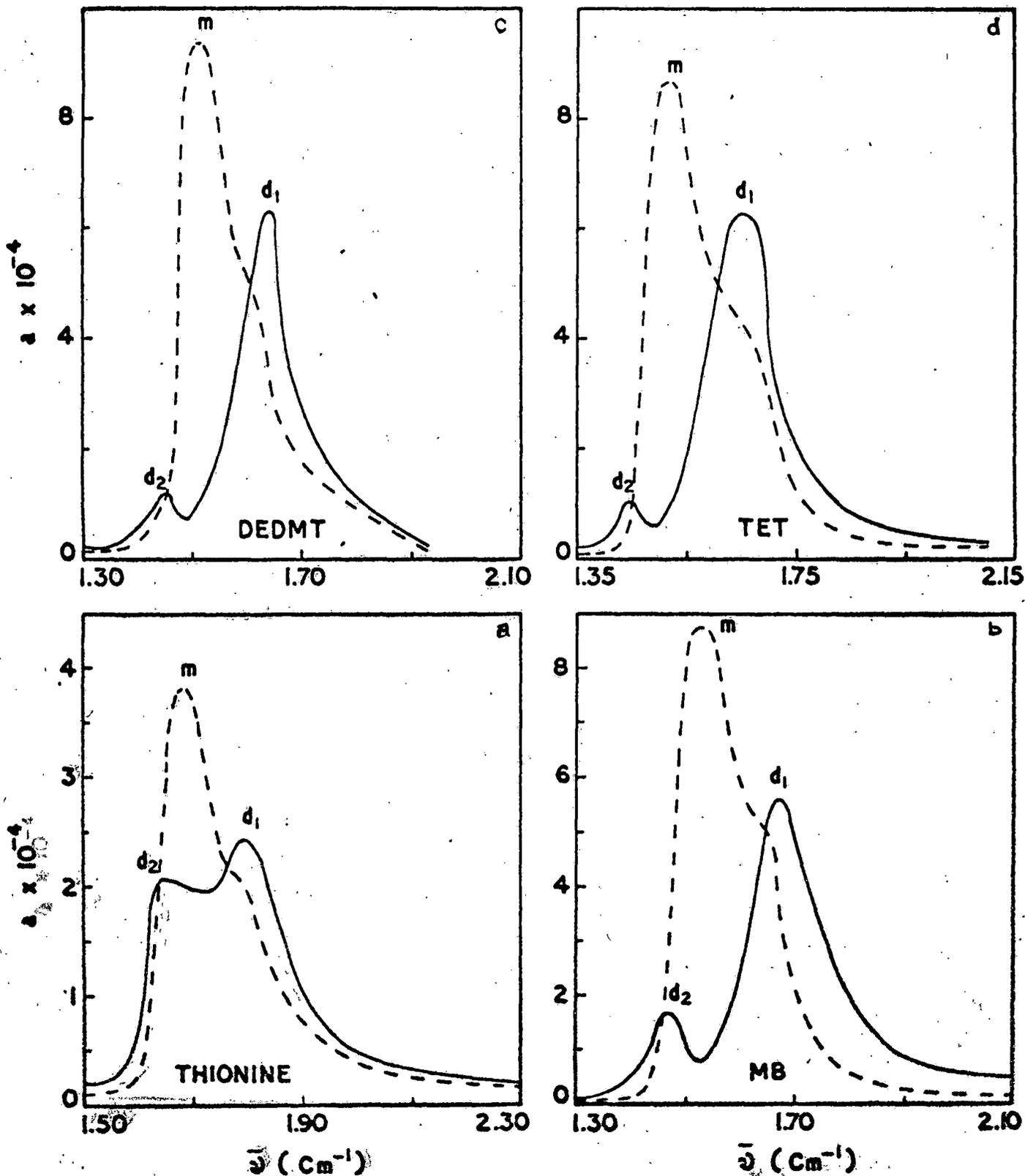


FIG. 6. MOLAR ABSORBANCY INDICES OF PURE MONOMER (a_m) AND DIMER (a_d) OF AQUEOUS DYE SOLUTIONS. (a) THIONINE; (b) MB; (c) DEDMT AND (d) TET SOLUTION.

$$R = \frac{9 n_0}{(n_0^2 + 2)^2}$$

(Lorentz internal field)

$$R = n_0 \cdot \frac{(2 n_0^2 + 1)}{3 n_0^2}$$

(Onsagar internal field)

n_0 , is the refractive index of the solvent. Results of the four dyes are given in the following table.

Table - 5

Oscillator Strengths:

Dyes	Monomer (f_m)	Dimer (f_{d_1})	Dimer (f_{d_2})
Thionine	0.28 (L) 0.38 (O)	0.21 (L) 0.38 (O)	0.08 (L) 0.11 (O)
MB	0.59 (L) 0.80 (O)	0.44 (L) 0.59 (O)	0.06 (L) 0.09 (O)
DEDMT	0.59 (L) 0.79 (O)	0.41 (L) 0.55 (O)	0.046 (L) 0.06 (O)
TET	0.60 (L) 0.81 (O)	0.42 (L) 0.57 (O)	0.03 (L) 0.07 (O)

It has been proposed earlier that the London dispersion forces and the hydrophobic bonding give rise to the aggregation of two cationic dye molecules. Moreover, the principal axis of the monomeric dye molecule which lie along the long axis of the molecule parallels its transition dipole axis i.e., the transition dipole is placed along the long axis of the molecule. The nature of stacking of two molecules to each other in forming the dimer can be understood from the transition from the lower to the higher or from the higher to the lower excited state levels, which on the other hand depends on the angle (ϕ) between the transition dipoles i.e., between the principal axes of the aggregated molecules. The values of the angles can be evaluated by employing the values of transition dipoles in the relation given by Levinson, Simpson and Curtis (22)

$$\vec{q}_{d+} = 2^{-\frac{1}{2}} (\vec{q}_T + \vec{q}_B) \quad \text{IV - 9}$$

where q_{d+} and q_{d-} are dimer transition moments and \vec{q}_T , \vec{q}_B are the transition moments of the top and bottom molecules. The above also leads to the equation

$$q_{d+} = q_m (1 + \cos \phi)^{\frac{1}{2}} \quad \text{IV - 10}$$

$$q_{d-} = q_m (1 - \cos \phi)^{\frac{1}{2}} \quad \text{IV - 11}$$

where q_m is the monomer transition moment length. From the values of the transition moment lengths as listed in the Table - 4 above, the angles (ϕ) between the monomer transition dipoles in the aggregate are calculated employing the following equation (IV-12).

$$\text{Cos } \phi = \frac{(q_{d+}^2 / q_{d-}^2 - 1)}{(q_{d+}^2 / q_{d-}^2 + 1)} \quad \text{IV - 12}$$

In order to attain greater stability, the monomer units arranged their charged $-NH_2$ groups along opposite edges of the aggregate. The latter part overcomes the coulombic repulsion between the charged centres of the molecules. It has been found from the calculated values of the angles between the transition dipoles of monomer units in aggregate (Table - 6) that the value of the angle lowers when the chain lengths of the dyes are increased by substituting hydrogens of $-NH_2$ groups partially or completely by alkyl groups. Since there is no binding forces other than London dispersion forces or hydrophobic type of force between the monomeric molecules, it is expected a low restoring force constant for the torsional deformation about an axis perpendicular to the planar ring system and passing through the central rings. The transition dipole moments of the molecules causing this torsional

deformation in order to overcome the steric hindrance along the long bulky substituted amino groups of the sandwich is more or less the same for all the four dyes as there is no basic changes in the skeleton on the molecules. Consequently, we expect that the restoring force constants allowing the side chain to move apart is greater for the smaller molecules than the long chain molecules. And hence, torsional deformation is less for the long chain molecules than the smaller ones. The values of ϕ for the four dyes are given in Table - 6.

Table - 6

Dyes	Angles
Thionine	62.53°
MB	48.61°
DEDMT	43.45°
TET	31.78°

The steric hindrance caused by increase in the chain length, thus, explains the increasing value of the dimer dissociation constants of the substituted dyes.

The role of water in the formation of the aggregates, either as dielectric sandwiching between the dye molecules or as a former of the hydrogen bonds has been found to be

of great importance. In a series of articles Hillson and McKay (23,24), McKay and Hillson (25,26) and McKay (27,28) presented evidence to support their belief that the metachromasia of dyes observed in solvents of low dielectric strength was due to strong interactions between dye ions and counter ions to produce an undissociated dye in which the ions are in intimate contact and not separated by solvent molecules (25). In one article they suggest that aggregation probably does not occur at all in solvents of very low dielectric strength (27) even though metachromasia is evident. In a later article they imply that even when the dye is in the presence of a chromotrope in water, metachromasia occurs largely as a result of the interaction of the cations with polyanion, resulting in a perturbation of the charge distribution of the dye cation (24). In a subsequent paper they have, however, conceded that dimerization of the dye is an important cause of metachromasia in water and that this is due to the tendency of the water molecules to self associate, giving rise to strong hydrophobic bonding between dye molecules (29).

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