

Formation of Mixed Dimers in Solutions of Basic Dyes

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The formation of mixed dimers of basic dyes has been studied in aqueous solution at room temperature. These mixed dimers have absorption bands in the visible region at wavelengths shorter and longer than those shown by the self dimers of the dyes having higher and lower excited states, respectively. Both transitions are allowed according to exciton theory. The angle between the dye molecules in the sandwich-like dimers was calculated from the molar absorption coefficients and is in the range 140°–160°. The non-alignment of the molecules may be due to steric hindrance caused by the bulky side groups.

The aggregation of dyes is a phenomenon well known for many years. Most basic dyes, when in aqueous solution show deviations from Beer's law at higher concentrations. This behaviour has been attributed to the formation of dimers and higher aggregates of the dyes.^{1–3} In general, the dimerization equilibrium constants are in the range 100–10 000, corresponding to free energies of 2–5 kcal mol^{–1}. These are of the order of magnitude of formation of hydrogen bonds,⁴ as well as of other types of interactions such as hydrophobic,^{5,6} van der Waals or Π - Π interaction dispersion forces,^{1,6} all of which have been proposed as responsible for the formation of aggregates.

Aggregates of the same type, having similar spectroscopic properties, are also formed at lower concentrations when in the presence of natural or synthetic polymers^{7,8} or polyelectrolytes.^{9,10} In these cases the equilibrium constants are, at least, a factor 10 higher,¹¹ so that aggregation can be observed at concentrations which are well behaved in the absence of additives. It is claimed that in these cases the polymer spatial configuration may act as a pattern for the dye molecules, forcing them into a geometry appropriate for the interaction. This phenomenon has been referred to as metachromasy. Additional to the spatial arrangement effect, in the presence of anionic polyelectrolytes there will be an electrostatic attraction between the polyelectrolyte charged groups and the basic dye molecules, modifying the charge distribution at the latter, and decreasing its repulsion for a second dye molecule, which will eventually add on top of it, facilitating its aggregation.

Although a great number of studies have been made on the effects described above, practically all of them deal with systems of only one dye, whereas little has been reported on mixed-dye systems. In aqueous solutions, reports of mixed-dye systems have been made by Lemin and Vickerstaff¹² on methylene blue–direct dyes, Arvan¹³ on thionine–acridine, Pal and Schubert¹⁴ on methylene blue–acridine orange, Ghosh *et al.*¹⁵ on acridine orange–basic dyes and Vitagliano *et al.*¹⁶ on acridine orange–fluorescein systems. Studies of mixed-dye aggregation in the presence of polyelectrolytes have been reported by Pal and Schubert¹⁴ on methylene blue–acridine orange on polysaccharide polyions and by Shirai *et al.*^{17,18} on methylene blue with other dye systems on synthetic anionic polyelectrolytes. The relationship existing between mixed aggregation in the presence of polymers and that in pure aqueous solution is similar to that for self-aggregation.¹⁷

In view of some doubts existing on the interpretation of the effects observed in solutions of two basic dyes and the mechanism reported for the formation of mixed aggregates of basic

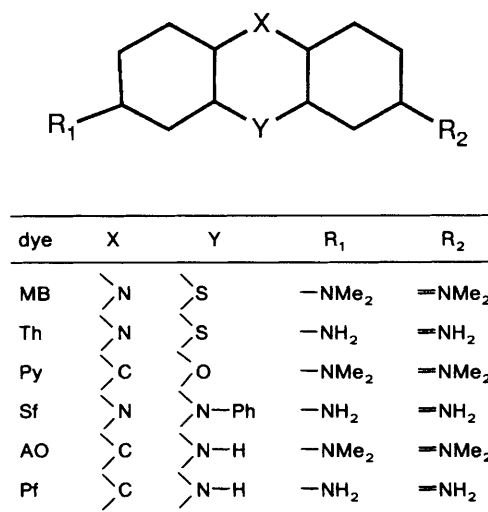
dyes,¹⁵ we have undertaken a study of various systems of this type.

Experimental

The following dyes, with structures shown in scheme 1, were used: methylene blue (C. Erba p.a., λ_{\max} 661 nm), MB; thionine (Aldrich cert., λ_{\max} 598 nm), Th; pyronine Y (Aldrich cert., λ_{\max} 553 nm), Py; safranine (Merck p.a., λ_{\max} 530 nm), Sf; acridine orange (Aldrich p.a., λ_{\max} 489 nm), AO and proflavine (Aldrich, λ_{\max} 456 nm), Pf. All the dyes were used as received and their spectra and molar absorption coefficients were similar to those reported in the literature. All the determinations were made in aqueous solutions at room temperature 25 \pm 1 °C, using bi-distilled, deionized water.

Absorption spectra were taken on a Beckman DU-7 spectrophotometer using different cells (0.1, 0.2, 1.0 and 5.0 cm pathlengths).

Self-dimerization equilibria were determined for some of the systems using 10^{–7}–10^{–4} mol dm^{–3} solutions, and the calculated constants were similar to those already reported.⁹ These were the values used in the calculations. The mixed-dimer systems were studied keeping the concentration of the dye absorbing at longer wavelengths constant at 3 \times 10^{–5} mol dm^{–3} and varying the other to obtain concentration relations of between 0.5 and 11.



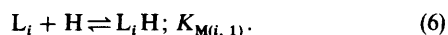
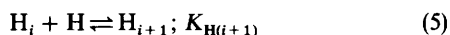
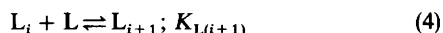
Scheme 1.

Results and Discussion

A mixed aggregation process can be assumed to involve the following equilibria



where L and H are two basic dyes, absorbing at low and high wavelengths, respectively, and K_i are the corresponding equilibrium constants. When the concentration of the dyes is high enough, a series of other equilibria must be taken into account



Otherwise, provided the dye concentrations are sufficiently low, reactions (4)–(6) can be neglected.

In fig. 1 are shown the differential absorption spectra for the pair AO–MB, in which the concentration of MB is kept fixed and that of AO is increased gradually up to the ratio 1 : 10. As can be seen, a decrease of the MB–monomer band at 660 nm is observed together with an increase at longer wavelengths, with an isosbestic point at around 695 nm. A quantitative interpretation of the spectrum at shorter wavelengths, in the absorbant region of AO, is more difficult. There is a decrease in AO concentration owing to its combination with MB, and its self-aggregation at higher concentration [reaction (2)]. Additionally, bands due to the mixed dimer and to the self-dimer and to higher aggregates (at the higher concentrations), appear in this region, generally overlapping each other.

Thus, assuming a system such as that described above, the absorption at any wavelength λ , will be

$$A_\lambda = \varepsilon_L^\lambda [L] + \varepsilon_{L_2}^\lambda [L_2] + \varepsilon_{L_n}^\lambda [L_n] + \varepsilon_H^\lambda [H] + \varepsilon_{H_2}^\lambda [H_2] + \varepsilon_M^\lambda [M] \quad (7)$$

where the ε_i^λ are the molar absorption coefficients of species i at wavelength λ , and M stands for the mixed dimer.

To obtain relevant data on the mixed aggregates using this equation, the molar absorption coefficients for the monomers

and self-dimers can be obtained from independent experiments performed on single-dye systems, the concentration of these species being calculated from the known dimerization equilibrium constants. Thus, in principle, only the mixed dimer molar absorption coefficient and its concentration are left as unknowns. Although an iterative method could be devised for the simultaneous determination of both quantities, similar to that used in the determination of the self-dimerization equilibrium constants,¹⁹ the mathematical relationships will involve third-order equations, which makes the procedure laborious and the error minimization process quite uncertain.

Several approaches can be adopted to facilitate the evaluation of the parameters needed to calculate the mixed-dimer equilibrium constant. The easiest way would be to make the measurements at the isosbestic point between the monomer and the dimer of the dye absorbing at higher wavelengths (H), assuming that the absorption of the other dye and its aggregates at this wavelength can be neglected. In this case the variations of the absorbance upon addition of the second dye would correspond to the formation of the mixed dimer. Unfortunately, in the cases studied the variation of the absorbance in this region was too small. Thus, monitoring was made at wavelengths above that point, near the maximum of the differential spectrum. So, if the concentration of H is low enough not to form higher aggregates, in the absence of L, the measured absorbance will be

$$A_0 = \varepsilon_H [H] + \varepsilon_{H_2} [H_2]. \quad (8)$$

Once dye L is added to the solution

$$A = \varepsilon_H [H]' + \varepsilon_{H_2} [H_2]' + \varepsilon_M [M]. \quad (9)$$

Remembering that

$$[H]_0 = [H] + 2[H_2] = [H]' + 2[H_2]' + [M] \quad (10)$$

where $[H]_0$ is the total concentration of dye H, the primed concentrations correspond to the system in the presence of dye L, and $[M]$ is the concentration of the mixed dimer, the equations above transform into

$$A_0 = \varepsilon_H ([H]_0 - 2[H_2]) + \varepsilon_{H_2} [H_2] \quad (11)$$

and

$$A = \varepsilon_H [H]_0 + (\varepsilon_{H_2} - 2\varepsilon_H) [H_2]' + (\varepsilon_M - \varepsilon_H) [M]. \quad (12)$$

The difference between both, measured from the differential spectrum, will be

$$\Delta A = \{\varepsilon_M - \varepsilon_H\} [M] + (\varepsilon_{H_2} - 2\varepsilon_H) ([H_2]' - [H_2]). \quad (13)$$

At the wavelengths used for the measurements, the molar absorption coefficients of the monomer and dimer are of the same order so that their difference will be small when compared to the difference between the molar absorption coefficients of the monomer and the mixed dimer; furthermore, $[M]$ will always be larger than the difference $[H_2]' - [H_2]$ (i.e. when MB is involved, it can be calculated from the dimerization constant of the latter that less than 10% of the dye will be present as dimer). This means that the second term in the right-hand side of eqn (13) will be smaller than the first. When this term is neglected, the equation reduces to

$$\Delta A = (\varepsilon_H - \varepsilon_M) [M]. \quad (14)$$

A simple relationship between both molar absorption coefficients is assumed in order to obtain the values of the two unknown parameters in eqn (14)

$$\varepsilon_M = n\varepsilon_H. \quad (15)$$

So, for a fixed value of n , concentrations of the mixed dimer $[M]$ can be calculated from the experimental data, when the

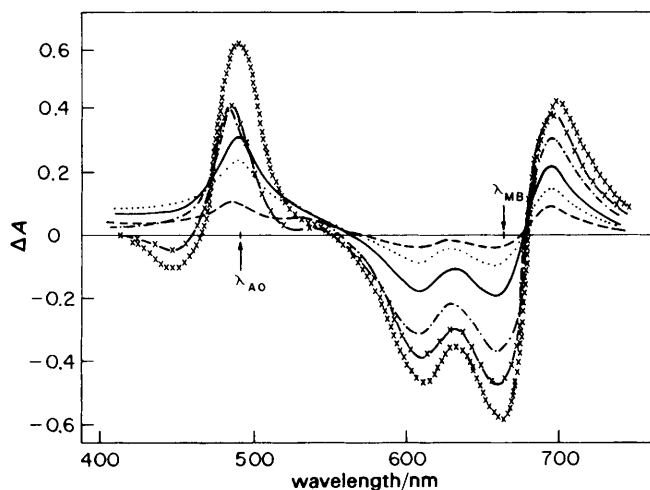


Fig. 1. Differential absorption spectra of MB–AO mixtures. $[MB] = 3 \times 10^{-5} \text{ mol dm}^{-3}$. Ratio $[AO]/[MB]$: 0.5 (—); 1.0 (···); 2.0 (---); 4.0 (- · - ·); 7.0 (× - × -); 11.0 (× × × ×). λ_{AO} and λ_{MB} are the wavelengths of the maxima of the AO and MB monomer peaks.

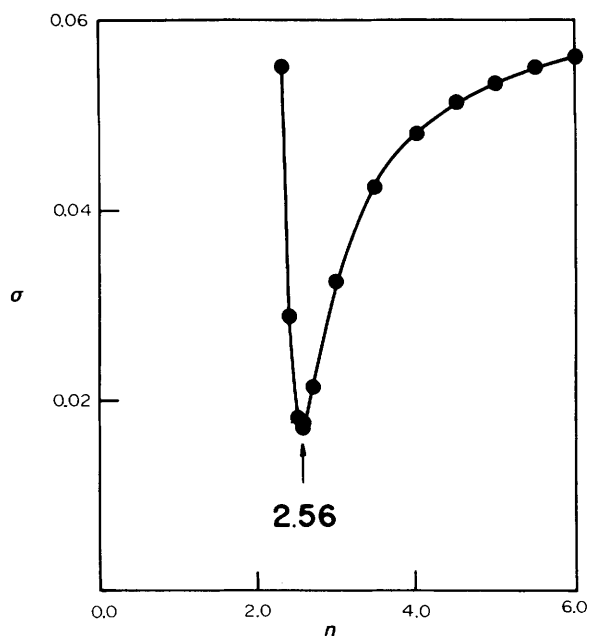


Fig. 2. Variation of the squared deviation as a function of the parameter n , for the MB-AO system.

initial dye concentrations, the molar absorption coefficient and the dimerization equilibrium constants of dye H are known. Therefrom, values for the equilibrium constant of the mixed dimer can be calculated for each L concentration. The constant corresponding to the n value which showed the least standard deviation (see fig. 2) was considered to be the best value. The equilibrium constants and the molar absorption coefficients obtained in this way are shown in table 1.

As can be seen from table 1, the values of the mixed dimer equilibrium constants are, in general, *ca.* five times larger than those reported for self-dimerization of this type of dye (from 10 000 for AO down to 600 for Pf),⁹ similar to the effect reported by Lemin *et al.*¹² For the systems studied here, the existence of hydrogen bonds does not seem to be very plausible, so both dispersion forces and hydrophobic effects seem to be acting to hold the dye molecules together. These forces should not be very different for self and mixed dimers, and cannot explain by themselves the differences found between the two sets of values. The factor of 5 corresponds to a free-energy difference of *ca.* 1 kcal mol⁻¹. These values can be explained assuming a charge-transfer-type interaction. When similar molecules dimerize this kind of effect will not be

Table 1. Mixed-dimer equilibrium constants and molar absorption coefficients at the calculation wavelengths

| system ^a | K_M^b | $\epsilon_H^{\lambda}/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} c$ | $\epsilon_M^{\lambda}/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ |
|---------------------|---------|---|---|
| MB-Pf (690) | 49 000 | 11 700 | 22 500 |
| MB-Sf (695) | 20 400 | 11 200 | 27 400 |
| MB-AO (695) | 22 300 | 11 200 | 23 000 |
| Th-Pf (630) | 14 200 | 6 550 | 10 400 |
| (460) | 11 700 | 23 400 ^d | 41 000 |
| Th-Sf (630) | 1 600 | 6 550 | 16 700 |
| Th-AO (630) | 1 500 | 6 550 | 64 300 |
| Th-Py (630) | 1 400 | 6 550 | 19 300 |
| Py-AO (575) | 28 500 | 11 300 | 14 100 |
| Py-Pf (570) | 1 700 | 11 300 | 26 600 |

^a Wavelengths used for the calculations (in nm) are given in parentheses; ^b K_M values are $\pm 15\%$; ^c ϵ^{λ} values are $\pm 2000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$; ^d corresponds to ϵ_L^{λ} .

active as the electron densities of both molecules are equal. In mixed aggregates, both molecules are different and will, therefore, have different electron densities, as well as different polarizabilities allowing that interaction. This can be shown from the order of the equilibrium constants for MB and Th with Pf, Sf and AO. It can be seen that for both series the mixed dimers are more effectively formed with Pf, whereas with Sf and AO the equilibrium constants are lower and more or less equal.

Knowing the mixed dimerization equilibrium constants, the spectra of these dimers in the high-wavelength region can be calculated from eqn (9), assuming no absorbance of species involving dye L

$$Y \equiv A^{\lambda} = \epsilon_H^{\lambda}[H] + \epsilon_{H_2}^{\lambda}[H_2] + \epsilon_M^{\lambda}[M]. \quad (15)$$

The plot shown in fig. 3 has been obtained by modifying the above equation

$$\frac{A^{\lambda} - \epsilon_{H_2}^{\lambda}[H_2]}{[H]} = \epsilon_H^{\lambda} + \epsilon_M^{\lambda} \left(\frac{[M]}{[H]} \right). \quad (16)$$

The extrapolation to the origin corresponds to the monomer molar absorption coefficient, whereas the mixed-dimer molar absorption coefficient is obtained from the slope of the straight lines. The left-hand term in eqn (16) and the ratio of the concentrations were calculated using the self- and mixed-dimer equilibrium constants and the self-dimer molar absorption coefficients from the literature. When the latter were unknown they were calculated by a similar method using data of single-dye experiments. For systems in which the absorption spectra of the dyes were close together (Th-Py, Th-Sf and Py-AO) the left-hand side of eqn (16) was corrected for the contribution to the absorption by dye L and its self dimer. The spectra of the mixed dimers are shown in fig. 4, and their molar absorption coefficients and maxima are listed in table 2.

The maxima are all near or at longer wavelengths than the maxima of the monomeric dyes, as would be expected from

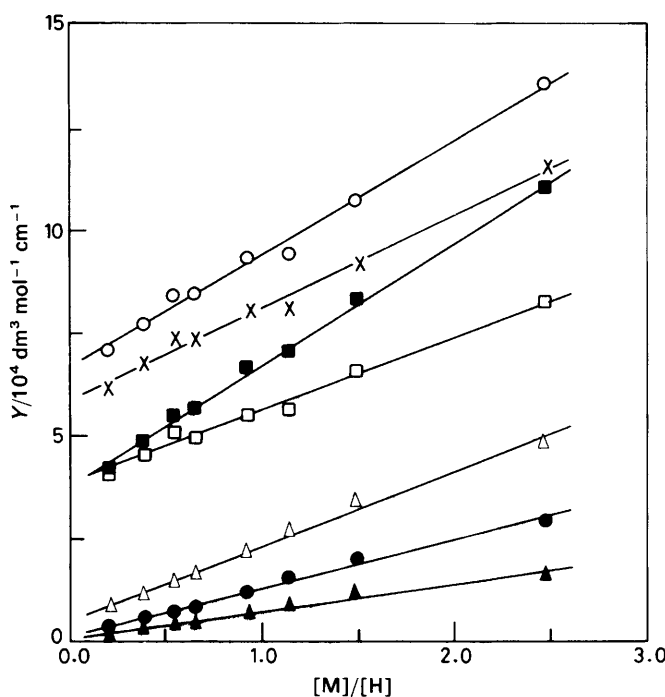


Fig. 3. Plots for the determination of dimer molar absorption coefficients for the MB-AO system, according to eqn (16). (○) 665 nm; (×) 650 nm; (■) 680 nm; (□) 620 nm; (△) 700 nm; (●) 710 nm; (▲) 720 nm.

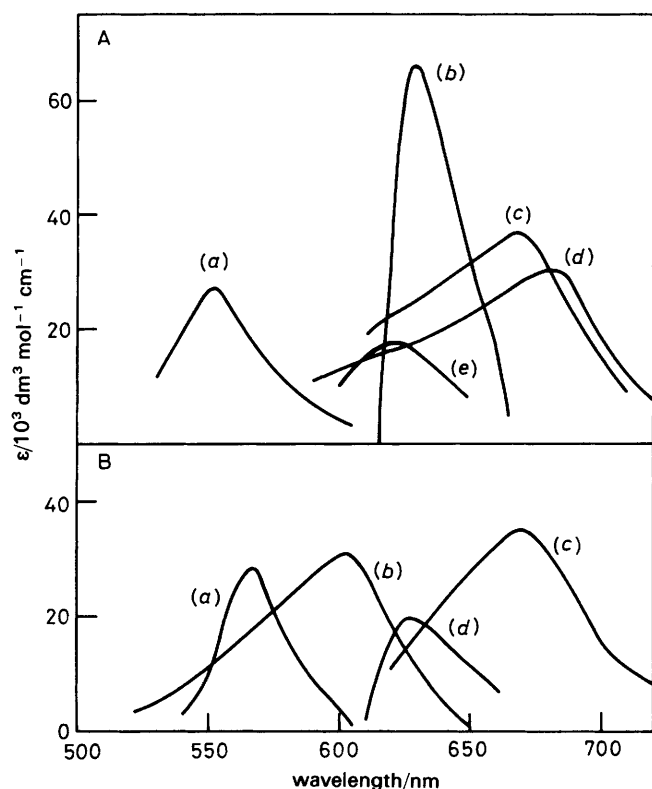


Fig. 4. Low-energy mixed-dimer spectra. A, (a) Py-AO, (b) Th-AO, (c) MB-Sf, (d) MB-AO, (e) Th-Sf. B, (a) Py-Sf, (b) Th-Pf, (c) MB-Pf, (d) Th-Py.

the interaction between two molecules with different electronic energy states. In the same way, the absorption corresponding to the higher energy dimer level has to be found at wavelengths below that of the L-dye. The total spectrum of the mixed dimer Th-Pf has been calculated and is shown in fig. 5 compared with those of the monomeric dyes. It can be seen that both bands appear at the appropriate wavelengths, below and above the L- and H-monomer peaks.

According to the exciton theory, the transition moment for the excitation of the dimer is²⁰

$$M = \frac{1}{\sqrt{2}} \iint \Psi_E \left(\sum_i \mu_1^i + \sum_j \mu_2^j \right) \Psi_G d\tau_1 d\tau_2 \quad (17)$$

where Ψ_G and Ψ_E are the wavefunctions describing the dimer in its ground and excited states, and μ_1 and μ_2 are the

Table 2. Mixed-dimer absorption maxima and molar absorption coefficients

| dye system | $\lambda_M^{\max}/\text{nm}^a$ | $\epsilon_M^{\max}/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}^b$ |
|--------------|--------------------------------|--|
| MB (monomer) | 665 | 80 000 |
| MB-MB | 600 | 77 000 |
| MB-Sf | 665 | 36 700 |
| MB-AO | 680 | 30 500 |
| MB-Pf | 670 | 35 500 |
| Th (monomer) | 600 | 61 000 |
| Th-Th | 560 | 74 000 |
| Th-Sf | 620 | 17 000 |
| Th-Py | 630 | 19 300 |
| Th-Pf | 600 | 30 400 |
| | 445 | 50 500 |
| Pf (monomer) | 445 | 39 600 |

^a λ_M^{\max} values are ± 5 nm; ^b ϵ_M^{\max} values are $\pm 2000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$.

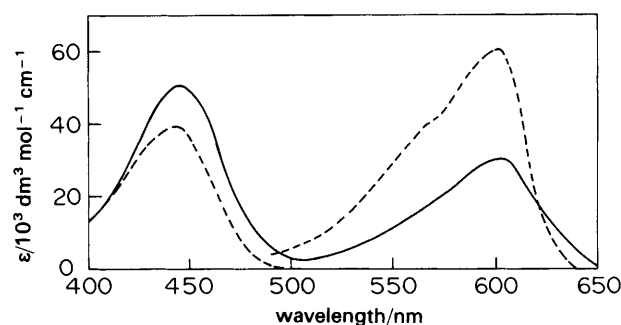


Fig. 5. Spectrum of the Th-Pf mixed dimer, compared with the spectra of the monomers (---).

induced dipole moments acting on both individual dye molecules. For a parallel dimer configuration (as found in the present case),^{6,21} the transition moment for the excitation of the dimer will be

$$M = \frac{1}{\sqrt{2}} (M_1 \mp M_2) \quad (18)$$

where M_1 and M_2 are the transition moments for the excitation of the individual dye molecules. According to eqn (18) only the transition to the high-energy state (in-phase transition moments) is allowed for self dimers, due to the cancellation of the induced dipole moments in the out-of-phase low-energy state. In the case of mixed dimers no such cancellation will exist, as both moments will be different, in general. Therefore, the molar absorption coefficients for the low-energy transitions should be lower than those for the self-dimer peaks, but not zero. On the other hand, they also must be lower than those of the high-energy dimers, if the transition moments add up. This can be seen in table 2.

The angle between the dye molecules in the dimer can be calculated from the molar absorption coefficients at the maximum-wavelength absorption peak and the peak widths, assuming that

$$M_i^2 \propto \epsilon_i^m \Delta \bar{\nu}_i^{1/2} \quad (19)$$

where M_i , ϵ_i^m and $\Delta \bar{\nu}_i^{1/2}$ are, respectively, the transition moment, the molar absorption coefficient at the maximum wavelength and the width of the absorption peak at half-height of species i .

The angle between both molecules is the same as that between the individual transition moments, as shown in fig. 6, and has been calculated from

$$M_M = M_1 + M_2 + 2\sqrt{M_1}\sqrt{M_2} \cos \theta. \quad (20)$$

These angles are shown in table 3 for the low- and high-energy dimers.

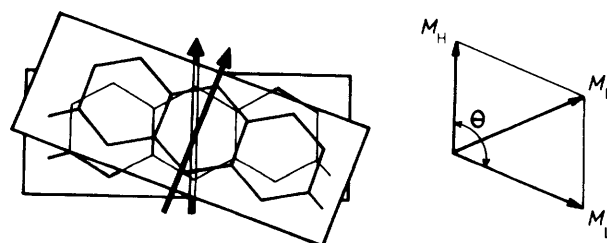


Fig. 6. Geometry of the mixed dimers.

Table 3. Angles between the main axes of the individual molecules in dye dimers

| system | angle/° | |
|--------|-------------------|------------------|
| | high-energy dimer | low-energy dimer |
| MB-MB | 126 | |
| Th-Th | 119 | |
| Th-Pf | 109 | 138 |
| MB-Pf | | 148 |
| Th-Sf | | 159 |
| MB-Sf | | 151 |
| MB-AO | | 141 |
| Th-Py | | 161 |
| Py-AO | | 150 |
| Py-Pf | | 145 |

As can be seen from table 3, the dye molecules in the mixed dimers are not perfectly aligned, but form angles of 120°–150° with one another. Although our results are not good enough to allow us to calculate the distances between the molecules, the distances can be assumed to be not very different from those for other xanthenic dyes, i.e. around 3–5 Å.^{22,23} This distance will not permit accommodation of the dyes one exactly on top of the other owing to steric hindrance due to the side groups. Similar geometries have been found for dimers of various other types of dyes, such as rhodamines,²⁴ fluoresceins¹⁹ and azonaphthols,²⁵ which also do not have aligned components.

Conclusions

The formation of mixed dimers of basic dyes shows spectroscopic properties in accordance with exciton theory. The bands of these dimers can be found at wavelengths shorter and longer than those of the forming dyes, with higher or lower excited states, respectively. These bands correspond to the transitions of both the in-phase (high energy) and out-of-phase (low energy) transition-moment geometries, none of which are prohibited.

The angles between the induced dipole moments, calculated from the molar absorption coefficients of the mixed dimers, show that the molecules in the aggregate are not aligned but form angles of 140°–160° with one another, possibly due to the need to accommodate the bulky side groups. Similar angles can also be calculated for self dimers.

The mixed-dimer formation equilibrium constants are, in general, larger than those for self dimers, the differences corresponding to ca. 1 kcal mol⁻¹. These are ascribed to a charge-transfer contribution to the interaction as a result of the difference in the electron densities of the different dyes.

No evidence could be found for induced aggregation or deaggregation of self dimers for the dyes under the conditions used in this work.

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