Effects of Colloidal SiO_2 and Cyclodextrins on the Aggregation of Dyes in Aqueous Solutions

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Aggregation of two positively charged dyes, Methylene Blue, MB^+ (1), and pinacyanol chloride, PC^+, (2) is induced in aqueous SiO_2 colloids. In colloidal SiO_2, MB^+ aggregates into the dimeric form, and PC^+ is aggregated into H-assemblies. The aggregation processes are a result of high local dye concentrations in the vicinity of the colloid owing to binding of the positively charged dyes to the negatively charged colloidal interface. De-aggregation of thionin, (3), is accomplished in water by the addition of cyclohexa-amylose, α-CD, and cyclohepta-amylose, β-CD. Monomeric thionin binds to α-CD and β-CD ($K_{ass.}$ = 6.67×10^2 M^{-1} and 1.78×10^3 M^{-1}, respectively). Conversion of thionin dimer into the monomeric form, upon addition of α-CD or β-CD, is attributed to association of the monomeric form to the hydrophobic cavity of CD. This conversion is accompanied by changes in the absorption and fluorescence spectra of the dye.

Thionin and cyanine dyes play an important role as sensitizers in photographic processes, laser components, photographic filter layers, and chemotherapy.[1,2] Recently, these dyes have also been studied extensively as sensitizers in photoelectrochemical solar energy conversion systems.[3-5] Interestingly, thionin and cyanine dyes are present in solution with the monomeric forms in equilibria with dimeric aggregates and/or poly-aggregated forms (H-mers and J-aggregates, respectively),[6] [equation (1)]. The concentration of the dyes in

$$kD \rightleftharpoons m[D.D] \rightleftharpoons n[DD\ldots D] \qquad (1)$$

solution strongly affects these equilibria. The monomeric form persists in dilute solutions while the aggregated forms predominate in concentrated solutions. The photophysical properties of the dyes, *i.e.* absorption spectra and fluorescence characteristics, are strongly affected by the aggregation state of the dye. For example, thionin in its monomeric form is strongly luminescent, while the fluorescence of the thionin dimer is intramolecularly quenched. A variety of environmental factors such as hydrophobic dispersions and electrostatic forces affect dye–dye interactions.[6] It has been found that surfactant micelles promote the de-aggregation of dyes to their monomer forms.[7,8] In contrast to the de-aggregating activity of micellar microenvironments, polyelectrolytes often induce aggregation.[9] Electrostatic interactions of the dye with the charged sites of the macromolecules are considered to be active in the aggregation process induced by the polyelectrolytes.

Here we wish to report on a study that examines the effects of colloidal SiO_2 on the aggregation of positively charged dyes in an aqueous environment. The silanol groups of colloidal SiO_2 particles are ionized in basic aqueous solutions (pH > 7).[10,11] Consequently, a diffuse double layer is produced in the vicinity of the colloidal interface and the particles are characterized by a high electric surface potential (*ca.* −170 mV).[12] Positively charged dyes are thus expected to be adsorbed to the charged colloidal interface, and their aggregation should be affected.

Similarly, we report on the effect of addition of cyclo-amyloses (cyclodextrins, CD) on the monomer–dimer equilibrium of thionin in an aqueous solution. Cyclodextrins (CD) are cyclic polysugars composed of glucose units linked by 1–4 glycoside bonds.[13,14] These cylindrical polysugars include in their cyclic structure a hydrophobic cavity capable of binding organic substrates.[15] This binding property of CD has been utilized in recent years in studying CD as models for substrate–receptor interactions of natural enzymes.[16] The dimensions of the CD cavity depend on the number of sugar units linked in the cyclic structure.[13] For cyclohexa-amylose (α-CD) the cylindrical cavity height corresponds to 6.7 Å while its diameter is 4.5 Å. Cyclohepta-amylose (β-CD) includes a cavity with a height of 7.0 Å and a diameter of 7.0 Å. Thus, relatively small organic molecules, *i.e.* benzene rings, or parts of a molecular backbone, are capable of entering into the CD cavity. Certainly, the dimensions of the CD cavities exclude the possibility of the association of molecular aggregates. Consequently, the equilibrium of dye monomers–aggregates in aqueous solution is expected to be shifted upon the introduction of cyclodextrins that bind the monomeric dye form only.

Experimental

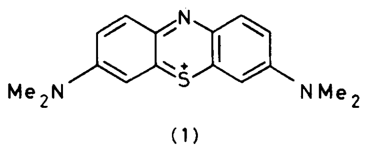
Absorption spectra were recorded on a Uvikon-820 (Kontron) spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer (MPF-2A) fluorescence spectrophotometer.

Complexation of thionin hydrochloride (Aldrich) to β-CD (Aldrich) was studied by successive additions of β-CD to 25 ml of an aqueous thionin solution (5.69×10^{-6}M). The changes in the absorption spectra upon addition of weighed amounts of β-CD (11.0, 4.68, 9.6, 12.05, and 15.5 mg) relative to the pure dye spectrum were recorded. For precise determination of the absorbance differences (ΔA) (see later) the original (pure dye) spectrum (stored in the memory of the spectrophotometer) was subtracted from the modified spectrum obtained after addition of β-CD. Complexation to α-CD (Aldrich) was studied in the same manner. The effect of β-CD on the monomer–dimer equilibrium of thionin was examined by following the absorption spectrum of an aqueous thionin solution (8.1×10^{-4}M) after adding weighed amounts of β-CD (6.65, 7.25, 7.15, and 9.45 mg).

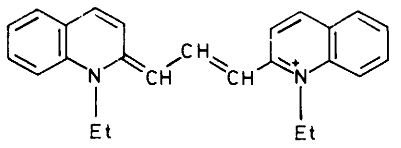
Association of Methylene Blue, MB^+ (Aldrich) and pinacyanol chloride, PC^+ (Aldrich) to colloidal SiO_2 40 Å diameter particles (Nalco No. B-1015) was determined spectroscopically. To an aqueous PC^+ solution (1.87×10^{-5}M) were added, successively, 10 µl portions of a SiO_2 colloid stock solution (7.25 mg ml^{-1}), prepared from commercial 14.5% SiO_2 colloid. The absorption spectra of the resulting dye colloid suspension were recorded and compared with the original aqueous dye spectrum. Similarly, binding of MB^+ to SiO_2 colloid was determined by successive addition of 10 μl portions of the stock SiO_2 colloid to an MB^+ aqueous solution (2.86×10^{-5}M). The absorption spectrum of pinacyanol chloride, PC^+, was also determined at 8.9×10^{-6}M and 8.9×10^{-5}M dye concentrations (in 1 cm and 1 mm glass cells, respectively) and at 3.57×10^{-3}M using a 0.05 mm Teflon ring placed between 2 glass microscope slides.

Results and Discussion

Aggregation of Dyes in SiO_2 *Colloids*.—The effect of the negatively charged SiO_2 colloid on the aggregation of dyes has been investigated in aqueous solutions that include positively charged dyes, *i.e.* Methylene Blue, MB^+ (1), or the cyanine dye, pinacyanol chloride, PC^+ (2).



(1)



(2)

The absorption spectrum of methylene blue, MB^+ (1), in water at a concentration of 2.86×10^{-5}M shows two bands, one at 664 nm attributed to monomeric MB^+, and a second at 614 nm that corresponds to the dimeric form of MB^+ ($A_{664}/A_{614} = 1.78$).[6] Introduction of the SiO_2 results in significant changes in the absorption spectrum of the dye that are displayed in Figure 1. The absorption bands at 664 and 614 nm decrease in their intensities and a new relatively broad absorption band at $\lambda = 598$ nm is formed. The changes in the spectrum upon increasing the concentration of the colloidal SiO_2 reveal two isosbestic points at 698 and 598 nm. This indicates that the monomeric and dimeric dye forms in solution are adsorbed to the colloidal interface and the SiO_2-associated dye forms are in equilibrium with free dye forms [equation (2)]. The spectrum of the dye at an SiO_2 particle

$$MB_m^+ + MB_d^+ + SiO_2 \rightleftharpoons (MB_m^+ \cdots SiO_2 \cdots MB_d^{2+}) \quad (2)$$

concentration of 2.9×10^{-6}M (Figure 1E) represents a saturation effect on the dye adsorption process, and further addition of SiO_2 does not substantially affect the spectrum pattern. Namely, even at very high SiO_2 colloid concentrations, the free monomer dye form is visible, indicating that the free and associated dye forms are in equilibrium under these conditions. Since the size of the SiO_2 particles is known (*ca.* 40 Å) the molar concentration of particles could be estimated and the association constant of the dye to colloidal interface [equation (2)] was calculated ($K_{ass} = 1.06 \times 10^5 M^{-1}$).

Several conclusions can be drawn from the spectral changes observed upon addition of the SiO_2 colloid. (i) The absorption band of the SiO_2-associated dimeric form is red shifted by 16 nm as compared with the absorption of the dimer solution, while no noticeable effect on the colloid-adsorbed monomeric form is observed. Theoretical considerations predict that excitonic transitions in the dimer are sensitive to the relationship of the transition dipoles with the molecular axis of the dimer.[17] Any perturbation in the dimer molecular axis, *i.e.* owing to the adsorption to the SiO_2 colloid, is thus expected to affect its optical absorption spectra. (ii) At high SiO_2 concentrations, the adsorbed dimeric dye is the predominating form in the monomer–dimer equilibrium, (A_{598}/A_{664} *ca.* 2). The ratio of monomer–dimer dye forms in solution depends on the dye concentration. In dilute dye solutions monomeric dye prevails, while in concentrated solutions the dimeric assembly predominates. The original dilute, aqueous, MB^+ solution (2.86×10^{-5}M) includes the monomer as the major component, and addition of SiO_2 colloid induces its dimerization. This process is attributed to the adsorption of positively charged dye to the negatively charged colloid, resulting in an increase in the local concentration of the dye at the colloidal interface. Consequently, the dimeric form becomes the predominating component in its associated state.

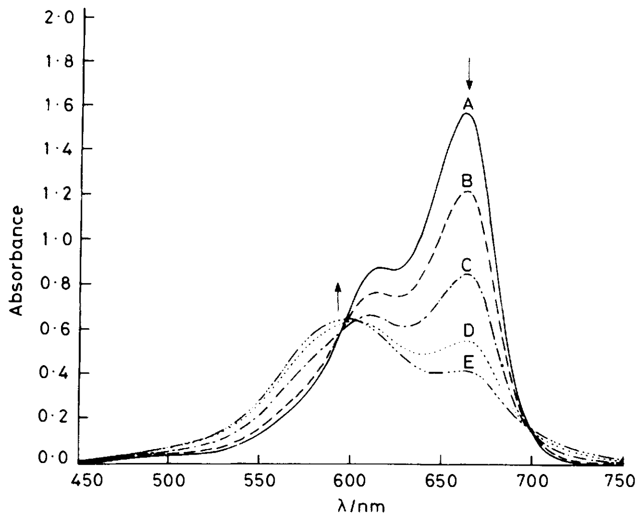


Figure 1. Aggregation of MB^+ to SiO_2 colloid, $[MB^+] = 2.86 \times 10^{-5}$M. A, No added SiO_2; B, with addition of colloid, $[SiO_2] = 7.38 \times 10^{-7}$M; C, with addition of colloid, $[SiO_2] = 1.47 \times 10^{-6}$M; D, with addition of colloid, $[SiO_2] = 2.2 \times 10^{-6}$M; and E, with addition of colloid, $[SiO_2] = 2.9 \times 10^{-6}$M

Similarly, aggregation of the positively charged cyanine dye, pinacyanol chloride, PC^+ (2), is induced in colloidal SiO_2. The absorption spectrum of PC^+ (2) (1.87×10^{-5}M), shows two absorption bands at $\lambda = 600$ and 548 nm (Figure 2), that are assigned to electronic transitions of the monomeric and dimeric dye, respectively. Addition of colloidal SiO_2 to the dye solution results in a significant change in the absorption spectrum of the dye (Figure 2). The monomeric and dimeric bands decrease in their intensities and a new absorption band at 500 nm appears. The spectrum D in Figure 2 represents the saturation effect of the SiO_2 colloid upon the absorption spectrum, and further addition of colloid does not affect the absorption spectrum of the dye noticeably. It can be seen that under these conditions the dye is mostly present in the aggregated form absorbing at 500 nm, together with minor amounts of the dimeric and monomeric dye forms. Without SiO_2 colloid the optical absorption spectrum of PC^+ (2), in water strongly depends on its concentration (Figure 3). A dilute solution (8.9×10^{-6}M) shows the spectrum displayed in Figure 3A. Under these conditions the monomeric band at 600 nm predominates and the dimeric dye absorbing at 548 nm is in equilibrium with the dye monomer

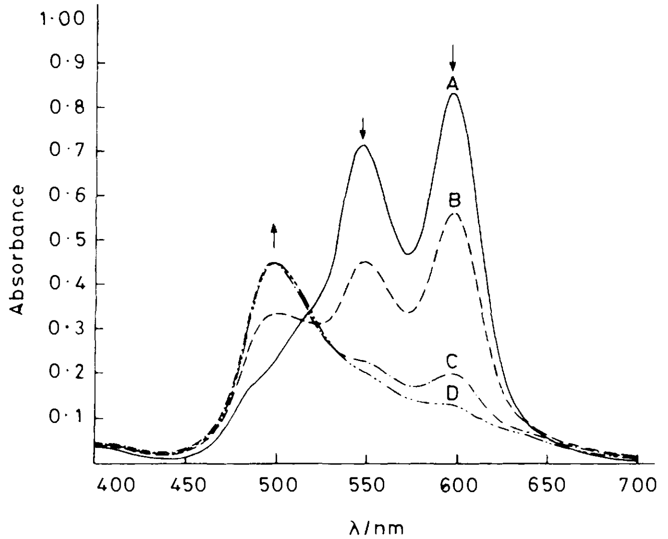


Figure 2. Aggregation of PC^+ to SiO_2 colloid, $[PC^+] = 1.87 \times 10^{-5}$M. A, No added SiO_2; B, with colloid, $[SiO_2] = 2.8 \times 10^{-7}$M; C, with colloid, $[SiO_2] = 8.2 \times 10^{-7}$M; and D, with colloid, $[SiO_2] = 1.4 \times 10^{-6}$M

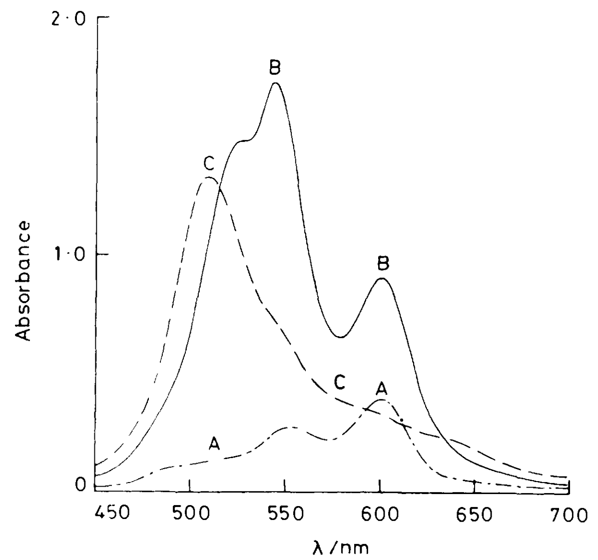
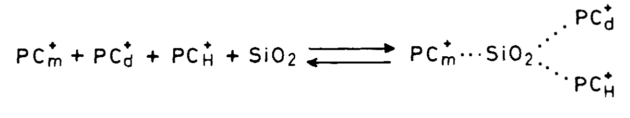


Figure 3. Absorption spectra of PC^+ in water, at different concentrations. A, $[PC^+] = 8.9 \times 10^{-6}$M; B, $[PC^+] = 8.9 \times 10^{-5}$M; C, $[PC^+] = 3.6 \times 10^{-3}$M

($A_{600}/A_{548} = 1.52$). At a higher dye concentration of (2), (8.9×10^{-5}M), the dimeric dye aggregate becomes the major component in the spectrum ($A_{600}/A_{548} = 0.51$) and a clear new absorption band at 500 nm is developed. Further increase in the dye concentration (3.57×10^{-5}M) results in reduction of the intensity of the dimeric band absorption, leaving the absorption band at 500 nm as the major component in the spectrum. This band, absorbing at 500 nm, has been assigned [6] to H-aggregates (polyaggregates) of the dye. A saturated dye solution shows, in addition to the H-aggregates, a blue-shifted band absorbing at 646 nm. This low-energy absorption band has been assigned to J-aggregates of cyanine dyes.[6]

Comparison of the aggregation properties of the dye in the homogeneous aqueous phase with the spectral changes occurring in a dilute dye solution upon addition of colloidal SiO_2, implies that PC^+ aggregates in colloidal SiO_2 at a low dye concentration. From Figure 2 it can be seen that in the homogeneous aqueous phase at a concentration of 1.87×10^{-5}M PC^+ appears in its dimeric and monomeric forms only (spectrum A). Addition of SiO_2 results in the dye aggregation into H-aggregates that coexist with minor amounts of the monomeric and dimeric PC^+ forms (spectra B and C). Thus, in the presence of colloidal SiO_2, PC^+ (2) is mostly present in the H-aggregated assembly adsorbed to the SiO_2. The spectral changes observed with the addition of SiO_2 colloid indicate that the adsorbed H-aggregates are continuously formed upon addition of the colloid. Ultimately, we see that these H-aggregates are in equilibrium with minor amounts of the monomeric and dimeric forms. To account for this behaviour we assume the equilibrium outlined in equation (3).

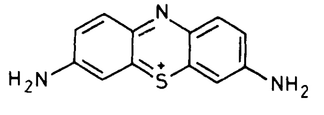


$$PC_m^+ + PC_d^+ + PC_H^+ + SiO_2 \rightleftharpoons PC_m^+\cdots SiO_2\begin{matrix}\cdot\cdot\, PC_d^+\\ \cdot\cdot\, PC_H^+\end{matrix}$$

Saturated aqueous solutions of the dye PC^+ show, in addition to H-aggregates, the blue-shifted J-mers. Addition of SiO_2 colloid to such a saturated dye solution prevents the formation of the J-assemblies, and only H-mers are present. Since it is known that the J-assemblies are of a considerably ordered ' brickwork ' structure,[18] we conclude that the ' rough colloidal interface ' prevents the formation of such ordered forms.

It is evident from these results that the SiO_2 colloid induces the effective aggregation of (1) and (2). These processes are attributed to electrostatic interactions of the dyes with the negatively charged colloidal interface. The colloid is characterized by a high negative surface potential (−170 mV) [12] and adsorbs the positively charged dyes. Consequently, their local concentration in the vicinity of the colloidal interface is substantially higher than in the homogeneous phase, and their aggregation is favoured. Therefore, we can conclude that the colloid provides a unique environment to control the processes of dye aggregation, and simultaneously affect the photophysical properties of the dyes.

De-aggregation of Thionin by Cyclodextrins.—Thionin (3),



(3)

has been intensively studied in recent years as a sensitizer for solar energy conversion fuel cells.[4,5] Thionin appears in dilute aqueous solutions in its monomeric form absorbing at λ = 598 nm. In concentrated solutions it aggregates into a dimeric assembly, absorbing at λ = 558 nm, which is in equilibrium with the monomeric form [equation (4)]. Monomeric thionin

$$TH_m^+ \rightleftharpoons TH_d^{2+} \qquad (4)$$

has a strong fluorescence at λ = 623 nm, while the dimeric form is non-luminescent due to self quenching of the excited aggregate. For effective solar energy conversion, concentrated dye solutions are required for efficient light absorbance. These

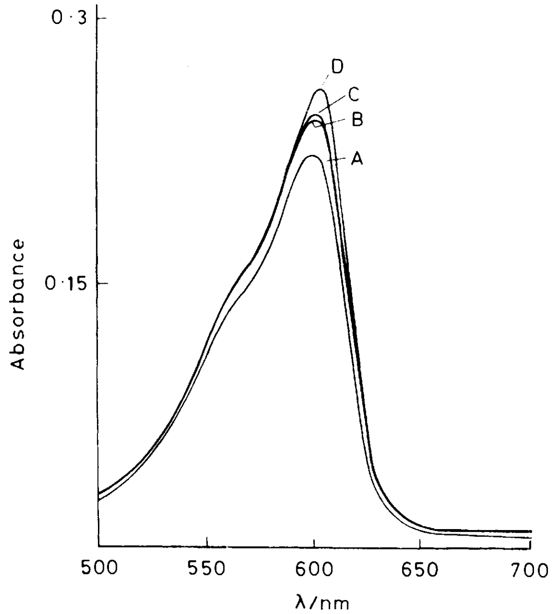


Figure 4. Complexation of thionin to β-CD in H_2O. $[TH^+] = 5.69 \times 10^{-6}$M. A, Without addition of β-CD; B, with [β-CD] = 3.9×10^{-4}M; C, with [β-CD] = 5.5×10^{-4}M; D, with [β-CD] = 1.9×10^{-3}M

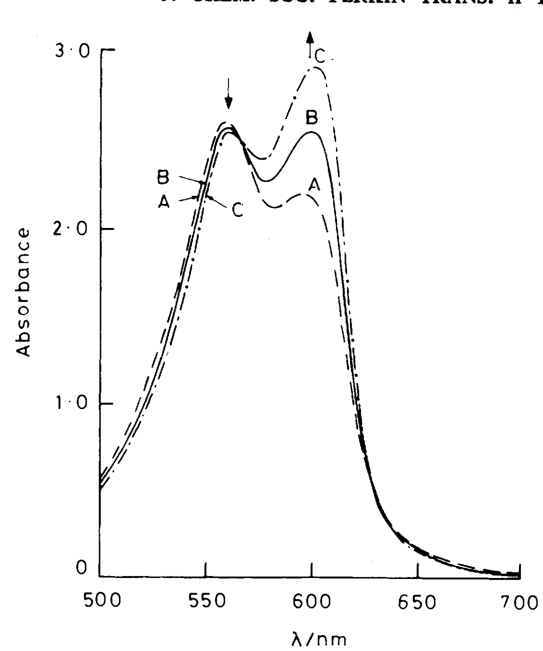


Figure 5. Effect of β-CD on the aggregation of thionin $[TH^+] = 8.1 \times 10^{-4}$M. A, Without addition of β-CD; B, with [β-CD] = 1.2×10^{-3}M; and C, with [β-CD] = 2.7×10^{-3}M

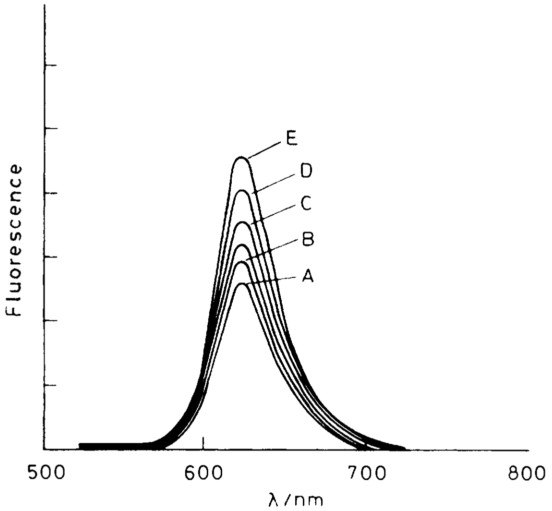


Figure 6. Effect of β-CD on fluorescence spectra of thionin; λ excitation 595 nm, $[TH^+] = 4.5 \times 10^{-6}$M. A, Without added β-CD; B, with [β-CD] = 1.66×10^{-4}M; C, with [β-CD] = 3.1×10^{-4}M; D, with [β-CD] = 8.3×10^{-4}M; and E, with [β-CD] = 1.9×10^{-3}M

dye solutions must also be strongly luminescent, in order to allow the utilization of the excited species in electron-transfer reactions. Thus, the use of thionin (3) as a sensitizer in photoelectrochemical cells confronts a basic limitation where concentrated solutions of the monomeric thionin are needed.

We have examined the effect of added cycloamyloses (cyclodextrins, CD) on the monomer–dimer equilibrium of thionin (3). Cyclodextrins (CD) are water-soluble cyclic polysugars capable of binding organic molecules into their hydrophobic cavity.[13-15] The dimensions of cyclohexa-amylose, α-CD (diameter 4.5 Å, cavity height 6.7 Å) and cyclohepta-amylose, β-CD (diameter 7.0 Å, cavity height 7.0 Å) allow only one molecule to be associated with the hydrophobic cavity. The addition of β-CD to a dilute solution of the monomeric thionin dye (5.69×10^{-4}M) results in the spectral changes displayed in Figure 4. From the changes in the absorption spectrum of the dye upon addition of β-CD, the association constant (K_{ass}) of monomeric thionin to β-CD was estimated [equation (5)],[19] where TH^0 and CD^0 are the

$$\frac{TH^0 \,.\, CD^0}{\Delta A} = \frac{1}{K_{ass}\,.\,\Delta\varepsilon} + \frac{1}{\Delta\varepsilon}(TH^0 + CD^0) \qquad (5)$$

concentrations of thionin and cyclodextrin introduced into the system, ΔA is the absorbance difference upon addition of CD, and $\Delta\varepsilon$ the difference in the molar extinction coefficient of the associated and free dyes. The association constant of (3) to β-CD is $K_{ass} = 1.78 \times 10^3$M^{-1}. Similarly, we find that thionin binds to α-CD ($K_{ass} = 6.67 \times 10^2$M^{-1}). Binding of monomeric thionin to CD receptors is expected to affect the monomer–dimer equilibrium. The effect of addition of β-CD to an aqueous solution of thionin (8.1×10^{-4}M) is shown in Figure 5. At this concentration, dimeric thionin predominates without added β-CD ($A_{558}/A_{598} = 1.2$). Addition of β-CD results in a continuous decrease of the dimeric band accompanied by an increase in the monomeric dye form. These results are attributed to the association of monomeric thionin to the hydrophobic cavity of β-CD. Consequently the concentration of free thionin dye in solution decreases and the equilibrium is shifted toward the monomeric form [equation (6)]. Namely, association of thionin to β-cyclodextrin allows

$$TH_m{}^+ \cdots \beta\text{-CD} \rightleftharpoons TH_m{}^+ + \beta\text{-CD} \rightleftharpoons TH_d{}^{2+} \qquad (6)$$

the appearance of the dye in its monomeric form, despite its high concentration. Similar results are observed when α-CD is added to the thionin solution. However, owing to the lower association constant of thionin to α-CD as compared with β-CD, higher concentrations of α-CD are required to shift the dimer aggregate into the monomeric dye form. The fluorescence spectra of thionin are strongly affected by the addition of β-CD (Figure 6). It can be seen that addition of β-CD results in the increase in the fluorescence intensity at $\lambda = 623$ nm. This is attributed to the shift in the monomer–dimer equilibrium. As the dimeric thionin is non-fluorescent, the increase in the monomeric component upon addition of β-CD enhances the luminescence of the system.

These results demonstrate that the introduction of CD receptors to the thionin solution affects the monomer–dimer equilibrium. Association of the monomer dye is accompanied by de-aggregation of the dimeric form. Thus, the introduction of the cyclodextrins allows the protection of the monomer form even at high dye concentrations. As a result the preparation of highly fluorescent dye solutions is feasible.

Conclusions

The effects of two additives in controlling dye aggregation have been examined. Colloidal SiO_2 provides a negatively charged interface that induces the electrostatic aggregation of dyes. In turn, the cyclodextrins provide molecular hydrophobic cavities that induce the de-aggregation of dyes by selective association of the monomeric form. The effects of these environments in controlling the absorption and fluorescence properties of the dyes have been discussed. We believe that applications of colloid-aggregated dyes in photographic processes, photographic filter layers, and laser components are feasible. Similarly, the increase of photochemical conversion quantum yields in thionin–β-CD-sensitized photoelectrochemical cells is anticipated.

Acknowledgements

The support of the Stein Foundation (Drexel University, Philadelphia) for this co-operative research is gratefully acknowledged.

References

1 F. M. Hamer, ' The Cyanine Dyes and Related Compounds,' Wiley, New York, 1964.
2 F. P. Schafer, ed. ' Dye Lasers,' Spring-Verlag, Berlin, 1973.
3 (*a*) W. Arden and P. Fromherz, *Ber. Bunsenges. Phys. Chem.*, 1978, **82**, 868; (*b*) W. Arden and P. Fromherz, *J. Electrochem. Soc.*, 1980, **127**, 372; (*c*) O. Inacker, H. Kuhn, D. Mobius, and G. Debuch, *Z. Phys. Chem. (Frankfurt am Main)*, 1976, **101**, 337.
4 (*a*) M. Z. Hoffman and N. N. Lichtin, ' Solar Energy Chemical Conversion and Storage,' eds. R. R. Hautala, R. B. King, and C. Kutal, The Human Press Inc., New Jersey, pp. 153—187; (*b*) W. D. K. Clark and J. A. Eckert, *Sol. Energy.*, 1975, **17**, 147.
5 (*a*) W. J. Albery and A. W. Foulds, *J. Photochem.*, 1979, **10**, 41; (*b*) W. J. Albery and M. D. Archer, *Nature (London)*, 1977, **270**, 399; *J. Electroanal. Chem.*, 1978, **86**, 19.
6 (*a*) A. H. Herz, *Adv. Colloid Interface Sci.*, 1977, **8**, 237; (*b*) A. H. Herz, *Photogr. Sci. Eng.*, 1974, **18**, 323.
7 S. Herzfeld, M. Corrin, and W. Harkins, *J. Phys. Chem.*, 1950, **54**, 217.
8 C. Williamson and A. Corwin, *J. Colloid. Interface Sci.*, 1972, **38**, 567.
9 (*a*) F. Quadrifoglio and V. Crescenzi, *J. Colloid. Interface Sci.*, 1971, **35**, 447; (*b*) V. Vitagliano and L. Constantino, *J. Phys. Chem.*, 1976, **80**, 959.
10 R. K. Iller, ' The Chemistry of Silica,' Wiley, New York, 1979.
11 R. K. Iller, ' The Colloid Chemistry of Silica and Silicates,' Cornell University Press, Ithaca, NY, 1955.
12 C. Laane, I. Willner, J. W. Otvos, and M. Calvin, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 5829.
13 W. Saenger, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 344.
14 I. Tabushi, *Acc. Chem. Res.*, 1982, **15**, 66.
15 R. J. Bergeron, *J. Chem. Educ.*, 1977, **54**, 204.
16 R. Breslow, *Science*, 1982, **218**, 532.
17 M. Kasha, ' Physical Processes in Radiation Biology,' Academic Press, New York, 1964, p. 17.
18 (*a*) H. Bucher and H. Kuhn, *Chem. Phys. Lett.*, 1970, **6**, 183; (*b*) J. Maurus and G. Bird, *J. Phys. Chem.*, 1972, **76**, 2982.
19 F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Am. Chem. Soc.*, 1967, **89**, 14.

Received 16*th May* 1983; *Paper* 3/784