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Spectroscopic Effects of Organized Media on a Cyanine Dye/Pyrene Derivative

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Abstract. The absorption and fluorescence spectra of a cyanine dye/pyrene derivative are studied in the presence of γ-cyclodextrin, Brij 35, cetyltrimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS). Benesi–Hildebrand type equations are used to estimate the apparent association constant of the dye/cyclodextrin complex. In addition, an estimate of the dimerization constant for the dye is examined.

Key words. Fluorescence, cyanine dye, γ-cyclodextrin, surfactant.

1. Introduction

Cyanine dyes are useful as sensitizers in photographic processes, laser components, chemotherapy and as indirect indicators of electrical potential differences in cells and organelles [1–3]. These dyes are characterized by their increased fluorescence in low polarity solvents and an intense absorption at longer wavelengths [1, 3, 5]. Other features associated with these dyes are self-aggregation, solvatochromic effects and a high lipid/water partition coefficient [1, 5–7].

The self-aggregation of hydrophobic dyes such as thionine, methylene blue and acridine orange in aqueous solution at higher concentrations is well established [8, 9]. These aggregates result in changes in absorbance profiles and reduced fluorescence efficiency [8, 9]. In the case of cyanine dyes, fluorescence quenching has been reported when the dyes are in the aggregate state [6]. The formation of these aggregates is reported to be influenced by changes in solvent polarity [2]. For instance, aqueous alcoholic solutions have been shown to decrease the aggregation of thionine by forming relatively strong solvation complexes [8].

In the presence of organized media such as the cyclodextrin cavity and surfactants, deaggregation of dyes have been reported to occur [4, 11, 12]. Cyclodextrins (CDs) are torus shaped polysaccharides that possess a hydrophobic interior which includes molecules of appropriate size and polarity [13]. The CDs are available with varying inner diameters of 5.7, 7.8 and 9.5 Å for α-, β-, and γ-CD, respectively. Considerable attention has been given to CDs because of their use in areas of analytical separation and pharmaceutics [13]. In addition, the ability to alter the photophysical properties of a guest molecule upon complexation is also of significant interest.

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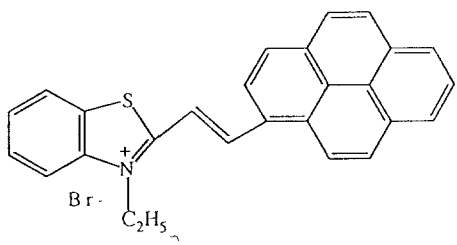
The use of CDs has been reported to increase the fluorescence efficiency of xanthene dyes [4]. Dan *et al.* [1] have reported on the enhanced fluorescence efficiency of cationic dyes such as methylene blue and pinacyanol chloride by use of CDs. The ability of CDs to enhance the fluorescence intensity of these dyes has been attributed to the selective inclusion of the monomeric form, thus shifting the equilibrium away from aggregate formation [4, 11]. Furthermore, West *et al.* [6] have found that the aggregation of cyanine dyes may result in fluorescence quenching. Therefore, the selective shift of the monomer–dimer equilibrium towards the monomer should result in enhanced fluorescence.

Surfactants have also been used to promote the deaggregation of dyes to their monomeric forms [10, 12, 14]. But in some instances, at certain concentrations, surfactants were found to enhance self-association of certain dyes. For example, the use of sodium dodecyl sulfate (SDS) has been known to induce aggregate formation of cationic dyes below the critical micelle concentrations (CMC). Above the CMC, cationic dyes are individually bound to micelles preventing the formation of dye aggregates [10].

In this paper, the effects of cyclodextrins and surfactants on a cyanine dye/pyrene derivative, 1-(1-pyrenyl)-2-(3-ethylbenzothiazolium) ethylene bromide (PBT) are examined. The structure of PBT is illustrated below.



1-(-1-Pyrenyl)-2(3-ethylbenzothiazolium) ethylene bromide.

The association constant (K_{dim}) for dimer formation of PBT in aqueous media is estimated using absorbance measurements. The apparent association constant (K_{ass}) for the monomeric PBT/CD complex is estimated by use of Benesi–Hildebrand plots of absorbance and fluorescence data. Changes in the vibronic band intensity ratio is also used to estimate K_{ass} for the complex. In addition, the effects of various micellar media on PBT fluorescence are evaluated.

2. Experimental

2.1. APPARATUS

Steady state fluorescence measurements were acquired with a SPEX Fluorolog model F2T21I fluorescence spectrophotometer equipped with a thermostated cell housing and a cooled photomultiplier tube. The emission spectra were taken at an excitation wavelength of 330 nm, using respective excitation and emission bandwidths of 5 and 3 nm. Absorbance measurements were obtained using a Cary 3 UV/vis spectrophotometer. All measurements were performed at 25 ± 0.1°C.

2.2. MATERIALS

The PBT was prepared as follows: the starting material was 3-ethyl-2-methylbenzothiazolium bromide, obtained by heating a solution of ethyl bromide and 2-methylbenzothiazole in dimethylformamide at 150°C for 12 h. Workup of this reaction mixture included cooling to 23°C and slow addition of diethyl ether to crystallize the product. The crystals were filtered, washed with diethyl ether and dried under reduced pressure to give analytically pure 3-ethyl-2-methylbenzothiazolium bromide, m.p. 241–243°C. A mixture of 1 g (3.8 mmol) of 1-pyrenecarboxaldehyde and 0.726 g (3.9 mmol) of dry triethylamine was refluxed in 5 mL of ethanol for 30 min. The reaction mixture was cooled and solvent removed under reduced pressure. The crude solid thus obtained was dissolved in dichloromethane, precipitated with ether and recrystallized from methanol. The m.p. of the analytically pure compound is 225–228°C, decomposing. The structure of the dye given above is fully consistent with the measured NMR spectrum ($CDCl_3$): δ 1.8 (*t*, 3H); 4.9 (*q*, 2H) and 6.9–8.7 (*m*, 15H).

Cyclodextrins were obtained from American Maize Products (Hammond, IN). Cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and polyoxyethylene-(23)-lauryl ether (Brij 35) were obtained from Sigma. All reagents were used as received.

2.3. METHOD

A stock solution of PBT was prepared in methanol at a concentration of 1.0×10^{-3} M and stored at 0°C. To prepare an aqueous PBT solution, a measured aliquot of the stock was transferred into a volumetric flask. The methanol was evaporated using dry nitrogen and the PBT residue was redissolved in deionized water. The final PBT solutions contained approximately 0.1% v/v methanol which was necessary to aid in dissolution. It should be noted that the influence of a small amount of methanol (up to 20% v/v) does not have any significant influence on the fluorescence spectra of PBT. The PBT concentration was 2.0×10^{-5} or 2.0×10^{-6} M, depending on the desired experiment.

2.3.1. *Determination of Self Association of the Dye*

A. 5.0×10^{-5} M solution of aqueous PBT was prepared as described above. A series of dilutions ranging from 5.0×10^{-6} to 5.0×10^{-5} M were made for absorbance measurements. The experiments were conducted using cells of different pathlengths and varying dye concentrations.

2.3.2. *Influence of Cyclodextrins*

To prepare PBT in aqueous CD solutions, a 5 mL aliquot was transferred into 10 mL flasks and a measured amount of aqueous CD solution was added to achieve the desired concentration. The flasks were filled to the mark with deionized water, yielding a final PBT concentration of 1.0×10^{-5} or 1.0×10^{-6} M. Cyclodextrin concentrations ranged from 5.0×10^{-4} to 1.0×10^{-3} M. The PBT/cyclodextrin solutions were allowed to stand overnight at room temperature prior to analysis.

2.3.3. *Influence of Surfactants*

To prepare PBT in aqueous surfactant solution, a 5 mL aliquot of the aqueous PBT was transferred into a 10 mL flask. A measured amount of surfactant solution was added to achieve the desired concentrations. The flasks were filled to the mark with deionized water to give approximately 1.0×10^{-6} M PBT in all cases. Surfactant concentrations ranged from 2.0×10^{-3} to 0.2 M for SDS, 1.0×10^{-4} to 5.0×10^{-3} M for CTAB, and 2.0×10^{-5} to 1.1×10^{-4} M for Brij 35. All aqueous PBT solutions were freshly prepared and protected from light whenever possible.

3. Results and Discussion

3.1. DETERMINATION OF SELF-ASSOCIATION

It is well established that many dyes aggregate in aqueous solutions. To better understand the influence of cyclodextrins and surfactants on PBT, the aggregation of PBT in aqueous solution was first evaluated. Figure 1 shows the absorbance spectra of PBT in aqueous solution. The absorbance spectra taken using different cells of varying pathlengths indicate the possible formation of PBT aggregates at higher concentrations. The formation of aggregates was investigated by assuming that PBT dimerizes in solution. Taking into account the formation of a dimer, the following expression could be written:

$$2C_m \rightleftharpoons C_d$$

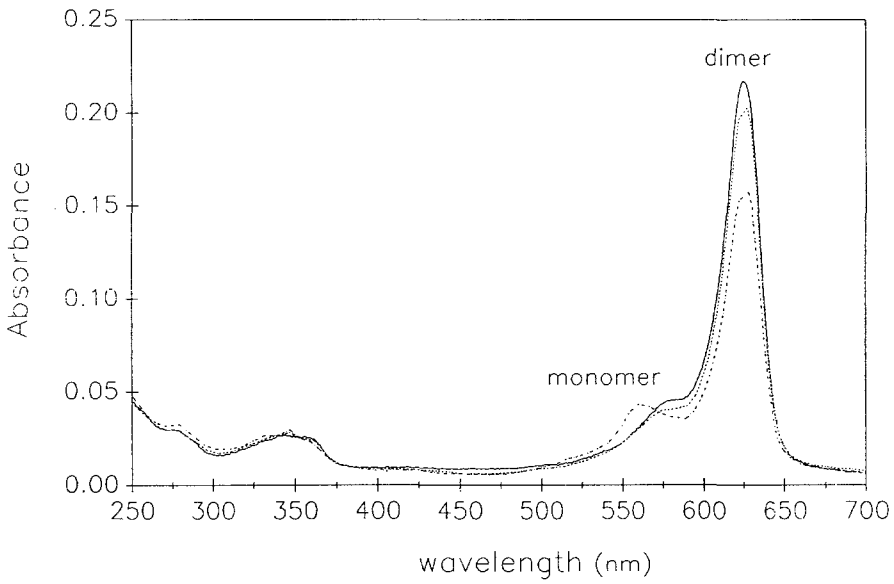


Fig. 1. Concentration dependence of the absorbance spectra of PBT in water: 1.0×10^{-4} M PBT, 0.1 cm path length (———); 5.0×10^{-5} M, 0.2 cm path length (— — —); 1.0×10^{-5} M, 1 cm pathlength (·—·—·—).

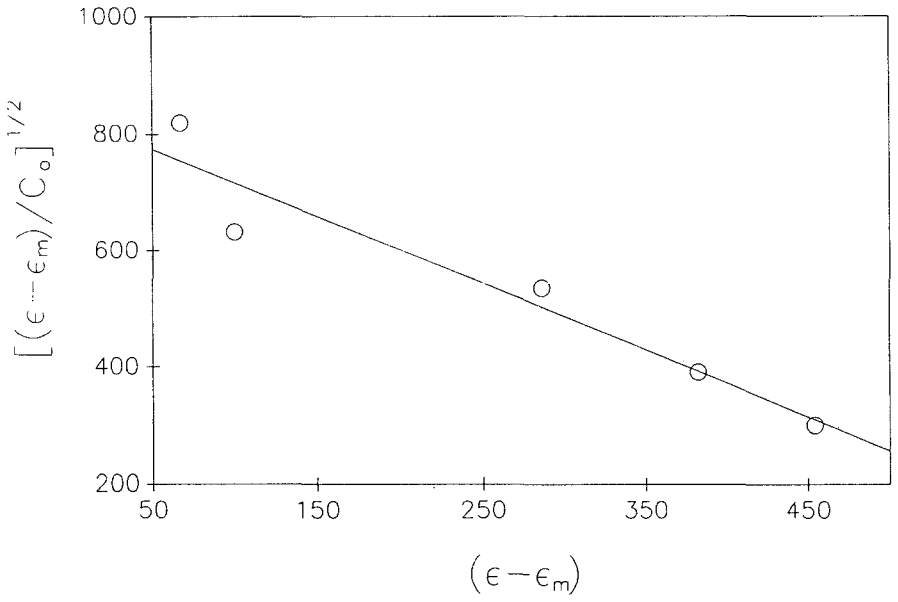


Fig. 2. Plot of $[(\epsilon - \epsilon_m)/C_o]^{1/2}$ vs. $(\epsilon - \epsilon_m)$ for PBT.

where C_m and C_d is the concentration of monomer and dimer, respectively. The dimerization constant (K_{dim}) is obtained from the following equation:

$$K_{dim} = C_d/C_m^2 \tag{1}$$

Taking into account the law of mass action, Equation (2) requires that

$$C_o = C_m + 2C_d \tag{2}$$

where C_o is the total concentration of the dye. Therefore, the fraction of PBT in the dimer form is expressed by the following equation:

$$C_d/C_o = (\epsilon - \epsilon_m)/2(\epsilon_d - \epsilon_m) \tag{3}$$

where ϵ_m and ϵ_d are the respective molar absorptivities for the pure monomer and the pure dimer. The measured analytical molar absorptivity is given by ϵ. By combining Equations 1, 2 and 3 and rearranging, we obtain Equation 4, from which we derive an estimate of K_{dim}.

$$[(\epsilon - \epsilon_m)/C_o]^{1/2} = -[2K_{dim}/\Delta\epsilon]^{1/2}\ (\epsilon - \epsilon_m) + [2K_{dim}\Delta\epsilon]^{1/2} \tag{4}$$

where $\Delta\epsilon = \epsilon_d - \epsilon_m$.

A plot of $[(\epsilon - \epsilon_m)/C_o]^{1/2}$ vs. $(\epsilon - \epsilon_m)$ should give a straight line if the molecule exists predominately as a dimer. Figure 2 shows the results of this study and a linear relationship reveals that at these concentrations, PBT exists as a dimer. The value for ϵ_m was estimated by extrapolations of ϵ to zero PBT concentration. This value was consistent with a value obtained from a PBT solution containing 0.1 M SDS (see discussion on surfactants). The dimerization constant (K_{dim}) for PBT in

aqueous solution is estimated to be $5.1 \times 10^4\ M^{-1}$. This value is considered to be a very conservative estimate since typical cyanine dyes adsorb strongly to glassware in aqueous solution [6].

3.2. INFLUENCE OF CYCLODEXTRIN

3.2.1. *Absorbance Measurements*

In the absence of CD, the absorbance contributed by the pyrenyl portion of the molecule occurs between 250 and 400 nm while the absorbance due to the dimer of the heterocyclic moiety is present at 620 nm (Figure. 1). Upon addition of cyclodextrin, a band appears at 560 nm. This band is attributed to the monomeric form of PBT. Increasing the concentration of CD promoted the increase of the monomer absorbance band while a subsequent decrease in the dimer absorbance band was observed. The increase in the monomer band is attributed to the deaggregation of the self-associated PBT molecules, and a subsequent incorporation of free PBT inside the CD cavity. This will in turn result in a decrease of the dimer band which is consistent with the observations of Dan *et al.* [11] and Politzer *et al.* [4] who reported a decrease in aggregation of dyes such as methylene blue, pinacyanol chloride, rhodamine 6G, rhodamine B and the disodium salt of fluorescein upon addition of CDs. This was rationalized in terms of a shift of monomer–dimer equilibrium towards the monomeric form upon addition of CD. The monomer–dimer equilibrium is represented by the following equation:

$$2(\text{PBT} \cdots \gamma - \text{CD}) \rightleftharpoons 2\,\text{PBT} + 2\,\gamma\text{–CD} \rightleftharpoons (\text{PBT})_2$$

Therefore, it could be inferred that the deaggregation in this study is a result of the intracavity inclusion of the monomeric PBT and γ–CD. The formation constant of monomeric PBT/γ–CD complex may then be estimated by use of the Benesi–Hildebrand equation. The apparent formation constant is determined from the following expression.

$$[\text{PBT}]_o[\text{CD}]_o/\Delta A = 1/K_f\Delta\epsilon + 1/\Delta\epsilon([\text{PBT}]_o + [\text{CD}]_o), \tag{5}$$

where ΔA is the absorbance difference of the monomer band upon addition of γ–CD; $\Delta\epsilon$ is the difference in the molar absorptivity of the complexed and free PBT. Figure 3 shows a linear plot of $1/\Delta A$ as a function of cyclodextrin concentration ($[\text{CD}]_o$) suggesting that the system is predominately 1:1. The apparent formation constant (K_f) for the monomeric PBT/complex was estimated to be $656\ M^{-1}$.

3.2.1. *Fluorescence Measurements*

The fluorescence spectrum of PBT consists of peaks appearing at 376 nm (band I), 396 nm (band II) and a broad structureless band around 475 nm which is due to the excimer formation of the pyrenyl group. The heterocyclic group fluoresces very little in water. Upon addition of γ–CD, the increasing fluorescence intensity at 580 nm is attributed to the heterocyclic group of PBT.

Figure 4 shows the changes in the fluorescence spectra upon increasing γ–CD concentration. In addition, increasing the γ–CD concentration results in an increase

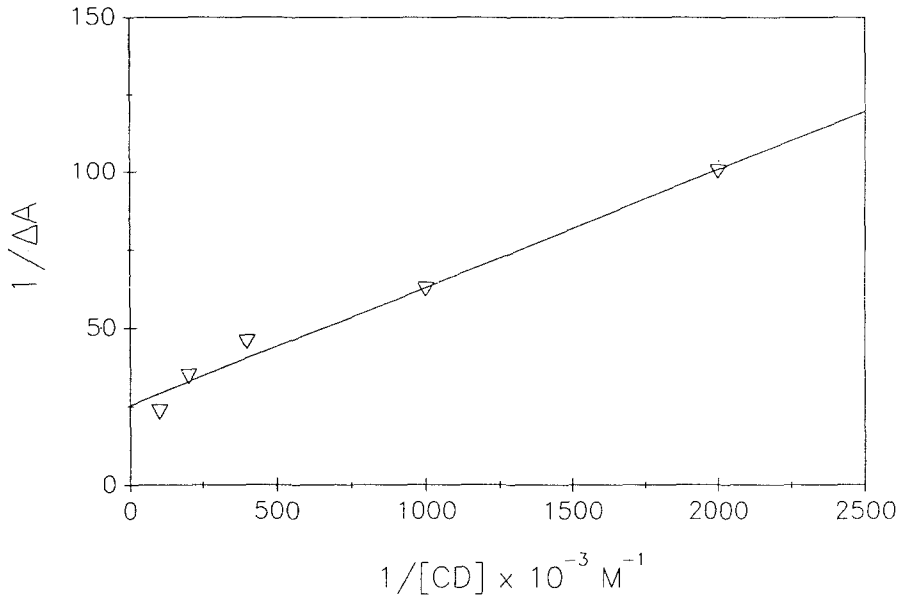


Fig. 3. Double reciprocal plot for the γ–CD : PBT complex.

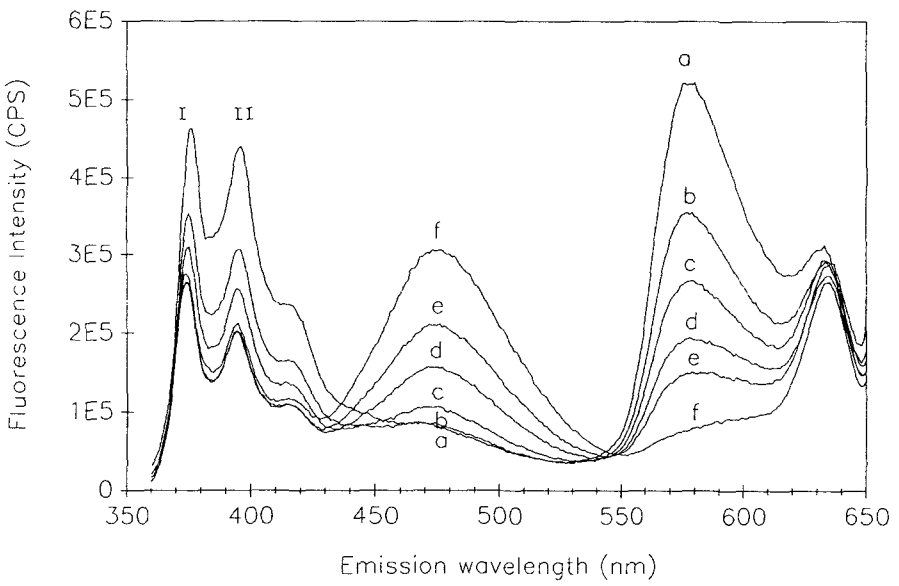


Fig. 4. The influence of varying concentrations of γ–CD on the fluorescence emission spectra of PBT: (a) 1.0×10^{-2} M; (b) 5.0×10^{-3} M; (c) 2.5×10^{-3} M; (d) 1.0×10^{-3} M; (e) 5.0×10^{-4} M; (f) 0 M.

in fluorescence intensity of bands I and II and a subsequent decrease of the broad structureless band at 475 nm. Along with other photophysical effects associated with cyclodextrin complexation, the increase in the fluorescence intensity of the 580 nm band is suggested to be primarily the result of increased fluorescence

efficiency as the equilibrium is shifted towards the monomeric form of the dye with increasing γ–CD concentration.

It is well established that solvent polarity influences the aggregation of certain dyes [2, 8]. For instance, Rabinowitch *et al.* [8] has shown that aqueous alcoholic solutions will decrease the aggregation of thionine by forming relatively strong solvation complexes. The results were an increase in the relative fluorescence intensity of thionine with increasing alcohol concentration. The same fluorescence spectral changes observed for PBT with increasing γ–CD concentration can be correlated with the changes observed with increasing methanol concentration. This, in turn suggests the formation of the monomer as the common denominator.

Assuming the fluorescence enhancement of the 580 nm band to be a function of the monomeric PBT/γ–CD complex, the apparent association constant, K_f can be estimated from a modified version of the Benesi–Hildebrand equation [15], i.e.

$$1/F\text{–}F_o = 1/K_f G Q_{PBT}[PBT]_o[CD]_o + 1/G Q_{PBT}[PBT]_o \tag{7}$$

where F_o and F represent the fluorescence signal of PBT in water and at a given CD concentration. The constant, G, is a characteristic of the fluorophore and instrumental parameters and Q_{PBT} is the quantum yield of the PBT monomer. The value of K_f (415 M^{-1}) was estimated from the linear plot of Equation 7.

The fluorescence characterized by the pyrenyl group of PBT was also used in determining K_f. Relative changes in the vibronic bands I and II were shown to be dependent upon solvent polarity. The use of vibronic band intensities in determining the apparent association constant is well established. For example, the vibronic band fine structure of pyrene exhibits a strong dependence on the changes in solvent polarity [16]. This characteristic change has been used as an indicator of the polarity of the microenvironment surrounding the pyrene molecule. In this study, a similar dependence of band I and II (I/II ratio) is shown to take place with increasing concentrations of γ–CD (Figure 5), suggesting a strong interaction between PBT and γ–CD. Wood [17] has observed that the addition of polysaccharides can enhance the fluorescence emission of certain dyes. This may suggest that the changes observed in the presence of γ–CD are not necessarily due to intracavity inclusion. In this study, an enhanced fluorescence intensity was observed upon adddition of α–CD to PBT while the I/II ratio remained relatively constant. The constant I/II ratios suggest no change in the microenvironment surrounding the PBT molecule, hence it is unlikely that PBT has entered the hydrophobic microenvironment of the α–CD cavity. Therefore, the changes in the I/II ratio of PBT in the presence of γ–CD provides the rationale that PBT is included in the γ–CD cavity.

The apparent formation constants of PBT/γ–CD complexes will be determined from the relative vibronic band ratios by employing a modified version of the Benesi–Hildebrand equation as described in Reference [15], i.e.

$$1/R_o - R = 1/K_f(R_o - R_1)[CD]_o + 1/R_o - R_1, \tag{8}$$

where R_o and R represent the measured I/II ratio of PBT in water and at a given CD concentration, R_1 denotes the ratio of PBT in the complexes. Assuming a 1 : 1 host–guest stoichiometry, a plot of $1/R_o - R$ vs. $[CD]_o$ should yield a straight line from which K_f of the PBT/CD complex was determined to be 529 M^{-1} (Figure 6).

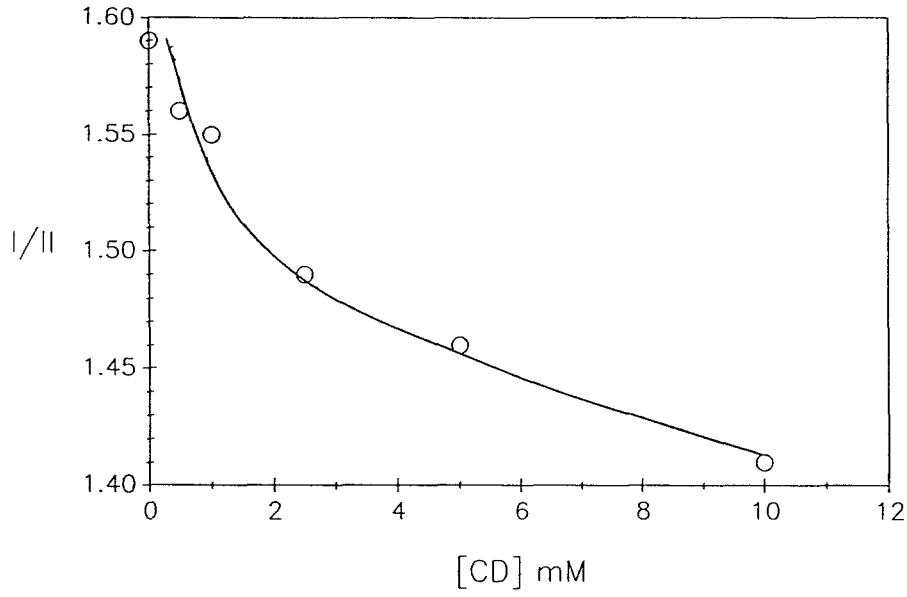


Fig. 5. Changes in the I/II vibronic band ratio of PBT with varying concentrations of γ-CD.

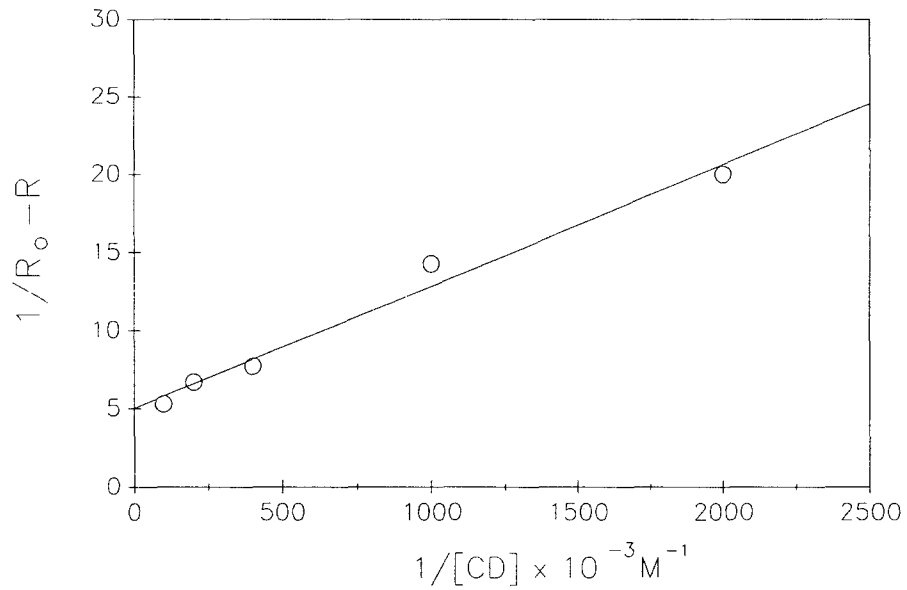


Fig. 6. Double reciprocal plot for the γ-CD : PBT complex calculated from the changes in I/II ratios.

The K_f obtained using both methods, from the I/II ratio and fluorescence intensity increase of the heterocyclic band are comparable. It should be noted that the changes in I/II ratios for PBT is concentration dependent. In this case, the PBT concentration was fixed at 1.0×10^{-6} M. At this concentration, no observed increase in the fluorescence intensity of the 580 nm band was observed upon increasing γ–CD concentrations. This is consistent with the observation of Politzer *et al.* [4] who reported that the inclusion complexes formed between dyes and CD is concentration dependent. These effects were rationalized in terms of structural differences of the dyes at low concentrations.

The broad structureless band appearing at 475 nm is attributed to excimer formation of the pyrenyl group of PBT. Increasing amounts of γ–CD resulted in a decrease of the excimer band, which suggests the separation of the excimer as γ–CD concentration is raised. This decrease was not observed with the addition of α–CD. The relative fluorescence intensity of the pyrenyl monomer (band II) was plotted as a function of the square of the excimer band. The linear relationship obtained suggests that the excimer is the result of the association of two pyrenyl groups.

To better understand the excimer/dimer relationship of the pyrenyl group, a plot of the excimer to monomer fluorescence intensity ratio versus excitation wavelength was constructed. The plot reveals a dependence of the excimer/monomer ratio on the excitation wavelength. Reynders *et al.* [18] have shown that an excimer/monomer ratio dependence on excitation wavelength is indicative of the presence of a ground state dimer. It is possible that the large dimerization constants, characteristic for this dye, enhances the formation of pyrenyl excimer.

3.3. PBT FLUORESCENCE IN MICELLAR MEDIA

Figure 7 shows a dramatic increase in the dye monomer fluorescence intensity at concentrations corresponding to a reported CMC value of 9.2×10^{-4} [19] for the

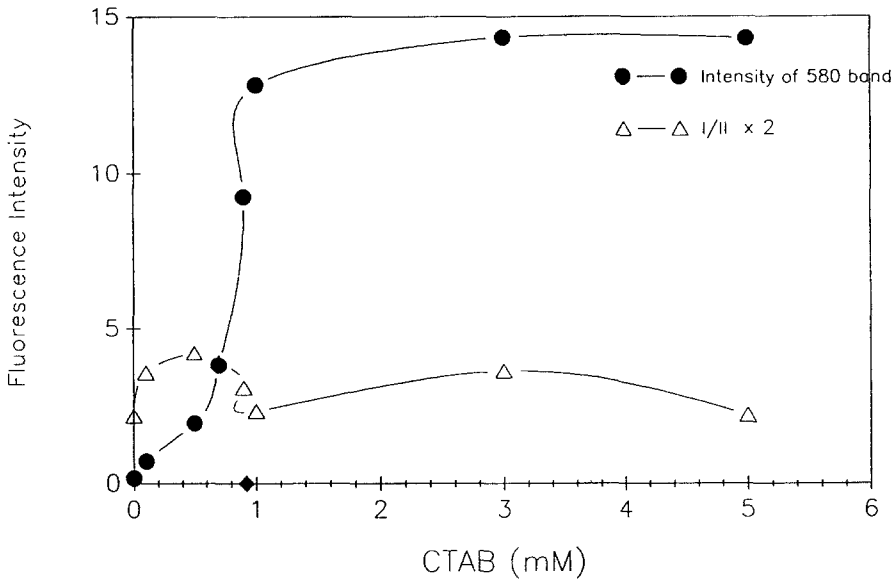


Fig. 7. The influence of CTAB on fluorescence intensity and the changes in the I/II ratios.

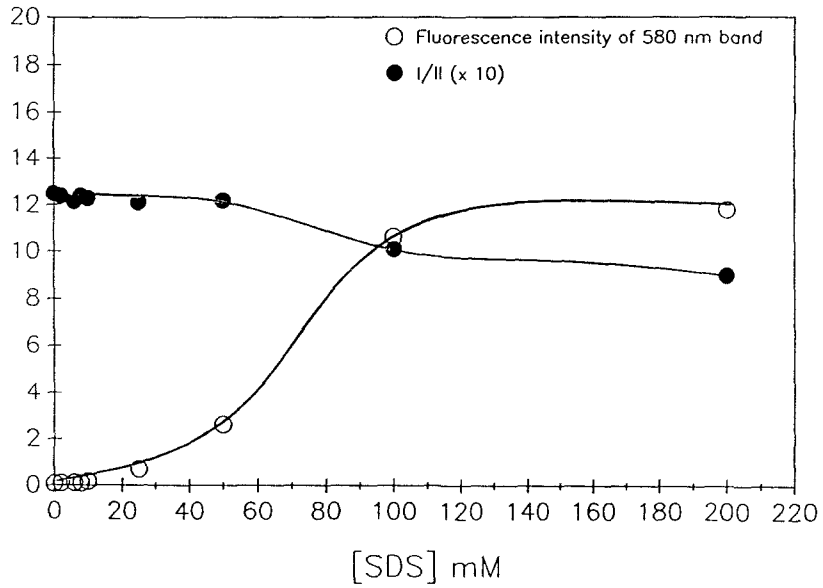


Fig. 8. The influence of SDS on fluorescence intensity and the changes in the I/II ratios.

cationic surfactant CTAB. The I/II values reveal a sharp increase and a subsequent decrease in the original value observed in water before the CMC was reached. The increase in the I/II values before the CMC, suggests a highly polar environment resulting from electrostatic repulsion of PBT and the cationic surfactant. As the surfactant begins to organize into a micellar structure, the I/II values decrease to their original values observed in water.

A gradual increase in the monomer fluorescence intensity was seen with increasing SDS concentrations between a reported CMC value of 8.1×10^{-3} M [19] and the maximum at 1.0×10^{-2} M (Figure 8). This result is in agreement with reports that at premicellar concentrations, SDS can induce the aggregation of cationic dyes by neutralizing the coulombic repulsion encountered between the charged dye molecules [10].

In contrast to the anionic surfactant SDS, the nonionic surfactant, Brij 35, is shown by Figure 9 to deaggregate the PBT dimer at concentrations well below the reported CMC of 6.0×10^{-5} [19]. The induced aggregation observed at premicellar SDS concentrations, is not evident with Brij 35. Also, there is little change in the I/II ratios with increasing Brij 35 concentration, suggesting that the aqueous microenvironment surrounding the pyrenyl group does not change appreciably.

The vibronic intensity ratios of pyrene have been successfully used in fluorescent probe studies of micelles. The changes in the vibronic band ratios was shown to correspond with microenvironmental changes as a result of the formation of micelles surrounding the pyrene molecule. These changes have been shown to correspond with the published CMCs for many surfactants [16]. Changes in I/II ratios for PBT do not correspond to reported CMCs for the surfactants analyzed. This observation is probably due to the ionic nature of the dye. It is noted that CMCs are affected by the type of fluorescent probe used in a particular micellar system [20].

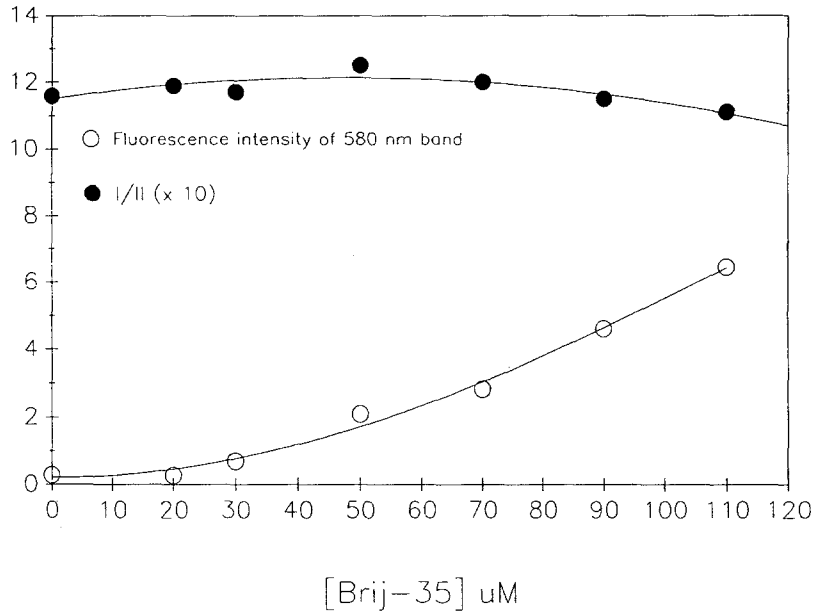


Fig. 9. The influence of Brij 35 on fluorescence intensity and the changes in the I/II ratios.

4. Conclusions

The PBT possesses several characteristics of both pyrene and cyanine dyes. For example, excimer formation and relative vibronic band intensity changes associated with microenvironment polarity are characteristics of PBT as well as pyrene. Aggregation, solvatochromatic effects and an intense absorbance at longer wavelengths are features which are also associated with cyanine dyes.

The monomer absorbance band is overshadowed by the large absorbance band of the PBT dimer at the concentrations used. An equation was derived using the changes in dimer absorbance to estimate the association constant.

Handa *et al.* have shown that dimerizing cationic dyes are useful in evaluating lipid membranes [21]. Also, fluorescence quenching resulting from dimerization can be used in evaluating respiring mitochondria [22]. Atik *et al.* have estimated the CMC for SDS and cetyltrimethylammonium chloride (CTAC) by observing the diffusion limited pyrene monomer/excimer reaction under intramicellar conditions [23]. The PBT may have potential applications in these areas.

Acknowledgements

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References

1. F. M. Hamer: *Cyanine Dyes and Related Compounds*, Wiley, N.Y. (1964).
2. H. Zollinger: *Color Chemistry*, VCH Publishers, N.Y. (1987).

3. D. L. Taylor: *Applications of Fluorescence in the Biomedical Sciences*, A. R. Liss, N.Y. (1986).
4. I. R. Politzer, K. T. Crago, T. Hampton, J. Joseph, J. H. Boyer, and M. Shah: *Chem. Phys. Lett.* **159**, 258 (1989).
5. A. Osman and M. S. K. Yousef: *Spectrochim. Acta* **37A**, 811 (1981).
6. W. West and S. Pearce: *J. Phys. Chem.* **69**, 1894 (1965).
7. P. J. Sims, A. S. Waggoner, C. Wang and J. F. Hoffman: *Biochem.* **13**, 3315 (1974).
8. E. Rabinowitch and L. F. Epstein: *J. Am. Chem. Soc.* **63**, 69 (1941).
9. L. Costantino, G. Gennaro, O. Ortona and V. Vitagliano: *J. Chem. Eng. Data* **29**, 62 (1984).
10. M. G. Neumann and M. H. Gehlen: *J. Colloid Interface Sci.* **135**, 209 (1990).
11. P. Dan, I. Willner, N. S. Dixit and R. A. Mackay: *J. Chem. Perkin Trans. 2* 455 (1984).
12. A. Yamagishi: *J. Colloid Interface Sci.* **86**, 468 (1982).
13. J. Szejtli: *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiado, Budapest (1982).
14. M. E. Diaz Garcia and A. Sanz-Medel: *Talanta* **33**, 255.
15. A. M. Munoz de la Pena, T. Ndou, J. B. Zung and I. M. Warner: *J. Phys. Chem.* **95**, 3330 (1991).
16. K. Kalyanasundaran and J. K. Thomas: *J. Am. Chem. Soc.* **99**, 2039 (1977).
17. P. J. Wood: *Carbohydr. Res.* **85**, 271 (1980).
18. P. Reynders, W. Kuhnle and K. A. Zacharlasse: *J. Am. Chem. Soc.* **112**, 3929 (1990).
19. J. H. Fendler and E. J. Fendler: *Catalysis in Micellar and Molecular Systems*, Academic Press (1975).
20. M. Rosen: *Surfactants and Interfacial Phenomena* (2nd ed.), John Wiley and Sons, N.Y. (1989).
21. T. Handa, I. Chikako, I. Yamamoto and M. Nakagaki: *Bull. Chem. Soc. Jpn.* **56**, 2548 (1983).
22. Bunting *et al.*: *Biophys. J.* **56**, 981 (1989).
23. S. S. Atik, M. Nam and L. A. Singer: *Chem. Phys. Lett.* **62**, 75 (1979).