Multiple Equilibria of Phenothiazine Dyes in Aqueous Cyclodextrin Solutions†

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The dimerization and inclusion complexation equilibria of six phenothiazine (PN) dyes with cyclodextrins (α-, *β*-, and *γ*-CDs) in aqueous media have been studied using absorption and fluorescence spectroscopy. The PN dyes used in this study are thionine (TH), azure A (AZA), methylene blue (MB), toluidine blue (TB), new methylene blue (NMB), and 1,9-dimethylmethylene blue (DMMB). The dimerization constants (K_D) of the dyes having two methyl substituents at the phenothiazine ring (NMB and DMMB) are much greater than those of other dyes having unsubstituted rings, and the presence of methyl groups on the amine groups affects little the K_D values. The positions of the monomer/dimer equilibria do not change with the presence of α-CD, while the addition of *β*-CD suppresses and *γ*-CD enhances the dimerization of the dyes except DMMB. The equilibrium constants for the inclusion complexation of the dye monomers and dimers with CDs are determined from the analysis of the dependence of the absorption spectra of the dye solutions on the concentrations of the CDs using a multiple-equilibrium scheme. The results indicated that, except DMMB, which has methyl groups at the 1-position of the fused phenothiazine ring, the dye monomers fit better to *β*-CD and the dimers fit snugly to *γ*-CD. The DMMB monomer is too large to fit in *β*-CD but forms stable complexes with *γ*-CD. It appears that the inclusion complexes of the dye monomers and dimers are formed by deep insertion of the phenothiazine rings into the cavities of the CDs, with the 2-methyl groups (in TB and NMB) and the amine groups protruding from the cavities of the CDs. Fluorescence spectroscopic studies indicate that the dye dimers are not fluorescent and inclusion of the monomer in *β*-CD results in a 3−5 times enhancement of fluorescence intensity. The determined equilibrium constants of the multiple-equilibrium scheme of the dyes in CD media and fluorescent properties of the dyes can be used to control the dye aggregation and the photophysical and photochemical properties of the phenothiazine dyes for various applications.

Introduction

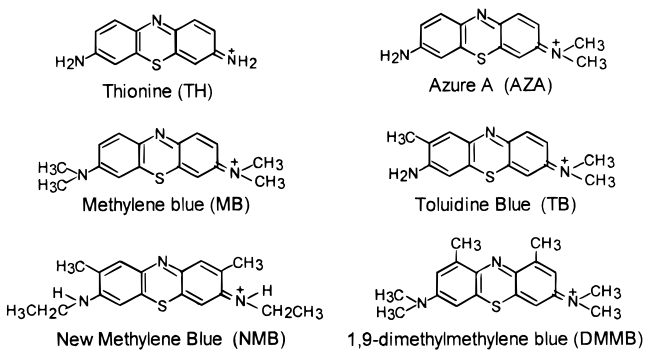
A wide variety of applications of the phenothiazine (PN) dyes have been reported, for example, as sensitizers in solar energy conversion,[1,2] redox mediators in catalytic oxidation reactions,[3] active species in electrochromism[4] and dye lasers,[5] ingredients in pharmaceutical preparation,[6] and candidates for cancer therapy by intercalating between DNA base layers.[7] All of these applications including the redox chemistry of PN dyes are, however, often complicated by the dimerization of dye molecules in aqueous media:[8−10]

$$2M \rightleftharpoons D \qquad K_D = [D]/[M]^2 \tag{1}$$

where K_D is the equilibrium constant for the monomer (M)/dimer (D) equilibrium. There are several reports for K_D values of PN dyes, but considerable differences are found between them presumably due to the ambiguity of the UV−vis spectroscopic parameters and the complications arising from adsorption of the PN dyes on glassware.[8−10]

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six, seven, or eight glucose units and called α-, *β*-, or *γ*-CD, respectively.[11] CDs have hydrophobic cavities and are capable of forming inclusion complexes by admitting the guest molecules into their cavities. This property can be used to control the position of monomer/dimer equilibria and aggregation behavior of dyes by CDs, affecting electronic absorption and fluorescence spectra[12−15] and photochemical[12−16] and electrochemical[17] properties of dyes. It has been reported that dye monomer−CD complexes predominate for some selected PN (methylene blue and thionine)[12,13,17] and structurally related oxazine[14] dyes with *β*-CD, whereas the dye dimers are preferentially included in the cavity of *γ*-CD.[13,17] This clearly shows the size selectivity of CDs. The dimerization behavior of PN dyes is also controlled by varying the substituents of phenothiazine. The variation is expected to result in changes of stability and stoichiometry of dye−CD complexes.

In this study, we describe quantitative analysis of the monomer/dimer and dye/CD complexation equilibria of six PN dyes by absorption and fluorescence spectroscopy.



These dyes were selected due to their difference in position

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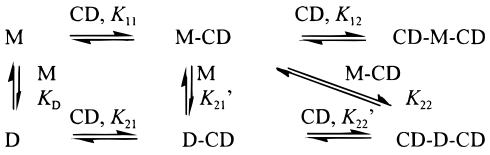
SCHEME 1



and number of methyl substituents on the same phenothiazine skeleton. They could provide useful information on the factors governing dimerization of the dyes and inclusion complexation with CDs. The UV−vis spectroscopic parameters and fluorescence characteristics of the monomers and dimers of the dyes as well as K_D values were deduced. The association constants of the monomer and dimer with β- and γ-CDs were obtained by fitting the dependence of the apparent dimerization constants (K_D') on the concentration of CDs to the multiple equilibrium system shown in Scheme 1.

Experimental Section

The PN dyes were obtained from Aldrich and recrystallized from $CH_2Cl_2-CH_3OH$ before use.[2] α- and β-CDs were obtained from Aldrich, and γ-CD was purchased from Tokyo Kasei. Solutions were prepared from reverse-osmosed water that was further purified by passage through a Millipore purification train. All solutions contained 0.01 M HCl to minimize the adsorption of dyes,[8] and a constant ionic strength was maintained with 0.1 M KCl. The concentrations of CD solutions were calculated from the reported optical rotation data.[11]

Absorption spectra were measured using a Hewlett-Packard model 8452A or GBC model 920 spectrophotometer equipped with a thermostated cell holder. The temperature was maintained at 25 °C unless otherwise specified. Three quartz cells with light path lengths (b) of 0.20, 1.0, and 10.0 cm were used to maintain the absorbances below 1. Fluorescence spectra were taken with a Hitachi model F3010 spectrofluorometer at 25 °C.

Results and Discussion

We first describe characteristics of absorption spectra of monomers and dimers of the PN dyes and determination of K_D values of the PN dyes from absorption spectra. We then present the effects of CDs on the absorption spectra of the dyes and association constants of the dye monomers and dimers with CDs. Finally, we report the effects of CDs on fluorescence spectra of the dyes and fluorescence characteristics of the dye−CD complexes.

Absorption Spectral Characteristics of the Dye Monomers and Dimers and Determination of K_D values. The absorption spectra of PN dyes are highly dependent on the concentration of the dyes, as exemplified in Figure 1 for MB. This is due to the monomer/dimer equilibria of the dyes.[8−10,13] Figure 1 clearly shows the MB monomer (664 nm) and dimer (604 nm). The normalization of spectra gave an isosbestic point (λ_i) of the MB monomer/dimer equilibrium at 620 nm with a molar absorptivity (ϵ_i) of 3.6×10^4 M^{-1} cm^{-1}. The isosbestic point was also observed with temperature dependence of the spectra at a given dye concentration (not shown).

We used the concentration-dependent spectra to deduce the spectral characteristics of the dye monomer and dimers and to determine K_D values. The spectra of the mixtures of M and D as in Figure 1 can be decomposed into individual monomer and dimer components by the linear combination of the respective spectra.[15,18,19] For this, it is necessary to have either a monomer or dimer spectrum. We used the spectrum of 1.0×10^{-6} M

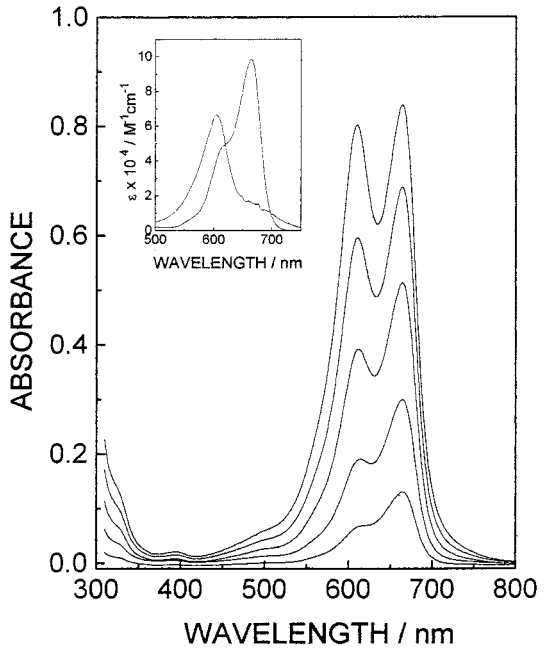


Figure 1. Absorption spectra of 1.0×10^{-5}, 2.6×10^{-5}, 5.2×10^{-5}, 7.7×10^{-5}, and 1.0×10^{-4} M MB solutions (from bottom to top) containing 0.1 M KCl and 0.01 M HCl (light path length 1.0 cm). The inset shows the resolved monomer and dimer spectra of MB.

(for MB, AZA, TH, and TB) or 2.0×10^{-7} M solutions (for NMB and DMMB) at 40 °C as the pure monomer spectrum of the dye: the spectrum showed no appreciable temperature dependence above 30 °C. Since the spectrum for higher dye concentration exhibits a larger fraction of monomer than that for lower dye concentration, the dimer spectrum can be obtained by subtracting the first spectrum (multiplied by an appropriate factor) from other spectrum. The multiplication factor is adjusted until the resulting spectrum does not show any hints of the contribution from monomer absorption near the absorption maximum of monomer. Then the monomer component in a monomer−dimer mixture spectrum is obtained by subtracting the dimer spectrum generated (multiplied by an adjustable factor) until the resolved monomer spectrum is indistinguishable from that of the pure monomer spectrum. The dimer component in the mixture spectrum is then obtained by subtracting the monomer component. The resolved monomer and dimer spectra of MB generated by this method are shown in the inset of Figure 1. Using ϵ_i values at λ_i, the concentrations of monomer (C_M) and dimer (C_D) are calculated. For C_D, we used the molar absorptivity of the dimer at λ_i as twice ϵ_i since the isosbestic point is that of the monomer/dimer equilibrium. The spectroscopic parameters are determined from the resolved spectra using the concentrations. Since we have the spectroscopic parameters of the monomers and dimers, the concentrations of the monomer and dimer in a mixture are calculated from the observed absorption spectra using the spectroscopic parameters of the monomers and dimers by solving simultaneous equations relating absorbance and concentration:

$$A_\lambda = \epsilon_{M,\lambda} b C_M + \epsilon_{D,\lambda} b C_D \qquad (2)$$

The dimerization constants K_D were calculated at each dye concentration and averaged. The same procedures were applied to obtain the spectroscopic parameters of other PN dyes. The results are summarized in Table 1. The K_D values are in the order TH < MB < AZA ≤ TB < NMB < DMMB. Apparently, the K_D values increase sensitively with the presence of methyl

TABLE 1: Spectroscopic Parameters of Monomers (M) and Dimers (D) and Dimerization Constants (K_D) of Phenothiazine Dyes in Acidic Aqueous Solutions at 25 °C[a]

	in the absence of CDs				isosbestic point for M/M−β-CD		
dye	$10^{-4}\epsilon_i$ (λ, nm)	$10^{-4}\epsilon_M$ (λ, nm)[b]	$10^{-4}\epsilon_D$ (λ, nm)[b]	$10^{-3}K_D$ M[c]	λ, nm	$10^{-4}\epsilon_M$	$10^{-4}\epsilon_D$
TH	3.7 (566)	6.1 (598) 3.3 (559)	2.5 (598) 8.0 (559)	3.2 ± 0.1	598	6.1	2.5
AZA	3.7 (598)	5.6 (634) 2.8 (586)	3.1 (634) 7.9 (586)	16 ± 0.8	630	5.5	3.3
MB	3.6 (620)	7.2 (664) 2.8 (604)	2.5 (664) 9.7 (604)	9.6 ± 1.1	664	7.2	2.4
TB	3.4 (596)	4.8 (634) 2.5 (582)	1.9 (634) 8.5(582)	18 ± 2.1	622	4.5	2.6
NMB	4.3 (594)	8.3 (632) 3.7 (586)	2.8 (632) 9.5 (586)	110 ± 12	624	7.9	3.2
DMMB	3.1 (600)	6.0 (652) 2.5 (590)	1.8 (652) 7.0 (590)	160 ± 30	648[d]	5.8[d]	1.8[d]

[a] The last three columns show the parameters at the isosbestic points for M/M−β-CD equilibria. [b] The longer wavelength is the absorption maximum of monomer, and the shorter wavelength is that of dimer. [c] Average ± SD for data taken at ≥4 different concentrations. [d] For the M/M−γ-CD equilibrium.

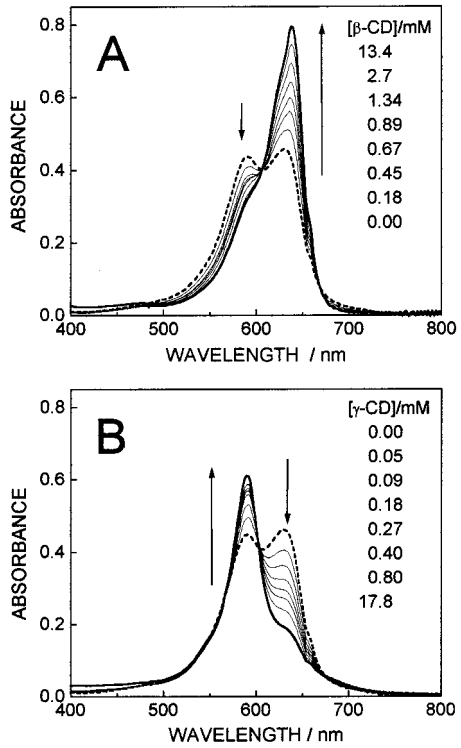


Figure 2. Effects of β-CD (A) and γ-CD (B) on absorption spectra of a 1.0×10^{-5} M NMB solution containing 0.1 M KCl and 0.01 M HCl (light path length 1.0 cm). Arrows point the direction of increasing concentration of CDs.

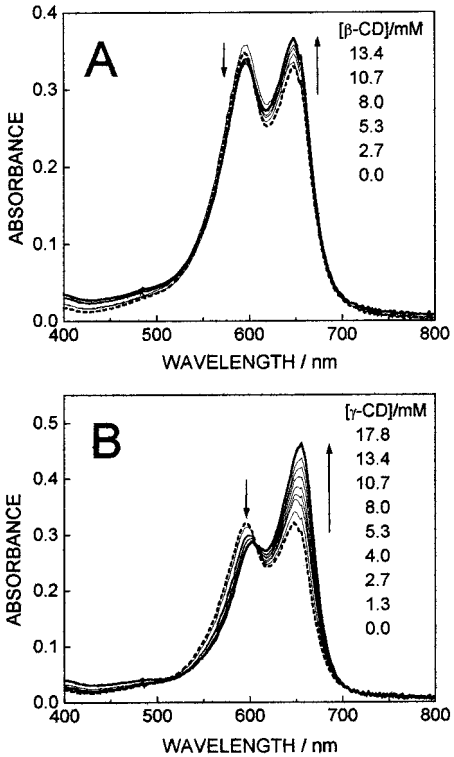


Figure 3. Effects of β-CD (A) and γ-CD (B) on absorption spectra of 1.0×10^{-5} M DMMB. Other conditions are the same as in Figure 2.

substituents on the phenothiazine skeleton, but the methyl groups on amine groups affect little the K_D values. This implies that the hydrophobic interaction between the phenothiazine fused rings contributes mainly to the stability of the dimer.[14]

Inclusion Complexation of PN Dyes with CDs. Figures 2 and 3 show the effects of β- and γ-CDs on the spectra of NMB and DMMB solutions, respectively. Such spectral changes in PN dye solutions were not observed upon addition of α-CD, presumably because the PN dyes are too large to fit inside the α-CD cavity. This was confirmed by CPK models. A large increase in the NMB monomer peak (632 nm) with a concomitant decrease in the NMB dimer peak (586 nm) is observed with increasing concentration of β-CD (Figure 2A), whereas the opposite tendency is observed with γ-CD (Figure 2B). This clearly indicates suppression of the dimer formation by inclusion of the NMB monomer in the β-CD cavity preferentially, while the NMB dimer is included in the cavity of γ-CD, stabilizing the dimer. The destabilization and stabilization of the dimers by the addition of β-CD and γ-CD, respectively, were also observed with TH, AZA, MB, and TB. However, the effects of CDs on DMMB (Figure 3) are quite different from those on other PN dyes. For DMMB, γ-CD as well as β-CD destabilizes the dimer and the destabilization effect of β-CD is much less pronounced than that of the other dyes. Generally, proper size matching between the host cavity and guest leads to a substantially larger association constant. Unlike the other PN dyes, the DMMB monomer is better fitted to the cavity of γ-CD, and the dimer is too large to be included selectively in the γ-CD as well as in the β-CD cavity.

The monomer/dimer equilibria of dyes in the presence of CDs can be represented by Scheme 1. The apparent dimerization constant (K_D') in the presence of CD is defined in eq 3.

K_D' is related to the microscopic equilibrium constants in Scheme 1 and [CD] by eq 4.

$$K_D' = \frac{[D] + [D-CD] + [\{D - CD\}_2]}{([M] + [M - CD] + [CD-M-CD])^2} \quad (3)$$

$$K_D' = \frac{K_D + K_{21}K_D[CD] + K_{11}^2K_{22}[CD]^2}{(1 + K_{11}[CD](1 + K_{12}[CD]))^2} \quad (4)$$

To evaluate K_D' values, we first checked the spectral difference between M and the M−β-CD complex. Small but noticeable changes in spectra of the dye solutions of low concentration, which give virtually pure monomer spectra, were observed upon the addition of β-CD up to 10 mM presumably due to the change in the environment of MB more hydrophobic upon binding with β-CD: for DMMB, we used γ-CD instead of β-CD. Since the monomer is the only species present in the solutions, the observed isosbestic point is regarded as that between M and the M−CD complex. The isosbestic points for the monomer and its CD complex equilibria and the molar absorptivities of the monomeric and dimeric species at the isosbestic points are included in Table 1. We assume that the molar absorptivity of CD−M−CD is the same as that of M−CD. In agreement with a report on oxazine dye,[14] we could not observe any noticeable changes of the resolved dimer spectra upon CD complexation. From the resolved monomer and dimer spectra in the presence of CDs, we calculated the concentrations of total monomeric and dimeric species of the dyes by using molar absorptivities at isosbestic points between M and M−CD. The K_D' values were calculated from eq 3 and are shown in Figure 4 as functions of [β-CD] and [γ-CD].

The thermodynamic parameters in Scheme 1 except K_{21}' and K_{22}' are determined from the dependence of K_D' on [CD] by nonlinear least-squares fitting of the data to eq 4. For this analysis, we used K_D values (in Table 1) as a fixed parameter and the other equilibrium constants as fitting parameters. From the cyclic loop in Scheme 1, K_DK_{21} is equal to $K_{11}K_{21}'$. Since K_D, K_{21}, and K_{11} have been determined from the regression analysis, the K_{21}' values are calculated from the relationship. Then the K_{22}' values are calculated from the relationship K_{21}' $K_{22}' = K_{22}$. The equilibrium constants obtained are listed in Table 2. For all dyes listed in Table 1, the K_{11} values, which are the association constants of dye monomer−CD complexes with a second CD molecule, were calculated to be less than 5 M^{-1}. This agrees with a report of the corresponding association constant 2.6 M^{-1} for an oxazine−γ-CD system.[14] Similarly, the association constant of dimer−CD complexes with β-CD were calculated to be less than 7 M^{-1}.

For the PN dyes which do not have substituents on the phenothiazine fused ring system (TH, AZA, and MB), the K_{11} and K_{21} values depend little on the presence of methyl substituents on the amine groups. This indicates that the fused rings are certainly included in the cavities of CDs and that the amine groups protrude from the CD cavity in the dye monomer and dimer−CD complexes.[20] Table 1 shows that the stability of β-CD complexes of the dye monomers is about 2 orders of magnitude greater than that of the dye dimers. In contrast to this, the dimers of the dyes make much more stable complexes with γ-CD than the monomer. These observations clearly suggest that dye monomers fit snugly into β-CD but rattle around inside the γ-CD cavity, while the dimers are better fitted to γ-CD but too large to be fitted snugly into β-CD. Another dye molecule is easily accommodated in the monomer−γ-CD complex, but it is not in monomer−β-CD complexes. This can explain the large difference in K_{21}' values between β-CD and γ-CD.

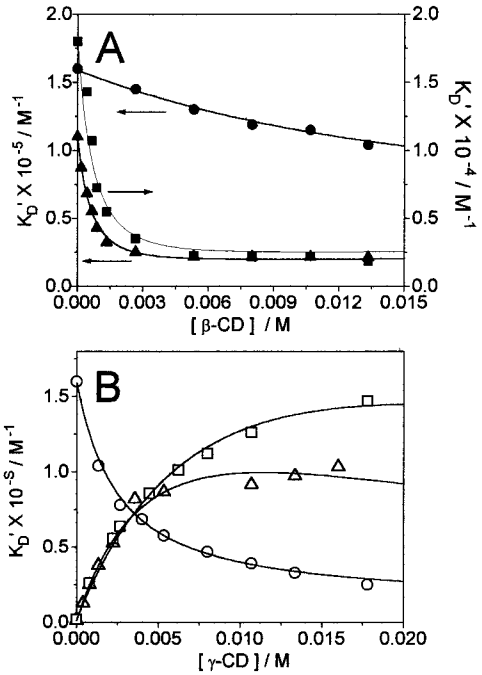


Figure 4. Plot of the apparent dimerization constants (K_D') of TB (■, □), NMB (▲, △), and DMMB (●, ○) against initial concentrations of β-CD (A) and γ-CD (B). Solid lines are fitted to eq 4 to give the equilibrium constants listed in Table 2. (*s* values in the ordinate of part B are 6, 7, and 5 for TB, NMB, and DMMB, respectively.)

Attachment of methyl groups to the 2-positions of the fused aromatic rings TH and NMB results in only a minor increase in K_{11} and K_{21} values with CDs. This finding supports our conclusion of deep penetration of the fused ring into the cavity: the 2-methyl groups also protrude from the CD cavity and interact with rims of CDs, giving minor increases in the stability of the CD complexes. However, the presence of a 1-methyl group on the fused ring (DMMB) seems to make the molecule too bulky to penetrate β-CD but provide for the better fit to γ-CD. The dimension of the unsubstituted phenothiazine ring is estimated as 0.67 (width) × 1.28 (length) × 0.32 (thickness) nm from a CPK model. The presence of a 1-methyl group on the ring increases the width to 0.76 nm. The cavity diameters of β-CD and γ-CD are ∼0.7 and ∼0.85 nm, respectively, and the depths of the cavities are about 0.7 nm for both CDs.[11] These dimensions justify aforementioned selectivities of CDs for binding of monomers and dimers of the PN dyes.

Formation of the 1:2 dimer−CD complexes, for which the equilibrium constant is K_{22}, is not considered by other investigators for oxazine−CD[14] and phenothiazine−β-CD complexation.[17] However, we found that the spectral data are poorly fitted to Scheme 1 without consideration of complex formation. Since the depth of the CD cavity is much shorter than the length of the dyes, the formation of 1:2 complexes is structurally feasible. The present work suggests that the monomer−$(CD)_2$ complexes are very weakly stable and the dimer−$(\beta\text{-CD})_2$ complexes have moderate stability.

Using the equilibrium constants in Table 1, we can calculate the fraction of monomeric species of the PN dyes as a function of CD concentrations. Figure 5 shows the results obtained at 1.0×10^{-5} M total dye concentration. By properly choosing the type of PN dye, CD, and CD concentration, we can have virtually any fraction of dye monomer. This may provide a convenient means for designing PN dye solution systems with CDs for applications such as in lasers.

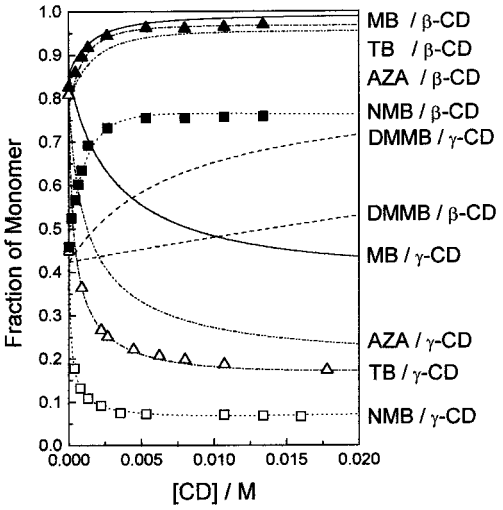


Figure 5. Dependence of the fraction of monomeric species of PN dyes on the concentrations of CDs at the formal concentration of 1.0×10^{-5} M PN dyes. The lines are calculated fractions from equilibrium constant data in Table 2, and symbols represent experimental data. Other experimental data points are omitted to avoid complexity.

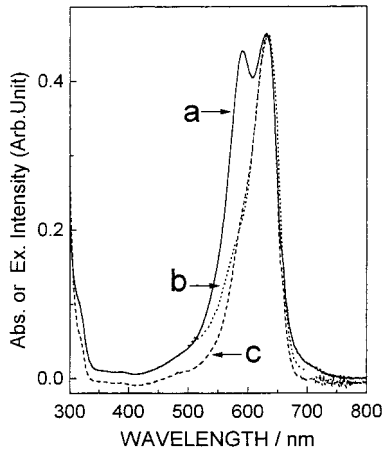


Figure 6. Absorption (a) and excitation spectra (uncorrected) (b) of 1.0×10^{-5} M NMB and the resolved absorption spectrum of NMB monomer (c). The spectra are normalized to give the same value at 632 nm.

Effects of CDs on Fluorescence Properties of Phenothiazine Dyes. Figure 6 compares absorption and excitation spectra of NMB. The excitation spectrum exhibits little response at the wavelength region of dimer absorption ($\lambda = 586$ nm) and resembles that of the resolved monomer spectrum of the dye. Other dyes showed similar behavior. This suggests strongly that the monomers of the PN dyes are fluorescent while the dimers are virtually nonfluorescent.[12,14] To obtain further evidence for this, we excited the NMB solutions in the concentration range $(5-40) \times 10^{-7}$ M at the isosbestic point of the monomer/dimer equilibrium (594 nm) and absorption maximum (632 nm) of the dye. No appreciable changes in spectral shape were observed with the concentration change. Also, the intensity ratio of the fluorescence spectra taken at two different excitation wavelengths was independent of concentrations and was about 2, which accords with the ratio of molar absorptivities of the NMB monomer at the exciting wavelengths. The plot of emission intensity (after correcting the innerfilter effect[21]) vs monomer concentration calculated by using the K_D values in Table 1 gave good linearity (plot is not shown). This is unequivocal evidence that the emitting species is a monomer and the dimer is nonfluorescent. The nonfluorescent nature of these PN dye dimers is probably due to self-quenching of dimers,[22] as the transition from lowest excited singlet state to the ground state is forbidden.[14]

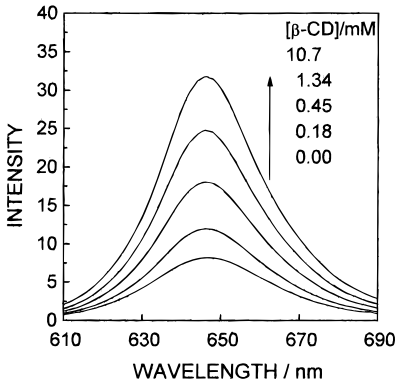


Figure 7. Fluorescence spectrum of 1.0×10^{-6} M NMB solutions at various concentrations of β-CD at $\lambda_{ex} = 594$ nm.

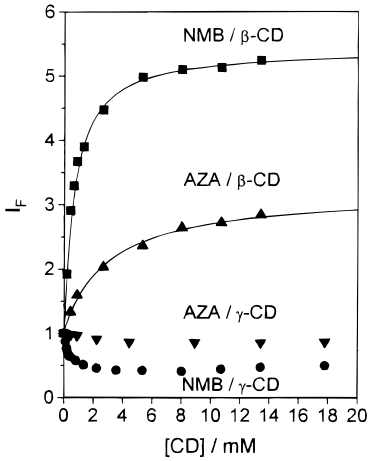


Figure 8. Plot of the fluorescence intensity against the concentration of CDs at the formal concentration of 1.0×10^{-6} M NMB and AZA. Symbols are from experimental data, and solid lines are fitted to eq 6 for β-CD media. Excitation wavelengths are the isosbestic points of monomer/monomer$-\beta$-CD equilibria.

Addition of CDs to PN dye solutions results in large changes of fluorescence intensity. We present this in Figure 7 for the effects of β-CD on the fluorescence spectra of NMB and in Figure 8 for the dependence of the fluorescence intensities of AZA and NMB on the concentrations of β-CD and γ-CD. The effects of CDs on the fluorescence intensities can be attributed at least in part to the change in the fraction of the fluorescent dye monomer due to CDs: β-CD increases the fractions of AZA and NMB monomers (Figure 5), and the fluorescence intensity of the dyes is enhanced by β-CD, while γ-CD shows the opposite effect. However, this alone cannot explain the dependence of the fluorescence intensity of PN dyes on concentrations of CDs. For example, the fraction of NMB monomer in 1.0×10^{-6} M solution is about 0.84 and the molar absorptivities of the NMB monomer and its β-CD complex are insignificantly different, but the fluorescence intensity of the dye increases more than 4 times with β-CD. The fluorescence quantum yields of many fluorescent dyes are very sensitive to the environment, and a striking enhancement is observed upon transferring the dye molecule to a nonpolar or rigid environment from aqueous solution.[21] The dyes bound to CDs experience nonpolar and rigid environments, and the fluorescence intensities of the dyes are greatly enhanced.[16,23,24] The greater enhancement of fluorescence intensities of the PN dyes as compared to the increase of the

TABLE 2: Equilibrium Constants for the Scheme 1 Association of Phenothiazine Dye Monomers and Dimers with Cyclodextrins in Acidic Aqueous Solutions at 25 °C[a,b]

dye	CD	$10^{-2}K_{11}$ M	$10^{-3}K_{21}$ M	$10^{-5}K_{21}$ M	$10^{-3}K_{22}$ M
TH	β	3.0 (3.8)[d]	0.010	0.0011	0.76
	γ	0.25	5.2	6.7	*c*
AZA	β	3.6 (3.3)[d]	0.029	0.013	2.8
	γ	0.21	4.4	34	*c*
MB	β	4.0 (3.5)[d]	0.035	0.0084	0.60
	γ	0.25	1.7	6.5	*c*
TB	β	6.5 (5.3)[d]	0.025	0.0069	1.8
	γ	0.48	15	56	*c*
NMB	β	7.4 (16)[d]	0.090	0.13	22
	γ	0.87	31	390	*c*
DMMB	β	0.20	*c*	*c*	*c*
	γ	2.42	0.14	0.93	21

[a] K_{12} values were less than 5 M^{-1} for all dyes with β-CD and γ-CD. [b] K_{21}' values are K_{22}/K_{21}' and are found to be less than 7 M^{-1} for β-CD. [c] Too small to have physical meaning. [d] From fluorescence titration data.

fraction of monomer indicates that above trend is also applied to PN dyes.[16]

Since the dimers are not fluorescent, the total fluorescence intensity measured in the presence of CDs becomes the sum of the contributions from the monomer and the monomer−CD complex, the contribution from CD−M−CD being neglected as the fraction of the species is negligible due to the small K_{12} value:

$$I_F = I_{F,M}[M] + I_{F,M-CD}[M-CD] \quad (5)$$

where $I_{F,M}$ and $I_{F,M-CD}$ are the expected fluorescence intensities when all of the dye molecules exist as monomers or monomer−CD complexes, respectively. For β-CD media, the monomer, the monomer−β-CD complex, and the dimer are predominant species under our experimental condition of total PN dye concentration (C_T) of 1.0×10^{-6} M and other species are estimated to be less than 1% from the calculation using the parameters in Table 2. In this case, eq 5 is transformed into eq 6 from the definitions of equilibrium constants and mass balance of the dye.

$$I_F = \frac{[-(1 + K_{11}[CD]) + ((1 + K_{11}[CD])^2 + 8K_D C_T)^{1/2}]}{4K_D} \times (I_{F,M} + I_{F,M-CD}K_{11}[CD]) \quad (6)$$

We then obtained K_{11}, $I_{F,M}$, and $I_{F,M-CD}$ from the dependence of I_F on [β-CD] (Figure 8) by regression analysis similar to that described earlier. The K_{11} values obtained by fluorescence analysis (Table 2) show reasonably good agreement with those from absorbance analysis. Enhancement of fluorescence intensities of monomers upon complexation ($I_{F,M-CD}/I_{F,M}$) was 2.9 for TH, 3.0 for AZA, 2.8 for MB, 3.9 for TB, and 5.1 for NMB. The enhancement is greater for the dye with higher K_{11} values, indicating the tighter binding to β-CD results in greater enhancement.

In conclusion, the absorption and fluorescence spectroscopic studies on the six phenothiazine dyes in aqueous media in the presence of cyclodextrins and analysis of the spectral behaviors in terms of the multiple-equilibrium scheme give the following conclusions: (1) The presence of methyl groups on the phenothiazine fused ring results in a large increase of the dimerization constant (K_D), but the substitution at amine groups affects little the K_D values. (2) Except for that of DMMB, which has methyl groups on the 1-position of the fused ring, the dimerizations of dyes are facilitated by the presence of γ-CD but inhibited by β-CD. This is due to the better fit of the phenothiazine monomer into β-CD, whereas dimers fit snugly into the γ-CD cavity. (3) The phenothiazine rings of the dyes are deeply inserted into the CDs, and the 1-substituents of the fused rings and amine groups protruded from the CD cavities. (4) The dye dimers are not fluorescent, and inclusion of the monomer into β-CD results in a 3−5 times enhancement of fluorescence intensity. The determined equilibrium constants of the multiple-equilibrium scheme of the dyes in CD media and fluorescent properties of the dyes can be used to control the dye the aggregation and the photophysical and photochemical properties of the phenothiazine dyes for various applications.

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