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Influence of *tert*-Butyl Alcohol on Cyclodextrin Inclusion Complexes of Pyrene

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Abstract. The effect of *tert*-butyl alcohol on complexes of pyrene and various cyclodextrins is investigated. The equilibrium constant for the complexation is derived from the fluorescence decay parameters. A greater than twofold enhancement of pyrene lifetime is observed in the presence of *tert*-butyl alcohol and β-cyclodextrin or γ-cyclodextrin. As the number of hydroxyl groups decreases, substituted β-cyclodextrins show smaller enhancements to both the fluorescence lifetime and the formation constant. These observations are explained by proposing that alcohol molecules are associated with the inclusion complex. This association increases the apparent hydrophobicity of the cyclodextrin cavity, protects the molecule from collisional quenching and deactivation, and provides additional rigidity to the system.

Key words. Cyclodextrin, pyrene, *tert*-butyl alcohol, cyclodextrin inclusion, fluorescence spectroscopy, fluorescence lifetime.

1. Introduction

Cyclodextrins (CDs) have received attention over the past several years because of their unique physical properties [1, 2]. The ability of a CD to include appropriately sized molecules in its hydrophobic interior makes it well suited as an organizing medium in aqueous systems. This molecular organizing property has been shown to have a marked effect on the photophysical properties of an included guest molecule [3–5].

Fluorescence spectroscopy has found wide application in the study of CD systems [6, 7]. Changes in the spectral characteristics of a probe molecule such as pyrene allow investigations of the immediate microenvironment of the CD cavity [8]. Fluorescence intensity effects can be utilized to calculate formation constants for inclusion compounds [9, 10]. In addition, the temporal nature of processes occurring in the vicinity of the CD and equilibrium concentrations may be observed using fluorescence lifetime measurements [11].

Recently, the interaction of a third component with CD inclusion complexes has been of interest. Much attention has been focused on intramolecular and intermolecular excimer formation in the CD cavity [4, 12]. Other studies have investigated retardation and enhancement of fluorescence quenching due to inclusion of the quencher, fluorophore, or both in the CD cavity [13–15]. In addition, mixed systems of CDs and surfactants have been demonstrated to have useful properties [16, 17].

In the present study, the effects of a bulky aliphatic alcohol, *tert*-butyl alcohol, on CD properties are investigated. Alcohols have previously been shown to change inclusion and spectral characteristics of a guest molecule [18, 19]. A large change in the apparent hydrophobicity of the CD cavity has been previously observed in the presence of alcohols. Also, significant changes in the fluorescence lifetime of pyrene have been shown to accompany

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complexation in the presence of alcohols [20]. These changes were shown to be dependent on the size of the aliphatic portion of the alcohol molecule. These enhanced properties may offer useful insight into the binding characteristics of CDs, while providing a new set of properties to be exploited in CD systems. The origin and nature of these interesting interactions in the presence of *tert*-butyl alcohol (*t*-BuOH) are investigated in β-cyclodextrin and γ-cyclodextrin systems using pyrene as a fluorescence probe.

2. Experimental

2.1. MATERIALS

Pyrene, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (di-β-CD) and heptakis(2,5,6-tri-*O*-methyl)-β-cyclodextrin (tri-β-CD) were obtained from Aldrich and were of 99+% purity. The γ-cyclodextrin (γ-CD) and β-cyclodextrin (β-CD) were obtained from Astec Separations and *tert*-butyl alcohol (ACS grade) was obtained from Fisher. A 12.5 μM stock solution of pyrene was prepared in *t*-BuOH.

The fluorescence lifetime of pyrene : CD complexes in the absence of *t*-BuOH was measured by recording the fluorescence decay of an aqueous 0.5 μM pyrene solution in the presence of various CDs. The curves were measured at a CD concentration of 2.5 mM.

Two types of experimental methodologies were employed to investigate the effects of *t*-BuOH on pyrene : CD inclusion complexes. In the first, pyrene and *t*-BuOH concentrations were held constant for all experiments. Samples of varying CD concentrations were prepared by pipeting 1.00 mL of the pyrene stock solution and an appropriate volume of an aqueous CD solution and diluting to the mark with deionized water in a 10.0 mL volumetric flask. The presence of 10 volume percent *t*-BuOH allowed experiments to be carried out at a pyrene concentration of 1.25 μM, slightly above the aqueous solubility limit of 0.5 μM. This significantly enhanced the pyrene fluorescence intensity, which was critical in single-photon counting lifetime experiments, due to low light throughput in our system. Also, we verified experimentally that pyrene fluorescence lifetime was independent of pyrene concentration for the described experimental conditions. Also, no measureable pyrene adsorption to the glassware was observed.

In the second experimental methodology, CD concentrations were held constant at 1.00 mM and pyrene at 0.5 μM and *t*-BuOH concentration varied from 0.1 to 20% by volume. Pyrene solutions were prepared by pipeting an appropriate volume of a stock pyrene solution into a volumetric flask and evaporating the cyclohexane solvent. Alcohol was then added and finally an appropriate volume of freshly prepared stock CD solution. This precedure ensured that all the pyrene was solubilized at low *t*-BuOH concentrations [24].

2.2. FLUORESCENCE SPECTROSCOPY

Fluorescence spectra were recorded using a Perkin-Elmer 650-10S fluorometer interfaced to an Apple II+ microcomputer. Fluorescence lifetimes were obtained using a Photochemical Research Associates System 3000 Fluorescence Lifetime Spectrometer coupled to a MicroVax II minicomputer by a Tandy TRS-80 microcomputer based intelligent interface [25]. The sample solutions were not degassed prior to the measurement of steady state or fluorescence decays. The excitation source for the fluorescence lifetime experiments was a low pressure (15 mm Hg) hydrogen flash lamp operated at 6 kV with a repetition rate of

30 kHz. The full-width half-maximum of the lamp was approximately 2 nanoseconds. All lifetimes were greater than 100 nanoseconds and the excitation source was treated as a δ-pulse, thus providing minimal convolution errors. The excitation wavelength was controlled by an interference filter with a 25% peak transmission at 340 nm and a 10 nm bandpass. Fluorescence emission was monitored at 395 nm. All samples were checked for pyrene excimer fluorescence. The formation of the pyrene excimer would change the equilibrium concentrations of free and complexed pyrene and represent an interference. The excimer band was not observed.

2.3. DATA ANALYSIS

Similarly to the naphthalene : CD system [11], the fluorescence decay of pyrene : CD systems has been shown to follow a biexponential decay of the form [20]:

$$F(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} \tag{1}$$

where τ_1 and τ_2 are the fluorescence lifetimes of complexed and free pyrene, respectively, and the A_is are pre-exponential factors. These parameters were obtained from experimentally measured fluorescence decay curves using nonlinear least-squares curve fitting of equation (1) and a Marquardt gradient search method [26, 27]. A global linking algorithm previously described by Knutson *et al.* [28], was also coupled with this numerical procedure. This algorithm entails the fitting of parameters common to several data sets simultaneously, reducing the overall dimensionality of the curve fitting. In the present case, the lifetimes of the pyrene : CD complex and the free pyrene are assumed to be the same in a series of experiments where only the CD concentration is varied. This assumption is reasonable and affords the application of the global analysis method. For all lifetimes obtained through global analysis, eight to ten data sets representing different CD concentrations were input to the algorithm. All data sets were well fitted as judged by the χ^2 statistic and visual inspection of weighted residual plots.

The pre-exponential A_is are related to the concentrations of each component [29]. If we write an expression for the pyrene : CD equilibrium in terms of the ratio of included to free pyrene and the A_is, then [20]:

$$\frac{A_1\tau_1}{A_2\tau_2} = K_f[\mathrm{CD}]\frac{\varepsilon_1\Phi_1}{\varepsilon_2\Phi_2} \tag{2}$$

where K_f is the formation constant of the complex, the ε_is are molar absorptivities, and the Φ_is are the quantum yields of the complexed and free pyrene (subscripts 1 and 2, respectively). Thus, parameters obtained from the fit of fluorescence decay curves may provide both fluorescence lifetime and equilibrium information.

The use of parameters obtained from fluorescence lifetime measurements to explore equilibrium concentrations of components in CD systems is well documented. Hasimoto and Thomas [17] have used such measurements to study pyrene and naphthalene in CD-surfactant systems. Herkstroeter *et al.* [4], have measured the concentration ratios of free and γ-CD complexed pyrenylbutyrate. The use of all of the parameters of the fluorescence lifetime experiment can simultaneously provide useful information concerning both the photophysical and equilibrium parameters of a CD or any system of interest.

3. Results and Discussion

Figure 1 demonstrates the change in the pyrene fluorescence decay curve with β-CD concentration. Similar curves were observed for all CDs studied. Upon the addition of CD to a solution of pyrene and t-BuOH, a longer lifetime component of fluorescence appears. This observation is consistent with those made for other fluorophore : CD systems [4, 11, 16, 20]. As the concentration of CD increases, the relative contribution of this long-lived component increases, but not its fluorescence lifetime. Global analysis of a series of curves such as presented in Figure 1 yields the fluorescence lifetime and data necessary to calculate the formation constant of the pyrene : CD complex.

3.1. PYRENE : CD EQUILIBRIUM

Equation 2 shows the expected dependence of fluorescence decay parameters on CD concentration for an equilibrium system of free and complexed pyrene. This equation may be difficult to apply in many instances due to its dependence on molar absorptivity and flourescence quantum yield parameters. Several researchers have applied useful assumptions which can simplify an equation such as (2). Nakajima [10] found the ratio of molar absorptivities for pyrene and complexed pyrene at 337 nm to be approximately unity. This observation was verified in this study for systems where t-BuOH was also present. No perceptible absorption wavelength shift or change in molar absorptivity occurs. Hashimoto and Thomas [17] have assumed that the ratio of fluorescence lifetimes for complexed and free pyrene are a resonable approximation to the ratio of the quantum yields. Following this reasoning, we can write:

$$\frac{\Phi_1}{\Phi_2} = \frac{\tau_2^0 \tau_1}{\tau_1^0 \tau_2} \tag{3}$$

where the τ_1^0 and τ_2^0 are the intrinsic fluorescence lifetimes of the complexed and free pyrene, respectively. Since the fluorescence of complexed and free pyrene arise from the same moiety, a reasonable approximation to the ratio of the intrinsic lifetimes would be unity. Applying this approximation to (3) and substituting into (2), we find that:

$$\frac{A_1}{A_2} \approx K_f [\mathrm{CD}] \tag{4}$$

This approximation would be expected to fail in two cases. First, if the fluorescence lifetimes of free or complexed pyrene varied with CD concentration, equation (3) would be a nonlinear function and each parameter would need to be determined for every observed CD concentration. We analyzed each fluorescence decay curve individually and found no systematic variation of either pyrene or pyrene : CD lifetime with CD concentration. This assured that the global technique was not masking an important trend in the lifetimes by averaging across such an effect. Second, if the ratios of the intrinsic lifetimes were not unity, but constant with CD concentration, then this deviation would be incorporated in the determined K_f in a linear fashion. Thus, K_f values determined from equation (4) would be either high or low by the ratio of the intrinsic lifetimes.

The system under consideration here involves three components: CD, pyrene, and t-BuOH. The formation constant, K_f, in equations (2) and (4) will be a pseudo formation constant, incorporating the t-BuOH concentration. The concentrations of alcohol utilized in this study produced a significant excess of t-BuOH over both CD and pyrene. Thus, the

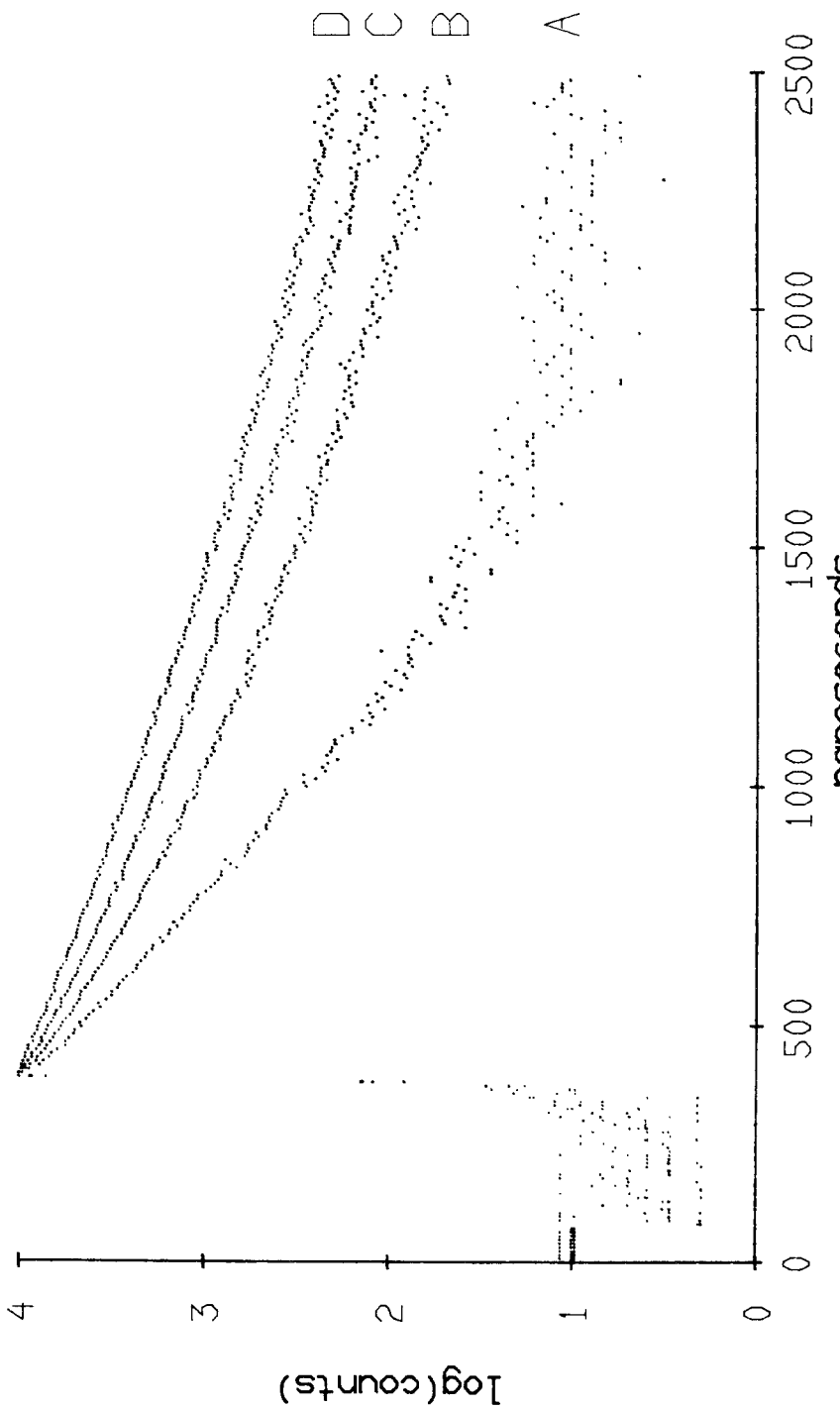

Fig. 1. Pyrene fluorescence decay curves measured in the presence of various concentrations of β-CD at 23°C: (A) [β-CD] = 0.00; (B) [β-CD] = 0.15 mM; (C) [β-CD] = 0.30 mM; (D) [β-CD] = 1.05 mM;

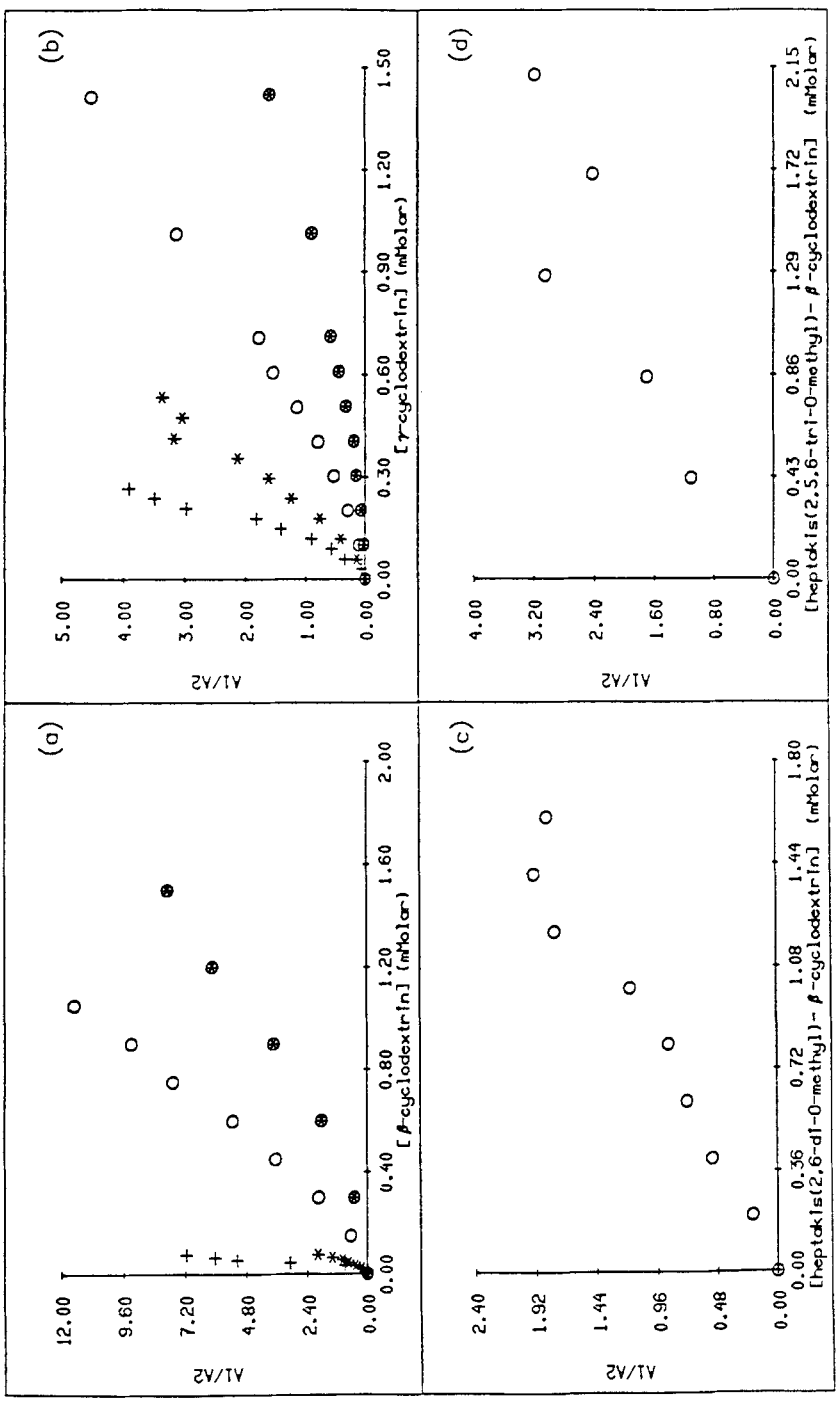

Fig. 2. Plot of A_1/A_2 versus concentration of various cyclodextrins: (a) β-CD at 2°C (+), 11°C (*), 23°C (○), and 34°C (⊛); (b) γ-CD at 2°C (+), 12°C (*), 23°C (○), and 35°C (⊛); (c) di-β-CD at 22°C; (d) tri-β-CD at 22°C. These values were computed from preexponential factors obtained through global analysis of fluorescence decay curves recorded at various CD concentrations. The associated lifetime parameters are reported in Table III.

formation constants determined from the experimental data represent only the equilibrium at the condition of 10% by volume of *t*-BuOH. Also, pyrene : CD complexes are understood to involve *t*-BuOH molecules.

Figure 2 deomonstrates this relationship for β-CD and γ-CD at various temperatures and for di-β-CD and tri-β-CD at 23°C. The first noticeable feature of these plots is the deviation from linearity at higher concentrations of CD. Several explanations for this behavior are possible. For example, this non-linearity may arise from fitting errors in the experimental parameters. As the [CD] increases, the divisor in the plotted ratio becomes increasingly small and the error becomes more important. Such errors would cause deviations in the directions observed.

Another explanation for non-linearity observed in some of the graphs presented in Figure 2 may be that the 1 : 2 pyrene : CD complexation becomes important in the equilibrium at higher CD concentrations. This interpretation is equally plausible, since such complexation would decrease the amount of free pyrene relative to the total amount of complexed pyrene. Since no pyrene excimer fluorescence was observed, we rule out the presence of 2 : 1 or 2 : 2 complexes. Complexes containing one pyrene would likely have similar lifetime characteristics to the 1 : 1 complex and thus may not be resolvable by lifetime measurements. Since the lifetime of the pyrene : CD complex is quite long, complexation with a second CD would not appreciably enhance the lifetime. The global analysis of the data using three components of fluorescence did not reveal a third component of fluorescence for any of the experiments. Therefore, the 1 : 2 complex, if present, is not resolvable from the 1 : 1 complex using fluorescence lifetime measurements. Thus, the concentration of such a complex would become incorporated in the A_1 factor of equation (4). The presence of a 1 : 2 complex could not be confirmed by fluorescence lifetime measurements. Therefore, only the initial linear portions of these curves were taken as a measure of the formation constant for the 1 : 1 complex. This procedure assured that the A_1/A_2 ratios contained only information for the equilibrium between pyrene and 1 : 1 pyrene : CD complex, in the presence of *t*-BuOH.

The formation constants estimated by this method at various temperatures are presented in Table I. The formation constant for the pyrene : γ-CD complex in the presence of 10% *t*-BuOH at 10°C has been previously reported to be 6760 M^{-1} [20]. The formation constant for the same complex at 12°C is reported in Table I and shows good agreement with the previous value. From the formation constants reported in Table I, we estimate that the heat of formation for the pyrene : β-CD complex in the presence of 10% *t*-BuOH is -34 kJ M^{-1} and for the pyrene : γ-CD complex is -53 kJ M^{-1}.

Previous studies have estimated the formation constant for the pyrene : γ-CD complex to be 50 M^{-1} [17] and 200 M^{-1} [10]. The formation constants of the complex in the presence of *t*-BuOH are an order of magnitude greater than those results. This enhancement indicates that the alcohol is present in the microenvironment of the complex. Such an increase would not be expected if the *t*-BuOH only changed the bulk properties of the solvent. In fact, a decrease in the formation constant would be expected since the presence of *t*-BuOH would likely make the solvent more favorable to pyrene than a simple aqueous system.

Using Table I, we also note that the formation constant for the complex decreased in going from β-CD to di-β-CD to tri-β-CD. This may indicate that the CD hydroxyl groups are involved in the association of *t*-BuOH with the complex. The methyl substituted CDs, especially tri-β-CD, cannot hydrogen-bond to the same extent with *t*-BuOH molecules as β-CD. Therefore, if the formation constant of the pyrene : CD complex is dependent on the extent of association of *t*-BuOH molecules, the observed decrease is reasonable.

Table I. Formation constants for pyrene and various cylodextrins in the presence of *tert*-butyl alcohol computed using fluorescence lifetime data and equation (5).

Temperature (°C)	Formation Constant (M^{-1})
γ-CD	
2	10500
12	5250
23	2630
35	833
β-CD	
2	26200
11	19500
23	11300
24	5540
di-β-CD	
22	1600
tri-β-CD	
22	1350

Patonay *et al.* [9], have found that for aqueous systems, the formation constant of the pyrene : γ-CD complex is larger than the pyrene : β-CD complex. In the presence of alcohols, this order was found to be reversed [18]. The formation constants reported in Table I are in reasonable agreement with those results, and show the same trends in the presence of *t*-BuOH.

3.2. MICROENVIRONMENTAL EFFECTS

Not only is equilibrium information available from the fitting of equation (1) to the observed decay curves, but also the decay times. The fluorescence lifetime of a probe such as pyrene gives an excellent indication of changes taking place in the microenvironment of the CD complex. Previous work has shown that alcohols substantially increase the lifetime of the pyrene : γ-CD complex [20]. In that study, the lifetime enhancement was found to increase with increasing bulkiness of the alcohol. Also, the hydrophobicity of the complex was found by Patonay *et al.* [18], to increase with the chain length of the alcohol present in the system. This change in hydrophobicity is most likely due to the interaction of pyrene with the aliphatic end of the alcohol, and decreased interaction with the aqueous solvent.

The lifetimes of the pyrene : CD complexes in the absence of *t*-BuOH are reported in Table II. The lifetimes of the pyrene : β-CD and pyrene : γ-CD complexes are in good agreement with those measured by Yorozu *et al.* [8]. These values indicate that CDs alone can produce a substantial change in microenvironment, as measured by included pyrene. Both γ-CD and β-CD produce approximately a twofold enhancement in pyrene fluorescence lifetime. The substituted β-CDs do not show such a large degree of enhancement. Yet complexation of these CDs lengthen the lifetime of the included pyrene. The lifetime enhancement observed in this study is similar to that reported by Nelson, *et al.* [11], for naphthalene complexes with CDs.

Table III is a tabulation of the fluorescence lifetime of the pyrene : CD complexes, in the presence of 10% *t*-BuOH, measured at various temperatures for the CDs used in the study.

Table II. Fluorescence lifetime parameters for systems of pyrene and various CDs in the absence of *tert*-butyl alcohol. These lifetimes were obtained by least-squares curve fitting of a single fluorescence decay measured for pyrene in the presence of 2.5 mM CD at 21°C.

Cyclodextrin	τ_{free} ns	$\tau_{complex}$ ns	χ_r^2
γ-CD	166	274	1.19
β-CD	149	344	0.94
di-β-CD	145	210	1.02
tri-β-CD	150	226	1.24

The lifetimes of the complexes in the presence of *t*-BuOH reported here are significantly greater than those reported in Table II. The enhancement of the complex lifetime by *t*-BuOH indicates that the alcohol molecule is involved in the inclusion complex. The lifetimes of the pyrene complexes with γ-CD, β-CD, and di-β-CD are over 1000 nanoseconds longer in the presence of *t*-BuOH. This magnitude of enhancement is quite remarkable for the interaction of a relatively small aliphatic alcohol molecule. It is interesting to note that the lifetime of the pyrene : tri-β-CD complex is slightly diminished in the presence of *t*-BuOH. This may indicate that the CD hydroxyls are important in the binding of the alcohol to the complex, or that *t*-BuOH at the studied concentration is in competition with pyrene for inclusion in tri-β-CD.

Several important trends can be noted from the data reported in Table III. In the case of β-CD and γ-CD, the lifetime of the complex is well over two times greater than that of

Table III. Fluorescence lifetimes for systems of pyrene and various CDs in the presence of 10% *tert*-butyl alcohol. The lifetimes were computed by global analysis of 6-10 fluorescence decay curves measured at different CD concentrations. The associated preexponential factors are presented in Figure 2.

Temperature (°C)	τ_{pyrene}[a] ns	τ_{free}[b] ns	$\tau_{complex}$[c] ns	χ_r^2
γ-CD				
2	188	191	481	1.22
12	172	179	448	1.21
23	163	172	414	1.12
35	149	151	359	1.21
β-CD				
2	198	197	485	1.01
11	174	181	469	1.10
23	191	188	457	1.02
34	138	146	426	1.10
di-β-CD				
23	152	196	313	1.05
tri-β-CD				
23	153	132	187	1.05

[a] Lifetime of pyrene in aqueous solution in the absence of CD.
[b] Lifetime of the shorter of two pyrene decay components observed in the presence of CD.
[c] Lifetime of the longer of two pyrene decay components observed in the presence of CD.

the free pyrene. This is particularly remarkable for the β-CD system, where the cavity diameter is smaller than the width of the pyrene molecule. It must also be noted that the pyrene : β-CD complex has a longer lifetime at all temperatures studied than the pyrene : γ-CD complex, in the presence of *t*-BuOH.

The lifetimes of these complexes also shorten with increasing temperature. Since the formation constant decreases with increasing temperature, it is more probable that an excited pyrene will diffuse away from the CD within its lifetime. This would effectively shorten the lifetime of the pyrene due to increased solvent interactions, such as thermal deactivation of the excited pyrene. Temperature also changes the vibrational frequencies, which could result in a change in the transition probabilities. Either one or both of these explanations could play a role in the observed behavior.

The magnitude of the lifetime enhancement indicates a substantial alteration of the rate constants for the various deactivation pathways of the first excited singlet state. Two important deactivation processes, quenching by molecular oxygen and collisional deactivation are likely to be affected by CD complexation and the participation of *tert*-butyl alcohol in that complex. Cyclodextrin complexation has been shown by a number of researchers to protect pyrene from interaction with dynamic quenchers such as molecular oxygen [30–33]. The association of the bulky *t*-BuOH with the pyrene : CD complex may further restrict the diffusion of a quencher to an excited pyrene molecule. In addition, encapsulation of the pyrene by CD and *t*-BuOH is expected to decrease the interaction of the pyrene with the aqueous solvent, resulting in a decreased number of interactions of the pyrene with surrounding molecules.

Complexation of pyrene by CD in the presence of *t*-BuOH also provides a very hydrophobic microenvironment for the pyrene. The lifetime enhancement of pyrene upon CD complexation has been attributed to an increase in hydrophobicity around the included pyrene [8]. Kalyanasundaram and Thomas [34] demonstrated that the lifetime of pyrene in sodium lauryl sulfate micelles is approximately 330 ns. This change in lifetime was also attributed to a change in the hydrophobicity of the microenvironment of pyrene. The lifetimes for pyrene : γ-CD and pyrene : β-CD complexes in the presence of *t*-BuOH are 100 ns longer than either aqueous CD systems or sodium lauryl sulfate micelles. We conclude that the alcohol molecules offer additional hydrophobicity to the CD cavity. This conclusion is reasonable since Patonay *et al.* [18], reported that the III/I pyrene fluorescence vibronic band ratio for these complexes is similar to that observed in cyclohexane. The inclusion of a *t*-BuOH molecule in the CD may displace water from the cavity. Such a displacement would produce the observed longer lifetime of the pyrene : CD complex by diminishing interactions of pyrene with co-included water molecules. The increase in hydrophobicity observed by Patonay *et al.* may also be understood in these terms. The formation constants of the complex in the presence of alcohol would increase, since the pyrene would complex to a more hydrophobic environment in the CD cavity.

The importance of *t*-BuOH in this complex is evidenced by the lifetimes of complexes of pyrene with substituted β-CDs. Table III demonstrates that the lifetime of the pyrene : CD complex decreases as an increasing number of CD hydroxyl hydrogens are substituted with methyl groups. In the case of tri-β-CD, very little enhancement to the lifetime of pyrene occurs. When fewer hydrogen bonding sites on the CD are available, the interaction of t-BuOH molecules is apparently decreased. With less favorable participation of alcohol molecules in the complex, more interaction of the pyrene with the aqueous environment would be possible. Also, the rate of pyrene diffusion out of the CD

may increase, if the bulky *t*-BuOH molecule is not involved in the *O*-methyl substituted complexes to the same extent as in the unsubstituted CDs.

3.3. EFFECT OF *tert*-BUTYL ALCOHOL CONCENTRATION

In order to further clarify the role of *t*-BuOH in the pyrene : CD complex, a series of experiments were conducted in which the CD and pyrene concentration were held constant at 1 mM and 0.5 μM respectively, while the *t*-BuOH concentration was varied. The results are presented in Figure 3. The A_1/A_2 ratio is an estimate of the ratio of complexed to free pyrene at a given CD concentration. This ratio reaches a maximum for β-CD in the region of 100 alcohols per CD. For di-β-CD, the maximum is reached in the region of 10 alcohols per CD. For tri-β-CD no apparent complex is observed under the experimental conditions. In the case of tri-β-CD, the lifetimes of the free and complexed pyrene are similar. If the amount of complex is small, there is no significant difference in a single and double exponential fit of the data. Despite the similarity of the formation constants for the di-β-CD and tri-β-CD complex, the lifetime difference allows a definitive double exponential fit only in the case of the di-β-CD system.

The trend observed in Figure 3 suggests that there exists a concentration of alcohols needed for maximum enhancement. The formation constant for a 1 : 1 *t*-BuOH : β-CD complex has been measured to be 54 M^{-1} [35]. Taking this value and the concentration of β-CD utilized in the experiments, 10^{-3} M, it can be shown that the maximum observed in Figure 3 corresponds to the complexation of over 90% of the β-CD molecules with

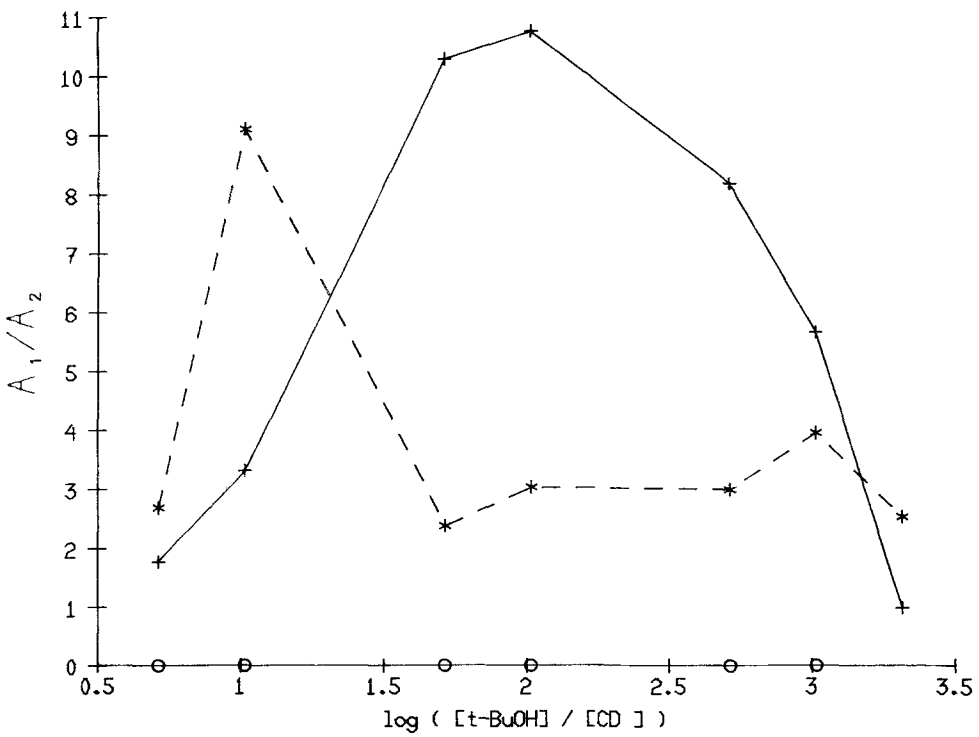

Fig. 3. Plot of A_1/A_2 versus log (*tert*-butyl alcohol concentration/cyclodextrin concentration) for β-CD (+), di-β-CD (*) and tri-β-CD (○).

t-BuOH. This infers that the majority of CD molecules encountered by pyrene contain at least one alcohol. The position of this maximum is at a lower alcohol concentration for di-β-CD, possibly indicating that the formation constant for *t*-BuOH : di-β-CD is larger than that of the β-CD complex. This is likely since the cavity of the methyl substituted CDs is more hydrophobic than the unsubstituted β-CD. In the case of tri-β-CD, no statement can be made, since no complexation was observed in the alcohol : CD concentration range studied. It must be noted that the further substitution present in tri-β-CD would make the cavity more hydrophobic, yet does not necessitate an increased formation constant with *t*-BuOH. The CD hydroxyls are intimately involved in the complexation of alcohols to CDs [36]. The absence of hydroxyls in the tri-β-CD may cause a decrease in the formation constant for alcohols, with respect to β-CD and di-β-CD. This observation is evidenced by the work of Patonay *et al.* [18], where the pyrene III/I vibronic band ratio for the fluorescence of pyrene : tri-β-CD complex in the presence of 10% *t*-BuOH is significantly smaller than for the corresponding pyrene : β-CD and pyrene : di-β-CD complexes.

At concentrations of *t*-BuOH below the point of this maximum, a mixture of free pyrene, pyrene : CD, and pyrene : CD : alcohol exist. In this case, the lifetimes of both free and pyrene : CD complex are incorporated in the A_1 factor. At higher alcohol concentrations, all the CD molecules are complexed with *t*-BuOH. The fall off of the A_1/A_2 ratio at high alcohol concentrations may be described by several processes. If higher complexes of *t*-BuOH : CD are formed, alcohol may displace pyrene from the cavity. Also, the polarity of the bulk solvent begins to change substantially at high alcohol concentrations. Thus the difference in hydrophobicity in the solvent and CD cavity would become smaller, making pyrene complexation with CD less favorable.

4. Conclusions

Large changes in the equilibrium and spectral properties of complexes between pyrene and CDs are produced by *t*-BuOH. These changes are likely to be due to interactions between the CD molecule and the alcohol. Apparently, *t*-BuOH molecules become associated with the complex and enhance the complexation between the CD and the pyrene molecule. This association likely diminishes the interaction of pyrene with the aqueous solvent, producing a greater than two-fold enhancement in the fluorescence lifetime. The alcohol also normalizes the size of the CD cavity such that β-CD offers a stronger interaction than γ-CD with the pyrene molecule. This is a direct reversal of the phenomena observed in purely aqueous solutions of CDs. These enhanced properties make CDs a more effective organization medium and offer interesting possibilities in the development of HPLC, extraction, and fluorescence methods.

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