FLUORESCENCE STUDIES OF CYCLODEXTRIN POLYMERS: A STUDY OF THE BINDING OF VARIOUS FLUORESCENCE PROBES TO CYCLODEXTRIN POLYMERS

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ABSTRACT

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Cyclodextrins (CDs) are cyclical oligosaccarides which exist as 6, 7, or 8 glucopyranose units joined by α- 1,4 linkages, referred to as α, β and γ CDs, respectively. Their structure resembles that of a truncated cone in which the interior cavity is hydrophobic while the exterior remains hydrophilic. This allows CDs to form inclusion complexes with nonpolar molecules in an aqueous environment. Polymerization of CDs (CDPs) has been carried out using epichlorohydrin which connects the CD cavities by an average of 12-15 repeating glyceryl linker units.

The binding of various fluorescence probes to CD monomers has been studied. In particular, pyrene (Py), which is very sensitive to the polarity of its microenvironment, has been widely used to study the binding site of the CDs. Py fluorescence emission spectra have a I/III band ratio which reflects the polarity of the Py surroundings when bound to CDs. These data, along with fluorescence lifetime data on bound Py, suggest that Py binds in the hydrophobic CD cavity. Moreover, in the presence of a fluorescence quencher, iodide ion, the CD cavity acts to protect the Py. This protection is further enhanced by the addition of additives such as alcohol.

We have found much different results for pyrene bound to CDPs. The I/III fluorescence band ratios, as well as, the fluorescence lifetime data of Py when bound to CDPs, suggest a binding which is much more hydrophilic and affords less protection to the pyrene. Thus, Py binding appears to be involving the long glyceryl linking units in the CDPs rather than the CD cavity. We have carried out similar I/III ratio and fluorescence lifetime tests on synthesized CD polymers of various shorter lengths of linker units. These data indicate that Py binding with the glyceryl linkers of CDPs is highly dependent upon the length of the glyceryl linker units and, at certain linker lengths, this binding can be tuned to a more inclusional type upon the addition of alcohol.

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Introduction

Cyclodextrins (CDs) are cyclical carbohydrates which are well known for their ability to form inclusional complexes with a variety of organic molecules. Their physical structure resembles that of a hollow torus in which the interior cavity is relatively hydrophobic due to the presence of the glycosidic oxygen bridges connecting the monomeric units.[1] The edges of the torus are lined with hydroxyl groups which causes the exterior of the CD cavity to remain hydrophilic. Thus, CDs are able to form inclusion complexes with various nonpolar solutes in an aqueous, or polar, environment.

CDs are made up of glucopyranose units joined by α-1,4 linkages. The three most common cyclodextrins are α-CD, β-CD and γ-CD, which exist as six, seven and eight glucopyranose units, respectively. The internal cavity diameter of α-CDP is the smallest (0.57 nm), followed by β-CDP (0.78 nm) and γ-CD (0.95 nm).[1] The CDs display varying degrees of solubility and reactivity with different solutes. β-CD, which is the most versatile of the CDs, has the lowest solubility.

In attempts to increase the solubility of CDs, methods have been devised to synthesize water-soluble CD polymers(CDPs). The most common procedure involves the use of epichlorohydrin, which produces CD units joined by repeating glyceryl linkers ($(-(CH_2\text{-}CHOH\text{-}CH_2\text{-})_n)$), where n=12-15.[7] All three of these polymers, α-CDP, β-CDP and γ-CDP, are highly water soluble and are now commercially available.

Much work has been done studying the binding of the CD monomers with various guest molecules. In addition, some effort has been put into a

comparison of the binding of guests to the CD monomers to that of the CDPs. There has been evidence which suggests several differences and similarities between CD and CDP complexation with guest molecules.[7,11]

Our work has included the use of various fluorescence probes, 2-acetylnapthalene (2-AN), 2-(N-methylanilino) naphthalene-6-sulfonic acid (2,6-MANS) and pyrene (Py), to study the binding by CDPs. In the presence of CDs, the fluorescence of 2-AN is quenched, and the extent of binding can be measured based upon the decrease in fluorescence.[11] The 2-AN probe forms a 1:1 complex with the three CDs, the strongest of which is formed with β-CD because the probe and the β-CD cavity form the best fit.[4] The 2,6-MANS probe forms a very strong 1:1 complex and a moderately strong 2:1 complex with β-CD.[12] The 2,6-MANS probe is useful in studying the polarity of the binding site on the CD or CDP because when bound in a hydrophobic environment the fluorescence intensity increases and blue shifts. For example, the maximum wavelength of fluorescence of 2,6-MANS in the presence of β-CD occurs at 563 nm, while in the presence of the polymer it decreases to 430 nm, indicating a more hydrophobic environment.[11] Both of these probes provide a range of binding interactions with the CDs. Thus, they are very useful when investigating the nature of the binding with CDPs.

Pyrene (Py) is an especially suitable fluorescence probe due to the sensitivity of its fluorescence vibronic structure and lifetime to its microenvironment.[2] Thus, use of Py s fluorescence emission spectra and lifetime have been instrumental in studying the pyrene binding sites on CDs. Py has a I/III (373nm/384nm) vibronic band ratio which reflects the polarity of its surroundings. In particular, when Py is in a polar solution,

such as water, the I/III ratio is about 1.8, but, when added to a non-polar solvent like cyclohexane, this ratio is substantially decreased (0.6).[2,7] Similarly, when Py is in the presence of 5×10^{-3} M β-CD, the I/III ratio is decreased to approximately 0.6.[2,7] This indicates that the environment in which the Py is located is hydrophobic in the presence of β-CD. This fact has been used to suggest that Py, which is too large to fit in a single β-CD cavity, forms a 2:1 complex with β-CD in which the Py is doubly capped by two CD cavities.[7] This proposed arrangement can be envisioned as a clam shell structure.[2,7] The I/III ratio of Py in the presence of γ-CD (I/III = 0.8) indicates a hydrophobic binding site as well. However, due to the larger diameter of the γ-CD cavity, which allows an entire Py molecule to fit inside, a 1:1 γ-CD:Py complex is the proposed arrangement.[6]

The fluorescence lifetime of pyrene in water has been shown in the literature to be approximately 130 nsec.[7] Upon the addition of β-CD, the decay data must be fit by a double exponential of which one lifetime component is always near that of pure Py (130 ns), while the other is substantially longer (300 ns).[7] Results with γ-CD:Py also show a longer lifetime component (215 ns).[5] The longer lifetime component clearly arises from the interaction of Py with the CD cavity. The dramatic enhancement in the lifetime suggests that pyrene is bound in the hydrophobic, protective interior of the CD cavity and virtually sealed off from the quenching effects of the solvent.[7]

Moreover, certain additives have been found which give additional support to the inclusional binding of pyrene with β-CD and γ-CD.

Potassium iodide in solution with pyrene is known to quench the fluorescence of pyrene and, thus, shorten the fluorescence lifetime. For example, the lifetime of Py in water is shortened to 95 nsec with the addition of 0.024 M KI.[3] However, when β-CD or γ-CD are added to the solution, the fluorescence of Py exhibits two lifetime components with the major component being over 300 nsec. This provides further evidence that Py is binding in the interior of the CD cavity, which acts to protect the Py from quenching effects of the iodide.

This protection by the CD cavity is further enhanced by the addition of alcohol to the solution. It has been suggested that alcohol forms a ternary complex with pyrene and a CD and acts to essentially cap off the opening of the CD cavity by binding to the hydroxyl groups on the exterior of the cavity lining.[5] Thus, pyrene in the presence of β-CD and alcohol is further shielded from the solvent, and, hence, the fluorescene lifetime of pyrene is enhanced. An enhanced lifetime of 493 nsec is reported for a solution of pyrene in 1 X 10^{-2} M β-CD and 1%(v/v) *t*-BuOH.[3]

The strength of this ternary complex is based on the ability of the alcohol to aid in the shielding of the fluorophore from solution interactions. It has been reported that fluorinated alcohols have a greater effect upon the shielding of the fluorophore when compared to non-fluorinated alcohols.[5] This increase in protection ability is due to the stronger hydrogen bonds which fluorinated alcohols form with the hydroxyl groups of the CD cavity.[5] This additional protection by the alcohol can be evidenced by a decrease in the I/III ratio values of pyrene when alcohol is added to a Py:β-CD system.[5] Warner reports a I/III ratio of 1.6 in the presence of 5 X 10^{-3} M β-CD and pyrene. Upon the addition of

0.025 M 2,2,3,3,3-pentafluoro-1-propanol (PFP) this value is dramatically decreased to 0.38, which is the lowest ratio reported thus far. This provides further evidence that pyrene is shifted to a more hydrophobic environment, in which the pyrene bound in the CD cavity is being blocked from the hydrophilic effects of the solvent. Thus, in the presence of β-CD and certain alcohols, pyrene is additionally shielded from the solvent.

CDP:pyrene binding can be studied with similar methods as discussed above. Xu et al. suggest that the β-CDP:pyrene complexes exists as 1:1 and 2:1 stoichiometry. For the latter, they suggested that the two β-CD cavities are from the same polymer chain. This intra-chain 2:1 complex formation should create a hydrophobic environment for the pyrene, similar to that observed with β-CD. However, they report the I/III ratio of this complex as 1.5, which is much larger than that with β-CD and indicative of a more polar binding environment. The reason they provide for this discrepancy is that the glyceryl linkers of the polymer can act as polar co-solvents for bound pyrene.[7]

In addition, Xu et al. have synthesized various β-CD oligomers, using epichlorohydrin (EP), with various ratios of β-CD to glyceryl linker units. They suggest that the length of the linker units will affect the ability of pyrene to bind in the CD cavity. They claim that a minimum spacer length of three glyceryl units is required to permit intrachain clam-shell binding. When CD cavities are any closer together than this, double capping the pyrene can only occur using CD units from two separate chains.[7]

We have focused most of our study on understanding the binding of the CDPs. We have obtained data for pyrene binding to two CDPs: β-CDP and γ-CDP. We have also tested the effects of additives such as iodide and

alcohols on the Py:CDP complex. We have found that the additives have different effects on the CDPs than that of the CDs. Trends in all of our data point to a CDP binding which is non-inclusional, non-cooperative and involving the glyceryl linker units of the CDPs rather than the CD cavity. This form of binding is different from the proposed clam shell binding observed by Xu et al.

We present here a thorough investigation of the binding of three fluorophores to commercially available CDPs. Through the use of three fluorophores, 2-AN, 2,6-MANS and pyrene, as well as data obtained from synthesizing CD polymers having shorter linker units than the commercial CDPs, we provide an investigation which includes a look at the strength, behavior and limitations of CDP binding. Our work will provide a comprehensive view of the binding of both β-CDP and γ-CDP.

Experimental

Apparatus

Absorption spectra for stock solutions of 2-AN, 2,6-MANS and pyrene were recorded using a Hewlett-Packard 8452A Diode Array Spectrophotometer. Absorption of the three fluorophores was viewed over the range of 300-400 nm. Absorption data over time were also collected on samples of β-CD and recrystallized β-CD over a range of 250-400 nm.

Fluorescence data were obtained with a Perkin-Elmer Lambda 5B Spectrofluorometer. This instrument automatically corrects emission spectra for the wavelength dependence of the emission monochromator and detector combination. Corrected emission spectra were obtained for CDP:Py solutions at an excitation wavelength of 320nm, over an emission range of 350-500 nm using excitation and emission slitwidths of 3 nm.

The spectra were plotted on a Perkin Elmer GP 100 Graphics Printer. Fluorescence emission intensities for β-CDP:2-AN were taken using an excitation wavelength of 340 nm and an emission wavelength of 437nm, while utilizing excitation and emission bandwidths of 10 nm. Temperature control during the 2-AN fluorescence experiments was provided by circulating water at the desired temperature ($10^{o}C$-$50^{o}C$) through a thermostated cell block using a Neslab Endocal Refrigerated Circulating Bath. The fluorescence emission spectra for the 2,6-MANS:β-CDP complex were taken using excitation wavelength of 340 nm, an emission wavelength range of 370-500 nm and slit widths of 5 nm. These spectra were recorded on a Perkin Elmer R100 Recorder. Aggregation of β-CD and recrystallized β-CD solutions was also studied using the fluorometer to measure light scattering at excitation and emission wavelengths of 600 nm and slit widths of 3 nm.

Fluorescence lifetime data were obtained with a LS 100 fluorescence lifetime system from Photon Technology International, Inc. The excitation wavelength employed for pyrene was 337 nm and the emission wavelength was 393 nm. Measurements for 2,6-MANS were taken at an excitation wavelength of 337nm and emission wavelength of 440 nm. The luminescence data are fitted with a fluorescence decay curve by a fitting procedure which can utilize from one to four exponentials. A single exponential fit to the lifetime data was deemed appropriate if a double exponential fit gave no improvement in the χ^2 parameter. All pyrene and 2,6-MANS data were analyzed using double exponential fits. Spectra were recorded on a Hewlett Packard Color Pro Plotter. All measurements were made at room temperature($23^{o}C$).

Analysis of β-CDPH, β-CDPL, as well as, synthesized β-CDP (2:1) and β-CDP (1:1) solutions were carried out on a Perkin Elmer Gel Permeation Chromatograph with a PE LC 30 RI detector. The column used was a gel permeation chromatography column and was purged with deionized water and 1%MeOH at a flow rate of 0.5 mL / min. This instrument was attached to an IBM Model 50Z computer and an Epson EX-800 printer.

β-CD polymers were dialyzed in various dialysis tubing (Spectra/Por CE Molecularporous Dialysis Membrane from Spectrum) with molecular weight cut-offs of 3500 and 2000. Solutions were then freeze dried utilizing dry ice and a vacuum pump.

Materials

The water used in all experiments was deionized, doubly distilled, and passed through a Millipore Milli-Q Water System. The 2-AN, Py and 2,6-MANS were obtained from Aldrich Chemical Company, Inc. The 2-AN was recrystallized twice from both hexane and ethanol, while the pyrene was recrystallized twice from ethanol. The 2,2,2-trifluoroethanol (TFE), 2,2,3,3,3-penta-fluoro-1-propanol (PFP), 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) and potassium iodide (KI) were also obtained from Aldrich Chemical Company, Inc. The *tert*-butyl alcohol (*t*-BuOH) was purchased from Fisher Scientific Company.

CDPs were purchased from Cyclolab R&D Laboratory Ltd. of Budapest, Hungary. The general formula of the polymers is

$$[CD\text{-}(CH_2\text{-}CHOH\text{-}CH_2\text{-}O)_n X]_p$$

where CD is α–, β–, or γ-CD; X is H or CD; p is > 1 but < 6-8 and n is > 1 but < 18 (α-CD), <21 (β-CD), <24(γ-CD). The average n value is 12-15, and the reported % CD is 54(α-CDP), 55 (β-CDP) and 57 (γ-CDP).

The γ-CD and the β-CD which was used in the synthesis of 1:1 and 1:2 β-CD:epichlorohydrin (EP) polymers was donated by Amaizo Labs. The EP was obtained from Aldrich Chemical.

Procedures

Stock Preparation:

The fluorophore stock solutions of 2-AN, Py and 2,6-MANS were prepared by adding a small amount of a given solid to water or buffer (2,6-MANS) and stirring the solution overnight for complete saturation. The stocks were then passed through a 0.2μ disposable syringe filters (Anotec) and their absorption spectra were taken in order to determine the fluorophore concentration. 2-AN stock absorbances were in the range 0.3-0.5 (1 cm cell) at 340 nm, while pyrene stock absorbances were approximately 0.02 (1 cm cell) at 337 nm. Absorbance of 2,6-MANS stock at 318nm was about 0.8 (1 cm cell). Fluorophore stock solutions were used only on the same day they were prepared.

The desired CDP stock concentration was obtained by calculating the required amount and then adding this known amount of CDP to a known volume of water. This stock was allowed to stir for several hours until all CDP was dissolved.

Solution Preparation:

a) CD monomer solutions were prepared by adding the required, known amount of CD to aliquots of prepared and filtered Py stock solution. The solutions were allowed to stir for several hours for CD to fully dissolve. If CD still remained undissolved in solution, it was not filtered out. Additives, such as KI and *t*-BuOH, were then added directly to these

solutions and the solutions were left standing for at least one hour.

b) The 2,6-MANS solutions were prepared by a 1:10 dilution of 2,6-MANS stock solution. A 1.00 mL aliquot of 2,6-MANS stock was diluted with 1.00 mL of a 1M phosphate buffer (pH = 6-7) and 8.00 mL of deionized water. The desired concentration of CDP (β-CDP, β-CDPL or β-CDPH) was acquired by adding a known amount of CDP directly to the 10 mL solution and allowing it to stir for at least one hour.

Table A: 2,6-MANS solution Preparation

CDP	mL buffer	mL 2,6-MANS stock	mLH2O	γ-CDP(g)	[CDP]
β-CDPL	1.00	1.00	8.00	0.0422	2.04×10^{-3} M
β-CDPH	1.00	1.00	8.00	0.0423	2.05×10^{-3} M
β-CDP	1.00	1.00	8.00	0.0423	2.05×10^{-3} M

c)The 2-AN solutions were prepared by adding various amounts of the β-CDP stock solution to a constant amount of stock 2-AN and then diluting the solution to 10.00 mL with deionized water. The concentrations of β-CDP ranged from 0-1.5 X 10^{-3} M.

Table B: Dilution Table for 2-AN:CDP solutions

standard	mL CDP stock	mL 2-AN stock	
0	0	1.00	
1	1	1.00	
2	2	1.00	(All solutions
3	4	1.00	were diluted to
4	6	1.00	10 mL with de-
5	8	1.00	ionized water).
6	9	1.00	
7	10	0	

The extent of the quenched fluorescence of 2-AN was measured for each of these solutions at various temperatures ranging from 10^{o}-50^{o}C.

d) Typical Py solutions for measurement of I/III ratios and fluorescence lifetime data were prepared as follows:

Table C: Preparation of Py:CDP solutions

$[\beta\text{-CDP}]_{stock} = 4.0 \times 10^{-3}$M

Solution	[β-CDP] (M)	mL β-CDP stock	mL Py stock	
0	0	0	5.00	
1	1.0×10^{-5}	0.025	5.00	(All
2	4.0×10^{-5}	0.100	5.00	solutions
3	2.0×10^{-4}	0.500	5.00	were diluted
4	4.0×10^{-4}	1.000	5.00	to 10mL with
5	8.0×10^{-3}	2.000	5.00	DI H_2O.)
6	1.0×10^{-3}	2.500	5.00	
7	2.0×10^{-3}	5.000	5.00	

These solutions were allowed to stand for at least one hour for equilibration. When additives such as alcohols or potassium iodide were used, known amounts were added directly to fully equilibrated CDP:fluorophore solutions and let stand for an additional hour. The solutions used for the Py I/III ratio data had [β-CDP] ranging over 0 - 2.0 X 10^{-3} M or [γ-CDP] ranging from 1.0 X 10^{-5} M to 4.0 X 10^{-3} M. The effects of alcohols (PFP and TFE at 1%v/v) were also measured using these ranges of [CDP]. In tests in which alcohol concentration was varied and [CDP] was held constant, the amount of alcohol was varied from 0 to 4%. For these experiments, [β-CDP] was held constant at 2.0 X 10^{-3} M and [γ-CDP] was maintained at 5.0 X 10^{-3} M. To observe the effects of I^-, with and without the presence of *t*-BuOH, the [I^-] ranged from 0-0.15 M and *t*-BuOH was fixed at 1%(v/v) when present.

e) β-CD and recrystallized β-CD solutions, used for comparing their solubility behavior over time, were prepared by adding 0.114 g of CD to 10 mL of DI H_2O to obtain a β-CD concentration of 0.010 M in both solutions. Solutions were allowed to stir for at least one hour and then placed in a quartz fluorescence cell for observation over the next 10 days.

Procedure for synthesis of β-CDPs:

Two different β-CD polymers were synthesized by changing the molar ratios of β-CD to EP (1:1 and 1:2).

Table D: Synthesis of β-CD 2:1 and 1:1 polymers

CD Polymer	g β-CDP	mL EP	yield (g)
β-CD (1:1)	5.03	0.344	5.4 X 10^{-3}
β-CD (2:1)	4.96	0.688	3.3 X 10^{-1}

The reactions were carried out in 20% NaOH with around 25-30 wt % of reactants at 50°C for about 3hrs. The reaction mixtures were cooled to room temperature and neutralized with 4 M HCl to pH 7. These solutions were diluted 3 times by pure water. We found this dilution step to be crucial in order to avoid aggregation of solids in the reaction mixture. We then transferred the mixture into dialysis tubing (MW cutoff= 2000 was used for the 1:1 and MW cutoff = 3500 was used for 1:2). The tubing was spun in deionized water over a magnetic plate. The deionized water was changed every 4-5 h for 7-8 times. After five dialysis sessions no Cl^- was detected using $AgNO_3$ addition to the outside solution. After dialysis, the solid polymer was isolated by freeze drying. This procedure was similar to that used by Xu et al.[7]

Results and Discussion

Temperature Dependence of Binding Constant of β-CDP:2-AN

2-Acetylnapthalene (2-AN) is a naphthalene probe which fluoresces only in the presence of a strong hydrogen-bonding environment, such as water.[8,9] Thus, when 2-AN binds to the hydrophobic interior cavity of the CDs, its fluorescence is quenched.[4] The quenching of 2-AN by CD has been determined to be static in nature due to the fact that the fluorescence lifetime, emission wavelength maximum and spectral shape are unaffected by the presence of CD molecules.[4] Therefore, if the 2-AN:CD complex is 1:1, the fluorescence intensity data should conform to the

modified Stern-Volmer equation:

$$Fo/F = 1 + K[CD] \quad (1)$$

In this equation, Fo and F are the intensities at the 2-AN fluorescence emission maximum in the absence and presence of a CD, respectively, [CD] is the CD concentration and K is the binding constant for the 1:1 complex. Therefore, by plotting Fo/F vs. [CD] , K can be obtained from the slope. Obtaining these data at various temperatures allows one to determine such thermodynamic parameters as changes in entropy(ΔS^0) and enthalpy (ΔH^0) by utilizing the van t Hoff plot. The equation for this plot is:

$$\ln K = -\Delta H^0/RT + \Delta S^0/R \quad (2)$$

Thus, by plotting the lnK vs. 1/T, one can obtain the values for ΔS^0 and ΔH^0 from the intercept and slope, respectively.

If the binding of β-CDP with 2-AN is also in a 1:1 ratio, this procedure can be utilized to study the CDP:2-AN binding as well. Our results with β-CDP show a much stronger binding constant (1320 at 294 K) than that which is reported for β-CD (536 at 294 K) (Our results are slightly lower than previous results reported with β-CDP, K = 1425 at 294 K).[11] The average binding constant values at various temperatures are listed in Table II (The individual values are given in Table I; each of which resulted from good linear Stern Volmer Plots). The linearity of the Stern Volmer plots and a y-intercept near unity indicate that 2-AN forms a 1:1 complex with β-CDP.

The average binding constant values at each temperature range were used in equation 2 to produce the van't Hoff plot (Figure 1). Thus, we were

Table I: Individual values for Temperature Dependence of Binding Constants of β-CDP:2-AN

Temp. Range(K)	K(values)	Notebook page
282-283	1489	9
	1465	26
	1365	31
	1406	33
	1429	40
290-291	1342	26
	1518	28
	1322	31
	1319	33
292-293	1331	9
	1323	40
	1262	42
301-302	1234	9
	1344	28
303-304	1181	40
	1112	42
308	1111	9
	1032	26
	1141	28
	1125	31
	1003	33
313	949	40
	983	42
314-315	996	9
	914	26
	1029	28
	1018	31
	958	33
322-323	810	9
	790	26
	925	28
	834	31
	818	33
	818	40
	823	42

Table II: Average K values for β-CDP[a] at various temperature ranges

Temperature(K)	Binding Constant	(+/-)[b]	Intercept	(+/-)
282-283	1460	38	0.91	0.04
290-291	1410	44	0.90	0.04
292-293	1320	27	0.96	0.03
301-302	1310	36	0.95	0.03
303-304	1150	32	0.96	0.03
308	1110	30	0.94	0.03
313	967	46	0.97	0.04
314-315	1020	29	0.91	0.03
322-323	854	23	0.94	0.02

[a] [β-CDP] ranges from 0 - 1.5 X 10^{-3} M.

[b] From Standard deviation of slope of Stern Volmer plot.

Table III: Van't Hoff Plot Data[a] for β-CDP:2-AN complex

slope = 1250 (+/- 137)

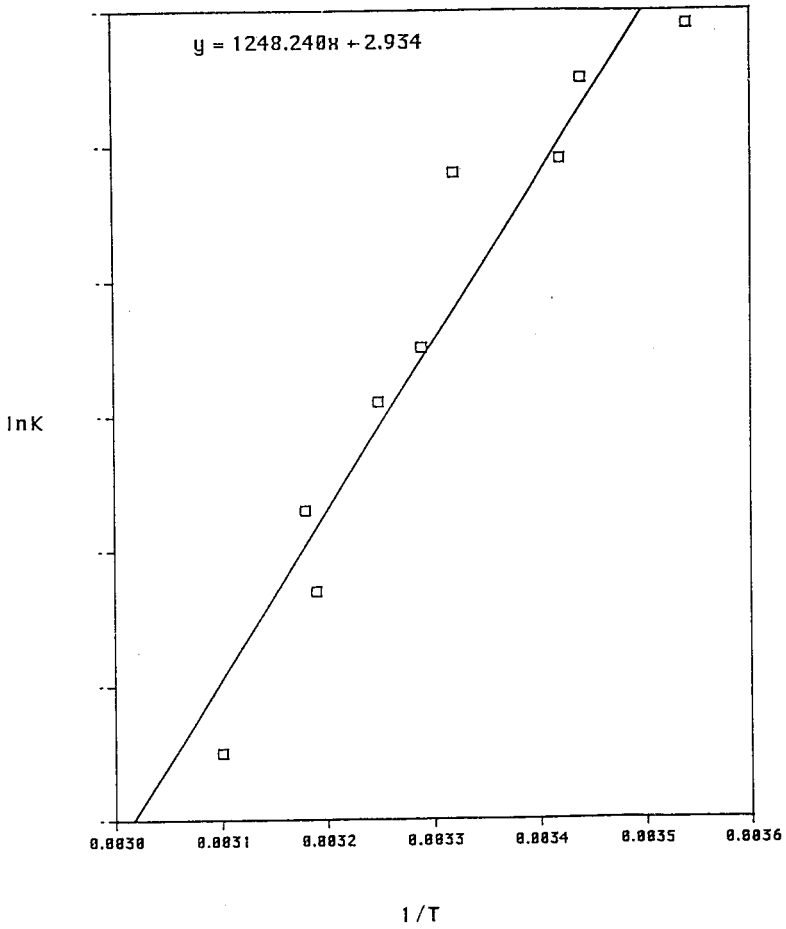
y-intercept = 2.93 (+/- 0.45)

ΔH = -10.4 (+/- 1.0) kJ
ΔS = 24.4 (+/- 4.0) J

[a] Van't Hoff Plot is included in Figure 1.

Figure 1: van't Hoff Plot for ΔH and ΔS values of 2-AN fluorescence (λ_{EM} = 437 nm) quencing with β-CDP.

Figure 1: van't Hoff plot for B-COP



able to obtain values for ΔH^0 and ΔS^0. These data are listed in Table II.

The large increase in the binding constant of β-CDP, compared to the binding constant of β-CD, indicates that the polymer exhibits a different type of binding. Since they share a common β-CD cavity, the only difference must be attributed to the presence of the glyceryl linker units in the polymer. Thus, the linkers must act in some way to enhance and strengthen the binding in β-CDP.

GPC Analysis of β-CDP, β-CDPL and β-CDPH

We wanted to see if the products which were obtained by dialyzing a commercial sample of β-CDP actually contained the high (β-CDPH, MW > 3500) and low (β-CDPL, MW < 2000) molecular weight components. The molecular weight distribution of these polydisperse CD polymers can be seen in the data supplied by Cyclolab (Figure 2). β-CDP displays a low molecular weight peak (<2000) which is due to a single CD unit per polymer chain. A high molecular weight peak at 9-10,000 is most likely due to the polymer which contains 4-5 β-CD units per polymer chain. Thus, β-CDPL, which contains molecular weights < 3500, should exhibit characteristics similar to the low molecular weight components of the commercial β-CDP, while β-CDPH should be expected to resemble the high molecular weight components of the native polymer.

The results (Figure 3) which we obtained using the GPC column were as expected. The native β-CDP chromatogram shows two distinct peaks, one at 12.7 min (I) and the other at 24.2 min (II). The first peak is due to

Figure 2: % Composition (%C) vs. MW in thousands for commercial samples of α-CDP, β-CDP and γ-CDP. Data obtained from Cyclolab.

Figure 2: MW Distribution of CDPS

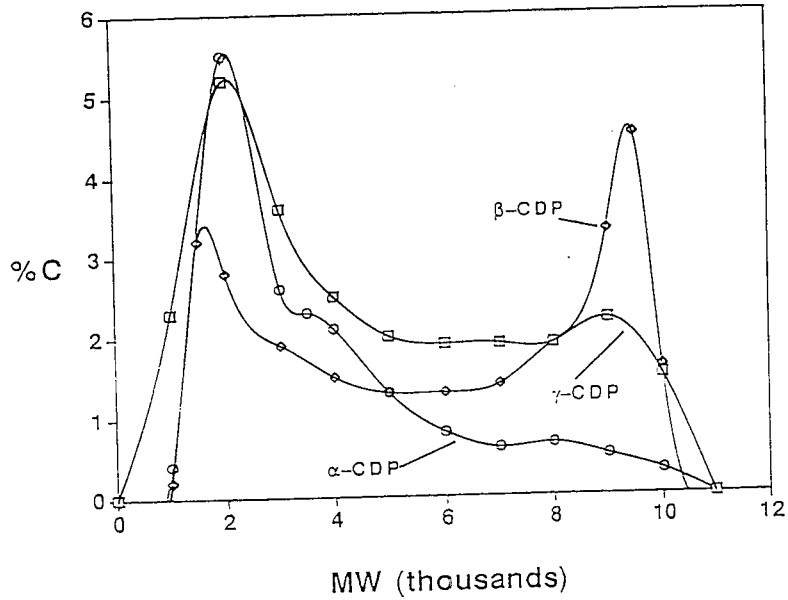
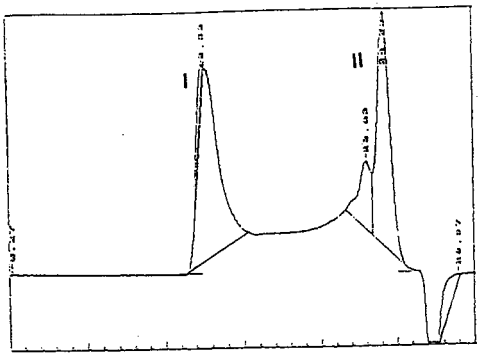


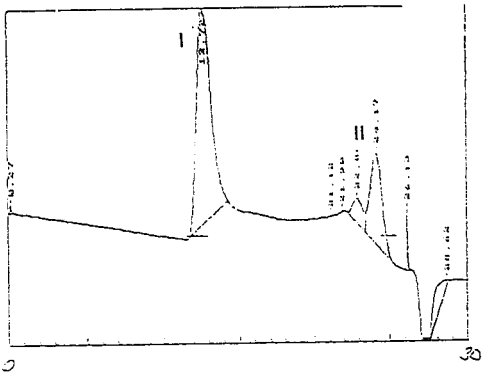
Figure 3: Gel Permeation chromatograph of β-CDP and of the low and high molecular weight components (β-CDPL, β-CDPH) of the polymer.

Low molecular weight β-CDP (β-CDPL)

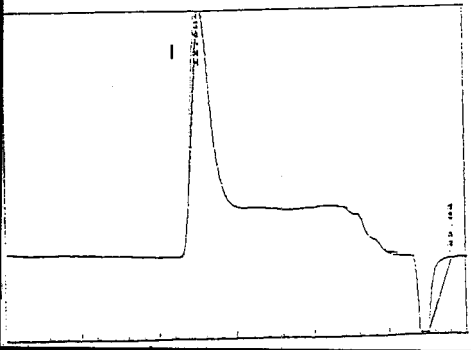
Figure 3



β-cyclodextrin polymer (β-CDP)



High molecular weight β-CDP (β-CDPH)



the high molecular weight components, while the second peak represents the remaining low molecular weight components.

The sample of β-CDPH produced only a peak at 12.6 min (I). Thus, we are convinced that the low molecular components were successfully removed by dialysis. The chromatograph of β-CDPL shows both peaks. However, the second (low molecular weight) peak at 24.2 min (II) was much higher in comparison to the first peak at 12.5 min (I) than is seen in the native β-CDP chromatogram. For example, from the β-CDPL chromatogram we calculated the I/II peak ratio of to be 0.79, while the I/II peak ratio for the native β-CDP was 2.68. Thus, we can be sure that results which we obtain with samples of β-CDPL are more affected by the binding behavior of the low molecular weight component than in the native polymer.

2,6-MANS Fluorescence Behavior in β-CDP

Fluorescence emission spectra were taken for solutions of 2,6-MANS in the presence of 2.0×10^{-3} M β-CDP, β-CDPL and β-CDPH. The wavelength maximum and relative intensities are listed in Table IV. The λ_{max} values are, within error, the same.

Since 2,6-MANS exhibits fluorescence blue shifts and intensification in a more hydrophobic environment, the similarity of these results indicates that the binding of 2,6-MANS with the high and low molecular weight components, as well as with the native polymer, are the same. These data allow us to rule out any chance of cooperative binding which may exist between two CD cavities on the same polymer chain.

β-CDPL and β-CDPH have different numbers of CD units per chain.

Table IV: Fluorescence Behavior of 2,6-MANS in the presence of 2 X 10^{-3} M β-CDP, β-CDPL and β-CDPH

Solution	λ_{max} (nm)	F_{rel}[a]
β-CDPL	431 (+/- 2)	1.07
β-CDP	430 (+/- 2)	1.00
β-CDPH	429 (+/- 2)	1.01

[a] λ_{EM} = 370nm , λ_{EX} = 340 nm

Note: The λ_{max} of 2,6-MANS in the presence of β-CD = 563 nm[11]

Table V: Fluorescence Lifetimes for 2,6-MANS in the Presence of β-CDP[a], β-CDPL and β-CDPH[a]

CDP	τ_1(nsec)[b]	τ_2(nsec)[b]	F_2[b,c]
β-CDP	4.6(0.2)	12.1(0.4)	0.45(0.04)
β-CDPL	4.8(0.2)	12.6(0.4)	0.43(0.03)
β-CDPH	4.4(0.2)	12.1(0.4)	0.50

[a] β-CDP and β-CDPH values are taken from Werner[11]

[b] Uncertainty in parentheses.

[c] F_2 is the fraction of the total fluorescence coming from the longer wavelength component.

The difference in the number of CDs/chain would affect the ability of the two CDs from the same chain to bind the probe. For example, if clam shell type binding were occurring from intrachain CD units, there should be a difference in the extent of binding and in the hydrophobicity of the binding site of 2,6-MANS with β-CDPL and β-CDPH.

The similarity in the fluorescence lifetime data (Table V) argues for similar binding behavior of 2,6-MANS to β-CDP, β-CDPL and β-CDPH. Both β-CDPL and β-CDPH appear to have at least two binding environments, as does the native polymer. The data indicate that the binding of β-CDP is similar among its different molecular weight components but different from that obtained for β-CD. The 2,6-MANS binding may not take place in the interior of the β-CD cavity but with the glyceryl linking units, in the case of the β-CDPs.

Behavior of Pyrene I/III ratios in β-CDP and γ-CDP

The results of the Py I/III ratio data in the presence of varying concentrations of both β-CDP and γ-CDP are listed in Tables VI-VII.

In the case of β-CDP, the I/III ratio decreases from 1.77 in the absence of β-CDP to 1.55 at the highest concentration of β-CDP (5.7×10^{-3} M) (Table VI). Results for γ-CDP are similar, ranging from 1.78 to 1.53 at the maximum γ-CDP concentration (5.7×10^{-3} M) (Table VII). This slight decrease in I/III ratio as the [CD] is increased suggests a slightly more hydrophobic environment for pyrene in the presence of both polymers than in the presence of water. However, this decrease is small when compared to the shift in I/III ratio which is seen for pyrene in the

Table VI: Effect of [β-CD] or [β-CDP] on Pyrene I/III Ratios in the presence of alcohol[b]

[β-CD] (M)[a]	I/III	I/III (PFP)	I/III (TFE)
1.0 X 10-3	1.48	0.42	0.96
2.0 X 10-3	1.39	0.38	0.72
3.0 X 10-3	1.24	0.38	0.72
4.0 X 10-3	1.12	0.38	0.72
5.0 X 10-3	1.05	0.38	0.72
6.0 X 10-3	0.98	0.38	0.72
[β-CDP] (M)			
0	1.78	1.78	1.78
9.7 X 10-6	1.74	1.76	1.68
3.8 X 10-5	1.73	1.73	1.65
1.9 X 10-4	1.64	1.64	1.65
3.9 X 10-4	1.62	1.61	1.65
7.8 X 10-4	1.59	1.59	1.65
9.7 X 10-4	1.58	1.61	1.59
1.9 X 10-3	1.58	1.56	1.60
5.7 X 10-3	1.55[c]		

[a] The β–CD data are taken from Elliot et al., J. Inclu. Phenom. **16**, 99 (1993).

[b] [PFP] = 2.4 $X10^{-2}$ M, [TFE] = 2.5 $X\ 10^{-2}$ M

[c] This data point is taken from TCW Book #2, p.32

Table VII: Effects of [γ-CD] or [γ-CDP] on Pyrene I/III ratios in the presence of PFP[b]

[γ-CD] (M)[a]	I/III	I/III (PFP)
2.0 X 10-3	1.40	1.23
3.0 X 10-3	1.10	0.93
4.0 X 10-3	0.93	0.86

[γ-CDP](M)	I/III	I/III (PFP)
0	1.78	1.78
3.0 X 10-5	1.79	1.74
6.0 X 10-5	1.69	1.65
3.0 X 10-4	1.58	1.58
6.0 X 10-4	1.57	1.54
1.8 X 10-3	1.56	1.51
3.3 X 10-3	1.53	1.55
5.7 X 10-3	1.53	

[a] γ-CD data are taken from Elliot et al., J. Inclu. Phenom. **1 6**, 99 (1993).

[b] [PFP] = 2.4 $X10^{-2}$ M, [TFE] = 2.5 X 10^{-2} M

[c] This data point is taken from TCW Book #2, p.42

Table VIII: Effects of %(v/v) Alcohol on the Pyrene I/III Ratios in presence of β-CDP[a,b]

% Alcohol	I/III(TFE)	I/III(PFP)	I/III(t-BuOH)[c]	I/III(HFP)[c,d]
0	1.58	1.58	1.58	1.58
0.2	1.55	1.54		
0.5	1.55	1.58		
1.0	1.56	1.57		
1.5	1.55	1.55		
2.0	1.59	1.62		
2.6	1.59	1.60		
3.2	1.58	1.62		
4.0	1.57	1.64	1.56	1.68

[a] [β-CDP] = 1 to $2X10^{-3}$M. There is no change in pyrene I/III ratio over this [β-CDP] concentration range.

[b] standard deviation for all values is (+/- 0.02)

[c] No change in I/III ratio is seen over this [β-CDP] range with either *t*-BuOH or HFP

[d] HFP = 1,1,1,3,3,3- hexafluoro-2-propanol

Figure 4: The effect of [β-CD] on the pyrene I/III ratio in the absence and presence of alcohols.

Triangles: β-CD alone

Blank circles: β-CD with 2.5 X 10^{-2} M TFE

Solid circles: β-CD with 2.5 X 10^{-2} M PFP

Plusses: β–CDP alone

Diamonds: β-CDP with 2.5 X 10^{-2} M TFE

Squares: β-CDP with 2.5 X 10^{-2} M PFP

All of the β-CD data are taken from Elliot et al., J. Inclus. Phenom. **16, 99** (1993).

Figure 4: The Effect of [β-CD] on Pyrene I/III ratios in the absence and presence of alcohols.

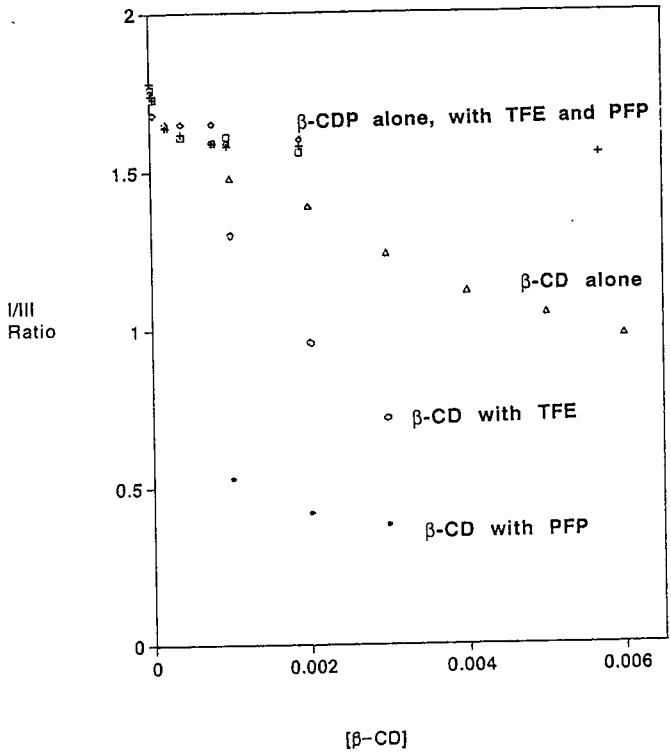


Figure 5: The effect of selected alcohols on the pyrene I/III ratio in the presence of 2.0×10^{-3} M β-CDP.

Figure 5: Effect of Alcohols on Pyrene I/III Ratio in Presence of 0.0020M β-CDP

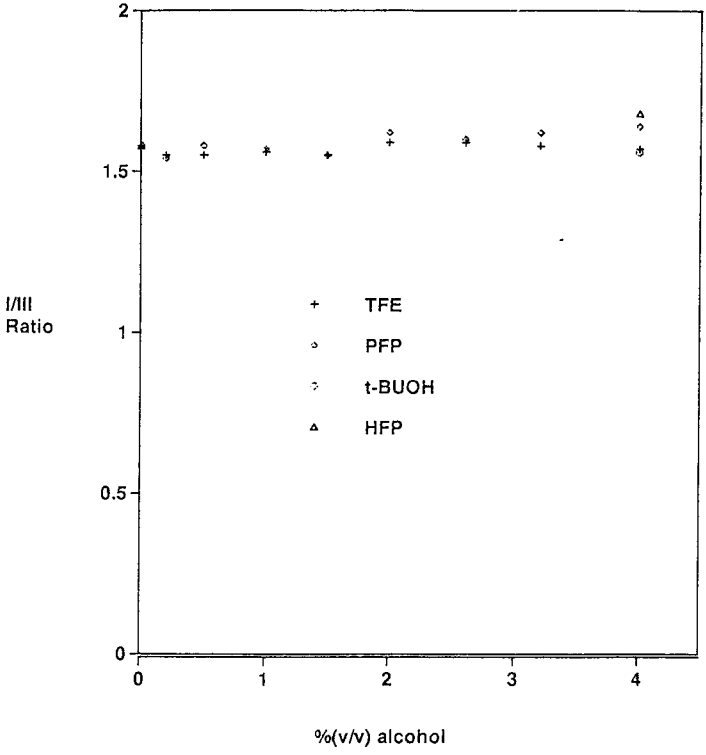


Figure 6: The effect of [γ-CD] on the pyrene I/III ratio in the absence and presence of alcohols.

Blank circles: γ-CD alone

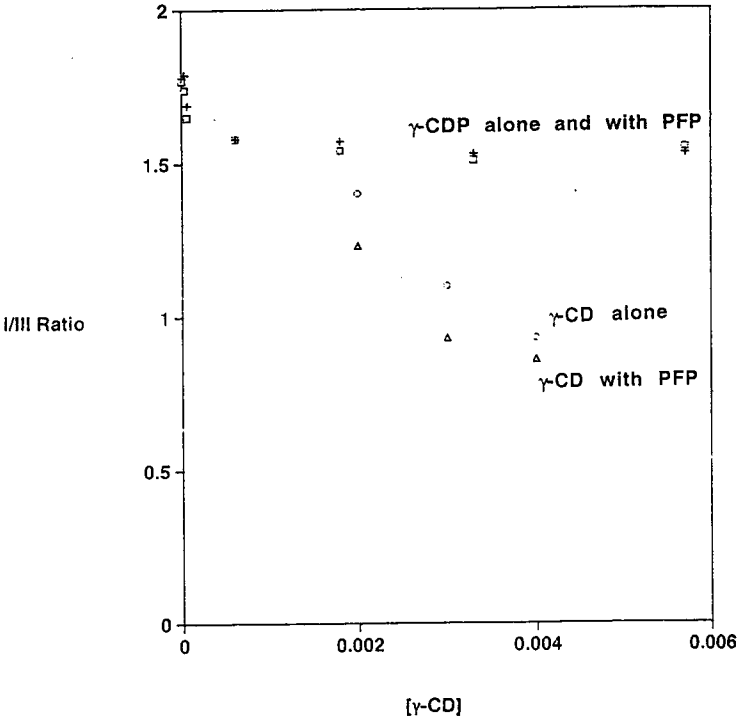
Triangles: γ-CD with 2.5 X 10^{-2} M PFP

Plusses: γ–CDP alone

Squares: γ-CDP with 2.5 X 10^{-2} M PFP

All of the γ-CD data are taken from Elliot et al., J. Inclus. Phenom. **16**, 99 (1993).

Figure 6: Effect of [γ-CD] and [γ-CDP] on Pyrene I/III Ratios in the absence and presence of PFP



presence of their parent monomers (0.98 for 6.0 X 10^{-3} M β-CD and 0.93 for 4.0 X 10^{-3} M γ-CD) in these tables.

Tables VI and VII also show the effects of TFE and PFP on the fluorescence of pyrene bound to β-CDP and γ-CDP. Fluorinated alcohols should have the most pronounced effect on the pyrene I/III ratio if the guests are binding in the CD cavity.[5] However, our results show no significant effect on the I/III ratios with the addition of either alcohol in solutions of both β-CDP and γ-CDP. Upon the addition of 2.5 X 10^{-2} M PFP or TFE, pyrene I/III ratios level off at approximately 1.55 even at very high concentrations of CDPs. In addition, Table VIII, which lists the I/III ratios for pyrene in the presence of β-CDP at various alcohol concentrations, suggests no significant change in the I/III ratio even at very high levels of alcohol. This effect can be seen for various alcohols, TFE, PFP, *t*-BuOH and HFP, in Figure 5. Thus, TFE and PFP, which have such dramatic effects on the pyrene I/III in the presence of CD monomers, exhibit little or no effect on the binding of pyrene to their polymer counterparts. This is especially striking in the case of PFP, where the pyrene I/III ratio is reduced to 0.38 in the presence of 1 X 10^{-2} M β-CD. Figures 4 and 6 show the contrasting results obtained for β-CDP and γ-CDP when compared to their parent monomers in both the absence and presence of PFP and TFE.

The results with the CDPs indicate a different type of binding than that which is occurring for the CD monomers. In the case of the monomers, the pyrene binding is inclusional, and the alcohol, which binds to the hydroxyl groups at the opening of the CD cavity, acts to shield the pyrene from the solvent.[7] Thus, pyrene is contained in a more hydrophobic

environment, which is evidenced by a decrease in the I/III ratios of pyrene. In contrast, our results with the CD polymers show no decrease in pyrene I/III ratios, indicating that the binding site of pyrene is remaining hydrophilic upon the addition of alcohol. This suggests that capping of the CD cavity by alcohol which occurs with CD monomers is not occurring with the CD polymers.

Since the pyrene remains in a rather hydrophilic environment when bound to the CDPs, the binding site is probably not that of the hydrophobic cavity. It appears that pyrene remains in contact with the solvent and thus, is bound by the glyceryl linker units. Therefore, it can be argued that the pyrene molecules are not residing in the cavities of the polymers.

Additionally, the fact that the values of the pyrene I/III ratios in the presence of both β– and γ–CDPs are so similar indicates that their binding sites are much alike. If the binding involved the CD cavities , the I/III ratios would reflect differences in the size of the cavities of β- and γ-CD. Different effects on the microenvironment of Py would be detected by its sensitive vibronic bands. Again, the similar results in the I/III data for the CDPs and the failure of the alcohol to have an effect on the I/III ratios allows one to argue that the binding is with similar portions of the two polymers: the glyceryl linker units.

In addition to these tests, we also carried out similar I/III pyrene ratio tests on the low molecular weight (β-CDPL) and high molecular weight components (β-CDPH) of the native β-CDP. The results are listed along with the other CD and CDP limiting Py I/III values in Table IX.

The limiting I/III values for both β-CDPL and β-CDPH are similar to the limiting value for native β-CDP (1.55). Furthermore, upon the addition of 1% (v/v) PFP to both β-CDPL and β-CDPH, the pyrene I/III ratio is not affected.

Table IX : Limiting values of Pyrene I/III Ratios

CD Environment (TFE, PFP = 2.5 X 10^{-2} M)	I/III Ratio(+/- 0.02)
β-CD	0.62[a]
β- CD + TFE	0.6[b]
β-CD + PFP	0.38[b]
γ-CD	0.82[b]
γ-CD + PFP	0.6[b]
β-CDP	1.55
β-CDP+ TFE	1.60
β-CDP + PFP	1.56
β-CDPH	1.55
β-CDPH + PFP	1.55
β-CDPL	1.52
β-CDPL + PFP	1.56
γ-CDP	1.53
γ-CDP + PFP	1.55

[a] = Munoz de la Pena, Ndou, Zung, Warner, J. Phys. Chem. **95**, 3330 (1991)

[b] = Elliot et al.

β-CDPL contains one CD unit per polymer chain and therefore, intrachain binding to pyrene is an impossibility for β-CDPL. Thus, the binding, which is hydrophilic in nature indicated by the rather high I/III value, must be involving something other than the CD cavity. In addition, the similarity of the β–CDPH results, indicates that the binding is not dependent upon the number of CD units per polymer chain. Once again, this shows that the binding is not with the CD cavity but rather with some other portion of the CD polymers, such as the glyceryl linker units.

Fluorescence Lifetime of Pyrene with CDPs and additives

Results showing the effects of β-CDP concentration on pyrene fluorescence lifetimes are listed in Table X. All solutions contain two lifetime components, which was determined by the lower χ^2 value for the double exponential fit over that of the single exponential fit value. As the concentration of β-CDP is increased, the contribution of the longer lifetime component is enhanced. Thus, we can infer that the second lifetime component at 250 nsec is due to pyrene bound to β-CDP. These results allow us to establish the minimum concentration of β-CDP at which virtually all of the pyrene present in solution is complexed with the β-CDP (F_2 = 0.97 at 2 X 10^{-3} M β-CDP). The concentration for γ-CDP at which most of the pyrene is bound was determined to be 5 X 10^{-3} M γ-CDP (F_2 = 0.99) (Table XIX).

Looking at Tables XIII and XIV for γ-CDP and β-CDP, respectively, you can see that the iodide ion, (I^-), considerably quenches the fluorescence lifetimes of pyrene. As the concentration of the iodide ion is increased,

Table X: Effects of [β-CDP] on pyrene lifetime measurements

(λ_{EX} = 337 nm, λ_{EM} = 395 nm)

Pyrene in Water $\tau_1 = 128$ (+/- 1) nsec

[β-CDP]	τ_1 (nsec)	τ_2 nsec) [a]	F_2 [b]
1.0 X 10-5	116	285	0.24
2.0 X 10-4	127	256	0.68
5.0 X 10-4	146	280	0.76
1.0 X 10-3	29	254	0.97
2.0 X 10-3	92	251	0.97

[a] The double fit gave significantly better $\chi 2$ values than the single fit data for the pyrene:β-CDP complex.

[b] F_2 is the fraction of the fluorescence which is due to the longer lifetime component

Table XI: Fluorescence Lifetime Quenching of Py by Iodide ion, I^-, in presence of β-CD[c]

quencher	*t*-BuOH	$[I^-]$, mM	τ_1(nsec)	τ_2(nsec)	F_2
KI[a]	absent	0		358	
KI[b]	absent	0	141	363	0.861
KI[a]	absent	13	23	350	
KI[b]	absent	15	48	328	0.944
KI[a]	1% (v/v)	38		493	
KI[b]	1% (v/v)	15	139	388	0.973

[a] Values taken from literature (Warner et al. J. Inclu. Phen. 1993, 16, 99).

[b] β-CD values taken from data in Colwell notebook #1, p. 87.

[c] All β-CD solutions = $1X10^{-2}$M.

Table XII: Fluorescence Lifetime Quenching of Py by Iodide ion, I^-, in presence of γ-CD[c]

quencher	*t*-BuOH	$[I^-]$, mM	τ_1(nsec)	τ_2(nsec)	F_2
KI[a]	absent	0		215	
KI[b]	absent	0	94	270	0.896
KI[a]	absent	43	29	130	
KI[b]	absent	27	25	171	0.736
KI[a]	1% (v/v)	16		427	
KI[b]	1% (v/v)	27	157	446	0.837

a Values taken from literature (Warner et al. J. Inclu. Phen. 1993, 16, 99).

b γ-CD values taken from data in Colwell notebook #1, p. 73.

c All γ-CD solutions = 1.0 X 10^{-2}M.

Table XIII: Fluorescence Lifetime Quenching of Pyrene and Pyrene:γ-CDP Complexes by Iodide

quencher	*t*-BuOH	[I⁻], mM	τ_1(nsec)	τ_2(nsec)[b]	F_2
KI[a]	absent	0	100	246	0.946
	absent	25	58	124	0.722
	absent	50	24	66	0.650
	absent	150	17	60	0.465
KI	1% (v/v)	0	42	259	0.967
	1% (v/v)	25	50	109	0.837
	1% (v/v)	50	32	103	0.650
	1% (v/v)	150	14	50	0.607

[a] [γ-CDP] = 5 X 10^{-3} M.

[b] double exponential fit gave signifigantly better χ^2 values than single exponential fits.

Table XIV: Fluorescence Lifetime Quenching of Pyrene and Pyrene:β-CDP Complexes by Iodide

quencher	*t*-BuOH	$[I^-]$, mM	τ_1(nsec)	τ_2(nsec)[b]	F_2
KI[a]	absent	0	92	251	0.974
	absent	25	29	87	0.788
	absent	50	11	53	0.849
	absent	100	15	58	0.588
KI	1% (v/v)	0	44	267	0.972
	1% (v/v)	25			
	1% (v/v)	50	23	100	0.745
	1% (v/v)	100	16	79	0.665

[a] [β-CDP] = 2 X10^{-3} M .

[b] double exponential fit gave signifigantly better χ^2 values than single exponential fits.

Table XV: Comparison of CDs and CDPs in presence of iodide quencher (I^-) and alcohol (*t*-BuOH)

CD	[CD]	[I^-]	[t-BuOH]	τ_1	τ_2	F_2
γ-CD[b]	0.010M			78	268	0.93
γ-CD[b]	0.010M	0.016M		39	251	0.87
γ-CD[b]	0.010M	0.016M	1%v/v	85	385	0.96
β-CD[a]	0.010M			141	363	0.86
β-CD[a]	0.010M	0.015M		48	328	0.94
β-CD[a]	0.010M	0.015M	1%v/v	139	388	0.97
γ-CDP[a]	0.0050M			130	246	0.95
γ-CDP[b]	0.0050M	0.015M		36	98	0.85
γ-CDP[b]	0.0050M	0.015M	1%v/v	64	170	0.57
β-CDP[a]	0.0020M			92	251	0.97
β-CDP[b]	0.0020M	0.015M		31	106	0.89
β-CDP[b]	0.0020M	0.015M	1%v/v	61	176	0.72

[a] Data taken from Colwell Notebook #1 (pgs. 87,66,53, respectively).

[b] Data taken from Colwell notebook #2 (pgs. 7, 8, 14, 13, respectively).

the fluorescence lifetime of the second lifetime component for both γ- and β-CDPs decreases. For example, as the concentration of I^- goes from 0 - 0.100 M, the fluorescence lifetime of the second pyrene component with β-CDP goes from 251 nsec to 158 nsec and, similarly, with γ-CDP this lifetime decreases from 246 nsec to 60 nsec. This indicates that bound pyrene to CDPs is highly exposed to the solvent, and thus, in contact with increasing amounts of iodide ion.

Tables XI and XII show a comparison of the lifetime results, which we obtained with the CD monomers, to the results which are given in literature. Our results are very similar to what was reported by Nelson and Warner.[3] Thus, we can be confident that the results which we obtained for the CDPs are reliable and can be accurately compared to our CD monomer results.

In Table XI, you can see that with β-CD the iodide quenching ($[I^-]$ = 0.015 M) of pyrene fluorescence is not large (τ reduced from 363 nsec to 328 nsec), while pyrene in the presence of γ-CD is more signifigantly quenched (τ reduced from 270 nsec to 171 nsec) by iodide (0.027M) (Table XII). This difference in quenching ability is due to the increased availability which iodide has to the pyrene in the larger γ-CD cavity. β-CD, which forms a strong 2:1 complex with pyrene, does not allow the pyrene as much exposure to the iodide. These differences are not evident in the quenching data of the two polymers, shown in Tables XIII and XIV. This suggests a similar binding environment for pyrene in both β-CDP and γ-CDP, which is different from those in the monomer.

Table XV lists the fluorescence lifetimes of pyrene in the presence of β- and γ-CD, as well as both β-CDP and γ-CDP. It also shows the effects

which KI (0.015M) and alcohol (1%(v/v) *t*-BuOH) have upon the lifetimes of pyrene.

The addition of alcohol to solutions of the CDPs causes only slight enhancement in the pyrene fluorescence lifetimes with both CDPs. This enhancement is much less than that seen for pyrene in CD monomer solutions. With the addition of 1%(v/v) *t*-BuOH, the longer lifetime component of pyrene in β-CD and 0.015 M KI increases from 328 nsec to 388 nsec, while with γ-CD a fluorescence lifetime enhancement from 251 nsec to 385 nsec is observed with the same amount of alcohol added. Once again, the difference observed with the monomers reflects the difference in the size of the cavity.

The enhancement of fluorescence is due to shielding from the solvent which the alcohol affords to the pyrene in the presence of CD monomers. The shielding effects observed with the CDPs are definitely not as great as the protection which alcohol gives to pyrene in the presence of the CD monomers. This variation in protection supports the premise that the binding of pyrene with the CD polymers is different than the binding seen with the CD monomers and is involving the linker units rather than the CD cavity. If the pyrene were bound in the CD cavity, the fluorescence enhancement would be much greater, as is observed with the CD monomers.

The enhanced quenching of pyrene by iodide in solutions of the CD polymers and the minimal effect which alcohol has on the enhancement of these fluorescence lifetimes, can be explained by pyrene binding in a location which is open to the solvent. Once again, this provides evidence for a non-inclusional type binding with the glyceryl linker units.

Behavior of Pyrene I/III ratios with the synthesized β-CD 2:1 and 1:1 polymers

Based on a procedure presented by Xu et al. [7], we were able to synthesize two variations of a β-CD polymer using epichlorohydrin in different molar ratios. The first polymer is a 1:1 β-CD:EP polymer for which there is only one glyceryl linker unit per CD cavity. In addition, we synthesized a β-CD polymer which contains two glyceryl linker units per CD unit (β-CD 2:1).[7] Both of these synthesized polymers have shorter glyceryl linker units than the comm ially available β-CDP, which has an average of 12-15 glyceryl linkers between CD cavities. The synthesized polymers have not yet been analyzed to determine their exact molecular weight. However, based on the synthesis procedure which we followed from Xu et al, we assume that the CD polymers which we obtained are in the ratios of EP to CD units that they report.

We have measured the I/III band ratios of pyrene with both of these polymers in the presence and absence of PFP. Table XVI lists the I/III ratios obtained for various mg/mL solutions of both the 1:1 and 2:1 β-CD polymers. Concentrations can not be listed in molarity until molecular weight data for the two CD polymers are obtained.

The pyrene I/III ratios for both polymers level off at 2.00 mg/mL of β-CD polymer solution. At this concentration of CD polymer, the 1:1 polymer limiting I/III value is 1.17 while the 2:1 levels off at a value of 1.27. These values indicate a trend towards a slightly more hydrophobic environment than observed with the commercial β-CDP (1.55). However, the environment still remains largely hydrophilic when compared to the limiting I/III ratios which are observed for β-CD (0.62) and γ–CD (0.82).

Pyrene in the presence of the synthesized polymers could possibly be binding with the glyceryl linkers as is suggested for pyrene when bound to the native polymer. However, the somewhat lower I/III values with the synthesized polymers suggest that binding may also include the CD cavity, although the double cappping or clam shell arrangement is still clearly not occurring. One possibility is that the pyrene is now binding in a 1:1 stoichiometry with a CD cavity, which is influenced by linker units. It appears that the length of the glyceryl linker units between cavities affects the nature of pyrene binding to the CD polymers.

This is supported by studies using alcohol addition. Upon the addition of 2.5 X 10^{-2} M PFP, the I/III ratio of pyrene in the presence of the 2:1 polymer is dramatically decreased to 0.54, while the 1:1 CD polymer produces a limiting I/III value of 0.50 (Table XVI).

This dramatic change in the I/III ratio indicates that the pyrene is bound in a much more hydrophobic environment upon the addition of alcohol. This suggests that, with a shorter linked polymer, the binding of pyrene is clearly involving the hydrophobic CD cavity to some extent. As the linker units get smaller, the behavior of the polymer begins to resemble that of the CD monomers. Additionally, in the presence of certain alcohols, it appears that the binding of pyrene to the CD polymers can be tuned to a more inclusional type.

Table XVI: Pyrene I/III ratios with 2:1 and 1:1 synthesized β-CD polymer

1:1 β-CDP (mg/mL)	I/III	I/III (PFP)	source[b]
0	1.78	1.74	p. 23-24
0.01	1.79	1.49	p. 23-24
0.05	1.71	0.96	p. 23-24
0.20	1.58	0.63	p. 23-24
0.40	1.47	0.55	p. 23-24
0.50	1.42	0.53	p. 23-24
1.00	1.17	0.50	p. 23-24
2:1 β-CDP (mg/mL)	**I/III[a]**	**I/III (PFP)**	
0.01	1.81		p. 20
0.02	1.73		p. 20
0.05	1.73		p. 20
0.10	1.68		p. 20
0.20	1.63	0.64	p. 21
0.30	1.59		p. 21
0.40	1.53	0.62	p. 21
0.50	1.42		p. 19
1.00	1.36	0.54	p. 19, 22
2.00	1.25	0.54	p. 19, 22
Native β-CDP(mg/mL)	**I/III**	**I/III (PFP)**	
0	1.78	1.78	p. 56, 49
0.12	1.64	1.64	p. 56, 49
0.24	1.62	1.61	p. 56, 49
0.49	1.59	1.59	p. 56, 49
0.61	1.58	1.61	p. 56, 49
1.19	1.58	1.56	p. 56, 49
3.56	1.55 TCW Book #2, p. 32		p. 56, 49

[a] Uncertainty in all ratio data is assumed to be +/- 0.02.

[b] All data is taken from Colwell Notebook #2, pages are listed.

Figure 7: The effect of 1% (v/v) PFP on pyrene I/III ratios in the preence of synthesized 1:1 and 2:1 β-CD polymers.

Blank Circles: Commercially-available β-CDP which has an average of 12-15 glyceryl linker units per CD cavity.

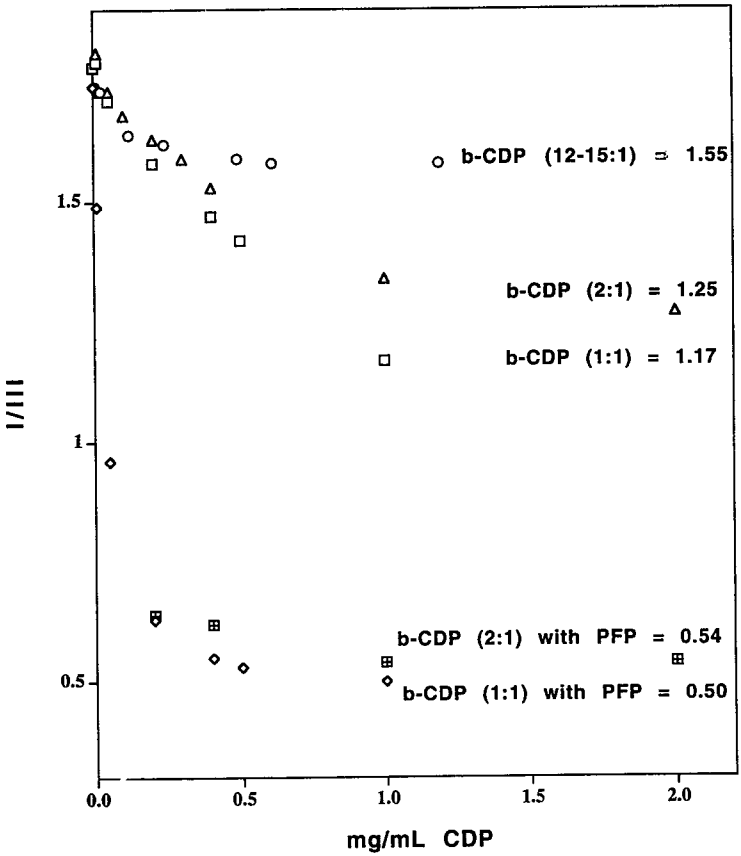
Blank triangles: β-CDP (2:1) alone

Checkered squares: β-CDP (2:1) with 2.5 X 10^{-2} M PFP

Blank squares: β-CDP (1:1) alone

Diamonds: β-CDP (1:1) with 2.5 X 10^{-2} M PFP

Figure 7. Effects of 1% (v/v) PFP on Pyrene I/III ratios in the presence of synthesized b-CD 1:1 and 2:1 polymers



Fluorescence Lifetime Measurements with 1:1 and 2:1 β-CD Polymers

Fluorescence lifetime measurements were taken for both the 1:1 and 2:1 β-CDPs. These results, along with results obtained from the addition of 1% (v/v) PFP, are listed in Table XVII. All solutions contain two lifetime components, determined by a better χ^2 value for the double exponential fit over that of the single fit.

The 1:1 β-CDP gave a fluorescence lifetime for bound pyrene at 269 nsec, while the second lifetime component for 2:1 β-CDP was similar (260 nsec). This fluorescence lifetime value is close, but slightly longer than the value reported for commercial β-CDP (251 nsec). However, upon the addition of PFP to the synthesized polymers, the second lifetime component is dramatically enhanced to 423 nsec for 1:1 β-CDP and 385 nsec for 2:1 β-CDP, while the same amount of PFP addded to regular β-CDP has very little effect on the fluorescence lifetime (267 nsec).

The fact that PFP had such an enhancing effect upon the fluorescence lifetimes of the synthesized polymers, indicates that pyrene binding is involving the CD cavity when certain alcohols are added. This effect is seen only with the short linked polymers; no evidence of CD cavity binding upon the addition of alcohol to regular β-CDP has been observed.

The fluorescence lifetime enhancement for 1:1 β-CDP upon the addition of alcohol was greater than the enhancement seen for 2:1 β-CDP. This suggests that the binding of pyrene in the presence of 1:1 β-CDP is slightly more dependent upon the CD cavity. This can be explained by the shorter linker length of the 1:1 polymer compared to that of the 2:1 β-CDP. The length of the linker units of the 1:1 β-CDP are so short that the

polymer is beginning to act like its CD monomer counterpart.

Thus, the nature of pyrene binding to CDPs is dependent upon the length of the glyceryl linker units. As the length of the linker units decreases, the binding of pyrene to the CDPs begins to involve the CD cavity to a certain degree. Moreover, upon the addition of alcohol, this binding can be tuned to an even more inclusional type of binding.

Table XVII: The effects of PFP on the Lifetime Measurements of pyrene in the presence of 1:1 and 2:1 β-CD polymer[a]

CDP	τ_1	τ_2	F_2
1:1 β-CDP	73	269	0.915
1:1 β-CDP + PFP	144	423	0.973
2:1 β-CDP	118	260	0.575
2:1 β-CDP + PFP	88	385	0.902

[a] 1:1 and 2:1 β-CDP solutions were at concentrations of 2 mg / mL. PFP concentration was 1% v/v = 2.5 $X10^{-2}$ M.

Solubility tests of β-CD vs. recrystallized β-CD

In the process of synthesizing various polymers, we observed that the short-linked 1:1 β-CD:EP polymer aggregated a great deal upon sitting at high concentrations. We found that the dilution step of the synthesis was crucial in avoiding this aggregation and therefore, could not be deleted. Since the 1:1 CD polymer most closely resembles β-CD monomer, it became evident that it would be useful to know the extent of aggregation in normal solutions of β-CD, as well as in more pure, recrystallized β-CD solutions.

The two solutions (0.010M) were observed over a period of days using both fluorescence and absorption measurements. It appears that the regular β-CD seemed to aggregate much quicker than did the recrystallized β-CD (Table XVIII). After one full day of allowing the solutions to sit, the regular β-CD had a scattering intensity of 970 at 600 nm (slit widths = 10 nm), while the recrystallized β-CD was only 233. After a period of 4 days, the aggregation appears to have reached a maximum for both solutions and seems to have leveled off. However, the maximum aggregate level for the recrystallized β-CD was much lower, giving a scattering intensity of 70 while the regular β-CD leveled off at a scattering intensity of 230 (slit widths = 3 nm).

The absorption spectra (250-400nm) were also observed. The initial spectra of the recrystallized solution has a broad range of absorption from 250-310 nm with little absorption beyond this. However, over time, the range of absorption broadened to include a range which extended up to 350 nm. This increase in absorption is due to aggregation occurring in the solution.

The absorption spectra of the uncrystallized β-CD was much less resolved and showed no distinct peaks. It showed a very high absorption at 250nm which decreased to about 400 nm. This indicates that there is much more interference aggregation absorption occurring in the uncrystallized β-CD solution compared to the recrystallized β-CD solution. From this information, it seems clear that in the future testing should be carried out with the more pure and stable, recrystallized β-CD monomer.

Table XVIII: Fluorescence data based on the solubilities of β-CD and recrystallized β-CD over time

Date/Time	β-CD	Recrystallized β-CD
Fluorescence was measured at λ = 600nm and slit widths = 10 nm		
2-21 / 2:00pm	37	9
2-22 / 10:00am	258	77
2-22 / 3:30pm	970	233
2-23 / 10:00am	999+	440
	At this point, slit widths were switched to 3 nm	
	265	60
2-24 / 2:00pm	212	50
2-28 / 9:00am	205	50
3-2 / 2:30 pm	230	70

Conclusion

The results which we have obtained from the work with all three of the fluorescence probes indicate that the binding of pyrene to the CDPs is much different than that to the CDs. They suggest a binding site which is more hydrophilic and non-inclusional and which binds pyrene more strongly than the monomer CD site.

The 2-AN probe has led us to conclude that the binding to the polymers has a much greater K value than that for the monomers, which indicates that the binding site is different. The 2,6-MANS data, carried out with the high and low molecular weight components of β-CDP, allow us to rule out the possibility of a cooperative binding of CD cavities on the same polymer chain, which was proposed by Xu et al.[7] In addition, the fluorescence studies with pyrene are useful in establishing the similarities in binding exhibited among the various polymers, despite the large differences which their parent monomers exhibit.

The CDP binding appears to involve the glyceryl linker units in such a way that they hold the guest molecule strongly and only minimally protect it from exposure to the solvent. This binding can be stronger and more favorable than binding which may occur in the CD cavity. Moreover, the pyrene I/III data, which we obtained from the synthesized β-CD polymers, suppports that CDP binding is also dependent on the length of the glyceryl linker units. Thus, guest molecule binding in the CD cavity of CDPs does occur to some extent when the linker units of the CD polymers are substantially decreased.

All in all, we present a solid argument that the commercially available CDPs show non-inclusional binding which is stronger, and more hydrophilic than their monomer counterparts. Moreover, the nature of CDP

binding with pyrene exhibits a dependence upon the length of the glyceryl linker units and at certain linker lengths, this binding can be tuned to a more inclusional type upon the addition of alcohol.

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Table XIX:

Pyrene Lifetime Data Summary

CD	[CD]	[I⁻]	[t-BuOH]	τ1	τ2	f2	Done by:
none[1]	—	—	------	128	----	----	TCW, Bk 2, page 38
none	—	0.0157M	------	39	----	----	KC Bk 2, page 5
none	—	0.0161M	------	38	----	----	KC Bk 2, page 5
α-CD[2]	0.00852M	—	-----	132	----	----	TCW, Bk 2, page 7
β-CD[3]	0.00805M	—	-----	140	321	0.573	TCW, Bk. 2, page 46
β-CD[3]	0.00988M	----	-------	141	363	0.861	KC, Bk 1, page 87
β-CD[5]	0.00988M	0.015M	--------	48	328	0.94	KC, Bk 1, page 87
β-CD[5a]	0.00988M	0.015M	1%(v/v)	139	388	0.97	KC, Bk 1, page 87
γ-CD[4]	0.00803M	----	--------	128	250	0.700	TCW, Bk 2, page 46
γ-CD[4]	0.010M	----	-------	94	270	0.896	KC, Bk 1, page 73
γ-CD	0.010M	-----	-------	78	268	0.93	KC, Bk 2, page 7
γ-CD	0.010M	0.016M	--------	39	251	0.87	KC, Bk 2, page 7
γ-CD	0.010M	0.016M	1(v/v)%	7	300	0.89	KC, Bk 2, page 7 (see below for better values)
γ-CD	0.010M	0.0167M	1(v/v)%	71	386	0.96	KC, Bk 2, page 8
γ-CD	0.010M	0.0160M	1(v/v)%	99	384	0.97	KC, Bk 2, page 8
γ-CD[6]	0.010M	0.027M	--------	25	171	0.736	KC, Bk 1, page 73
γ-CD[6a]	0.010M	0.027M	1%(v/v)	157	446	0.84	KC, Bk 1, page 73
α-CDP	0.0056M	-----	-------	100	250	0.95	TCW, Bk 2, page 36

CD	[CD]	[I⁻]	[t-BuOH]	τ_1	τ_2	t_2	Done by:
α-CDP	0.0056M	0.015M		35	105	0.85	TCW, Bk 2, page 37
α-CDPH	0.0067M	----	------		244		TCW, Bk 2, page 7
(Get 255, 241, 246 for 0.0069M α-CDPH in TCW, Bk 1 , page 151. Data all have low S/N ratio.)							
β-CDP	1.0 X10^{-5}M	----	-----	116	285	0.24	KC, Bk 1, page 53
β-CDP	2.0 x 10^{-4}M	----	------	127	256	0.68	KC, Bk 1, page 53
β-CDP	5.0 x 10^{-4}M	----	-------	146	280	0.76	KC, Bk 1, page 53
β-CDP	1.0 x 10^{-3}M	------	------	29	254	0.97	KC, Bk 1, page 53
β-CDP	1.86 x 10^{-3}M	--------	---------	4.9	241	0.97	TCW, Bk 2, page 37
β-CDP	2.0 x 10^{-3}M	----------	---------	92	251	0.97	KC, Bk 1, page 53
β-CDP	2.0 x 10^{-3}M	0.015	----------	31	106	0.89	KC, Bk 12, page 13
β-CDP	2.0 x 10^{-3}M	0.015	1%	61	176	0.72	KC, Bk 12, page 13
β-CDP	3.88 x 10^{-3}M	----------	---------	---	253		TCW, Bk 2, page 37
β-CDP	5.66 x 10^{-3}M	----------	----------	9	250	0.98	TCW, Bk 2, page 37
β-CDP	0.0020M	0.025M	----------	29	87	0.79	KC, Bk 1, page 83
β-CDP	0.0020M	0.050M	----------	11	53	0.85	KC, Bk 1, page 84
β-CDP	0.0020M	0.050M	1%(v/v)	23	100	0.75	KC, Bk 1, page 84
β-CDP	0.0020M	0.100M	---------	15	58	0.59	KC, Bk 1, page 84
β-CDP	0.0020M	0.100M	1%(v/v)	16	79	0.66	KC, Bk 1, page 84
β-CDP	0.0056M	0.015M	---------	29	97	0.39	TCW, Bk 2, page 37
β-CDPH	0.00196M	----	----------	1	240	0.99	TCW, Bk1, page 133

CD	[CD]	[I⁻]	[t-BuOH]	τ_1	τ_2	I_2	Done by:
β-CDPH	0.0020M	---	----------	111	250	0.95	KC, Bk 1, page 60
β-CDPL	0.0020M	---	----------	139	251	0.94	KC, Bk 1, page 60
γ-CDP	0.0049M	---	----------	130	246	0.95	KC, Bk 1, page 66
γ-CDP	0.0056M	---	----------	35	245	0.99	TCW. Bk 2, page 39
γ-CDP	0.0057M	0.0149M	----------	34	95	0.88	TCW, Bk 2, page 40
γ-CDP	0.0050M	0.015M	----------	36	98	0.85	KC, Bk 2, page 14
γ-CDP	0.0050M	0.015M	1%(v/v)	64	170	0.57	KC, Bk 2, page 14
γ-CDP	0.0050M	0.025M	----------	58	124	0.72	KC, Bk 1, page 79
γ-CDP	0.0050M	0.025M	1%(v/v)	50	109	0.84	KC, Bk 1, page 79
γ-CDP	0.0050M	0.050M	----------	24	66	0.65	KC, Bk 1, page 79
γ-CDP	0.0050M	0.050M	----------	19	57	0.76	KC, Bk1, page 82
γ-CDP	0.0050M	0.050M	1%(v/v)	32	103	0.58	KC, Bk 1, page 79
γ-CDP	0.0050M	0.050M	1%(v/v)	22	70	0.72	KC, Bk 1, page 82
γ-CDP	0.0050M	0.150M	----------	17	60	0.46	KC, Bk 1, page 79
γ-CDP	0.0050M	0.150M	1%(v/v)	14	50	0.61	KC, Bk 1, page 79

(1) 130 by Xu et al., JPC 97, 6546 (1993); 136 by Nelson and Warner, JPC 94 , 576 (1990).

(2) 130 by Xu et al.

(3) 358 in 0.010M β-CD by Nelson and Warner; 130, 300 by Xu et al. (I_2 depends on conc.)

(4) 215 in 0.10M γ-CD by Nelson and Warner

(5)23 and 350 with A_1/A_2 = 0.319 for 0.010M β-CD and 0.013M KI in Nelson and Warner

(5a) 493 for 0.010M β-CD, 0.038M KI and 1%(v/v) t-BuOH in Nelson and Warner

Note: KI is almost three times larger in 5a than in 5.

(6) 29, 130 with A_1/A_2 = 1.302 for 0.010M γ-CD and 0.0432M KI in Nelson and Warner

(6a) 427 for 0.010M γ-CD, 0.016M KI and 1%(v/v) t-BuOH in Nelson and Warner

Note: Rezik Agbaria gets three-exponential fits for pyrene with all three CDPs. The longest component is about 230 nsec in each case and f_3 is >0.8 for all three.