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Synthesis and Characterization of β-Cyclodextrin Polymers

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SYNTHESIS AND CHARACTERIZATION OF β-CYCLODEXTRIN POLYMERS

By

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ABSTRACT

Cyclodextrin polymers (CDPs) are water soluble polymers composed of either α, β, or γ cyclodextrin (CD) monomers. These commercially available polymers are synthesized using epichlorohydrin and consist of CD monomers joined by repeating glyceryl linkers (-(CH_2-$CHOH$-CH_2-)$_n$) with an average n value of 12-15. GPC analysis of these polymers indicate two major component peaks that have molecular weights (MW) of 2,000 (one CD/ polymer chain) and 9-10,000 (4-5 CDs/polymer chain). These polymers have been used to study the binding interactions of various fluorescence probes. It has been shown that the pyrene fluorescence lifetime increases and its emission I/III ratio decreases in the hydrophobic CD cavity. In addition, it has been reported that pyrene exists in a more open, hydrophilic environment when bound to the CDPs than that observed with the CDs. We have used these fluorescence properties to study the binding of pyrene to our synthesized β-CDPs with shorter linker units. We have shown that as the MW of the synthesized β-CDPs increases (increase in length of linker units), the pyrene I/III increases and the fluorescence lifetime decreases, indicating a more hydrophilic environment. Competitive experiments involving both β-CD and commercial β-CDP indicate that pyrene has a strong affinity for the commercial β-CDP despite the enhanced hydrophobic environment when complexed with β-CD. We have calculated a K value for the 1:1 β-CDP:pyrene complex to be 1.9×10^3 using competitive binding experiments.

We have also synthesized a polymer using EP and sucrose to produce a polymer that contains linker units but no CD cavities. This polymer causes a decrease in the pyrene I/III ratio and an increase in the fluorescence lifetime, indicating that the linker units are involved in the binding of pyrene. Furthermore, competition experiments involving β-CD and the sucrose polymer also indicate some interaction of the linker units. Hence, these data are further evidence to support pyrene binding to the linker units of the commercial β-CDP. We conclude that the binding occurring between pyrene and the commercial β-CDP involves both the linker units and the CD cavities.

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Introduction

Cyclodextrins (CDs) are cyclical carbohydrates which are composed of glucopyranose units joined by α-1,4 linkages. They are torroidal in shape with a hollow interior cavity which is hydrophobic relative to its hydrophilic exterior. Because of their physical structure, cyclodextrins are known to form inclusion complexes with various organic molecules in aqueous solutions.

Three CDs that are commonly studied are α-CD, β-CD and γ-CD, which have 6, 7, and 8 glucopyranose units, respectively. They have internal cavity diameters of 0.57 nm for α-CD, 0.78 nm for β-CD, and 0.95 nm for γ-CD.[1] These CDs also vary in their solubilities, with β-CD being the least soluble and unfortunately the most versatile of the three cyclodextrins.

In order to increase the solubility of cyclodextrins, water soluble CD polymers (CDPs) have been synthesized. The most common procedure involves the use of epichlorohydrin, which produces CD units joined by repeating glyceryl linkers (-(CH_2-CHOH-CH_2-)$_n$), where n= 12 - 15.[2] In addition to β-CDP, α-CDP and γ-CDP are also commercially available, and all are highly water soluble.

There have been many studies involving the use of fluorescence probes to study the binding by the CD monomers as well as by the commercially available CDPs. Pyrene was chosen as our guest molecule because its fluorescence vibronic structure and fluorescence lifetime are very sensitive to its microenvironment.[3] Specifically, pyrene has a I/III (373 nm/384 nm) vibronic band ratio which is affected by the polarity of its

surroundings. For instance, when pyrene is in water, the I/III ratio is about 1.80, but, when pyrene is in a non-polar solvent like cyclohexane, the I/III ratio is decreased to 0.60.[3,4] A I/III ratio of 0.60 is also observed when pyrene is in the presence of 5×10^{-3} M β-CD.[3,4] This value indicates that pyrene is in a very hydrophobic environment in the presence of β-CD. This fact has been used to suggest that pyrene, which is too large to fit completely into a single β-CD cavity, forms a 2:1 β-CD:pyrene complex in which the pyrene is doubly capped by two β-CD molecules in a "clam shell" arrangement.[3,4] In addition, the hydrophobicity of the pyrene environment is increased even more so upon the addition of alcohols. It has been suggested that alcohol forms a ternary complex with pyrene and CD and "caps off" the opening of the CD cavity by binding to the hydroxyl groups on the exterior of the cavity lining.[5]

The fluorescence lifetime of pyrene in water has been reported to be approximately 130 nsec.[4] When pyrene is in the presence of β-CD, the decay data are fit to a double exponential of which one lifetime component is always near that of pure pyrene (130 nsec), while the other is significantly longer (300 nsec).[4] This longer lived component arises from the interaction of pyrene with the CD cavity. This enhancement of the lifetime suggests that pyrene is bound in the hydrophobic interior of the CD cavity where it is protected from the quenching effects of the solvent.[4]

β-CDPs have been synthesized by Xu et al. starting with a range of mole ratios of epichlorohydrin to β-CD.[4] They have shown a range of pyrene I/III ratios: 1.6 and 0.63 for the 8:1 and 1:1 EP:β-CDP, respectively. In addition, they claim that the I/III values are dependant on the starting molar ratio of reactants. Our work has focused on the synthesis of β-CDPs which have shorter glyceryl linkers than the commercial polymers and on observing their binding behavior when combined with pyrene. In addition, we have

synthesized a polymer which contains gyceryl linker units, but no CD cavities. This polymer was synthesized to access the role of the linker units in the binding of pyrene to the commercial β-CDP. It is obvious that analytical characterization of these synthesized polymers is also an important goal.

Initially, the synthesis procedure was taken directly from Xu et al.[4] However, this procedure has been modified using a procedure from Cyclolab R&D Laboratory Ltd. because initial mass yields were low and the resulting products appeared to be largely unreacted β-CD.

To date, we have focused our study on synthesis refinement and the resulting polymer characterization through molecular weight determination and spectral analysis.

In addition, we have attempted to determine the % CD in β-CD samples with the intent to use the perfected technique on the synthesized polymers.

We have also observed the competitive binding effects of the β-CD monomer and the commercial β-CDP for pyrene.

Experimental

Instrumentation

Absorption spectra for pyrene stock solutions were recorded using a Hewlett-Packard 8452A Diode Array Spectrophotometer. Absorption spectra were collected over a range of 300-400 nm.

Fluorescence spectra were recorded using a Perkin-Elmer Lambda 5B Spectrofluorometer. This instrument automatically corrects emission spectra for the wavelength dependence of the emission monochrometer and detector

combination. Corrected emission spectra were collected using an excitation wavelength of 320 nm, over an emission range of 350-500 nm using excitation and emission slitwidths of 3 nm. Plots of the spectra were recorded on a Perkin-Elmer R100 Recorder.

Fluorescence lifetime data were obtained with a LS 100 fluorescence lifetime system from Photon Technology International, Inc. The excitation and emission wavelengths employed for pyrene were 337 nm and 393 nm, respectively. The lifetime 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 chosen if a double exponential fit gave no improvement in the $\chi 2$ parameter. Spectra were recorded on a Hewlett Packard Color Pro Plotter. All measurements were made at room temperature (23°C).

GPC data were obtained using a Perkin-Elmer Gel Permeation Chromatograph with a PE LC 30 RI detector. The column was a Perkin-Elmer TSK G2000SW column, which is to be used for molecules in the 500-100,000 molecular weight range. The mobile phase was initially water containing 3% methanol and was later changed to 0.10 M acetic acid/acetate buffer (pH 4) containing 3% methanol. The chromatographs obtained were always compared at the same flow rate on the same day. The flow rate of the mobile phase was initially 0.45 ml/min and then had to be decreased to 0.40 ml/min when we were exceeding the maximum column pressure of 300 psi. We reduced the pressure build-up by rinsing the guard column. When the pressure returned to a constant value of 230 psi., the flow rate was returned to 0.45 ml/min.

Occasionally, there would be air bubbles in the cell compartments of the RI detector which would prohibit us from obtaining a chromatogram

with a straight baseline. In order to correct for this problem, 2-3 ml of isopropyl alcohol were rinsed through the detector before performing any runs for the day. The isopropyl alcohol was directly injected through the detector with a syringe.

Materials

The water used in all experiments was deionized, doubly distilled and passed through a Millipore Milli-Q Water System. Pyrene (99+%), epichlorohydrin (EP), 2,2,3,3,3 pentafluoro-1-propanol (PFP) were obtained from Aldrich Chemical Company, Inc., and the β-CD was a gift from the American Maize-Products Company. The pyrene was recrystallized twice from ethanol, while the β-CD was recrystallized twice from water.

The commercial β-CDP was 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-CD), 55(β-CD), and 57(γ-CD). Gel Permeation Chromatography (GPC) data from Cyclolab show a broad range of molecular weights for these polydisperse CD polymers up to a maximum of about 11,000. For β-CDP, the GPC data show two major peak components at about 2,000 and 9-10,000; the former is due to polymers which contain a single CD unit per polymer chain, while the latter contains 4-5 CD units per polymer chain.[6]

Solutions

Stock solutions of pyrene were made by adding a small amount of solid pyrene to water and stirring the solution overnight. The stock solution was then passed through a 0.2 μm disposable syringe filter (Anotec). This step was later omitted because new 0.2 μm disposable filters from a different supplier extracted pyrene out of solution, resulting in low stock concentrations. The absorbance of the pyrene stock solution would typically be between 0.01 and 0.02 (1 cm cell) at 334 nm, which corresponds to a pyrene stock concentration of about 2×10^{-7} M. Pyrene stock solutions were only used on the day they were prepared.

Stock solutions of 2-(N-methylanilino)-naphthalene-6-sulfonic acid, sodium salt (2,6-MANS) were made by adding a small amount of solid to 0.1 M phosphate buffer, pH 6-8, and stirring the solution overnight. Solutions for fluorescence measurements were prepared by diluting the stock with 0.1 M phosphate buffer, pH 6-8, to give absorbances of 0.02 - 0.05 (1 cm cell) at the exciting wavelength of 350 nm.

The required β-CD or β-CDP concentration was obtained by adding a weighed amount of solid β-CD or β-CDP to a known volume of pyrene (or 2,6-MANS) stock solution. Solutions were stirred and allowed to stand for several hours before measurements were obtained to ensure equilibration. PFP additions were delivered by volume to a known volume of pyrene stock solution.

Solutions for GPC analysis were prepared by adding a known weight of β-CD or β-CDP to a known volume of water. The solutions were then allowed to stand until all the solid was dissolved. The resulting solution was then passed through a 0.2 μm disposable syringe filter (Anotec). Polymer

concentrations ranged from 0.0 - 25.0 mg/ml, depending on the synthesized polymer.

Synthesis Procedure of β-CDP's

We initially followed a procedure from Xu et al. which involved adding 31% weight % of β-CD to a 20% (wt/vol %) NaOH solution.[4] The required amount of EP was added all at once when all the β-CD was dissolved to avoid reaction with itself. The temperature was then increased to 50°C, and the solution was left to react for three hours. Once cooled, the mixture was neutralized to a pH of 7, diluted to three times the volume, and dialyzed in molecular weight cut off tubing of 3500 for 6-7 changes of water or until no Cl^- was observed upon the addition of Ag^+. The polymer was then recovered by freeze drying. Xu et al. report product yields of 30-56%.[4]

Our synthesized polymers which resulted from this procedure will be discussed in the following sections and are designated as follows: XX% (Y:1) β-CDP, where XX% is the % β-CD by mass/volume in the reaction mixture and (Y:1) is the molar ratio of EP/β-CD in the reaction mixture.

Determination of % CD in β-CD

Before we are able to analyze the % CD in the synthesized polymers, we had to perfect the following technique with β-CD. The method that we followed for the determination of % CD was obtained from the Cyclolab Cyclodextrin Research and Development Laboratory. The process begins with a two hour hydrolysis of 30 mg of sample, which breaks the β-CD into seven

glucose units with the addition of 1 M HCl. The solution is then neutralized (methylred indicator) with 2 M NaOH before the addition of excess 0.1 N iodine solution. The solution is then left to stand in the dark for one hour. After this period, the solution is acidified with 0.5 M H_2SO_4. The excess iodine is then back titrated with 0.1 N $Na_2S_2O_3$ using a starch indicator. Each run was performed in triplicate.

Our initial results yielded averages of 61 and 181 % CD. We devised a procedure which started with 100 mg of sample, increased hydrolysis to 2 1/2 hours, and increased reaction time with iodine to about 1 1/2 hours. These modifications results in more reasonable accuracy, 82-102 % CD, but the precision measured by standard deviation varies from 3 to 10 %. Further modifications of this procedure for determining % CD have been delayed until a later time.

Results

Chromatograms of β-CD and commercial β-CDP are shown in Figure 1.

Competitive Binding Study of β-CD and β-CDP for Pyrene

We conducted a study of the competitive binding that occurs when pyrene is in the presence of both β-CD and commercial β-CDP. This experiment was conducted to get an idea of the binding strength of pyrene to β-CDP. A binding constant has been determined for the 2:1 β-CD:Py complex to be 6.67×10^4. [5]

Nine solutions were prepared containing varying concentrations of β-CD as shown in Table I, and the I/III ratio was measured for each. Solid β-CDP was then added to each solution to a concentration of 2.5×10^{-3} M. This β-CDP concentration was chosen because, in solutions containing only β-CDP, the pyrene I/III ratio levels off at this value and doesn't change at β-CDP concentrations above this value. The I/III ratio was measured again, and the values are listed in Table I. Two separate experiments were performed, and the data were averaged. In addition, plots of the pyrene I/III ratio vs. [β-CD] in the absence and presence of β-CDP are shown in Figure 2.

As shown in Figure 2, as the [β-CD] increases, the I/III ratio decreases to a value of 0.82 at a β-CD concentration of 0.010 M. In the presence of 2.5×10^{-3} M β-CDP, the same trend is observed and the I/III ratio decreases with increasing [β-CD]. However, the limiting I/III ratio is much higher at 0.010 M β-CD in the presence of the β-CDP than in its absence (1.12). Apparently, a significant fraction of pyrene is still bound to β-CDP even at a β-CD concentration of 0.010 M, which is about 60% of the β-CD solubility limit.

In order to quantify the relative binding between β-CD and β-CDP for pyrene, the % pyrene bound to β-CD (%Py:β-CD) was calculated. First, we have determined the limiting I/III values for pyrene in water and in the presence of β-CD (~0.015 M) to be 1.75 and 0.78, respectively. The limiting I/III ratio in the presence of β-CD was verfied by taking fluorescence measurements of duplicate saturated solutions. We can then calculate the % Py:β-CD at a given [β-CD] in the absence of β-CDP using the following equation:

$$\% \text{ Py:β-CD} = \frac{(1.75 - (I/III)_{[\beta\text{-CD}]})}{(1.75 - 0.78)} \times 100. \qquad (1)$$

In the presence of β-CDP, the limiting I/III value is 1.55 if all the pyrene is bound to β-CDP and 0.78 if all is bound to β-CD. We can then write the following equation for the % Py:β-CD in the presence of β-CDP:

$$\% \text{ Py:β-CD} = \frac{(1.55 - (I/III)_{[\beta\text{-CD}]})}{(1.55 - 0.78)} \times 100. \qquad (2)$$

The calculated values for percent pyrene bound to β-CD are listed in Table II and show that when β-CDP is added at a concentration of 2.5×10^{-3} M to a solution containing β-CD (3.0×10^{-3} M - 1.0×10^{-2} M), the percent of pyrene bound to β-CD decreases by 50% or more. These results indicate that although pyrene's affinity for β-CD is large, pyrene's affinity for β-CDP is quite significant, despite the fact that binding with β-CDP may be significantly non-inclusional.

In addition, when PFP was added to the solutions containing β-CD and commercial β-CDP to a concentration of 2.5×10^{-2} M, a decrease in the measured I/III ratios was observed. It is important to note the resulting I/III ratios are similar to those observed when PFP is added to solutions containing only β-CD at [β-CD] > 0.005 M. The I/III values are listed in Table I, and a plot of the data in the presence of PFP is shown in Figure 3.

Estimation of the Pyrene (P):β-CDP Binding Constant (K')

From the competitive binding experiment, we have estimated a binding constant for the 1:1 β-CDP:pyrene complex (K') using the 2:1 β-CD:pyrene clam shell system (K) to be 1.9×10^3. The following assumptions must be made in order to perform the calculations:

1. The K value for the binding of pyrene to β-CD in the clam shell arrangement is 6.67×104.[5]

2. The concentration of the hosts ([CD], [CDP]) are in large excess over that of the guest ([P]). As a consequence, the equlibrium concentrations of the hosts are equal to their initial concentrations ($[CD]_o$,$[CDP]_o$).

3. The complex stoichiometry for the β-CD/pyrene complex is 2:1 ($P(CD)_2$) but 1:1 for the β-CDP/pyrene (PCDP).

Using these assumptions, we have derived the expression for K'.

Equilibrium:

$$P + 2CD \rightleftharpoons P(CD)_2 \qquad P + CDP \rightleftharpoons PCDP$$

$$K = \frac{[PCD_2]}{[P][CD]^2} = \frac{[P(CD)_2]}{[P][CD]_o^2} \qquad K' = \frac{[PCDP]}{[P][CDP]} = \frac{[PCDP]}{[P][CDP]_o}$$

$$\frac{K'}{K} = \frac{[PCDP]}{[P][CDP]_o} \times \frac{[P][CD]_o^2}{[P(CD)_2]} = \frac{[CD]_o^2}{[CDP]_o} \times \frac{[PCDP]}{[P(CD)_2]}$$

$$K' = \frac{[CD]_o^2}{[CDP]_o} \times \frac{[PCDP]}{[P(CD)_2]} \times K \qquad (3)$$

The ratio $[PCDP]/[P(CD)_2]$ is calculated from the pyrene I/III emission ratio data. When $[CD]_o = 0.010$ M and $[CDP]_o = 0.0025$ M, the ratio of pyrene bound to β-CD ($[P(CD)_2]/[P]_o$) is equal to 0.56 as shown by equation (1) and Table II. If we assume that the "lost" pyrene is now bound to β-CDP, then the ratio of pyrene bound to β-CDP ($[PCDP]/[P]_o$) is equal to 0.40. Therefore, the ratio $[PCDP]/[P(CD)_2]$ is equal to 0.71. When substituted into equation (3), we calculate a K' value of 1.9×10^3.

Figure 1: Gel Permeation Chromatograms of β-CD and Commercial β-CDP. The mobile phase flow rate is 0.45 ml/min.

Figure 1: GPC's of β-CD and Commercial β-CDP.

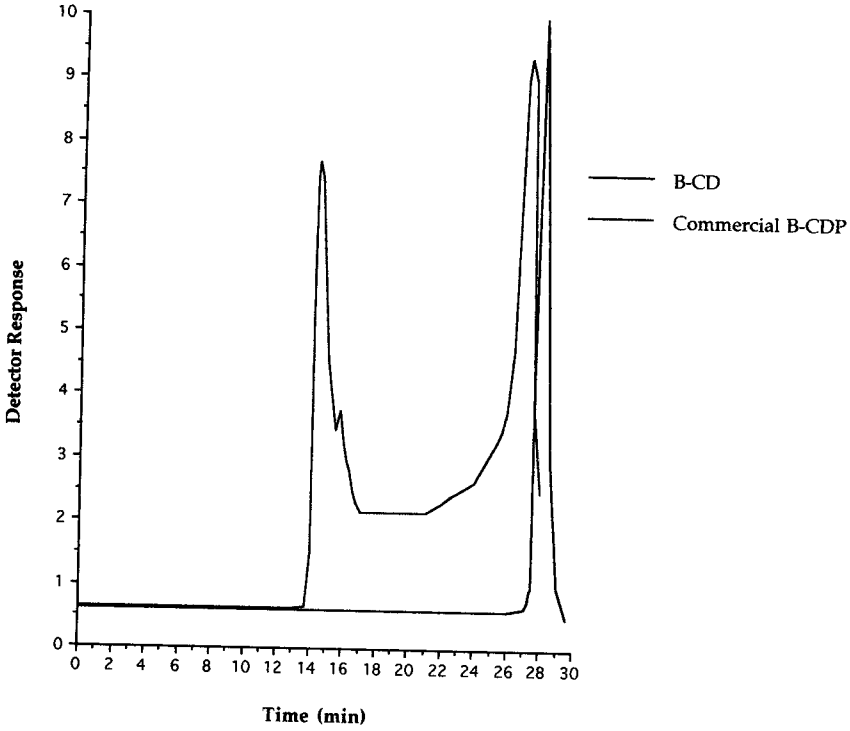


Table I: Pyrene I/III Ratios for β-CD in the Absence and Presence of 2.5×10^{-3} M Commercial β-CDP and 2.5×10^{-2} M PFP.

[β-CD] (M)	I/III(1)	[PFP] = 2.5×10^{-2} M I/III(1)
0.0	1.75	1.50
2.0×10^{-4}	1.73	1.01
4.0×10^{-4}	1.73	0.76
6.0×10^{-4}	1.71	0.65
1.0×10^{-3}	1.65	0.53
3.0×10^{-3}	1.31	0.45
5.0×10^{-3}	1.06	0.45
8.0×10^{-3}	0.94	0.46
1.0×10^{-2}	0.82	0.47

All the following solutions contain **2.5×10^{-3} M commercial β-CDP**.

[β-CD] (M)	I/III(2)	[PFP] = 2.5×10^{-2} M I/III(1)
0.0	1.55	
2.0×10^{-4}	1.53	1.40
4.0×10^{-4}	1.52	1.39
6.0×10^{-4}	1.54	1.27
1.0×10^{-3}	1.53	1.09
3.0×10^{-3}	1.49	0.59
5.0×10^{-3}	1.39	0.52
8.0×10^{-3}	1.22	0.49
1.0×10^{-2}	1.12	0.48

(1) Average of two experiments.
(2) Data from a single experiment

Figure 2: Pyrene I/III Ratios vs. [β-CD] in the Absence and Presence of 2.5×10^{-3} M Commercial β-CDP.

Solid Square: Pyrene I/III Ratio with β-CD
(Ave. of two Exp.)

Hollow Square: Pyrene I/III Ratio with β-CD and the
Addition of 2.5×10^{-3} M Commercial β-CDP
(Data from a single experiment.)

Figure 2: Competitive Binding Results in the Absence of 0.025 M PFP

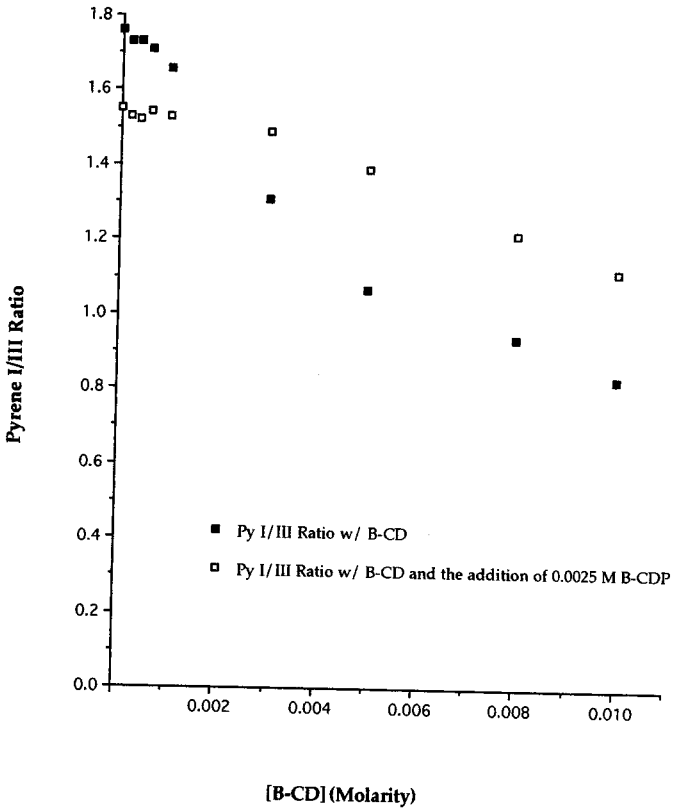


Figure 3: Pyrene I/III Ratios vs. [β-CD] with 2.5×10^{-2} M PFP in the Absence and Presence of 2.5×10^{-3} M Commercial β-CDP.

Solid Diamond: Pyrene I/III Ratio with β-CD and PFP (Average of two Exp.)

Hollow Diamond: Pyrene I/III Ratio with β-CD, PFP, and the Addition of 2.5×10^{-3} M Commercial β-CDP (Average of two Exp.)

Figure 3: Competitive Binding Results in the Presence of 0.025 M PFP

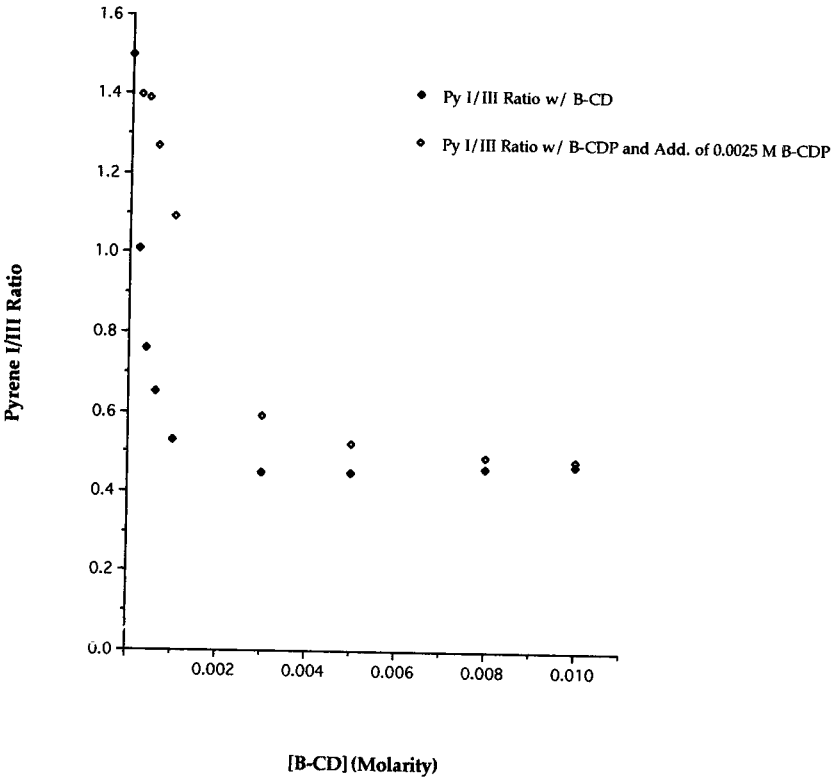


Table II: % Pyrene Bound to β-CD.

[β-CD] (M)	I/III (1)	% Py:β-CD (2)	I/III (3)	% Py:β-CD (3)
3.0×10^{-3}	1.31	45	1.49	7.8
5.0×10^{-3}	1.06	71	1.39	21
8.0×10^{-3}	0.94	84	1.22	43
1.0×10^{-2}	0.82	96	1.12	56

(1) Average of two experiments.

(2) Absence of β-CDP.

(3) Presence of 2.5×10^{-3} M β-CDP

Polymer Syntheses

The first two syntheses, in which we followed Xu's procedure, employed starting mole ratios of 1:1 (Exp. 1) and 2:1 (Exp. 2) EP:β-CD. The % mass yields are shown in Table III, which indicate little reaction for both mixtures. Analyses of these product mixtures by GPC show that there is no significant difference in the retention time of the major component peak from that of β-CD. These observations do not agree with those reported by Xu et al., which state MW determinations of 3700 (1:1) and 3800 (2:1) for their products. These product mixtures were then combined with pyrene, and I/III data were obtained. The concentration values for these product solutions of 31% (1:1) β-CDP (polymer derived from equal ratio of EP and β-CD) and corresponding I/III data are in Table IV. The data show that the I/III ratio levels off to the same value for 31% (1:1) β-CDP as for β-CD. We believe that the low % mass yields are an indication that the desired polymers are not being formed, resulting in characterizations that indicate the final mixtures contain unreacted β-CD and β-CD with a few added linker units. In fact, the GPC data for the 31% (1:1) β-CDP support this explanation because two peaks are observed with the bigger peak having a similar retention time as that of β-CD (Table V). Thus, we decided to modify the reaction procedure.

TableIII: Percent Mass Yields and Weight % of Reactants for Synthesized Polymers.

Exp. #	EP: β-CD Polymer	Wt. % β-CD	Wt. % EP	% MassYield
1	1:1	31	3	7
2	2:1	31	5	11
3	4:1	14	4	21
4	4:1	11	4	6
5	10:1	10	8	discarded
6	7:1	10	6	7
7	10:1	40	33	53
8	10:1	20	37	22
9	2:1	40	7	7
10	5:1	40	17	16

Table IV: Pyrene I/III Ratios with Product from 31% (1:1) β-CDP Synthesis (From initial synthesis following the method of Xu et al).[4]

31% (1:1) β-CDP (mg/ml)	I/III
0.00	1.72
0.10	1.72
0.20	1.62
0.50	1.55
1.00	1.42
2.00	1.14
5.00	1.00
10.00	0.83
15.00	0.65
20.00	0.64

The next set of reactions involved starting mole ratios of 4:1 EP:β-CD. Modifications included only partial neutralization, no dilution by three times the volume before the dialysis, increase in temperature (60°C), and adding EP dropwise only after the desired temperature was reached. The % mass yields for 18% (4:1) β-CDP and 15% (4:1) β-CDP (Exp. 3 and 4, respectively) are listed in Table III. The GPC data for both synthesized polymers show that most of the product mixture consisted of unreacted β-CD which remained in the dialysis bag.

The results were similar for the attempted polymer synthesis of 10% (10:1) β-CDP (Exp. 5) and 10% (7:1) β-CDP (Exp. 6). The product mixture from the 10% (10:1) β-CDP synthesis was discarded after dialysis because GPC data showed no reaction. Although the product mixture from the 10% (7:1) β-CDP synthesis was carried out to recovery, the GPC data showed similar results in which no reaction occurred. The % mass yield for this polymer is listed in Table III.

It was at this time that we decided to review our synthesis procedures for the polymers. When we consulted the method by Xu et al., they report starting the reaction with 25-30% wt. of reactants. We decided to run side-by-side reactions with the same starting mole ratios of 10:1 EP:β-CD but vary the % wt/vol of reactants. One reaction began with 40% (wt/vol) β-CD (4.0 g in 10.0 ml of 20% NaOH) and the other with 20% (wt/vol) β-CD (2.0 g in 10.0 ml of 20% NaOH). The reaction conditions were as follows: 50°C, addition of EP dropwise only after the desired temperature was reached and all the β-CD was dissolved, neutralization to a pH of about 7, dilution by three times the volume before dialysis, reduction of polymer solution by about half before freeze drying.

Table III shows the % mass yields of both 40% (10:1) and 20% (10:1) β-CDP (Exp. 7 and 8, respectively).

Under the conditions stated above, we were able to synthesize polymers that are different from both β-CD and the commercial β-CDP. Figure 4 shows that the 20% (10:1) β-CDP has one major GPC component peak similar to β-CD, however, its retention time is slightly shorter, indicative of a higher molecular weight. Figure 5 shows that the 40% (10:1) β-CDP is much like the commercial β-CDP; however, its major component peaks have shorter retention times indicative of mostly smaller molecular weight components than those found for commercial β-CDP. Table V lists the retention times for these β-CD polymers. We have proven that these retention times are reproducible under the given conditions. Table VI is an example of reproduciblity using the retention times of β-CD.

Figure 4: Gel Permeation Chromatograms of β-CD and 20% (10:1) β-CDP. The mobile phase flow rate is 0.45 ml/min.

Figure 4: GPC's of β-CD and 20% (10:1) β-CDP.

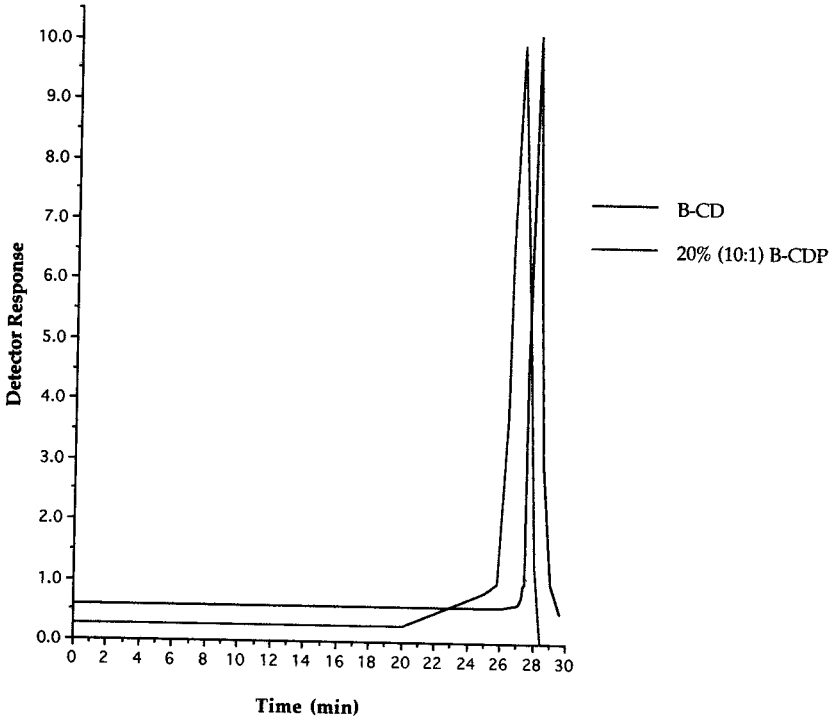


Figure 5: Gel Permeation Chromatograms of Commercial β-CDP and 40% (10:1) β-CDP. The mobile phase flow rate is 0.45 ml/min.

Figure 5: GPC's of Commercial β-CDP and 40% (10:1) β-CDP.

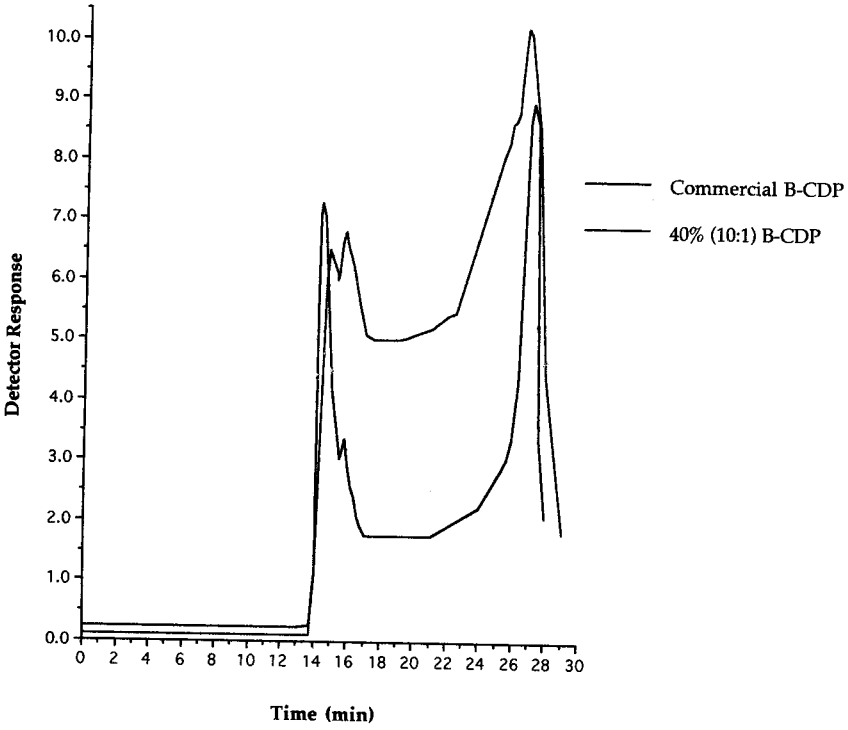


Table V: GPC Retention Times for β-CD Polymers.

Expt. #	Polymer	Flow Rate (ml/min)[1]	RT (min)	Relative Peak Area
1	1:1 β-CDP	0.50[2]	24.30	0.22
			25.13	1.00
4	4:1 β-CDP	0.45	27.88	1.00
7	40% (10:1) β-CDP	0.45	14.78	0.14
			15.78	0.15
			26.72	1.00
8	20% (10:1) β-CDP	0.45	27.05	1.00
9	40% (2:1) β-CDP	0.45	26.92	1.00
			27.83	0.96
10	40% (5:1) β-CDP	0.45	26.50	1.00
	Commercial β-CDP	0.45	14.32	0.38
			15.78	0.08
			27.08	1.00

(1) Rentention time of β-CD at 0.45 ml/min was 28.0 min.
(2) Retention time of β-CD at 0.50 ml/min was 25.2 min.

Table VI: Reproducibility of retention times using β-CD. Mobile phase is water with 3% methanol at a flow rate of 0.45 ml/min.

Date	Retention Time (min)	Concentration (mg/ml)
8/1	28.00	13.5
9/15	28.00	10.0
10/13	28.00	10.0

Our goal of synthesizing β-CDP's which are different from the commercial β-CDP's was now accomplished. The next step was to analyze how the 20% (10:1) and 40% (10:1) β-CDP's complex with pyrene and to compare the complexation with β-CD and commercial β-CDP. Table VII shows the I/III data that were measured for pyrene in the presence of 20% (10:1) β-CDP. The I/III value levels off at 1.41 with a [β-CDP] of 15.0 mg/ml or greater. This value is higher than 0.81 which we observed for β-CD at a concentration of 11.35 mg/ml. The higher I/III value means that the synthesized polymer is not β-CD, and it is not providing an environment for pyrene as hydrophobic as that of β-CD.

Table VIII also shows the I/III ratios that were measured when pyrene was in the presence of 40% (10:1) β-CDP. The lowest I/III value was 1.54 with a β-CDP concentration of 15.0 mg/ml or greater. This value is similar to that reported by Colwell for the commercial β-CDP, which had a I/III ratio of 1.55 at a concentration of 3.56 mg/ml.[3]

Figure 8 is a plot of pyrene I/III ratios vs. concentration for these synthesized polymers.

In addition, the I/III ratios were measured in the presence of 2.5×10^{-2} M PFP. It is at this concentration of PFP where the I/III ratio levels off at a fixed β-CD concentration.[5] These results are also listed in Table VIII and show the expected decrease in the I/III ratio upon the addition of PFP. It is important to note that a decrease in the I/III ratio of 40% (10:1) β-CDP after the addition of PFP proves that it is a different polymer than the commercial β-CDP. Colwell showed that there is no significant further decrease in the I/III ratio when the commercial β-CDP was complexed with pyrene and PFP was added.[3]

Figure 9 is a plot of pyrene I/III ratios vs. concentration for these synthesized polymers with the addition of 2.5×10^{-2} M PFP .

The two reactions that proved successful managed to give us a range of starting concentrations in which to synthesize new polymers. Our next step was to try reactions which produce polymers that fall within the range of the previous two polymers. One such reaction was performed with starting mole ratios of 2:1 and a β-CD concentration of 40% (40% (2:1) β-CDP).

Table VII: Pyrene I/III Ratios with the addition of 20% (10:1) β-CDP and 2.5×10^{-2} M PFP.

20% (10:1) β-CDP (mg/ml)	I/III	I/III (PFP)
0.00	1.73	----
0.20	1.69	1.16
0.50	1.67	0.83
1.00	1.60	0.66
2.00	1.56	0.60
5.00	1.48	0.55
10.00	1.41	0.52
15.00	1.41	0.51

Table VIII: Pyrene I/III Ratios with the addition of 40% (10:1) β-CDP and 2.5×10^{-2} M PFP.

40% (10:1) β-CDP (mg/ml)	I/III	I/III (PFP)
0.00	1.72	----
0.20	1.58	1.49
0.50	1.58	1.46
1.00	1.56	1.40
2.00	1.58	1.37
5.00	1.54	1.36
10.00	1.51	-----
15.00	1.54	1.35

Analysis of this polymer by GPC revealed two major component peaks; one which is most like β-CD and the other with a shorter retention time (Figure 6). The retention times for these peaks are listed in Table V. When the 40% (2:1) β-CDP was complexed with pyrene and PFP, the measured I/III values level off to values like those of β-CD (Table VII). The results are ploted in Figures 8 and 9.

The next synthesized polymer was 40% (5:1) β-CDP. Characterization of this polymer by GPC revealed that it was quite different from both β-CD and commercial β-CDP (Figure 7). However, when 2.5×10^{-2} M PFP was added to a solution containing pyrene and 40% (5:1) β-CDP, the polymer behaved more like β-CD. The limiting I/III values are listed in Table VII and the results are plotted in Figures 8 and 9.

Figure 6: GPC Chromatograms of β-CD and 40% (2:1) β-CDP. The mobile phase is 0.45 ml/min.

Figure 6: GPC's of β-CD and 40% (2:1) β-CDP.

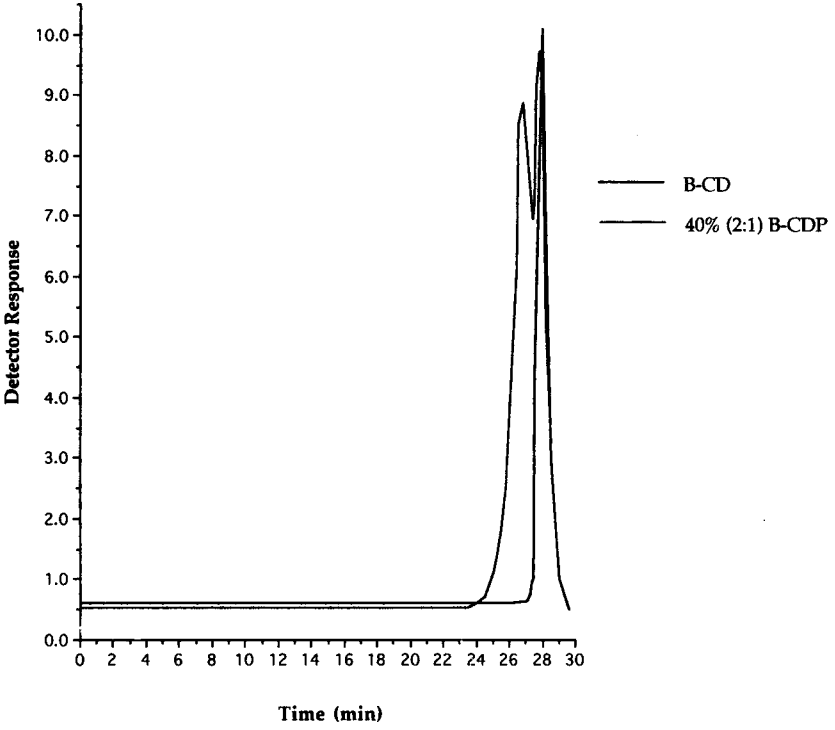


Figure 7: GPC Chromatograms of Commercial β-CDP and 40% (5:1) β-CDP. The mobile phase flow rate is 0.45 ml/min.

Figure 7: GPC's of β-CD and 40% (5:1) β-CDP.

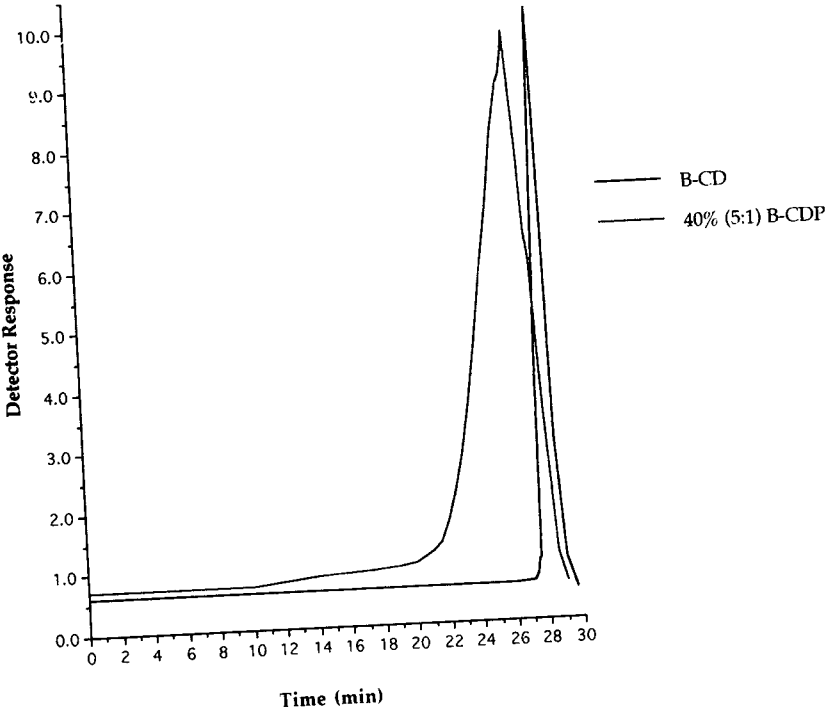


Figure 8: Pyrene I/III Ratio as a Function of β-CD Polymer Concentration.

Square with centered dot: Pyrene I/III Ratio with β-CD

Triangle: Pyrene I/III Ratio with 20% (10:1) β-CDP

Circle: Pyrene I/III Ratio with 40% (10:1) β-CDP

Cross: Pyrene I/III Ratio with 40% (2:1) β-CDP

Diamond: Pyrene I/III Ratio with 40% (5:1) β-CDP

Figure 8: Pyrene I/III Ratio as a Function of B-CD Polymer Concentration

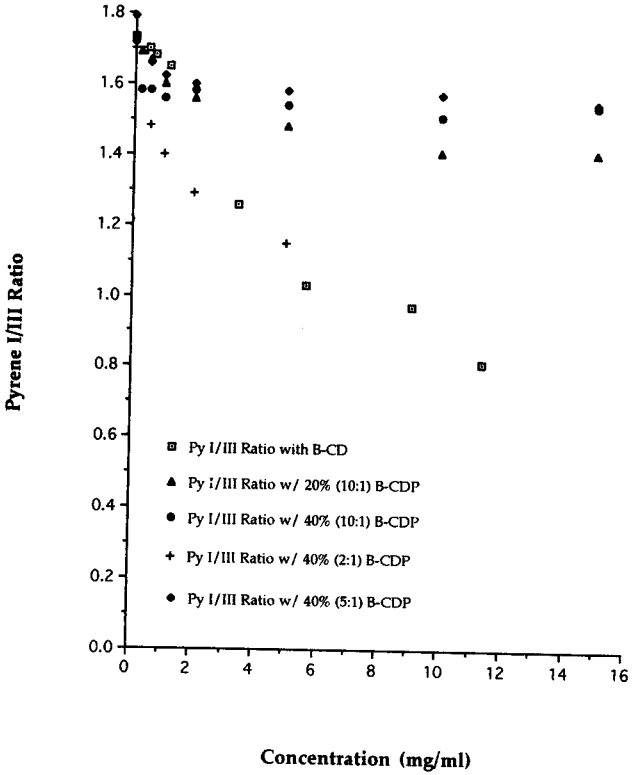


Figure 9: Pyrene I/III Ratio as a Function of β-CD Polymer Concentration with the Addition of 0.025 M PFP.

Square with centered dot: Pyrene I/III Ratio with β-CD

Triangle: Pyrene I/III Ratio with 20% (10:1) β-CDP

Circle: Pyrene I/III Ratio with 40% (10:1) β-CDP

Cross: Pyrene I/III Ratio with 40% (2:1) β-CDP

Diamond: Pyrene I/III Ratio with 40% (5:1) β-CDP

Figure 9: Pyrene I/III Ratio as a Function of B-CD Polymer Concentration with the Addition of 0.025 M PFP

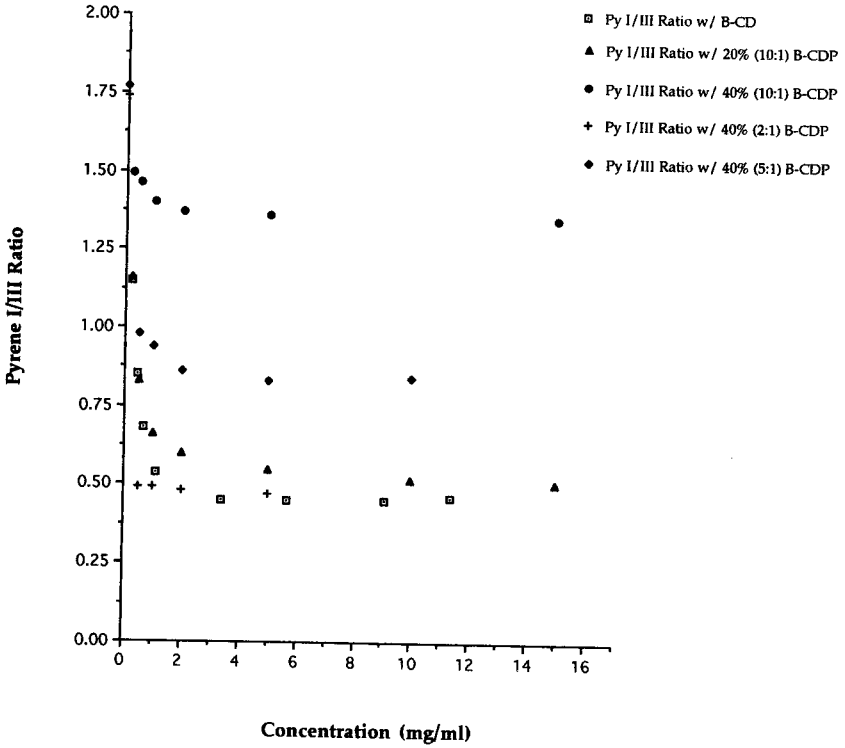


Table IX: Limiting Pyrene I/III Ratios for Synthesized Polymers in the Absence and Presence of 2.5×10^{-2} M PFP.

Polymer	Conc. (mg/ml)	Limiting I/III	Limiting I/III (PFP)
β-CD	11.4	0.81	0.45 (1)
40% (10:1)	5.0	1.54	1.35 (2)
20% (10:1)	10.0	1.41	0.51 (3)
40% (2:1)	5.0	1.15	0.47 (4)
40% (5:1)	15.0	1.55	0.83 (2)
Commercial β-CDP	3.56	1.55 (a)	1.55 (a)

(1) No significant change in value for [β-CD] > 3.4 mg/ml
(2) No significant change in value for [β-CD] > 5.0 mg/ml
(3) No significant change in value for [β-CD] > 10.0 mg/ml
(4) No significant change in value for [β-CD] > 0.5 mg/ml

(a) Values from reference 3.

Fluorescence Lifetime Measurements with Synthesized Polymers

In addition to the I/III ratios, fluorescence lifetime data were also collected for 40% (2:1) and 40% (5:1) β-CDPs. These results, along with results obtained from the addition of 0.025 M PFP, are listed in Table X. All the solutions contain two lifetime components which was determined by a better χ^2 value for the double exponential fit rather than a single fit.

As reported previously, the 40% (2:1) β-CDP is similar to that of β-CD as shown by GPC analysis and I/III data. Table X shows that the same trend is observed with the fluorescence lifetime measurements. Like β-CD, 40% (2:1) β-CDP has two lifetime components, one at 122 nsec (141 nsec for β-CD) and the other at 291 nsec (363 nsec for β-CD) with the second, longer lived component contributing 78% of the total decay signal. When PFP is added to the solutions to a concentration of 0.025 M, we observe an increase in the lifetime of the second, longer lived component in both cases, indicating further enhacement of the hydrophobicity of the CD sinding site.

In solutions containing 40% (5:1) β-CDP, which is different from both β-CD and commercial β-CDP as shown previously by the GPC and I/III data, we also observe two lifetime components. In this case however, the longer lived component (267 nsec) has a lifetime which is shorter than what is reported for β-CD (363 nsec), indicating a less hydrophobic environment for pyrene. However, upon the addition of PFP, the lifetime of the second component is increased to 337 nsec and contributes 98% to the total decay signal.

Table X: Fluorescence Lifetime Measurements for Pyrene in the Presence of Synthesized Polymers.

Condition	Conc.[a]	τ_1	τ_2	F_2
β-CD[b]	0.01 M	141	363	0.86
β-CD w/ 0.025 M PFP	0.01 M	407	427	0.72
40% (2:1) β-CDP	5.0 mg/ml	122	291	0.78
40% (2:1) β-CDP w/ 0.025 M PFP	5.0 mg/ml	194	552	0.92
40% (5:1) β-CDP	15.0 mg/ml	199	267	0.50
40% (5:1) β-CDP w/ 0.025 M PFP	15.0 mg/ml	63	337	0.98

(a) Concentration at which I/III is limiting.

(b) Values from reference 3.

Use of 2,6-MANS with Synthesized Polymers

We decided to complex our successful synthesized polymers with the naphthalene probe 2,6-MANS to observe the binding that occurs between them. This fluorophore exhibits fluorescence blue shifts and intensification in more hydrophobic environments.[9] Werner et. al. have reported a wavelength maximum of 496 nm for 2,6-MANS in the presence of β-CD and 434 nm in the presence of commercial β-CDP.[9] Table XI shows the wavelength maximum of 2,6-MANS in the presence of the different synthesized polymers. It is important to note the wavelength similiarities of 20% (10:1) β-CDP, 40% (2:1) β-CDP, and β-CD. In addition, the wavelength maximum for 40% (10:1) β-CDP resembles that of commercial β-CDP, while the 40% (5:1) β-CDP wavelength maximum is between that of β-CD and commercial β-CDP.

Table XI: The Fluorescence Behavior of 2,6-MANS in the Presence of Synthesized β-CDP's.

β-CDP	λmax (nm)
β-CD	496[a]
20% (10:1)	490
40% (2:1)	490
40% (5:1)	458
40% (10:1)	430
Commercial β-CDP	434[a]

(a) Values from reference 9.

Synthesis of Epichlorohydrin:Sucrose Polymer

In an attempt to better understand the relative interactions of the epichlorohydrin linker units with the guest molecules in the synthesized β-CDPs, we synthsized a polymer mixture containing epichlorohydrin and sucrose (used as a template for polymerization). Following the same synthesis procedure for the β-CDPs, we were successful in synthesizing 25% (10:1) SP; a polymer mixture with starting reactants of 25% (wt/vol) sucrose by weight and a 10:1 mole ratio of Epi:Sucrose.

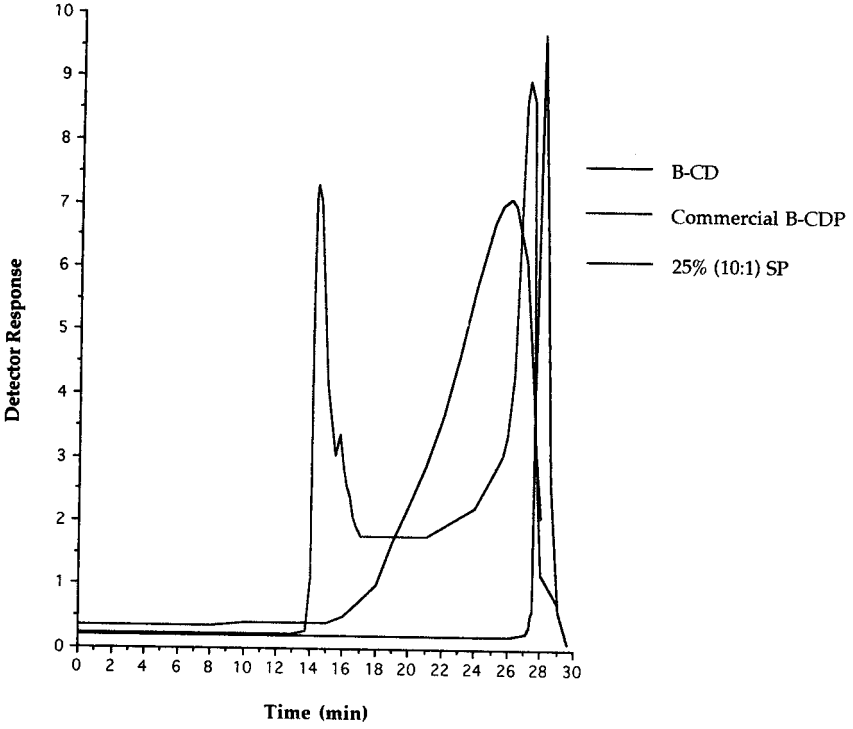
Characterization of this polymer by GPC showed one broad peak with a retention time of 26.02 min. This polymer mixture, therefore, has an average molecular weight that is between that of β-CD (1,100 MW) and the commercial β-CDP (9,000 MW) as shown in Figure 10.

When this polymer mixture was added to a solution containing pyrene, the limiting I/III ratio observed was 1.67 at a concentration of 10.0 mg/ml of 25% (10:1) SP. This result shows that, although the pyrene is exposed to a polar environment, it is slightly less polar than that of pyrene in water (1.75), suggesting that some binding to the linkers is occurring. Since the I/III ratio for pyrene in the presence of commercial β-CDP is 1.55, we can conclude the CD sites are probably also involved in pyrene binding.

In addition, we measured the fluorescence lifetime of pyrene in the presence of 25% (10:1) SP (15.0 mg/ml). Unlike the previous synthesized polymers, we observe a single exponential decay with a lifetime of 163 nsec. This value is slightly lengthened than that of pyrene in water (132 nsec), suggesting again, some interaction of the linker units. The fact that we do not observe two lifetimes is another indication that the CD sites must also contribute to the overall binding of pyrene to the commercial β-CDP.

Figure 10: Gel Permeation Chromatograms of β-CD, commercial β-CDP, and 25% (10:1) SP. The mobile phase flow rate is 0.45 ml/min.

Figure 10: GPC's of β-CD, commercial β-CDP, and 25% (10:1) SP.



Competitive Binding Study of β-CD and 25% (10:1) SP

We have conducted a study of the competitive binding that occurs when pyrene is in the presence of both β-CD and the synthesized sucrose polymer, 25% (10:1) SP. This experiment was conducted to get an idea of the relative binding strength of pyrene to the linker units.

Five solutions were prepared containing varying concentrations (mg/ml) of 25% (10:1) SP and a fixed concentration of β-CD of 0.010 M as shown in Table XII. Solutions were prepared in duplicate, and the I/III ratios were measured and averaged. A plot of the pyrene I/III ratio vs. the mg/ml of 25% (10:1) SP is shown in Figure 11.

As a reference, the same experiment as above was performed with commercial β-CDP in the presence of a fixed β-CD concentration of 0.010 M. The I/III data obtained from this experiment are also listed in Table XII and plotted in Figure 11. The concentration of β-CDP is also expressed as mg/ml for this comparison.

As shown in Figure 11, in the absence of any polymer, the I/III ratio for β-CD at its fixed concentration (0.010 M) is 1.22. As the mg/ml of β-CDP increases, the I/III ratio also increases to a value of 1.46 at a β-CDP concentration of 10 mg/ml. In solutions containing the sucrose polymer, the I/III also increases but only to a value of 1.36 at sucrose polymer concentration of 15.0 mg/ml. These data are further evidence that the linker units are involved in the binding of pyrene. However, they are not solely responsible for all the binding interactions that occur between pyrene and the commercial β-CDP.

Table XII: Pyrene I/III Ratios for 25% (10:1)SP and Commercial β-CDP in the Presence of a Fixed [β-CD] of 0.01 M.

25%(10:1)SP (mg/ml)	I/III(1)
0.0	1.21
1.0	1.26
5.0	1.31
10.0	1.35
15.0	1.36

Comm. β-CDP (mg/ml)	I/III(1)
0.0	1.23
1.0	1.38
2.0	1.39
5.0	1.43
10.0	1.46

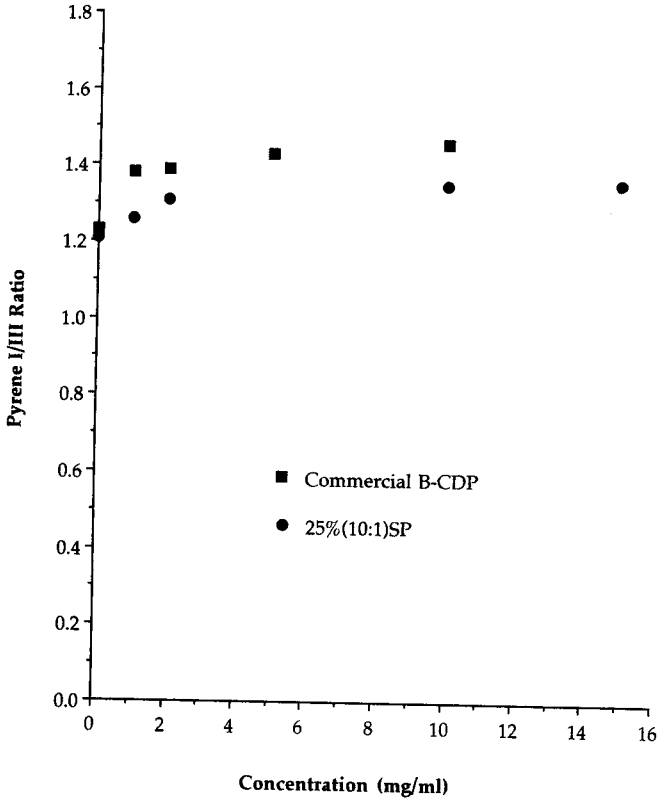
(1) Average of two experiments.

Figure 11: Competitive Binding of 25%(10:1) SP and Commercial β-CDP for Pyrene in the Presence of β-CD (0.01 M).

Solid Square: Pyrene I/III Ratio with Co. mercial β-CDP (Ave. of two Exp.)

Solid Circle: Pyrene I/III Ratio with 25%(10:1) SP (Ave. of two Exp.)

Figure 11: Competitive Binding for Pyrene with a Fixed [β-CD] of 0.010 M.



Discussion

Binding of Pyrene to Commercial β-CDP

It has been shown from I/III ratio data that pyrene exists in a more hydrophilic environment when in the presence of commercial β-CDP than β-CD. From the competitive experiments involving the synthsized sucrose polymer, we have shown that the linker units are involved in the binding of pyrene. However, we can not account for all the binding that occurs between pyrene and β-CDP by the linker untis units alone. Therefore, we conclude that the binding occurring between pyrene and the commercial β-CDP involves both the linker units and the CD cavities.

In addition, the competitive experiments involving β-CD and β-CDP have shown that the binding of pyrene to commercial β-CDP is competitive to the binding that is observed with β-CD. However, we suggest that the binding complex is 1:1 for β-CDP/pyrene in contrast and 2:1 for β-CD/pyrene. Using this assumption, we calculate a K value for the 1:1 β-CDP/pyrene complex to be 1.9×10^3, a value which is smaller than that of the 2:1 β-CD/pyrene complex (6.67×10^4).[5] This result is consistent with the assumption of a 1:1 complex, where the pyrene is only partially enveloped in a single β-CD cavity. Therefore, we conclude that pyrene is forming an inclusion complex with commercial β-CDP, but not the same one as the 2:1 β-CD/pyrene complex.

The picture that emerges from our results is of a pyrene molecue binding with a β-CD unit on the polymer such that about half of the pyrene molecule is not included in the cavity. The exposed "half" of the pyrene molecule would prefer to be in contact with the linker units than with solvent water.

Characterization of Synthesized Polymers

The discussion of the synthesized polymers will focus on the four polymers that were synthesized after the reaction modifications (Exp. 7-10). GPC data show that 20% (10:1) β-CDP is most like β-CD and 40% (10:1) β-CDP is most like commercial β-CDP. The two other synthesized polymers have MW distributions that are between that of β-CD and commercial β-CDP. Specifically, the chromatogram of 40% (2:1) β-CDP most resembles that of β-CD and that of 40% (5:1) β-CDP lies between those of β-CD and β-CDP.

The pyrene I/III data in the presence of these polymers supports the results shown by the GPC chromatogams. These synthesized polymers can be summarized in full by analysis of Figures 8 and 9. From Figure 8, it is observed that 40% (2:1) β-CDP is most like β-CD when complexed with pyrene. The same trend is observed upon the addition of alcohol (Figure 9). Similarities were also observed with fluorescence lifetime measurements as shown in Table X.

The polymer that is most like the commercial β-CDP is 40% (10:1) β-CDP. The limiting I/III values for both polymers are virtually the same (Table IX). However, when PFP is added, the I/III value for 40% (10:1) β-CDP decreases slightly, which is evidence that it is somewhat different than the commercial β-CDP (Table IX).

The 20% (10:1) β-CDP exhibits a higher limiting I/III value than β-CD in the absence of PFP (Table IX). This difference is then minimized when PFP is added as shown in Figure 9; evidence that supports the ability to "tune" the binding from somewhat non-inclusional to largely inclusional.

The polymer that is most intriging is the 40% (5:1) β-CDP. In the absence of PFP, the I/III limiting value is most like the commercial β-CDP (Table IX). However, when PFP is added, the limiting I/III value is between

that of β-CD and β-CDP as shown in Figure 9. Fluorescence lifetime measurements also show the same trends as shown in Table X. These results are further evidence to support the ability to "tune" the observed binding.

The results obtained by complexing 2,6-MANS with the four synthesized polymers follow the same trends that are observed when complexed with pyrene. As discussed earlier, the 20% (10:1) β-CDP and the 40% (2:1) β-CDP resemble β-CD in terms of their pyrene I/III values in the absence of PFP. Similarly, the wavelength maxima for 2,6-MANS with these two polymers (490 nm) are similar with that for 2,6-MANS with β-CD (496 nm).

In addition, we have characterized 40% (10:1) β-CDP to be similar to commercial β-CDP using the pyrene I/III values in the absence of PFP. This same trend holds true when the polymers are complexed with 2,6-MANS, since the 2,6-MANS fluorescence maxima are 430 nm and 434 nm for the 40% (10:1) β-CDP and commercial β-CDP, respectively.

One would predict that the wavelength maxima for 40% (5:1) β-CDP with 2,6-MANS should fall between those of β-CD and commercial β-CDP due to the reported trends of pyrene I/III values. In fact, this is exactly what is observed. The 2,6-MANS wavelength maxima with 40% (5:1) β-CDP is 458 nm, which is shorter than with β-CD (490 nm) but longer than with commercial β-CDP (434 nm).

In summary, we have synthesized polymers that appear to be intermediate in their properties between those of β-CD and commercial β-CDP. It is important to note that we can not completely rule out the contribution from the unreacted β-CD in the polymer mixtures. Further characteriztion of these polymers mixtures will involve analysis by Mass Spectrometry.

Appendix

Use f β-NTA with β-CD

We decidied to analyze another napthalene fluroescence probe and observe its binding behavior when complexed with β-CD. This probe is 4,4,4-trifluoro-1-(2-naphthyl)-1,3-butanedione (β-NTA).

A stock solution of β-NTA was prepared by adding a small amount of solid β-NTA to water and stirring the solution overnight. The stock solution was then passed through a 0.2 μm disposable syringe filter (Anotop). The absorbance of the β-NTA stock would typically be between 0.10 and 0.20 (1 cm cell) at 332 nm. The required β-CD concentration was obtained by adding a weighed amount of solid β-CD to a known volume of β-NTA stock solution.

Fluorescence spectra of solutions containing β-NTA in the presence of β-CD (5.0×10^{-3} M), show a red shift in the fluorescence wavelength maximum (488 nm with water/500 nm with β-CD) with relative fluorescence intensities of 6.60 and 1.00 in water and in β-CD, respectively. This shift is not what is expected for such naphthalene probes according to the polarity of the environment. Future work involves verifiying these claims with experiments of β-NTA and the synthesized β-CDPs.

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