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COMPARISON OF LIQUID CHROMATOGRAPHIC SELECTIVITY FOR POLYCYCLIC AROMATIC HYDROCARBONS ON CYCLODEXTRIN AND C_{18} BONDED PHASES

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SUMMARY

Selectivity towards polycyclic aromatic hydrocarbons (PAHs) was studied on cyclodextrin bonded phases and compared to selectivity observed on C_{18} phases. The study included the separation of eleven five-ring PAH isomers on each of three phase types; monomeric C_{18}, polymeric C_{18} and cyclodextrin. Retention of PAHs ranging in size from three to six condensed rings was also investigated. Retention on the cyclodextrin phase is based on inclusion complexing between the solute and cyclodextrin cavity, resulting in a strong shape dependence. However, the shape selectivity exhibited by the cyclodextrin phase is different from that exhibited by either the monomeric or polymeric C_{18} phases; retention on the cyclodextrin phase is strongly dependent on the shape and shows very little molecular weight dependence. Calculations of solute molecular widths were performed to predict the isomers' ability to enter the cyclodextrin cavity. The effect of sample solvent and injection volume was also investigated for the cyclodextrin phase. A retention model based on the solute shape is proposed for PAH isomers on β-cyclodextrin phase.

INTRODUCTION

Complex mixtures of polycyclic aromatic hydrocarbons (PAHs) are often encountered in environmental samples and their complexity is due to the numerous isomeric structures of PAHs. Considerable emphasis has been put into the separation of isomeric PAHs since certain isomers are more mutagenic and/or carcinogenic than others.

Differences in liquid chromatographic (LC) retention and selectivity towards PAH isomers among commercially available columns are well known, and although the retention mechanisms are not yet fully understood, models for retention have been presented for different types of stationary phases[1–5]. Sander and Wise have

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shown that the selectivity of C_{18} bonded phases for the separation of PAH is dependent on a number of parameters[2,6–8]. These parameters include phase type (monomeric or polymeric synthesis)[2,6], pore diameter and surface area of the silica substrate[7], and surface coverage or C_{18} ligand density[2,8]. Monomeric C_{18} phases are prepared by using monofunctional silanes whereas polymeric C_{18} phases are generally prepared using trifunctional silanes in the presence of water. The greatest selectivity for the separation of PAH isomers is achieved on polymeric C_{18} phases prepared on wide pore (>150 Å in diameter) silica substrates with low surface area (100 m^2/g)[2,6,7].

In investigations concerning the selectivity of PAH on different stationary phases, Wise and co-workers[1,2] found a relationship between retention on a polymeric C_{18} phase and the shape of the solute, defined as length-to-breadth ratio (L/B). The L/B value is determined by drawing the PAH molecule using the appropriate bond lengths and then constructing a box around the structure which provides the maximum length to breadth ratio. There is a high correlation between this ratio and solute retention for PAH isomers on polymeric C_{18} phases.

Armstrong and co-workers[9,10] suggested that cyclodextrin bonded phases could be an alternative to traditional C_{18} bonded phases for the separation of PAH isomers. Selectivity of the cyclodextrin bonded phase towards PAHs is based on inclusion complexing of the solute and the glucopyranose cavity and would thereby provide different selectivity than a C_{18} bonded phase. Armstrong and co-workers[9,10] suggested that cyclodextrin phases exhibit enhanced selectivity toward PAH isomers when compared to C_{18} phases. However, they showed the separation of only three pairs of isomers (benzo[*a*]- and benzo[*e*]pyrene, 1,2:3,4- and 1,2:5,6-dibenzanthracene, phenanthrene and anthracene) to illustrate this claim. Differences in selectivity of PAHs of different molecular weight on β- and γ-cyclodextrin have also been presented[9], although the observed changes in relative retention between the two phases were not explained.

In this work we compare PAH selectivity on monomeric and polymeric C_{18} bonded phase materials with the selectivity on a β-cyclodextrin bonded phase. PAH separations on a γ-cyclodextrin are also investigated briefly. Eleven PAH isomers of molecular weight 278 have been used by Wise and Sander[2] to illustrate the dependence of shape (L/B) for PAH retention on polymeric and monomeric C_{18} bonded phases. In this work, the same eleven isomers were used to compare retention mechanisms on cyclodextrin bonded phases with retention mechanisms on C_{18} bonded phases. PAHs of different molecular weights are studied, and a model of the retention of PAHs on cyclodextrin bonded phases is proposed.

EXPERIMENTAL[a]

Materials

Phenanthro[3,4-*c*]phenanthrene was obtained from Aldrich (Milwaukee, WI, U.S.A.), 1,2:3,4:5,6:7,8-tetrabenzonaphthalene was obtained from Rütgers (Castrop-

[a] Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Rauxel, F.R.G.) and benzo[*a*]pyrene was obtained from BCR (Community Bureau of Reference, Brussels, Belgium). PAH isomers of molecular weight 178 and 228 (three and four aromatic rings) were all obtained from commercial sources. The five-ring PAH isomers of molecular weight 278 were obtained as reported previously[2]. The six-ring PAH isomers were obtained from W. Schmidt (Ahrensburg, F.R.G). Methanol, acetonitrile and water (all HPLC grade) were obtained from J. T. Baker (Whippany, NJ, U.S.A.).

Columns

Separations of the different PAH mixtures were performed on commercial columns: Vydac™ 201 TP (polymeric C_{18}), The Separations Group (Hesperia, CA, U.S.A.); Zorbax® ODS (monomeric C_{18}), MAC-MOD Analytical (Wilmington, DE, U.S.A.); and Cyclobond™ I (β-cyclodextrin) and Cyclobond™ II (γ-cyclodextrin), Astec (Whippany, NJ, U.S.A.). The four columns were all 250 × 4.6 mm I.D. with 5-μm packing material.

Chromatography

A liquid chromatograph consisting of a reciprocating piston pump, a solvent programming system, a 20-μl (where not otherwise stated) loop injector and a 254-nm fixed-wavelength detector was used throughout the studies. Retention data were collected on a chromatography data system. All samples were run isocratically with aqueous methanol mobile phases and the solutes were dissolved in methanol prior to injection. Temperature of the column was maintained at 30°C during all chromatographic runs.

The molecular widths and lengths of the PAH solutes were calculated with a XIRIS molecular modeling program (XIRIS Co., New Monmouth, NJ, U.S.A.) on a personal computer.

RESULTS AND DISCUSSION

Polymeric C_{18} phases on wide pore (*e.g.*, 300 Å) silica provide very high selectivity for the separation of PAH isomers[2,6,7]. The enhanced selectivity for isomeric PAHs observed with polymeric C_{18} phases, compared to monomeric C_{18} phases, can be attributed to a shape recognition ability of the polymeric phase. Relationships between shape and reversed-phase LC retention, as described previously by Wise *et al.*[1], have also been observed in this work with the polymeric C_{18} phase as well as with the cyclodextrin stationary phases. However, the shape recognition of the cyclodextrin phases is different from that of the polymeric C_{18} phase.

The Cyclobond™ stationary phases consist of cyclodextrins chemically bonded to 5-μm spherical silica gel. The cyclodextrins are arranged in the shape of a hollow truncated cone[11]. β-Cyclodextrin consists of seven glucopyranose units, which provide a cone with an inner diameter of 7.8 Å. γ-Cyclodextrin consists of eight glucopyranose units and yields a truncated cone with an inner diameter of 9.5 Å. The interior of the cyclodextrin cavity is relatively hydrophobic with a high electron density, and the exterior is hydrophilic. In this case, with an aqueous organic mobile phase, the proposed separation mechanism is based on an inclusion complex between the solute and the cone[11–14]. Any organic modifier present in the mobile phase will compete

with the solute for the preferred location in the hydrophobic cavity. In this way, the modifier will reduce solute-bonded phase interaction, and thus decrease retention. Acetonitrile has a stronger affinity for the cavity than methanol and is a stronger eluent.

The choice of sample solvent was observed to be critical for separations carried out on the cyclodextrin phases. A similar observation was made by Wilson[15]. In general for reversed-phase chromatography, the best column efficiency and solute peak shape results when solutes are dissolved in the mobile phase. Stronger solvents are often required, however, particularly for solutes of low solubility. On conventional C_{18} columns, detrimental solvent effects are usually minor, as long as the solvent strengths (sample solvent and mobile phase) are not too dissimilar and injection volume is small. For the cyclodextrin columns, the effect is readily seen. Attempts to chromatograph solutes dissolved in acetonitrile with an aqueous methanol mobile phase yielded extremely poor peak shape. For solutes dissolved in methanol and chromatographed in an aqueous acetonitrile mobile phase, peak shape was not affected. To study the cause of the poor peak shape, pure acetonitrile was injected onto the column immediately prior to injection of the sample (dissolved in methanol), using an aqueous methanol mobile phase. No degradation in peak shape was apparent. From this observation, it appears that interactions become significant only when the sample is dissolved in acetonitrile. This was further investigated by preparing two PAH sample solutions of the same concentration, one in methanol and one in acetonitrile. Three different volumes (5, 20 and 50 μl) of the solutions were injected onto the β-cyclodextrin column, using a 55% aqueous methanol mobile phase (Fig. 1). The sample dissolved in methanol showed only a slight band broadening as injection volume increased, whereas chromatographic performance with the acetonitrile solution was strongly dependent on injection volume. Peak splitting was apparent at 20-μl injection volume, and at 50-μl extremely poor peak shape resulted with two peaks present for each component. Acetonitrile disturbs the column partitioning equilibria by initially decreasing solute retention. When the kinetics of solute transfer between the mobile and the stationary phase is slow, only a portion of the retained band is affected by the stronger solvent "plug". Thus, a gaussian peak is changed into a bimodal distribution, and the result is peak broadening or, in extreme cases, peak splitting. This effect was also seen when 20 μl of the methanol sample solution was injected followed by an injection of 20 μl pure acetonitrile, as shown in Fig. 2. The acetonitrile molecules are essentially unretained and "catch up" with the retained solutes. The result is the same as if the sample was dissolved in acetonitrile. Peak splitting occurs even when the acetonitrile plug is injected as late as 3 min (the approximate column void volume) after the sample injection is performed.

Three PAH isomers of molecular weight 228 were run on both a β- and a γ-cyclodextrin stationary phase (see Fig. 3A and B, respectively). The elution order of the three components is different on the two phases; on both phases the bulky triphenylene elutes first, but the elution of benz[*a*]anthracene and chrysene are reversed. In the case of the β-cyclodextrin phase, chrysene elutes before benz[*a*]anthracene because of the difference in shape of the solutes. The long narrow portion of the benz[*a*]anthracene structure can enter the cavity more deeply and form a more stable inclusion complex than the bulkier chrysene. A comparison of the dimensions of the cyclodextrin cavity and solute widths reveals that the β-cavity is wide enough only for

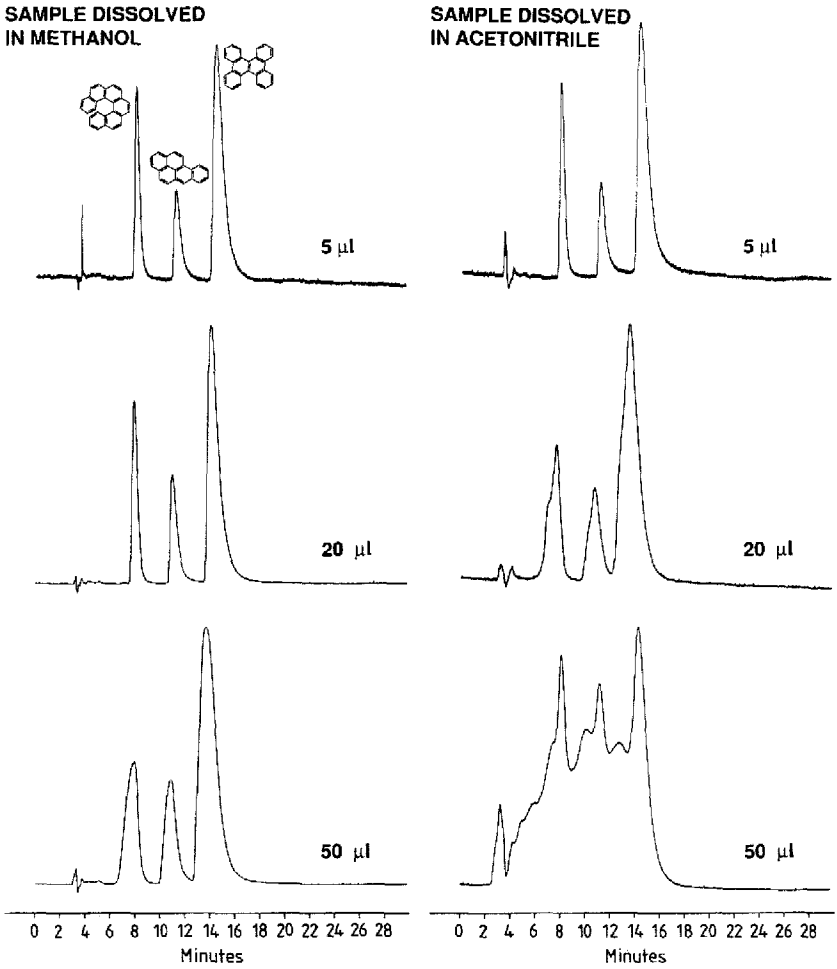


Fig. 1. Separation of phenanthro[3,4-*c*]phenanthrene, benzo[*a*]pyrene and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene on β-cyclodextrin (Cyclobond™ I). The mobile phase composition was methanol–water (55:45) and the flow-rate was 1 ml/min. Volumes indicate sample sizes injected.

a single chain of benzene rings to enter. In the case of the γ-cyclodextrin phase, the cavity is wide enough for chrysene to enter and thereby form a more stable complex (*i.e.*, resulting in longer retention) than the narrower benz[*a*]anthracene. On polymeric C_{18} columns these three PAH isomers elute in the same order as on the γ-cyclodextrin phase, but with much greater efficiency and resolution. This elution order is in agreement with increasing L/B ratios[1]. The low efficiency of the cyclodextrin phases for these solutes might be due to the low solubility of the solutes in the mobile phase. No increase in column efficiency has been observed using acetonitrile as the organic modifier. At comparable k' values of the solutes, the water concentration is higher compared to elution with an aqueous methanol mobile phase, and it seems likely that the solubility of the solutes is not enhanced.

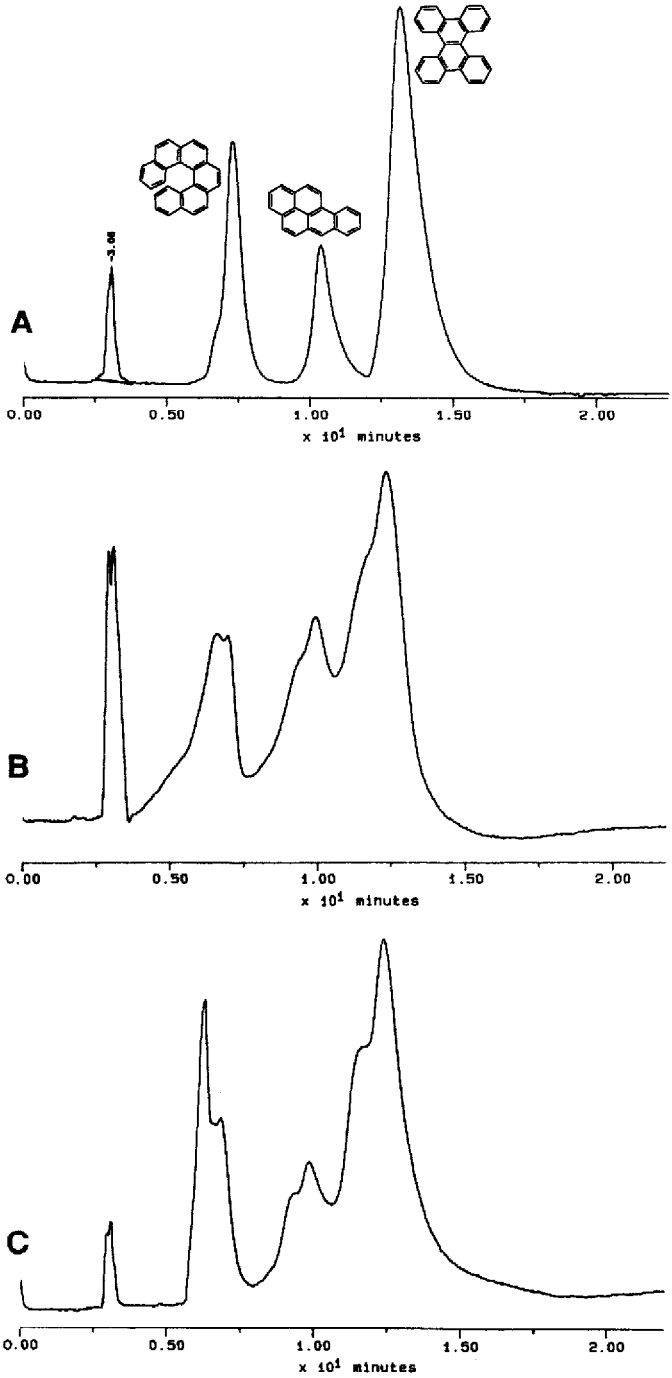


Fig. 2. Chromatographic conditions as in Fig. 1. The sample was dissolved in methanol and the injection volume was 20 μl. (A) Sample injected. (B) Sample injected followed by an immediate injection of 20 μl, pure acetonitrile. (C) Sample injected followed by an injection of 20 μl pure acetonitrile 3 min after sample injection.

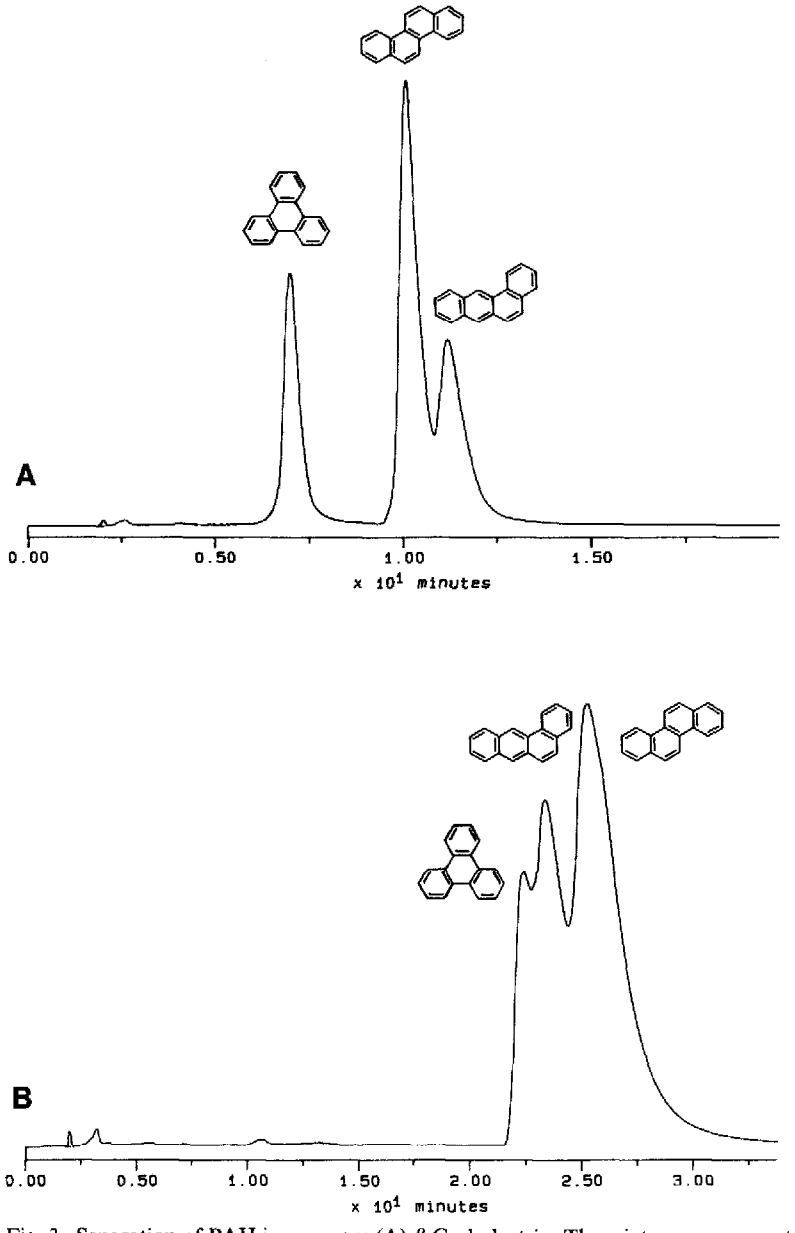


Fig. 3. Separation of PAH isomers on: (A) β-Cyclodextrin. The mixture was separated isocratically with methanol–water (50:50) at a flow-rate of 1.5 ml/min. (B) γ-Cyclodextrin. The mixture was separated isocratically with methanol–water (40:60) at a flow-rate of 1.5 ml/min.

The varying amounts of organic modifier needed to elute the components are indicative of the differences in binding energy of the inclusion complex for the two phases; with a mobile phase consisting of 50% aqueous methanol, chrysene elutes at 10 min on β- and at 7 min on γ-cyclodextrin. Less energy is involved in the interaction

of solute–γ-cyclodextrin than solute–β-cyclodextrin since these isomers are too small to interact strongly with the γ-cavity.

The efficiency and peak shape of the γ column is very poor for solutes of the size and shape discussed here. Six PAH isomers of molecular weight 278 (isomers 1, 3, 5, 8, 10 and 11, Fig. 4) that were completely resolved on the β-cyclodextrin phase gave only two peaks on the γ-cyclodextrin phase. This result is expected when widths of the solutes are calculated. The narrowest part of the molecules, a single chain of benzene rings, is about 7.3 Å wide. The widest part, which is 9.7 Å, is restricted from the 9.5-Å γ-cyclodextrin cavity. Only the narrow part of the molecule can enter. It seems likely that the inclusion complex formed with the narrow chain (7.3 Å) within the 9.5-Å cavity is relatively weak. It is obvious that the fit of the solute inside the cavity is crucial, and for the PAH molecules presented here, the β-cyclodextrin is more suitable than the γ-cyclodextrin.

The eleven five-ring PAH isomers of molecular weight 278 (listed in Fig. 4) showed the same retention behavior (*i.e.*, a molecule with a narrow shape will be

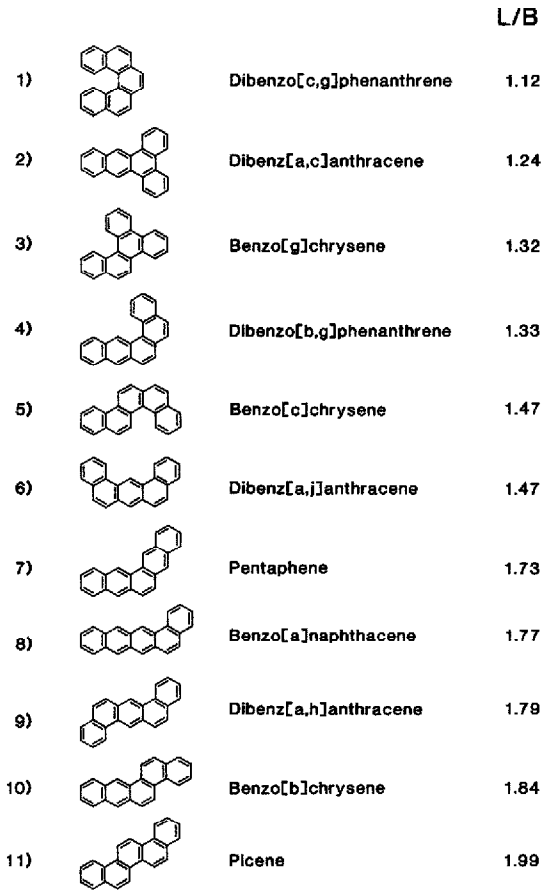


Fig. 4. Structures of five-ring PAH isomers used in this study. (L/B is the length-to-breadth ratio of the PAH).

TABLE I

RELATIVE RETENTION DATA; PAH OF MOLECULAR WEIGHT 278

Compound	*L/B*	*Relative retention*[a]		
		Polymeric C_{18}[b]	*Monomeric* C_{18}[c]	*Cyclodextrin*[d]
Dibenzo[*c,g*]phenanthrene	1.12	1.00	1.00	1.00
Dibenz[*a,c*]anthracene	1.24	3.37	1.53	2.57
Benzo[*g*]chrysene	1.32	1.98	1.40	1.91
Dibenzo[*b,g*]phenanthrene	1.33	2.41	1.57	1.77
Benzo[*c*]chrysene	1.47	3.37	1.67	2.30
Dibenz[*a,j*]anthracene	1.47	4.33	1.73	2.11
Pentaphene	1.73	5.50	1.92	3.57
Benzo[*a*]naphthacene	1.77	12.15	2.40	5.55
Dibenz[*a,h*]anthracene	1.79	7.01	1.87	2.99
Benzo[*b*]chrysene	1.84	12.62	2.22	4.10
Picene	1.99	16.84	2.26	3.82

[a] Retention relative to dibenzo[*c,g*]phenanthrene.
[b] Polymeric C_{18} (Vydac™ 201 TP) isocratic 95% aqueous methanol 1.5 ml/min 30°C.
[c] Monomeric C_{18} (Zorbax® ODS) isocratic 90% aqueous methanol 1.5 ml/min 30°C.
[d] β-Cyclodextrin (Cyclobond™) isocratic 50% aqueous methanol 1.5 ml/min 30°C.

retained longer than a bulky molecule) on the β-cyclodextrin as the four-ring PAHs above. Relative retention data of the isomers run on a polymeric C_{18}, a monomeric C_{18} and a β-cyclodextrin stationary phase are summarized in Table I. Relative retention data from Table I on each of the three phase types are also plotted *vs.* L/B ratios in Fig. 5. On the polymeric C_{18} column, the retention generally increases with increasing L/B ratio. In contrast, retention on the β-cyclodextrin is different than on either the polymeric or the monomeric C_{18} columns. Typical chromatograms of the isomer mixture run on each of the three columns are shown in Fig. 6. It is evident that a different retention mechanism is involved for the cyclodextrin phase when compared to traditional C_{18} phases.

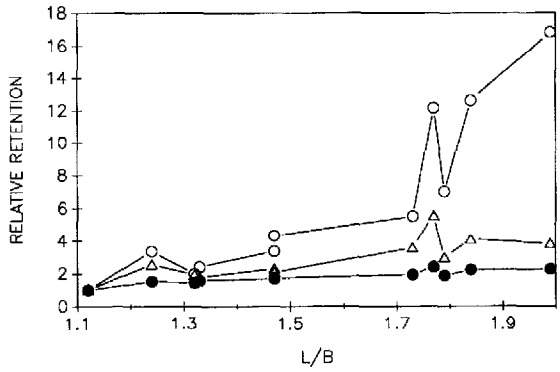


Fig. 5. Plots of relative retention *vs.* L/B ratios for five-ring PAH isomers on three different stationary phases. Retention is relative to dibenzo[*c,g*]phenanthrene. ○ = Polymeric C_{18}; ● = Monomeric C_{18}; △ = β-cyclodextrin.

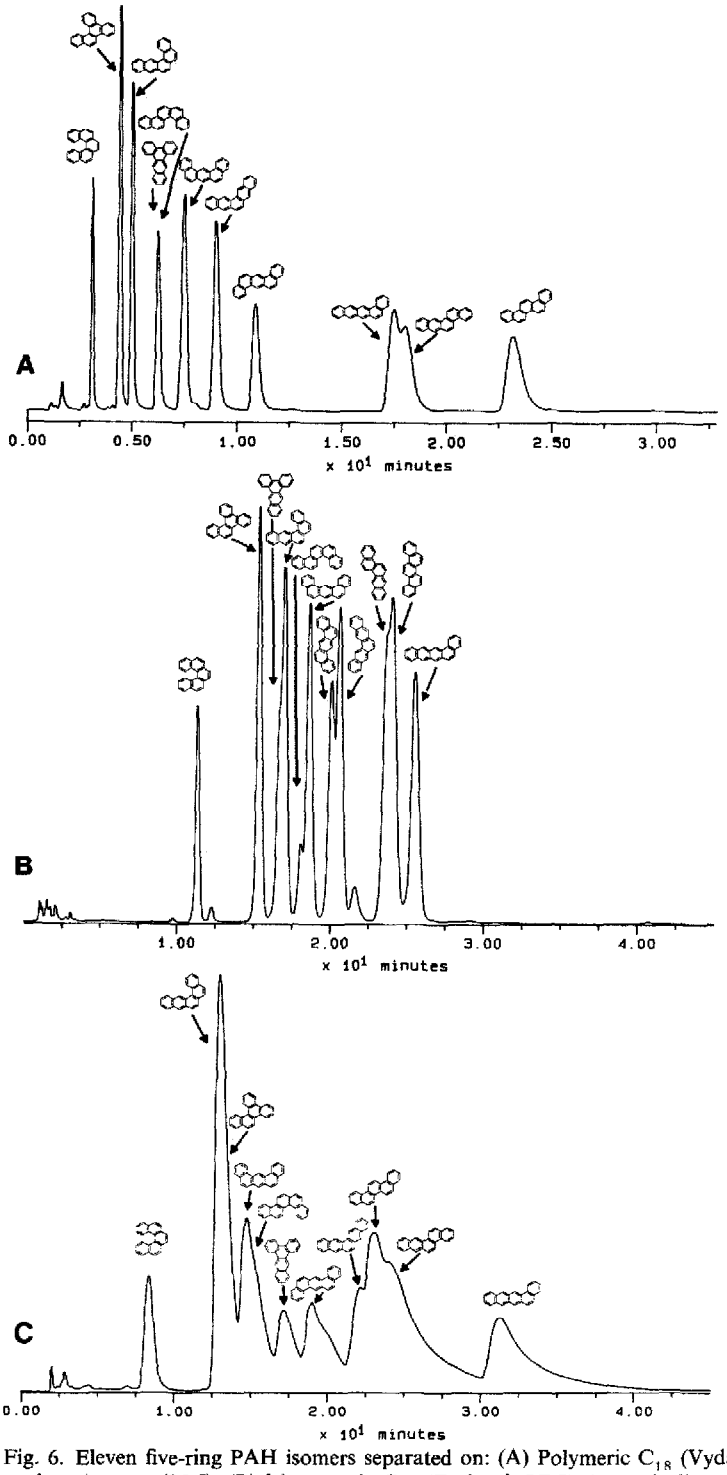


Fig. 6. Eleven five-ring PAH isomers separated on: (A) Polymeric C_{18} (Vydac™ 201 TP), isocratically methanol–water (95:5); (B) Monomeric C_{18} (Zorbax® ODS), isocratically methanol–water (90:10); (C) *β-cyclodextrin* (Cyclobond™ I), *isocratically methanol–water (50:50). All chromatographic runs were* performed at a flow-rate of 1.5 ml/min.

The retention of the pair benzo[*a*]naphthacene and picene clearly illustrates the difference between the mechanisms. The two molecules are narrow and well-retained on the polymeric C_{18}, monomeric C_{18} and cyclodextrin phases. Large differences do exist, however, in their relative retentions. Picene is the most rod-like of the eleven, as defined by the L/B ratio, and is thus retained the longest on the polymeric C_{18} phase. This trend has been observed by Wise and Sander[2] and is due to the high order of the polymeric phase, providing "slots" for long, narrow molecules to enter. On the cyclodextrin phase, picene cannot enter the β-cyclodextrin cavity longitudinally and therefore it will not be retained as long as benzo[*a*]naphthacene which has a single chain of four benzene rings in a row. The long narrow chain will enter deeply into the cavity and form a strong complex. This holds true for all of the eleven isomers; retention order is strongly dependent on how narrow the molecule is (*e.g.*, linear annelation of the benzene rings).

The elution order of these isomers (see Figs. 5 and 6) on the cyclodextrin column more closely resembles the elution order on the monomeric C_{18} than on the polymeric C_{18} column. This trend was observed primarily for solutes with L/B ratios of 1.7 and greater, *i.e.* later eluting solutes. However, elution order on the monomeric C_{18} of all eleven isomers is not identical to that on the cyclodextrin phase, and thus the mechanisms of retention for the two columns are different.

Differences in retention mechanisms are further illustrated by comparing the separation of PAHs of different molecular weights on the three types of columns. Retention data as k' values for several PAHs of varying molecular weight are summarized in Table II. On both monomeric and polymeric C_{18} columns, retention is largely dependent on the molecular weight of the solute. This trend is strongest for the monomeric C_{18} column where the three different molecular weights are divided into three distinct sections of the chromatogram. On the polymeric C_{18} column, retention is also dependent on the shape of the solute and the molecular weight dependence is less pronounced.

Retention on the cyclodextrin phase shows very little molecular weight dependence. The bulky five-ring benzo[*g*]chrysene elutes earlier than the long, narrow three-

TABLE II

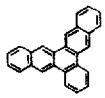
k' VALUES OF THREE-, FOUR- AND FIVE-RING PAH ISOMERS

Compound	*Molecular weight*	k'		
		Monomeric C_{18}[a]	*Polymeric* C_{18}[b]	*Cyclodextrin*[c]
Phenanthrene	178	1.91	0.95	2.79
Anthracene	178	2.16	1.26	6.15
Triphenylene	228	3.77	2.69	3.06
Chrysene	228	4.57	4.82	6.07
Benzo[*g*]chrysene	278	7.78	5.94	5.34
Dibenz[*a,j*]anthracene	278	9.65	13.08	6.25

[a] Monomeric C_{18} (Zorbax® ODS) isocratic 90% aqueous methanol 1.5 ml/min 30°C.
[b] Polymeric C_{18} (Vydac™ 201 TP) isocratic 85% aqueous methanol 1.5 ml/min 30°C.
[c] β-Cyclodextrin (Cyclobond™ I) isocratic 50% aqueous methanol 1.5 ml/min 30°C.


Phenanthrene


Anthracene


Benzo[c]naphtho[2,3-a]anthracene

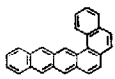

Phenanthro[4,3]anthracene

Fig. 7. Structures of PAHs listed in tables II and III.

ring anthracene. For the other PAHs in Table II, retention is dependent more on the ability of the solute to enter deeply into the cyclodextrin cavity than it is on the molecular weight of the solute. Only in cases of PAH molecules with similar shape and the same number of benzene rings in a row, will the heavier of the two molecules elute later (*e.g.*, three-ring phenanthrene elutes just before four-ring triphenylene). Several five- and six-ring PAH (for structures of six-ring isomers see Fig. 7) were also studied and the results are listed in Table III. The monomeric C_{18} column provides a separation based primarily on molecular weight with little differentiation between isomers. In contrast, the polymeric C_{18} is influenced profoundly by shape (L/B ratio) within an isomer group (*e.g.*, picene is the longest retained). For the cyclodextrin, the number of rings in a row acts as the dominant factor in determining retention, *e.g.*, benzo[*a*]naphthacene with four rings in a row is retained longer than the higher molecular weight six-ring isomers, whereas picene is the earliest eluting compound of the PAH in Table III.

An undesirable aspect of the cyclodextrin columns studied is the change in retention that occurs with time. Column equilibration times were found to be exces-

TABLE III

k' VALUES OF FIVE- AND SIX-RING PAH ISOMERS

For structures see Figs. 4 and 7.

Compound	*Molecular weight*	*k'*		
		Monomeric C_{18}[a]	*Polymeric* C_{18}[b]	*Cyclodextrin*[c]
Pentaphene	278	4.57	2.12	3.92
Picene	278	5.66	6.66	3.62
Benzo[*a*]naphthacene	278	5.84	4.65	5.64
Benzo[*c*]naphtho[2,3-*a*]anthracene	328	7.39	3.60	4.73
Phenanthro[4,3-*b*]anthracene	328	8.66	3.92	4.94

[a] Monomeric C_{18} (Zorbax® ODS) isocratic 97% aqueous methanol 1.5 ml/min 30°C.
[b] Polymeric C_{18} (Vydac™ 201 TP) isocratic 100% aqueous methanol 1.5 ml/min 30°C.
[c] β-cyclodextrin (Cyclobond™) isocratic 55% aqueous methanol 1.5 ml/min 30°C.

sive and frequent regenerations of the column were required to maintain column efficiency. Regeneration was accomplished by passing several column volumes of pure ethanol through the column, followed by pure water and methanol. Regeneration was found to be necessary even when aqueous organic mobile phases (no buffers) and pure standard solutions were used.

It can be concluded that in routine work, C_{18} columns provide better separation of PAH mixtures than cyclodextrin columns. Among C_{18} columns, the polymeric C_{18} column provides the highest selectivity, particularly for the separation of PAH isomers. However, cyclodextrin columns can offer unique selectivity and may be applied as an alternative method for separation of PAHs. The lack of retention dependence on molecular weight on the cyclodextrin phase may be advantageous for the separation of PAHs of different molecular weights. Chromatographic runs may be shortened and the use of solvent programming may be unnecessary in many cases. Another observation of using a cyclodextrin column is the lower amount of organic solvent needed for PAH separations compared to either monomeric or polymeric C_{18} phases.

RETENTION MODEL

Possible mechanisms of selectivity towards PAH on cyclodextrin bonded phases have been proposed by the column manufacturer[11]. Their results included retention data for a limited set of PAH isomers on the three types of columns, α- (6-glucopyranose units), β- and γ-cyclodextrin. In our work the mechanisms have been further investigated and the retention model for PAH isomers on β-cyclodextrin bonded phases has been extended. By using calculated widths of the different PAH isomers, we can determine whether the molecule is able to enter into and form a stable complex with the hydrophobic cavity. Width calculations were performed by adding two times the Van der Waal radius of hydrogen (1.2 Å) to the distance between hydrogen nuclei.

The inclusion complexing is illustrated in Fig. 8 using benz[*a*]anthracene as a model solute. The narrow part of the molecule is 7.3 Å. This portion of the molecule

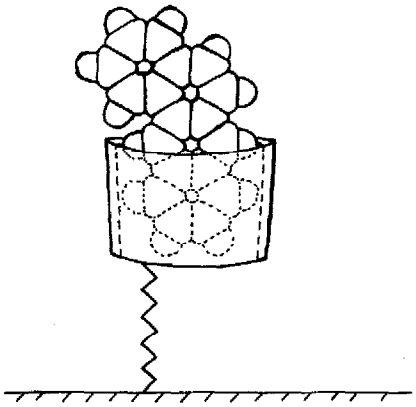


Fig. 8. PAH isomer benz[*a*]anthracene illustrates inclusion complexing with the β-cyclodextrin cavity. The narrow part of the molecule is 7.3 Å and able to enter the cavity, while the broader part (9.7 Å) is excluded.

will fit into the β-cavity of 7.8 Å while the broader part of the molecule, 9.7 Å, is excluded. A clear example of such exclusion results from molecules with an angular annelation of the benzene rings. As most PAHs are non-linear, only a portion of the molecule can participate in the complex. Molecules with linear annelation of the benzene rings ("rod-like" structures) will be able to enter deeper into the cavity.

This retention model takes into account only the shape of the molecule. Other interactions may also take place and affect solute retention. In this work it has been shown that the shape (the "rod-like" nature of the molecule) plays the most important role in solute retention. The longer the chain of single benzene rings (more narrow), the longer the PAH is retained on the cyclodextrin. Similar retention behavior was observed for larger PAH isomers with up to six condensed rings. For long narrow solutes, the molecule is able to enter the cavity completely. However, because the depth of the cavity is only 7.8 Å[13], the cavity is not deep enough for interaction along the full length of the solute. For example, the three-ring PAH anthracene has a molecular length of 11.5 Å and will not be completely covered by the cavity. Larger isomers containing such linear structures (*e.g.*, with the same stereochemistry as anthracene) will protrude, either at the mouth or at the bottom, from the cavity. Since retention is observed to increase with the addition of more aromatic rings in a row, the retention mechanism must include a solute-bonded phase interaction that occurs outside the cavity. Hydrophobic and electrostatic interactions may occur simultaneously with the inclusion complex, thus enhancing its stability.

REFERENCES

1 S. A. Wise, W. J. Bonnett, F. R. Guenther and W. E. May, *J. Chromatogr. Sci.*, 19 (1981) 457.
2 S. A. Wise and L. C. Sander, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 248.
3 K. Ogan and E. Katz, *J. Chromatogr.*, 188 (1980) 115.
4 K. Jinno and K. Kawasaki, *Chromatographia*, 17 (1983) 445.
5 R. J. Hurtubise, T. W. Allen and H. F. Silver, *J. Chromatogr.*, 235 (1982) 517.
6 L. C. Sander and S. A. Wise, *Anal. Chem.*, 56 (1984) 504.
7 L. C. Sander and S. A. Wise, *J. Chromatogr.*, 316 (1984) 163.
8 S. A. Wise and W. E. May, *Anal. Chem.*, 55 (1983) 1479.
9 D. W. Armstrong, A. Alak, W. DeMond, W. L. Hinze and T. E. Riehl, *J. Liq. Chromatogr.*, 8 (1985) 261.
10 D. W. Armstrong, W. DeMond, A. Alak, W. L. Hinze, T. E. Riehl and K. H. Bui, *Anal. Chem.*, 57 (1985) 234.
11 *Cyclobond™ Handbook*, Advanced Separations Technologies Inc., Whippany, NJ, 1987.
12 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.*, 22 (1984) 411.
13 *Cyclobond™ HPLC Column Operating Instructions*, Advanced Separations Technologies Inc., Whippany, NJ, 1988.
14 W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, A. Alak and T. Ward, *Anal. Chem.*, 57 (1985) 237.
15 T. D. Wilson, *J. Chromatogr.*, 448 (1988) 31.