A Study of Polynuclear Aromatic Hydrocarbons on an Amino Bonded Phase Liquid Chromatographic Column in the Normal and Reversed Phase

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A STUDY OF POLYNUCLEAR AROMATIC HYDROCARBONS ON AN
AMINO BONDED PHASE LIQUID CHROMATOGRAPHIC COLUMN
IN THE NORMAL AND REVERSED PHASE

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ABSTRACT

Experiments were run using an n-propyl amine polar bonded
phase (Chromosorb LC-9) liquid chromatographic column in both the
normal and reversed phase mode. Results confirm that the
mechanism of separation in the normal phase is due mainly to a
charge transfer interaction between the lone pair electrons on the
stationary phase nitrogen and the π electron cloud of the solute
PNAs. Elution order seems to depend upon a combination of π en-
ergy, and type of ring condensation of the solute. Plots of log I
versus number of aromatic carbons for catacondensed PNAs suggest
that while the specific interaction is different than that seen in
silica chromatography, the overall adsorption effect is
comparable. In the reversed phase there may be two types of
separation mechanisms: 1) a pure partitioning effect in highly
polar mobile phases (methanol/water), or 2) a mixture of
liquid-solid adsorption and liquid-liquid partition in less polar
solvent systems (acetonitrile/water).

INTRODUCTION

Polynuclear aromatic compounds (PNAs) constitute an important

class of chemical pollutants, many of which are known or suspect

carcinogens and mutagens (1, 2). Several industrial sources of

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these pollutants have been identified and include power plants, motor vehicles and oil refineries. Reliable analytical methods are needed to determine their presence since many of these compounds are on the EPA priority pollutant list. Due to the fact that PNAs are commonly found in complex matrices, it has been necessary in the past to combine several analytical techniques to provide complete characterization.

A typical analysis scheme for PNAs would include a pre-separation step such as solvent extraction or adsorption on silica or alumina, and then a subsequent analysis by GC and/or LC. However, solvent extraction and the use of "classical" adsorbents can be time consuming and tedious. Moreover, impurities such as water in the mobile phase can significantly modify the adsorbent, producing a non-reproducible separation (3). These adsorbents display a strong attraction for PNAs and often trace components will be strongly retained on the column. This results in decreased recovery and poor separation. As an alternative, gel filtration on Sephadex LH-20 has been incorporated into a separation procedure by Giger and Blumer (4). Novotiny et al. (5) used Sephadex LH-20 for initial purification and then an amino bonded phase for further fractionation of PNAs. Studies by others (6,7) have demonstrated that complex mixtures can be resolved by using adsorption or gel chromatography followed by reversed phase HPLC.

Considerable interest has been focused on the class separation of PNAs on alkyl amine chemically bonded phase columns.

Galya and Suatoni (8) have reported using a μBondapak NH_2 column to separate saturates, aromatics, resins, and asphaltenes (SARA) from crude oils. Wise and co-workers (9) have used the same column for the HPLC separation of crude oils and marine sediment samples. Chmielowiec and George (10) in a subsequent study of several polar stationary phases found superior PNA selectivity for a diamino bonded phase column. Similarly, a chemically bonded pyrrolidone substrate has been used to separate PNAs in shale oil samples (11). These three studies reported separations which were performed in the normal phase and which exhibited an elution order generally based on ring number and the type of ring condensation. The separation mechanism on the amino, diamino, and pyrrolidone columnm packings, in the normal phase mode, is postulated to be a charge-transfer interaction between the lone pair electrons of the stationary phase nitrogen and the π electron cloud of the PNAs. An interesting development in Mourey's work was the investigation of a reversed phase separation on pyrrolidone (11). These experiments demonstrated that normal and reversed phase separations are complementary in terms of selectivity with the latter technique more efficient for solutes with less than six aromatic rings.

Separation via adsorption chromatography is based on the competition of the solute and solvent molecules for active sites on the stationary phase. In classical adsorption, the active sites on the adsorbent generally interact with polar functional groups on compounds to be separated. The elution order in this

type of separation is usually predictable and follows the constituent polarity. In a charge transfer mechanism, the interaction between the adsorbent surface and solute is more specific. When the mobile phase is relatively non-polar, the lone pair electrons on the polar amine functionality of the stationary phase interact with the π cloud on the PNAs forming a weak bond. Elution order in this case may be dependent more on either the electron affinity or the total energy of the π electron system rather than its polarity. A modification of this explanation may be required, however, when considering the interactions involved when the mobile phase is more polar.

In order to explore further the separation mechanism of the n-propyl amine bonded phase column, this work reports the retention of PNAs on this stationary phase in both the normal and reversed phase modes. The selectivity and capacity factors are given for several examples of three, four, and five ring PNAs which can be considered as members of different ring classes. This data as well as theoretical considerations are discussed in terms of previously described mechanisms.

EXPERIMENTAL

Apparatus

All data was obtained on a microprocessor controlled Altex model 312 MP liquid chromatograph with a 100 μL sample loop. The PNAs were separated on a 30 cm X 10 mm i.d. Chromosorb LC-9 column (Supelco Inc., Belefonte, PA) and monitored with a UV absorption detector at 254 nm.

Reagents and Standards

All PNAs used were purchased at 95% purity or better and were used without further purification. Mobile phase eluents were glass distilled cyclohexane for normal phase chromatography, and spectrograde methanol, glass distilled acetonitrile, and type three reagent grade water obtained using a Milli-Q water purification system, for reversed phase chromatography. Reagent grade isopropanol, and 99+% dichloromethane were solvents used for converting from the normal to the reversed phase modes. Standards for the normal phase mode were prepared by dissolving the appropriate amount of each standard in cyclohexane to prepare solutions with concentrations of approximately 10^{-4} M. For the reversed phase work, standards were prepared in both acetonitrile and methanol.

Procedure

The PNA standards were run in the normal phase (100% cyclohexane) and the retention times recorded. The column was then converted to reversed phase by flushing with 20 column volumes each of isopropanol, dichloromethane and methanol. In the final step, equilibrium was attained with an additional 100 column volumes of methanol. The retention times for the PNA standards were measured in mobile phases consisting of varying percentages of methanol/water or acetonitrile/water. In both modes of chromatography, the flow rate was 2 mL/min. After all reversed phase work was complete the column was converted back to normal phase by flushing with the above solvents in the reverse order. Comparison of the number of theoretical plates before and after

conversion to reverse phase showed no significant changes indicating little or no irreversible effects.

RESULTS AND DISCUSSIONS

Chromosorb LC-9 packing consists of an n-propyl amino silane chain chemically bonded to 10 micron silica particles. Because the surface of this material is highly polar, it is useful for separating polar compounds. However, when used with a non-polar mobile phase, such as cyclohexane, non-polar aromatic hydrocarbon separation is possible, analogous to the separation obtained on silica or alumina. Generally, elution volumes seem to increase with the number of condensed rings.

Because the retention times of various solutes can change from run to run due to minor experimental differences, it is necessary to relate retention time to a parameter independent of these differences. One possibility is to use the retention index (I). This is a parameter derived in a manner analogous to the Kovat's index for gas chromatography. The calculation is based on a linear relationship between $\log V_r$ and the number of aromatic rings. The basic assumption is that slight variations in chromatographic conditions which cause a shift in retention of one PNA will also affect the retention of all other PNAs although their position relative to each other will not change. In other words, the observed V_r will change but I values will not.

The retention indices for several PNAs were calculated as previously described by Popl et al. (12). To compare the

retention characteristics of PNAs on several nitrogen containing

stationary phases, the logarithms of the retention indices (I) for

several PNAs on μBondapak NH_2 (13), diamine (10), pyrrolidone (11)

and Chromosorb LC-9 are given in Table I. The hydrocarbon

standards for the μBondapak NH_2, pyrrolidone, and diamine packing

material were assigned the following retention indices: benzene

10, naphthalene 100, phenanthrene 1000, 1,2-benzanthracene 10,000

and benzo (b) chrysene 100,000. For Chromosorb LC-9, the same

hydrocarbon standards were used with the following exceptions:

anthracene 1000, and 1,2,3,4-dibenzanthracene 100,000. As

reported by Popl et al. (14), the selection of the index compounds

for separation of PNAs on alumina was dictated by an observed pH

dependence. For PNAs with certain structural characteristics such

as an acidic hydrogen (fluorene) or low ionization potentials

(anthracene), it was found that retention increased on basic

alumina and decreased on acidic alumina. Popl chose index

compounds for which the log V_r vs log I relationship was linear

because of this acid-base effect.

Although we are dealing with charge transfer interactions,

the same criteria Popl used can be applied when choosing PNAs for

index compounds on amine polar bonded phases. That is, those

compounds for which the log V_r versus log I relationship is linear

would be suitable index compounds. Even though the compounds

chosen above are not necessarily charge transfer homologs, if a

linear relationship is satisfied, then the compounds are

satisfactory index compounds. Figure 1 shows a plot of log I vs

Table I

Comparison of the Log of Retention Index (log I) of PNAs on
Several Nitrogen Containing Liquid Chromatographic Packing
Materials

Compound	Chromosorb LC-9 (Cyclohexane)	µBondapak NH$_2$ (Hexane)	Pyrrolidone (Heptane)	Diamine (n-Heptane)
One Ring Aromatics	log I[a]	log I[b]	log I[c]	log[b]
Benzene	1.00	1.00	1.00	1.00
Two Ring Aromatics				
Biphenyl	1.93	2.16	1.40	2.25
Naphthalene	2.00	2.00	2.00	2.00
Acenaphthylene	2.64	2.59	2.26	3.20
Three Ring Aromatics				
Fluorene	2.59	2.55	2.26	2.50
p-Terphenyl	3.03	3.28	2.88	3.49
Anthracene	3.00	2.94	2.96	2.92
Phenanthrene	3.12	3.00	3.00	3.00
9,10-Diphenyl-anthracene	2.57	—	3.16	3.47
Dibenzothiophene	2.77	2.75	—	—
Four Ring Aromatics				
Fluoranthene	3.60	3.51	3.46	3.59
Pyrene	3.43	3.37	3.47	3.43
Chrysene	3.87	3.90	3.83	—
p-Quaterphenyl	4.05	4.50	3.90	—
Triphenylene	4.23	4.07	3.91	—
1,2-Benzanthracene	4.00	4.00	4.00	4.00
Five Ring and Larger Aromatics				
Perylene	4.70	4.61	3.92	—
Benz(a)pyrene	4.44	4.38	4.11	—
Benz(e)pyrene	4.60	4.46	—	—
Benz(g,h,i)perylene	5.00	4.83	4.45	—
1,2,5,6-Dibenz-anthracene	4.68	4.94	4.60	—
1,2,3,4-Dibenz-anthracene	5.00	4.93	4.64	—
Picene	—	5.03	4.63	—
Benze(b)chrysene	—	5.00	—	5.00

[a]Index compounds were assigned retention indices as follows:
benzene 10, naphthalene 100, anthracene 1000, 1,2-benz-
anthracene 10,000 and 1,2,3,4-dibenzanthracene 100,000.

[b]Index compounds were assigned retention indices as follows:
benzene 10, naphthalene 100, phenanthrene 1000, 1,2-benz-
anthracene 10,000 and benzo(b)chrysene 100,000, and are taken
directly from the references.

[c]Index compounds were assigned retention indices as follows:
benzene 10, naphthalene 100, phenanthrene 1000, and 1,2-benz-
anthracene 10,000, and were calculated from the k' values
given in reference 11. For compounds with higher elution
volumes, the linear dependance of log V_r versus log I was
extrapolated to the region of I $\geq$ 10,000.
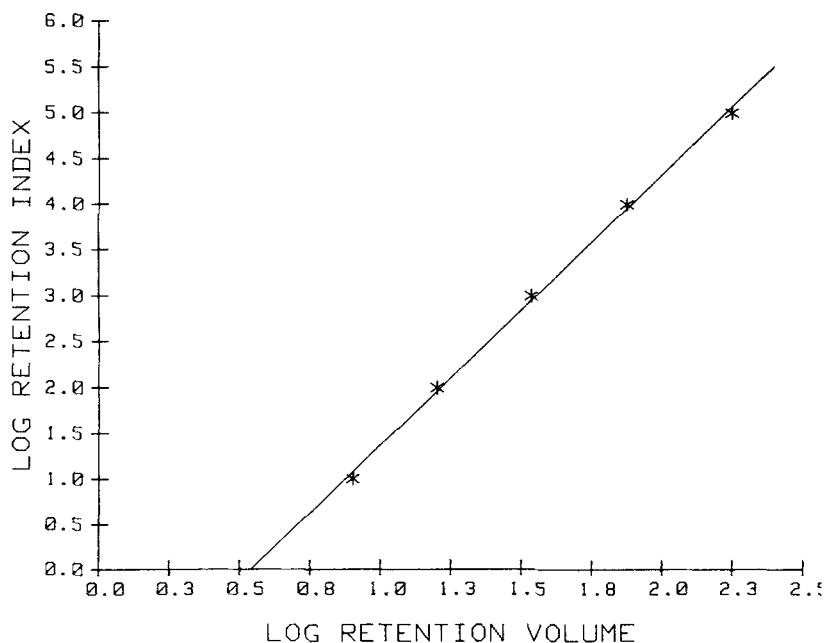
FIGURE 1.   Calibration plot for PNAs on Chromosorb LC-9.

log V_r' on Chromosorb LC-9 for the index compounds chosen.   The

plot is linear with a coefficient of determination, r^2, of .9984.

Although this r^2 is based on a small data set (only five points),

the probability of five unrelated data points giving an r value

this high would be .001 (15).   If the choice of index compounds is

not critical, then one would suspect that the log I values of the

same PNAs on similar columns should yield similar relationships,

even if the index compounds chosen to calculate the log I values

are slightly different.   Figure 2 shows a plot of log I vs log V_r'

(the corrected retention volume) for the same compounds on

Chromosorb LC-9.   The log I values were calculated using the
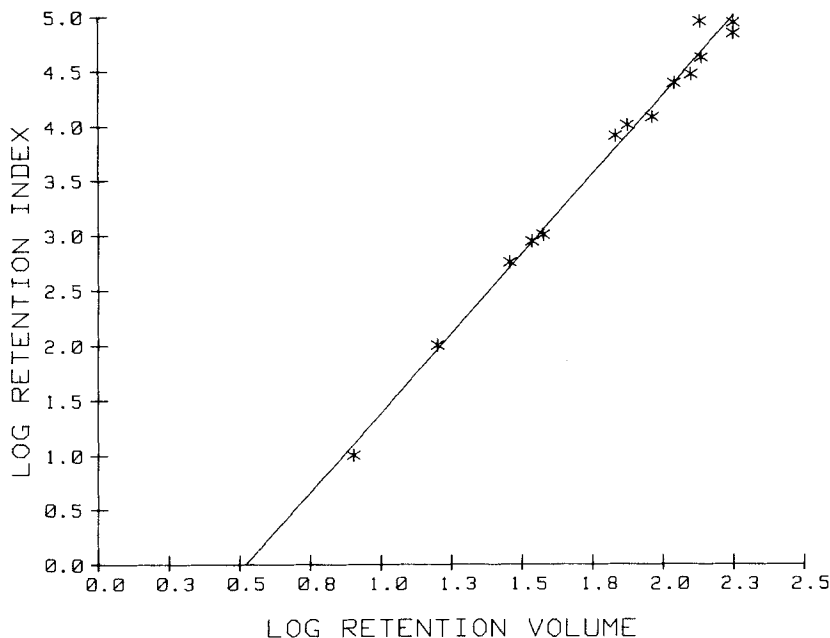
FIGURE 2. Comparison calibration plot.

retention volumes of the index compounds on μ Bondapak NH$_2$.

Phenanthrene was used as a three ring standard and benzo (b)

chrysene for a five ring index standard. This relationship is

also linear with a coefficient of determination of .9908. The

probability of obtaining this r value with 14 unrelated points is

.001 (15). It should be noted that the new index standard will

not produce identical retention indices for both amine columns;

however, the relationships between individual PNAs is preserved.

Anthracene has a log I value of 3.00 and 2.94 on the Chromosorb

LC-9 and μ Bondapak NH$_2$ columns respectively. Likewise,

phenanthrene has log I values of 3.12 and 3.00 Although these

values are not identical, phenanthrene has a larger log I value than anthracene on both columns. Figure 2 shows that the relationship of each individual PNA to any other is the same using both sets of standards. Therefore, one may conclude that choosing anthracene instead of phenanthrene for a three ring index standard, and 1,2,3,4-dibenzanthracene in place of benzo (b) chrysene for a five ring index standard will not significantly affect the data comparison, even if the index compounds are not charge transfer homologs.

If a polar bonded phase amine packing material separates primarily via a charge transfer mechanism, then one would expect that the elution order of PNAs would be dictated by some parameter of the solute PNA which reflects the electron transfer interaction. Some possible choices would be total π energy, electron affinity (EA), the reduction potential $(E_{1/2\ red})$, ionization energy (IE), the energy of the lowest unoccupied molecular orbital (LUMO) or the highest occupied molecular orbital (HOMO). Some values for these constants for several PNAs are given in Table II. Plots of log I versus each of these parameters were made for each different type of packing material. The coefficients of determination for these plots are given in Table III. The parameter r^2 is the coefficient of determination which was determined for each plot, n is the number of data points used and P is the probability of obtaining or exceeding the given r using completely unrelated data (15). Ranking of r^2 indicates that the plot of log I vs π energy is the best fit to the data,

Table II

Solute Parameters Used in Adsorption Mechanism Study

Compound	Π Energy [16]	EA(eV) [15]	IE(eV) [18]	$E_{1/2red}(V)$ [17]	LUMO [16]	HOMO [16]
Benzene	5.87	——	9.22	-2.30	0.800	-0.800
Biphenyl	11.85	0.085	8.36	——	0.599	-0.599
Naphthalene	8.12	0.152	8.28	-1.54	0.535	-0.534
Acenaphthylene	11.87	——	8.21	——	0.550	-0.555
Fluorene	11.85	——	——	——	0.599	-0.596
p-Terphenyl	17.84	——	——	——	0.514	-0.519
Anthracene	13.81	0.552	7.76	-1.09	0.375	-0.370
Phenanthrene	13.94	0.308	8.18	-1.50	0.526	-0.520
Fluoranthene	16.04	——	8.14	-1.45	0.535	-0.403
Pyrene	16.01	0.579	7.85	-1.16	0.400	-0.404
Chrysene	17.99	0.419	8.17	-1.35	0.460	-0.460
p-Quaterphenyl	23.83	——	——	——	0.473	-0.470
Triphenylene	18.07	0.284	——	-1.55	0.584	-0.580
1,2-Benz-anthracene	17.90	0.696	7.79	-0.85	0.320	-0.326
Perylene	20.06	——	7.49	-0.85	0.320	-0.326
Benz(a)pyrene	20.04	0.829	——	——	0.340	-0.340
Benz(e)pyrene	20.15	0.486	7.85	-1.24	0.486	-0.483
Benz(ghi)pery-lene	22.25	——	7.76	-1.01	0.396	-0.396
1,2,5,6-Dibenz-anthracene	21.99	0.676	——	-1.19	0.423	-0.426
1,2,3,4-Dibenz-anthracene	22.05	——	——	-1.25	0.444	-0.444
Picene	22.03	.0490	7.23	-1.33	0.446	-0.446

ionization energy is next and all others show a much poorer fit.
This correlation supports the theory that the separation mechanism
involves a charge transfer interaction between the nitrogen
containing stationary phase and the solute PNA. This conclusion
is also supported by the fact that both Wise (9) and Mourey (11)
noticed that the addition of alkyl substituents does not
significantly shift the retention of a PNA on either an amine or

TABLE III

Comparison of Coefficients of Determination, r^2, for Plots of Log I versus Solute Parameters[a]

Solute Parameter[b]	Chromosorb LC-9			μBondapak NH$_2$			Pyrrolidone			Diamine		
	r^2	n	P	r^2	n	P	r^2	n	P	r^2	n	P
π Energy	0.880	20	0.001	0.948	21	0.001	0.863	11	0.001	0.891	11	0.001
IE (eV)	0.702	13	0.001	0.757	14	0.001	0.712	13	0.001	0.726	9	0.01
EA (eV)	0.526	11	0.02	0.457	12	0.02	0.548	11	0.02	0.836	6	0.01
$E_{1/2red}$(V)	0.590	16	0.001	0.535	15	0.01	0.531	14	0.01	0.708	7	0.01
LUMO	0.550	20	0.001	0.530	21	0.001	0.557	20	0.001	0.618	11	0.01
HOMO	0.381	20	0.01	0.347	21	0.01	0.369	20	0.01	0.669	11	0.01

[a] See Tables I and II for data

[b] All πEnergies, LUMO and HOMO numerical data were obtained using the Huckel molecular orbital theory. n is the number of data points and P is the probability of obtaining or exceeding the given r with unrelated data.

r^2 is the coefficient of determination,

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Table IV

Coefficients of Determination for Plots of Log I Versus II Energy for Different
N-Containing Packings[a]

Ring Condensation Type	Pyrrolidone		Chromosorb LC-9		μBondapak NH$_2$	
Non-Condensed	0.928	n=5	0.946	n=5	0.982	n=5
Peri-Condensed	0.954	n=6	0.985	n=7	0.984	n=7
Cata-Condensed	0.993	n=9	0.979	n=8	0.989	n=9

[a]See Table I for data

pyrrolidone bonded phase.   This is because addition of an alkyl group does not appreciably change the total π energy.

Table IV gives the coefficient of determinations for log I vs π energy plots for different ring classes on different packing materials.   It appears that the coefficient for catacondensed PNAs is generally greater than that for pericondensed solutes.   This may be due in part because the retention index standards are catacondensed structures, but it also may imply that a steric effect should be considered as well.   Work done by Hammers, Spanjer, and DeLigny (19) on an amino butyl polar bonded phase column indicates the presence of a steric effect.   These authors noticed a discrepancy in the behavior of flat fused arenes and slightly twisted polyphenyls. Because the interaction is charge transfer, it follows that the greater the π conjugation the stronger the interaction.   All else being equal, the amount of π conjugation depends upon the arrangement of the molecules.   For example, interaction appears to be greater for flat molecules than for slightly twisted polyphenyls.

Popl et al. (12) found that on silica, there is a linear dependence of log I on the number of aromatic carbon atoms for parent catacondensed PNAs.   They found that the following equation describes the observed effect,

$$\log I = 0.25\ nC_{Ar} - 0.5$$

where $n\ C_{Ar}$ is the number of aromatic carbon atoms in the molecule.   Table V gives the equation of the line which describes

Table V

Equations Describing the Relationship Between Log I and Number
of Aromatic Carbons in Catacondensed PNAs

Pyrrolidone	$\log I = 0.230\ n\ C_{Ar} - 0.286$
μBondapak NH$_2$	$\log I = 0.249\ n\ C_{Ar} - 0.496$
Diamine	$\log I = 0.245\ n\ C_{Ar} - 0.497$
Silica	$\log I = 0.25\ \ n\ C_{Ar} - 0.5$

the relationship between carbon number and log I for catacondensed

PNAs on μBondapak NH$_2$, the diamine, and pyrrolidone packing

materials. In the monoamine, and especially the diamine systems,

the equations are nearly identical to the silica relationship.

This indicates that the interaction is similar to adsorption on

silica. However, this is not to say that all PNAs behave the same

on both the silica and the amine polar bonded phases. Alkyl

substitution of PNAs results in greater chromatographic

differences on silica than on the amine packing material as shown

by Wise et al. (9).   Therefore, the separation mechanisms or

"adsorption" interactions are different, although the overall

adsorption effects are similar.

     After examining the performance of Chromosorb LC-9 in the

normal phase, we converted the column to the reversed phase to

examine its behavior under these conditions. The capacity factors

for several PNAs in 100% CH$_3$CN and 100% MeOH are given in Table

VI.   The capacity factors are very small, indicating that this

particular column does not retain PNAs very strongly under these

Table VI

Capacity Factors, k', for Selected PNAs on the Amino Bonded
Column in the Reversed Phase

Compound	100% CH_3CN	100% MeOH
Two Rings		
Naphthalene	0.121	0.170
Three Rings		
Anthracene	0.165	0.188
Four Rings		
p-Quaterphenyl	0.123	0.136
Chrysene	0.170	0.180
Triphenylene	0.177	0.180
1,2-Benzanthracene	0.167	0.170
9,10-Dimenthyl-1,2-benzanthracene	0.165	0.165
Fluoranthene	0.170	0.121
Pyrene	0.159	0.175
Five Rings and More		
Benz(a)pyrene	0.177	0.234
Benz(e)pyrene	0.180	0.244
1,2,3,4-Dibenzanthracene	0.167	0.229
Pentacene	0.175	0.159
9,10-Diphenylanthracene	0.144	0.141
Perylene	0.203	0.252
Benz(g,h,i)perylene	0.196	0.275
Rubrene	0.139	0.149

conditions and would not be a practical way to chromatograph PNAs.
However, the relative retention (or lack of retention) of these
compounds in the reversed phase should reveal information about
the mechanism of separation of the N-containing packing materials.
With the more polar solvents used in reversed phase

chromatography, adsorption effects are minimized and thus retention is shorter. Mourey et al. (11) reported much higher k' values for the same PNAs in an even more polar mobile phase (60% methanol/water) on pyrrolidone, a cyclic amine bonded phase. The best explanation for this increased retention is a combination of two effects: 1) the bonded phase polarity and 2) the mobile phase polarity. Obviously, the pyrrolidone functionality is much less polar than the n-propyl amine in the reversed phase. Thus it is possible to chromatograph PNAs on pyrrolidone in the reversed phase but not on an amino bonded phase column.

Adding water to a silica column can greatly affect the activity of the column and cause irreproducible effects. However, when the silanol groups are chemically bonded with some polar functional group (such as these nitrogen containing moieties) the column becomes much more stable to water. Although it is likely that due to incomplete coverage there are some exposed silanol groups which can interact with water, the percentage of these is low, and reproducible results can be achieved if the column is allowed to come to equilibrium with the mobile phase.

Although the k' values are very small they seemed to be very reproducible. Table VII shows the retention of three PNAs (naphthalene, anthracene, and pyrene) on Chromosorb LC-9 using different mobile phases. The standard deviation of each point is listed along with the number of repetitions. This data is presented graphically in Figures 3 and 4. The limits of the standard deviation are marked on the last point of each plot since

Table VII

Retention of Three PNAs on Chromosorb LC-9 Using Varying
Percentages of Methanol/Water and Acetonitrile/Water

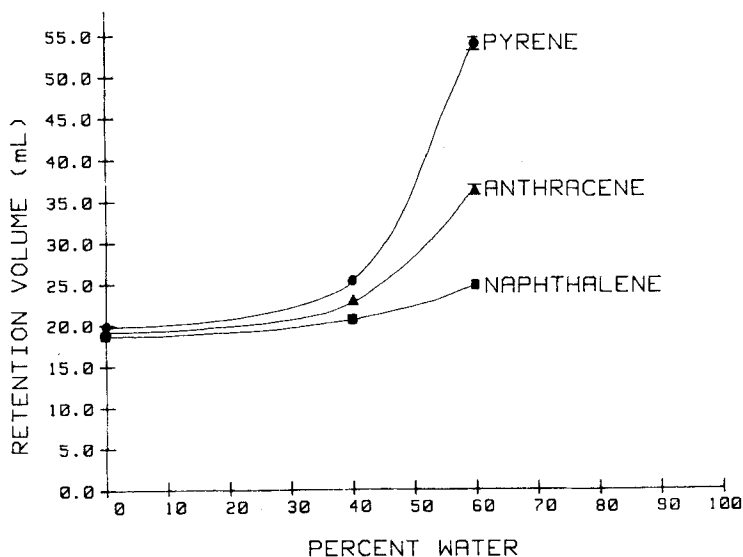| Percent Methanol	Number of Repetitions	Pyrene	Anthracene	Naphthalene
100	8	20.60 ∓ 0.01	19.92 ∓ 0.02	10.40 ∓ 0.02
60	8	26.14 ∓ 0.11	23.50 ∓ 0.05	21.33 ∓ 0.05
40	5	54.72 ∓ 0.68	37.14 ∓ 0.42	25.62 ∓ 0.14
Percent Acetonitrile				
100	5	19.25 ∓ 0.02	18.98 ∓ 0.03	18.83 ∓ 0.05
80	8	16.92 ∓ 0.03	16.72 ∓ 0.02	16.83 ∓ 0.05
60	8	16.10 ∓ 0.07	15.97 ∓ 0.17	15.81 ∓ 0.25
40	8	21.60 ∓ 0.09	20.55 ∓ 0.06	19.47 ∓ 0.05
20	5	56.51 ∓ 0.67	49.70 ∓ 1.00	26.04 ∓ 0.72

FIGURE 3.   Change in retention volumes of some polynuclear
aromatic hydrocarbons with water content in a methanol/water
solvent   system.   The retention volumes are not corrected for the
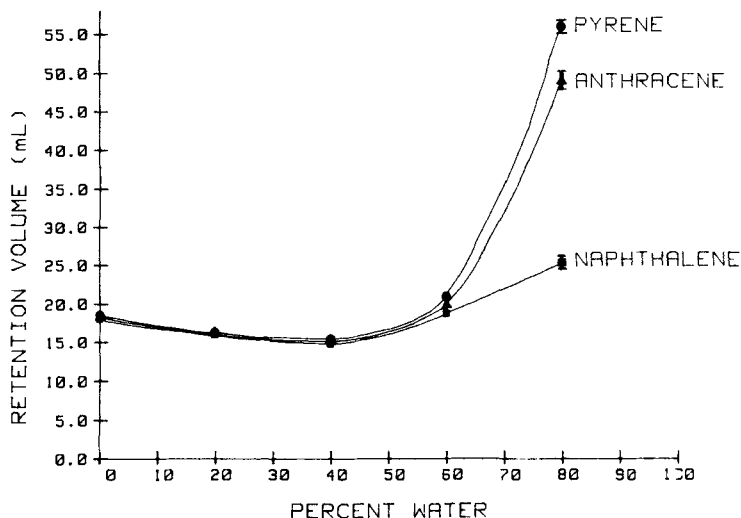column void volume (15.3mL).

FIGURE 4. Change in retention volumes of some polynuclear aromatic hydrocarbons with water content in an acetronitrile/water solvent system. The retention volumes are not corrected for the column void volume (15.3 mL).

this is the only place the deviation was large enough to visually observe. The upper limit of the percent water in the mobile phase is determined by the solubility of the PNAs. In the methanol/water system (Figure 3), the retention of each PNA seems to increase as the polarity of the solvent increases. For reversed phase chromatography, this is exactly what would be expected if the separation occurred according to a partition mechanism. In the acetonitrile/water system (Figure 4), however, results are a bit more interesting. The three compounds are strongly retained on the column in 80% water/acetonitrile. As the percent water is decreased the retention volumes decrease with a

minimum occurring at about 40% water/acetonitrile. At 100%
acetonitrile (0% water), however, the elution volumes again
increase. Similar effects have been observed by Goldstein (20) on
pyrrolidone in isopropanol, and have been compared to parallel
behavior observed on silica. As the composition of the mobile
phase changes from 100% acetonitrile to acetonitrile/water, the
stationary phase surface adsorbs an aqueous layer, prohibiting
solute interaction with the stationary phase and consequently
solute adsorption is decreased. As more water is added, the
aqueous layer becomes more extensive and the solutes slowly begin
to preferentially partition into the stationary phase. Thus,
there are two effects in competition here. At high percentage of
organic modifier, a liquid–solid adsorption effect seems to be
predominant which becomes less important as a more aqueous solvent
system is reached where a liquid–liquid partitioning effect seems
to be more important.

CONCLUSIONS

The separation mechanism for PNAs on the amino, diamino, and
pyrrolidone columns in the normal phase is postulated to be a
charge transfer interaction between the lone pair electrons on the
stationary phase and the π electron cloud.   This possible
mechanism is supported by the high correlation between the log I
values and the total π energy of the PNAs studied. Ranking of
coefficients of determination for these plots indicate that
π energy has the best fit to the data and the ionization potential

is the next best. These values were taken from Hückel molecular orbital calculations and possibly more exact calculations for π energy could give a better fit. According to the r^2 values, it would seem that pericondensed PNAs are retained differently than catacondensed PNAs, suggesting that there could be a geometric effect in addition to π energy and ionization potential.

Plots of log I versus number of aromatic carbons for catacondensed PNAs on the monoamine, diamine, and pyrrolidone materials suggest that the retention is due to interactions resulting in adsorption effects which are similar in both silica and amine separations. However, the specific inteactions are different than those seen in silica chromatography.

The reversed phase data show that the selectivity on Chromosorb LC-9, in general, for PNAs in 100% acetonitrile or 100% methanol is poor. It is difficult to classify bonded phase, reverse phase chromatography as either a strict partition or adsorption process. The data seems to point to both processes happening. With methanol/water systems the data seems to indicate a partitioning mechanism. On the other hand, in an acetonitrile water system the mechanism seems to be a mixture of liquid-solid adsorption and liquid-liquid partition, depending upon the polarity and water content of the mobile phase.

In general, our data support previously drawn conclusions concerning the separation mechanisms of PNAs on nitrogen-containing supports. However, to our knowledge, these conclusions have not been supported experimentally. We have

formulated these conclusions from both normal and reversed phase
studies using data from our experiments as well as the published
results of other studies.

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