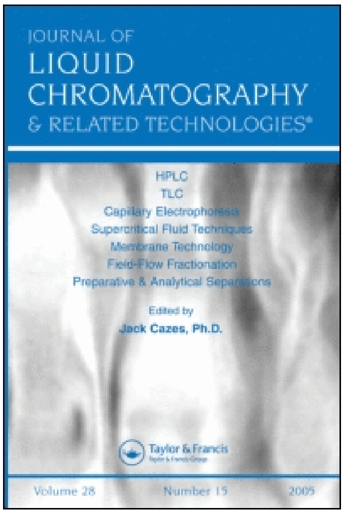

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Effects of π-π Interactions on the Separation of PAHs on Phenyl-Type Stationary Phases

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Abstract: Phenyl-type stationary phase surfaces are useful for the separation of highly aromatic compounds because of the extensive intermolecular forces between the π-electron systems. For this reason, we studied the retention behaviour and selectivity of polycyclic aromatic hydrocarbons (PAHs) on Synergi polar-RP and Cosmosil 5PBB chromatography columns using methanol/water, acetonitrile/water, benzene spiked (0.5%) methanol/water, and benzene spiked (0.5%) acetonitrile/water mobile phases. These four solvent systems were employed because π-π interactions between the aromatic solute (i.e., PAH) and the aromatic stationary phase should be inhibited in mobile phases that are also π electron rich, and hence a competitor for the analyte. Our results showed that the acetonitrile mobile phases were substantially stronger eluents than the methanol mobile phases, which was consistent with the premise that retention of aromatic compounds is sensitive to π-π interactions. Aside from changes in absolute retention, selectivity of the PAHs was also generally greater in methanol rather than acetonitrile mobile phases because the methanol did not attenuate the π-π bonding interactions between the PAH and the stationary phase; but, despite this, the retention behaviour of the Synergi polar-RP column was similar to that observed on C_{18} columns. The excessive retention times of the

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Cosmosil 5PBB column were decreased dramatically when acetonitrile was used as the mobile phase; however, selectivity between structural isomers was lost.

Keywords: π-π interactions, Synergi polar-RP, Cosmosil 5PBB, Stationary phase, PAH, Retention characteristics

INTRODUCTION

In recent years, there has been an increase in the design and development of new aromatic stationary phases for the separation of highly aromatic compounds such as polycyclic aromatic hydrocarbons (PAHs), nitroaromatic compounds, and polychlorinated biphenyls (PCBs). Phenyl-type phases have been undergoing a significant increase in popularity for the separation of aromatic compounds, and have been shown to have a greater specificity towards PAHs, indicating additional selectivity, presumably due to π-π interactions.[1–4] Kibbey and Meyerhoff[5] conducted separations on a number of aromatic hydrocarbon bonded stationary phases, such as phenyl phases, using PAHs (between 2–5 rings). They found that the dominant factors affecting retention on these types of phases were the size and shape of the PAHs, and that retention was mainly due to a combination of both π-π and shape interactions. Therefore, a number of aromatic stationary phases have been designed to exploit π-π interactions in order to provide increased separation selectivity. A few examples of these aromatic phases include the Synergi polar-RP (phenyl-oxypropyl bonded silica),[6–10] pyrenylethyl-(PYE),[11] pentaflurophenylpropyl-(PFP),[12] and Cosmosil columns.[6,8,13–15]

The Cosmosil stationary phases (manufactured by Nacalai Tesque, Japan) have been recently introduced into the market. The popularity of these stationary phases has been increasing due to their ability to separate closely related π-electron containing species. Murahashi and coworkers employed Cosmosil columns for the separation and determination of PAHs and other environmental contaminants from diesel and automobile exhaust particulate matter,[16,17] and have also taken advantage of their different retention behaviour and employed them in two dimensional high performance liquid chromatography (HPLC) separations of structurally related compounds.[18]

A number of Cosmosil columns are available, each having unique retention of aromatic compounds, however, the stationary phase of interest to us in the present work is the Cosmosil 5PBB phase, which is a pentabromobenzyl-oxypropyl bonded silica, and has been found to be useful in the separation of fullerenes[14,19] and offering very strong retention for PAHs.[6] The bromine atoms on the aromatic ring of the Cosmosil 5PBB column have been found to affect dispersion interactions (which may be due to the increased surface contact with the solute) and charge transfer interactions (where the electron-withdrawing bromine atoms can create an electron deficient zone in the ring centre), and therefore, this column favours charge transfer complexes with electron-rich solutes.[15] Hence the Cosmosil 5PBB stationary phase is highly retentive for PAHs providing also substantial selectivity with respect to the number of aromatic rings.[6,20] Despite significant retention being beneficial to gaining high resolution separations of PAHs, it is also a limitation of the Cosmosil 5PBB column, because traditional reversed phase solvents, such as methanol, offer poor elution strength on this column. Therefore, the addition of tetrahydrofuran or dichloromethane would be required for timely elution of PAHs with ring numbers that exceed 4 or 5 so that retention is not excessive.

Another column manufactured by Phenomonex is the Synergi polar-RP column. This column contains a propyl-ethoxy phenyl stationary phase, specifically designed to maximise the retention and selectivity of aromatic analytes. In a previous study,[6] we examined the retention behaviour of PAHs in an aqueous/methanol mobile phase on two C_{18} and three phenyl-type stationary phases. In general, the phenyl-type columns offered better selectivity for linear PAHs, while the C_{18} columns gave better selectivity for the non-linear PAHs. However, somewhat surprisingly, the performance of the Synergi polar RP column was similar to the C18, rather than the other phenyl-type columns. It is for this reason, that here we specifically compare the retention behaviour of PAHs on both the Synergi polar RP phase and the Cosmosil 5PBB phase in a variety of solvent systems, so that selectivity in separation can be optimised on either phase or in combination with a view towards two dimensional separations.

EXPERIMENTAL

Chemicals

HPLC grade methanol (MeOH) and acetonitrile (ACN) were obtained from Lab Scan Analytical Sciences (distributed by LOMB Scientific (AUST) Pty. Ltd. (Taren Point, NSW, Australia)). Tetrahydrofuran (THF) was obtained from Aldrich Chemical Company, Inc. (Castle Hill, NSW, Australia). Milli-Q water (H_2O) was obtained in-house and filtered through a 0.2 μm Teflon filter (Millipore Australia Pty Ltd, North Ryde, NSW, Australia).

PAH standards were purchased from Aldrich Chemicals (Sigma-Aldrich Chemical Company Inc., Castle Hill, NSW, Australia). The limited solubility of the PAHs in methanol or acetonitrile required that they be dissolved in a stronger solvent such as THF. In this work, each PAH standard (total of 12 linear and structural isomers) were dissolved in 100% THF and made up to concentrations of 10 mg/mL. Five microliter injections were then used for chromatographic studies. Some evidence suggests that injection mismatch between solvent plug and mobile phase may affect chromatographic performance and even enhance the phenomenon of viscous fingering. However, no such problems were encountered in this work, which is due to the very small injection volumes and the strong solute retention. Essentially identical retention times were observed for the PAH standards that were able to be dissolved in methanol or acetonitrile and then injected into each of these mobile phases, respectively, as they were when the sample solvent was THF.[20]

Equipment

All chromatographic separations were performed on a Shimadzu LC system (Shimadzu Scientific Instrumentation, Rydalmere, NSW, Australia), incorporating a LC-10AT*VP* pumping, SIL-10AD*VP* auto injector, DGU-14A online degasser, SPD-M10A*VP* diode array detector (set at 262 nm), SCL-10A*VP* system controller, and Shimadzu Class-*VP* version 6.12 SP2 software on a Pentium III 700 MHz processor. The instrumentation contained an on-line degasser, but all mobile phases were also continually sparged with Helium. The temperature of the stationary phase, mobile phase, and all precolumn tubing was thermostated at 40 ± 0.2°C using a water bath. The chromatography columns used in this study included the Cosmosil 5PBB (5 μm particle diameter, 150 × 4.6 mm, 120 Å), and the Synergi polar-RP (4 μm particle diameter, 150 × 4.6 mm, 80 Å) columns, both purchased from Phenomenex, Australia.

Linear Chromatographic Separations

The surface area of the Cosmosil 5PBB is 300 m^2/g, particle diameter 5 μm and the ligand density is 2.71 $\mu mol/m^2$.[6,20] The Synergi polar RP column is prepared on Luna silica, and incorporates a polar end-capping agent. The surface area is 475 m^2/g, particle diameter 4 μm and the phase has a ligand density of 2.02 $\mu mol/m^2$.[6]

The separation behaviour of the stationary phase was tested with four mobile phase systems: methanol/H_2O, methanol/0.5%benzene/H_2O, acetonitrile/H_2O, acetonitrile/0.5%benzene/H_2O. Five mobile phase compositions were prepared for each of these systems at a flow rate of 1.0 mL/min. Experiments undertaken in each mobile phase were randomised, and duplicates were performed for each injection. Pyconometry was used to measure the void volume for calculation of the retention factor.[6,21]

RESULTS AND DISCUSSION

The retention factor, k, varies as a function of the solvent composition Φ, and this relationship can be used to quantify retention characteristics according to Equation (1):[22]

$$\log k = \log k_w - S\Phi \tag{1}$$

where k_w is the extrapolated value of k in a poor solvent, such as water, and S is the gradient of the plot log k vs. Φ. S is an important parameter for tuning selectivity and optimising resolution during method development in HPLC.[23] S reflects the relative number of binding sites that can interact directly with a molecule having a specific surface area. The S term typically reflects the effect of organic modifier on retention, and it should depend only on the interactions between the organic modifier and the solute, but not the stationary phase.[23] However, previous work[6] has shown that S displays some significant level of dependency on the type of stationary phase surface.

Synergi Polar-RP Phase

Plots of log k versus Φ were constructed for both the linear and non-linear PAHs for all the mobile phase compositions and these plots are shown in Figures 1(a–d) (for the linear PAHs only), using (a) methanol, (b) benzene modified methanol, (c) acetonitrile, and (d) benzene modified acetonitrile. In all cases, the plots of log k versus Φ were linear over the entire retention factor range tested with correlation coefficients greater than 0.997.

Visual inspection of the plots shown in Figure 1 reveals substantial differences in the relationship of log k versus Φ for the methanol mobile phases and the acetonitrile mobile phases. There is a general trend that less polar mobile phases are required to bring about elution in the methanol systems than in the acetonitrile systems. The data displayed in Figures 1(c and d) show that anthracene and 2,3-benzanthracene undergo a change in elution order at approximately 80% methanol and there was very little selectivity difference between these homologues across the entire solvent composition range. The selectivity changes for anthracene and 2,3-benzanthracene were similar to the retention relationships that were observed for C_{18} columns in our previous work.[6] No change in the elution order was apparent in both of the methanol mobile phases (Figures 1a and 1b). The selectivity difference between these members in the series (anthracene and 2,3-benzanthracene) was more in accord with the other members.

The polarity of the mobile phase may be calculated using Equation (2):[7]

$$p\prime = \phi_a p_a + \phi_b p_b \qquad (2)$$

where p' is the polarity of the mobile phase, given the volume fraction of each constituent ϕ_a and ϕ_b for each of the pure solvents a and b having a polarity of p_a and p_b, respectively. The data presented in Table 1 and Figure 2 show the experimentally derived polarities of the mobile phases required to give retention factors of 2 for each of the PAHs. This data quite clearly illustrates that both of the acetonitrile solvent systems are much stronger eluents than either of the methanol mobile phases. Furthermore, the experimental elution polarity of the mobile phase is substantially greater than the theoretical polarity that would be calculated using the well known selectivity optimisation factor given in Equation (3) (data in brackets in Table 1):

$$\phi_b = \phi_c \frac{S'_a}{S'_b} \tag{3}$$

where ϕ_a and ϕ_b are the volume fractions of each organic mobile phase constituent and S'_a and S'_b are the respective solvent strength parameters (3.1 for acetonitrile and 3.0 for methanol).[8]

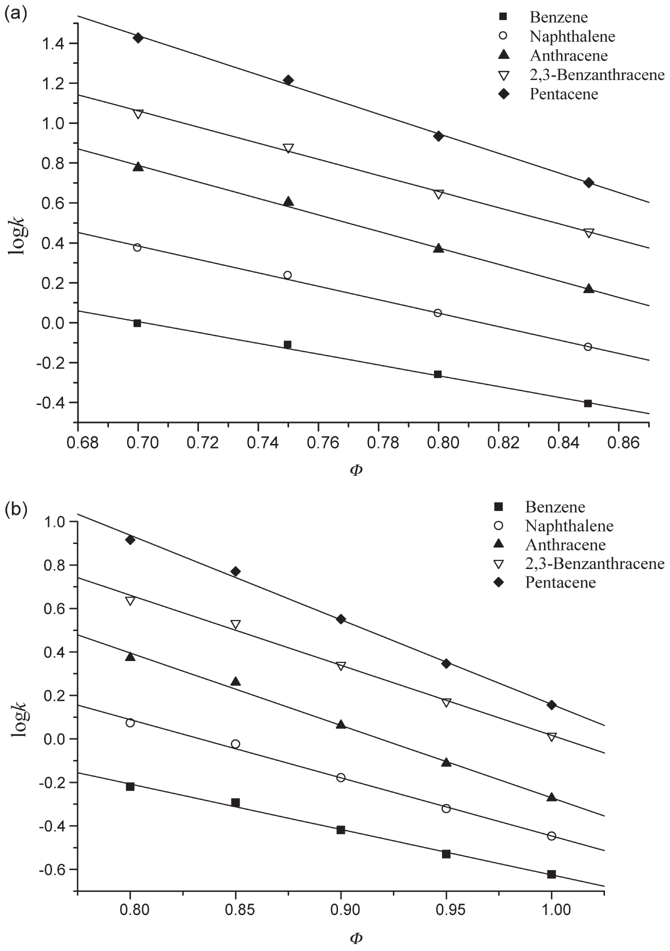

Figure 1. Plots of log k versus Φ at 40°C for the linear PAHs on a Synergi polar-RP column and mobile phases of: (a) methanol/water, (b) methanol (0.5% benzene)/water (c) acetonitrile/water, (d) acetonitrile (0.5% benzene)/water.

(*Continued*)

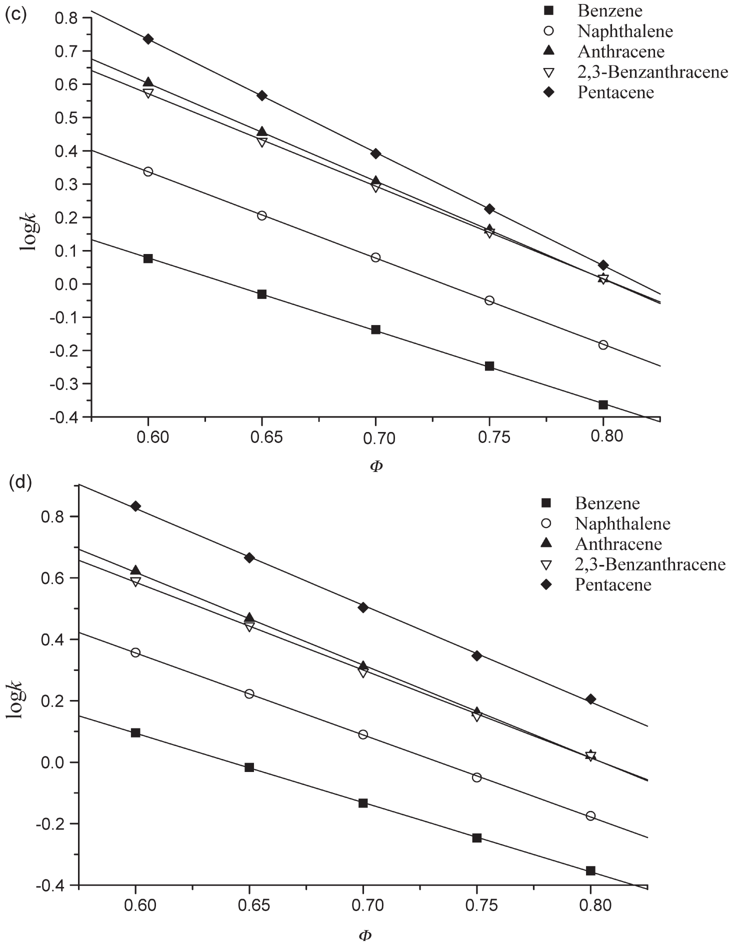

Figure 1. Continued

Since the polarities of the acetonitrile mobile phases found experimentally were consistently higher than the polarities of the methanol mobile phases and also the corresponding theoretical acetonitrile mobile phase polarity, acetonitrile therefore must be reducing the π-electron interactions between the PAH and the aromatic ring on the Synergi polar-RP phase or alternatively, the π-electron interactions between the PAH and the solvent have been increased. That is, proportionally less acetonitrile is required to bring about the elution of the PAHs and therefore it is a stronger eluent.

Phenyl phases are known to offer greater retention in methanol than acetonitrile,[9] but this does verify the significance of the π-π type interactions even on this phenyl type phase that was previously shown to have C_{18} characteristics. The molecular interactions between the solute and the stationary

Table 1. Experimentally derived polarities of the mobile phases that would yield retention factors equal to 2 for each of the linear PAHs on a Synergi polar-RP column

Linear PAHs	Methanol/ water	Methanol (0.5%benzene)/ water	Acetonitirle/ water	Acetonitrile (0.5%benzene)/ water
Benzene	7.2	7.4	8.0 (7.7)	8.0
Naphthalene	6.5	6.5	7.5 (7.1)	7.5
Anthracene	6.0	6.0	7.1 (6.7)	7.1
2,3-Benzanthracene	5.7	5.5	7.1 (6.4)	7.1
Pentacene	5.5	5.3	7.0 (6.2)	6.8

Data in brackets are values calculated from Equation 3.

phase are greater in the methanol systems than in the acetonitrile systems. Benzene was added as a modifier of these interactions and surprisingly, the benzene modifier had very little influence in the overall magnitude of retention.

Values of *S* derived from the plots illustrated in Figures 1(a–d) are given in Table 2. Similar to the polarity data given in Table 1, there are substantial differences in the magnitude of the *S* terms between both the methanol and acetonitrile mobile phases and in contradistinction to the polarity data there are also differences in the *S* data observed between the benzene modified and unmodified mobile phases. The magnitudes of *S* were generally greatest

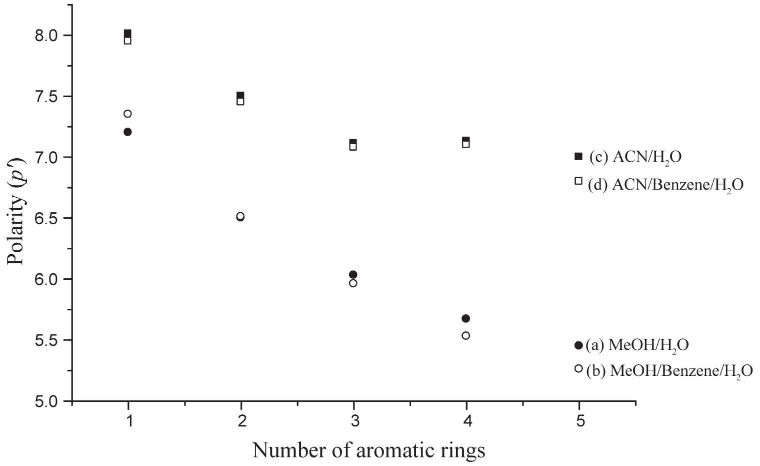

Figure 2. Plot of experimentally derived polarities of the mobile phases that would yield retention factors equal to 2 for each of the linear PAHs. (a) methanol/water, (b) methanol (0.5% benzene)/water, (c) acetonitrile/water, (d) acetonitrile (0.5% benzene)/water on a Synergi polar-RP column.

Table 2. S, log k_w and Φ_0 values for linear and non-linear PAHs with each of the mobile phase environments on a Synergi polar-RP column

	Methanol/ water		Methanol (0.5% benzene)/ water		Acetonitrile/ water		Acetonitrile (0.5% benzene)/ water	
	S	Φ_0	S	Φ_0	S	Φ_0	S	Φ_0
Linear PAHs								
Benzene	2.71	0.70	2.09	0.70	2.19	0.64	2.26	0.64
Naphthalene	3.37	0.81	2.68	0.83	2.59	0.73	2.67	0.73
Anthracene	4.13	0.89	3.33	0.92	2.94	0.80	3.02	0.80
2,3-Benzanthracene	4.04	0.96	3.22	1.01	2.78	0.81	2.86	0.80
Pentacene	4.91	0.99	3.89	1.04	3.40	0.82	3.15	0.86
Non-linear PAHs								
Acenaphthene	3.73	0.87	3.03	0.90	2.73	0.78	2.79	0.79
1,2-Benzanthracene	4.94	0.93	3.96	0.97	3.28	0.85	3.34	0.85
Benzo [a] pyrene	4.83	0.96	4.12	1.00	3.30	0.88	3.36	0.88
Benzo [e] pyrene	4.32	0.96	4.04	1.00	3.25	0.88	3.30	0.88
Chrysene	5.13	0.93	3.87	0.97	3.24	0.85	3.31	0.85
Pyrene	5.02	0.96	4.03	1.00	3.24	0.89	3.31	0.88
Perylene	5.02	0.91	3.44	0.95	2.98	0.83	3.04	0.83
Benz [e] acephenanthrylene	5.18	0.95	4.18	0.99	3.36	0.87	3.42	0.87

for each respective PAH in the methanol mobile phase. The corresponding S values for each PAH in the benzene modified methanol mobile phase were in effect 20 to 23% lower, and almost constant with molecular weight. The magnitudes of S in the acetonitrile mobile phase were between 20 to 30% lower than the S values in the methanol mobile phase. Interestingly, the difference became more significant as the size of the PAH increased. The results were even more significant for the benzene modified acetonitrile where the differences ranged between 17 to 36%, increasing as the molecular weight increased. The relative variation in the S term in the methanol mobile phase to that of the S term in each of the other mobile phases is given in Figure 3 and the data that list the percent relative deviation from that of the S values in the methanol mobile phase is given in Table 3. Similar trends in S values were also observed for the non-linear PAHs. For these non-linear PAHs the relative deviation was lower by between 7 and 32% in the benzene modified methanol mobile phase, 25 and 41% in the acetonitrile mobile phase and 24 to 40% on the benzene modified acetonitrile mobile phase.

Plots of S versus the aromatic ring number for the linear members of the series reveals distinct discontinuity after the 3 or 4 ring member, irrespective of the mobile phase as shown in Figure 4. The relationship in the S values for

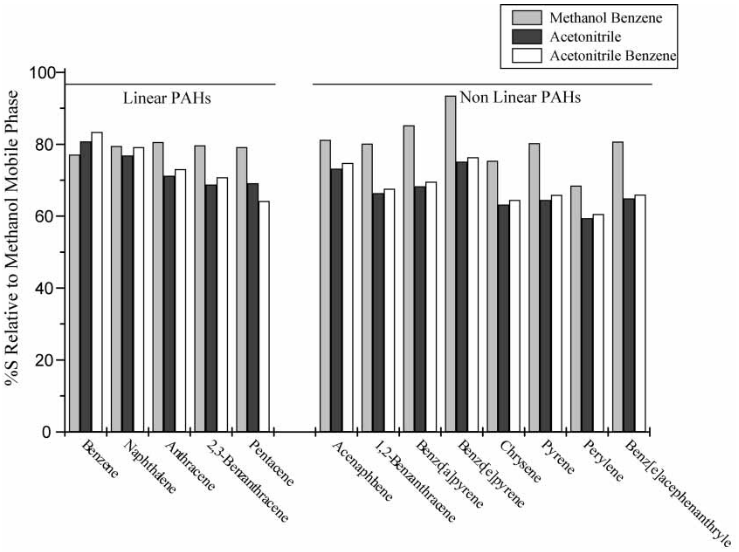

Figure 3. Histogram illustrating the relative variation in the *S* term of each linear and non-linear PAH for each mobile phase system in comparison to that of the methanol/water mobile phase on a Synergi polar-RP column.

the methanol (curve a) and the benzene modified methanol mobile phase (curve b) are very different, but the relationship between *S* and the ring number is very similar for the acetonitrile (curve c) and the benzene modified acetonitrile (curve d) mobile phases. Furthermore, the slopes of the *S* versus the ring number plot in the pre-discontinuity region (PAHs with less than four-rings) are 0.71 (methanol), 0.62 (benzene modified methanol), and 0.38 for both the acetonitrile and the benzene modified acetonitrile mobile phases.

The interpretation of the rate of change in these *S* values is very interesting because essentially the solvent environment is able to alter the molecular contact surface area of the PAH on the surface of the stationary phase. In a sense, the effect of a solvent change to either the benzene modified methanol or the acetonitrile systems is to mimic that of a more densely packed surface, where the slope of the *S* versus the ring number plot decreases from unity.[6] This was the case for both the benzene modified methanol and the acetonitrile mobile phases, although the change was more substantial for the acetonitrile systems. An explanation for this behaviour may be the adsorption of benzene to the stationary phase ligands and since the concentration of benzene is relatively high, it modifies that stationary phase to be more aromatic (i.e., should increase retention of PAH). The second scenario is that the benzene in the methanol mobile phase interacts with the PAHs, effectively changing the molecular species in solution, which in turn then alters the molecular bonding properties of

Table 3. Magnitude of the *S* values of the PAHs in each mobile phase system on a Synergi polar-RP column, relative to that of the *S* values measured in the methanol/water mobile phase

	S values				Relative% to MeOH		
	MeOH	MeOH/ benzene	ACN	ACN/ benzene	MeOH/ benzene	ACN	ACN/ benzene
Linear PAHs							
Benzene	2.71	2.09	2.19	2.26	77.1	80.8	83.4
Naphthalene	3.37	2.68	2.59	2.67	79.5	76.9	79.2
Anthracene	4.13	3.33	2.94	3.02	80.6	71.2	73.1
2,3-Benzanthracene	4.04	3.22	2.78	2.86	79.7	68.8	70.8
Pentacene	4.91	3.89	3.40	3.15	79.2	69.2	64.2
Non-linear PAHs							
Acenaphthene	3.73	3.03	2.73	2.79	81.2	73.2	74.8
1,2Benz anthracene	4.94	3.96	3.28	3.34	80.2	66.4	67.6
Benzo [a] pyrene	4.83	4.12	3.30	3.36	85.3	68.3	69.6
Benzo [e] pyrene	4.32	4.04	3.25	3.30	93.5	75.2	76.4
Chrysene	5.13	3.87	3.24	3.31	75.4	63.2	64.5
Pyrene	5.02	4.03	3.24	3.31	80.3	64.5	65.9
Perylene	5.02	3.44	2.98	3.04	68.5	59.4	60.6
Benz [e] acephenanthrylene	5.18	4.18	3.36	3.42	80.7	64.9	66.0

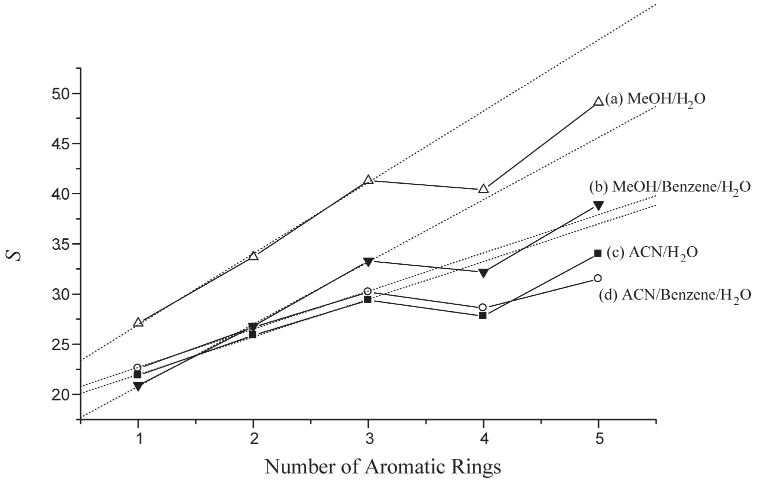

Figure 4. Plot of *S* versus the number of aromatic rings for the linear PAHs in (a) methanol/water, (b) methanol (0.5% benzene)/water (c) acetonitrile/water, (d) acetonitrile (0.5% benzene)/water on a Synergi polar-RP column.

the PAH. A third scenario is that the benzene reduces π-π type interactions between the PAH and the stationary phase ligand. It is also possible now to differentiate between the effect of the benzene and that of the acetonitrile; that is, at the concentrations that were employed for the spiking of the mobile phases with benzene, the acetonitrile was far more effective at altering the retention mechanism than was the comparatively low concentration of benzene. That is not to say, however, that through the addition of higher concentrations of benzene the effects would be more substantial, but this requires further assessment.

One factor that we can eliminate, however, is that the change in the mobile phase and its subsequent effect on the ligand orientation with respect to ‘wetting’ aspects is not responsible for the change in contact surface area of the PAHs with the ligands because the polarity of the mobile phase at elution remained constant for each respective type of organic solvent with and without the benzene component. Therefore, the effect is most certainly due to molecular orientations and the opportunity for bonding.

Selectivity

The selectivity factors (α) for each PAH in each mobile phase are given in Table 4. Also included in Table 4 are the product selectivity factors (α_p),[6] which were determined according to Equation (4).[6]

$$\alpha_P = \alpha_1 \times \alpha_2 \times \ldots \times \alpha_n \quad (4)$$

Table 4. Selectivity (α) and product selectivity (α_p) factors for the linear and structural isomer PAHs calculated for each mobile phase environment on a Synergi polar-RP column

	Methanol/water		Methanol (0.5% benzene)/water		Acetonitrile/water		Acetonitrile (0.5% benzene)/water	
	k	α	k	α	k	α	k	α
Linear PAHs								
Benzene	0.48	2.00	0.54	1.90	0.71	1.65	0.72	1.65
Naphthalene	0.96	2.04	1.03	1.94	1.18	1.64	1.19	1.62
2,3-Benzanthracene	1.96	1.92	3.71	1.81	1.93	1.03	1.93	1.04
Anthracene	3.77	1.87	2.00	1.86	2.00	1.22	2.00	1.56
Pentacene	7.06		6.71		2.43		3.13	
α_p (Linear PAHs)	14.70		12.40		3.41		4.34	
4 Ring isomers								
2,3-Benzanthracene	1.96	1.77	3.71		1.93	1.55	1.93	1.55
Chrysene	3.46	1.03	3.44	1.02	3.01	1.02	2.99	1.02
1,2-Benzanthracene	3.55		3.52	1.05	3.07		3.05	
α_p (4 Ring isomers PAHs)	1.81		1.08		1.59		1.58	
5 Ring Isomers								
Benzo [e] pyrene	4.90	1.03	4.79	1.03	3.82	1.03	3.78	1.03
Perylene	5.13		5.00		3.93	1.00	3.89	1.00
Benzo [a] pyrene	5.04	1.02	4.92	1.02	3.94		3.90	
α_p (5 Ring isomers PAHs)	1.05		1.05		1.03		1.03	
α_p (All PAHs)	14.70		12.40		5.52		5.42	

where α_1 is the selectivity factor for the first peak pair, α_2 is the selectivity factor for the second peak pair, etc. up to the *n*th peak pair α_n. The highlighted data represents changes in elution order that have taken place relative to the order in methanol, however, in the calculation of α_p the compounds were arranged according to their elution order. The data in Table 4 are separated into the selectivity of the linear PAHs, the four-ring isomers and the five-ring isomers. The product selectivity factor of the entire data set is also included in Table 4. For simplicity the selectivity factors for each mobile phase are discussed with respect to the methanol mobile phase.

With respect to the linear PAHs, α_p was greatest in the methanol mobile phase (14.7), followed by the benzene modified methanol mobile phase (12.4), the benzene modified acetonitrile mobile phase (4.34), and then lastly, the acetonitrile mobile phase (3.41). The difference in the α_p values for the linear PAHs between the methanol and acetonitrile mobile phases supports the previously discussed findings relating to the change in the retention process that is apparent in these two different solvents. Interestingly, the product selectivity factor for the four-ring isomers was greatest in the methanol mobile phase, but was the lowest in the benzene modified mobile phase, while both the acetonitrile mobile phases were almost identical and intermediate between the two methanol mobile phases. In this instance, the α_p value for methanol was 1.81, followed by 1.59 and 1.58 in the acetonitrile and benzene modified acetonitrile mobile phases, respectively. Lastly, α_p was 1.08 in the benzene modified methanol mobile phase. Also, elution order changes were apparent between the methanol and the benzene modified methanol mobile phases, which are highlighted in Table 4.

The α_p values again are different when examining the five-ring isomers, although in this instance, there is virtually no difference in α_p between the four different mobile phases. Overall, the α_p values for all the PAHs (both linear and non-linear) decrease in the following order: methanol (14.7), benzene modified methanol (12.4), acetonitrile (5.52), and benzene modified acetonitrile (5.42).

Cosmosil 5PBB Phase

Plots of log *k* vs. Φ were also constructed for all PAHs studied, at all mobile phase compositions on the Cosmosil 5PBB phase and are shown in Figures 5(a–d) (these plots display the data for the linear PAHs only). Figure 5(a) illustrates the relation between log *k* and Φ for the methanol/water mobile phases, while that in Figure 5(b) illustrates the relationship in a benzene modified methanol/water. Figures 5(c and d) illustrate the same relationships in acetonitrile/water and benzene modified acetonitrile/water mobile phases, respectively. In all cases, the plots of log *k* vs. Φ were linear over the entire retention factor range tested with correlation coefficients greater than 0.990, except for one instance (benzene in the acetonitrile mobile phase ($r = 0.958$)) where some degree of curvature is apparent.

There was no change in elution order apparent with any of the organic modifiers used for either the linear or non-linear PAHs, which was contrary to the retention behaviour of the PAHs on the Synergi polar-RP stationary phase,

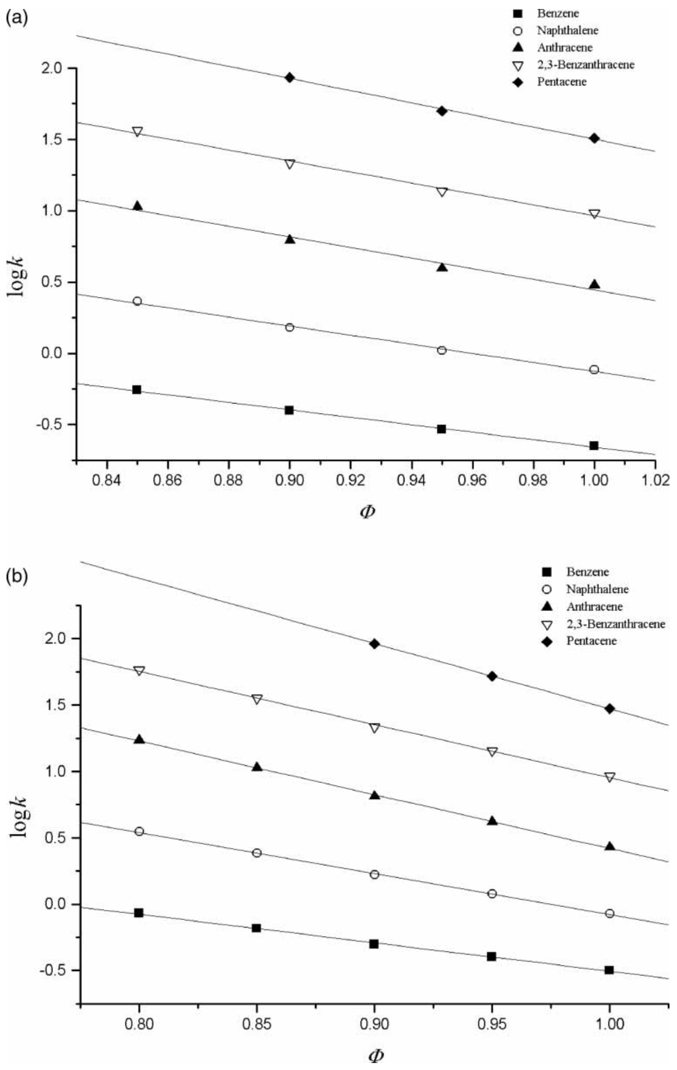

Figure 5. Plots of log k versus Φ at 40°C for the linear PAHs on a Cosmosil 5PBB column and mobile phases of: (a) methanol/water, (b) methanol (0.5% benzene)/water (c) acetonitrile/water, (d) acetonitrile (0.5% benzene)/water.

(*Continued*)

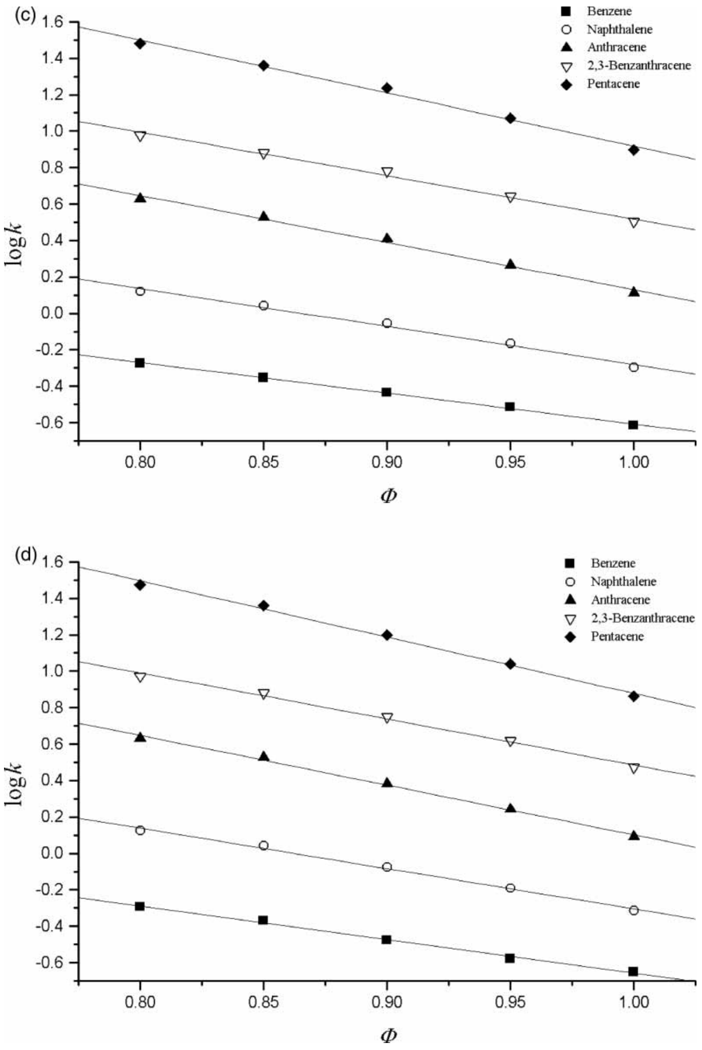

Figure 5. Continued.

where anthracene and 2,3- benzanthracene underwent an elution order change at approximately 80/20 acetonitrile/water, with very little selectivity differences between the other PAHs. While the substantial retention of the PAHs on the Cosmosil 5PBB stationary phase is beneficial to achieving high resolution separations, it is also a limitation of the Cosmosil 5PBB column, as traditional reversed-phase solvents such as methanol offer poor elution strength. However, the plots illustrated in Figure 5(a–d) indicate that when acetonitrile is used instead of methanol as the organic modifier on the Cosmosil 5PBB column there is still a large selectivity difference between the PAHs.

The values of S generated from Figures 5(a–d) are shown in Table 5. Significant differences in the S values are apparent between the values of S for the different mobile phase systems. This is more readily visualised by assessing the plots of S versus the PAH ring number for the linear PAHs shown in Figure 6. From these curves, it is immediately apparent that in only one system was there not a discontinuity observed at the 3–4 ring region of this relationship, that being the methanol mobile phase. In each of the acetonitrile and the benzene modified mobile phases there was distinct discontinuity, consistent with the results obtained on the Synergi polar-RP column. It is interesting that this discontinuity occurred only for the π-competitor mobile phases, an explanation for which we have yet to develop.

It is also noteworthy that the magnitude in the S values was lower in the acetonitrile systems than in the methanol systems, which was also consistent with the findings in the Synergi polar-RP phase. A more interesting feature of this work was found by evaluating the slopes of these curves, which were shown to be dependent on the mobile phase as shown in Figure 6. In the linear region of the curve, the rate of change in S was 0.55 for the methanol mobile phase, while for the benzene spiked methanol mobile phase the rate of change in S was 0.95. In both the acetonitrile mobile phases the rate of change in S was essentially constant at 0.45, illustrating that the high concentration of acetonitrile relative to that of the benzene essentially masked any affect benzene may be having in this mobile phase. It is also important to note that the magnitude of the rate of change in S reflects the number of bonding sites the PAHs can achieve during the retention process on the stationary phase, where as the rate of change in S approaches unity, the maximum number of bonding sites in proportion to the size of the solute is consequently achieved. Therefore, the addition of competitor π agents into the mobile phase vastly changes the nature of the interaction at the stationary phase interface. It is also unfortunate that there was discontinuity apparent at the 3–4 ring members in benzene spiked methanol mobile phase, because the selectivity would otherwise be very substantial, far exceeding that of the native methanol mobile phase. This is a very interesting aspect of the retention on this phase, which warrants further investigation.

The product selectivity factor (α_p) data is presented in Table 6 and is broken down according to the linear PAHs, the four ring isomers, and the five ring isomers. α_p when using methanol as the organic modifier on the Cosmosil 5PBB stationary phase is very high (147),[6] due largely to the continuous linearity in the relationship between S and the number of aromatic rings, which was unique to the methanol mobile phase. When benzene was added to the mobile phase, the discontinuity in the S versus n relationship (where n is the number of aromatic rings) at the 3–4 ring member effectively decreased the separative potential of the system with the product selectivity factor decreasing to 79 for the linear PAHs. As was the case for the Synergi polar-RP phase we found that using acetonitrile as the organic modifier the selectivity of this column decreased, so that the product selectivity factor

Table 5. S, log k_w and Φ_0 values for linear and non-linear PAHs with each of the mobile phase environments on a Cosmosil 5PBB column.

	Methanol/water		Methanol (0.5% benzene)/water		Acetonitrile/water		Acetonitrile (0.5% benzene)/water	
	S	Φ_0	S	Φ_0	S	Φ_0	S	Φ_0
Linear PAHs								
Benzene	2.60	0.80	2.15	0.76	1.69	0.64	1.85	0.64
Naphthalene	3.20	1.00	3.08	0.98	2.13	0.86	2.22	0.86
Anthracene	3.70	1.10	4.04	1.10	2.62	1.04	2.73	1.04
2,3-Benzanthracene	3.90	1.30	4.00	1.24	2.40	1.21	2.53	1.19
Pentacene	4.30	1.40	4.91	1.30	2.96	1.30	3.08	1.28
Non-linear PAHs								
Acenaphthene	3.20	1.10	3.60	1.08	2.36	0.99	2.51	0.98
1,2-Benzanthracene	4.30	1.20	4.95	1.17	3.06	1.15	3.19	1.14
Benzo [a] pyrene	4.50	1.30	5.10	1.24	3.20	1.25	3.32	1.24
Benzo [e] pyrene	4.40	1.30	4.97	1.24	3.14	1.25	3.27	1.24
Chrysene	4.20	1.20	4.70	1.18	3.00	1.15	3.12	1.14
Perylene	3.70	1.20	4.96	1.25	3.13	1.26	3.27	1.24
Pyrene	4.40	1.30	4.25	1.17	2.71	1.15	2.84	1.13
Benz [e] acephenanthrylene	4.50	1.20	5.15	1.21	3.23	1.21	3.36	1.20

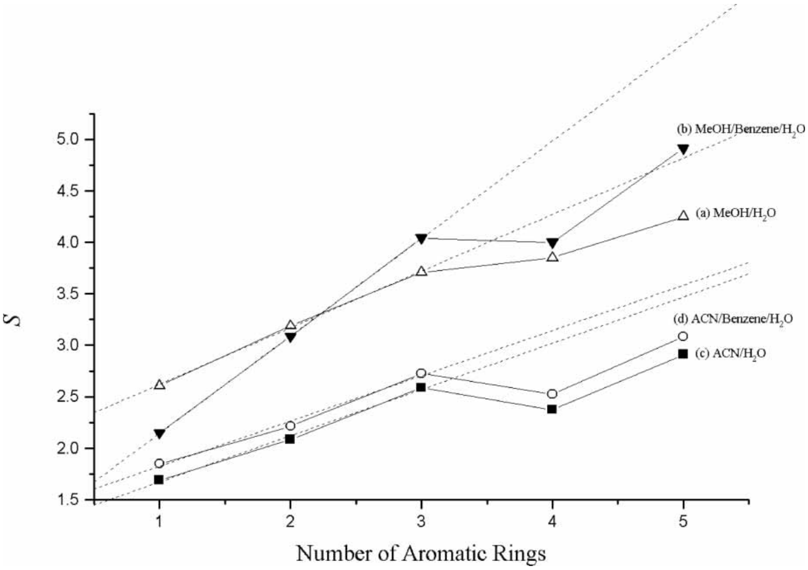

Figure 6. Plot of *S* versus the number of aromatic rings for the linear PAHs in (a) methanol/water, (b) methanol (0.5% benzene)/water (c) acetonitrile/water, (d) acetonitrile (0.5% benzene)/water on a Cosmosil 5PBB column.

was 49 and 42 for the acetonitrile and benzene spiked acetonitrile, respectively. Both these product selectivity factors far exceed any of the systems we have, to date, tested for these linear PAHs, and at the same time there is a substantial decrease in the extent of the retention of these PAHs, which bodes well for timely, high efficient separations in what could be considered 'preferred' reversed phase solvents. However, this reduction in retention comes at a cost: The separative potential for structural isomers is all but lost as the product selectivity factor in the acetonitrile mobile phases is almost unit, in comparison to the methanol systems where the product selectivity factor was around 1.4 for the four-ring isomers.

It is also interesting from a practical aspect to calculate the polarity of the mobile phase at elution. The data presented in Table 7 and Figure 7 show the experimentally derived polarities of the mobile phases required to give retention factors of 2 for each of the PAHs. This data quite clearly illustrates that both of the acetonitrile solvent systems are much stronger eluents than either of the methanol mobile phases. Furthermore, the experimental elution polarity of the mobile phase is substantially greater than the theoretical polarity that would be calculated using the well known selectivity optimisation factor given in Equation (3) (data in brackets in Table 7). Since the polarities of the acetonitrile mobile phases found experimentally were consistently higher than the polarities of the methanol mobile phases and also the corresponding theoretical acetonitrile mobile phase polarity, acetonitrile

Table 6. Selectivity (α) and product selectivity (α_p) factors for the linear and structural isomer PAHs calculated for each mobile phase environment on a Cosmosil 5PBB column

	Methanol/water		Methanol (0.5% benzene)/water		Acetonitrile/water		Acetonitrile (0.5% benzene)/water	
	k	α	k	α	k	α	k	α
Linear PAHs								
Benzene	0.22	3.5	0.27	2.52	0.32	2.25	0.30	2.40
Naphthalene	0.77	1.45	0.68	2.94	0.72	2.79	0.72	2.78
Antracene	1.12	8.60	2.00	3.42	2.00	2.36	2.00	2.34
2,3-Benzanthracene	9.63	3.36	6.83	3.10	4.73	2.72	4.67	2.72
Pentacene	32.32		21.18		12.85		12.70	
α_p (Linear PAHs)	146.91		78.54		40.14		42.45	
4 Ring isomers								
2,3-Benzanthracene	9.63		6.83		4.73		4.67	1.00
Chrysene	7.27	1.32	5.05	1.35	4.70	1.01	4.70	
1,2-Benzanthracene	7.24	1.00	4.84	1.04	4.68	1.00	4.68	1.00
α_p (4 Ring Isomers PAHs)	1.32		1.41		1.01		1	
5 Ring isomers								
Benzo [e] pyrene	17.58	1.05	11.72	1.05	10.29	1.03	10.26	1.03
Perylene	18.45		12.31		10.76		10.74	
Benzo [a] pyrene	17.53	1.00	11.63	1.01	10.62	1.01	10.61	1.01
α_p (5 Ring isomers PAHs)	1.05		1.06		1.03		1.03	
α_p (all PAHs)	146.91		78.54		40.44		42.33	

Table 7. Experimentally derived polarities of the mobile phases that would yield retention factors equal to 2 for each of the linear PAHs on a Cosmosil 5PBB column

	Methanol/water	Methanol (0.5% benzene)/water	Acetonitrile/water	Acetonitrile (0.5% benzene)/water
Linear PAHs				
Benzene	7.0	7.0	8.2 (7.5)	8.1
Naphthalene	5.8	5.7	7.0 (6.5)	7.0
Anthracene	4.9	4.9	6.1 (5.8)	6.1
2,3-Benzanthracene	4.2	4.2	5.4 (5.2)	5.5
Pentacene	3.7	3.9	4.9 (4.7)	4.9

Data in brackets are values calculated from Equation 3.

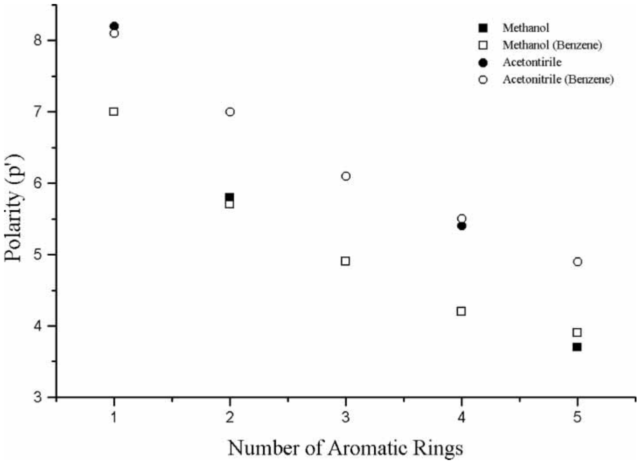

Figure 7. Plot of experimentally derived polarities of the mobile phases that would yield retention factors equal to 2 for each of the linear PAHs. (a) Methanol/Water, (b) methanol (0.5% benzene)/water, (c) acetonitrile/water, (d) acetonitrile (0.5% benzene)/water on a Cosmosil 5PBB column.

therefore must be reducing the π-electron interactions between the PAH and the aromatic ring on the Cosmosil 5PBB phase or alternatively, the π-electron interactions between the PAH and the solvent have been increased. As with the Synergi polar-RP phase, proportionally less acetonitrile is required to bring about the elution of the PAHs and, therefore, it is a stronger eluent.

CONCLUSION

Our intention in this work was to further investigate the retention behaviour of PAHs on Phenyl type stationary phases, i.e., the Synergi polar-RP, and

Cosmosil 5PBB columns. In previous work,[6] we observed that the Synergi polar-RP column was very 'C_{18} like' with respect to PAH retention and selectivity in methanol/water mobile phases. Following more detailed investigation in this work, we were able to show that acetonitrile mobile phases modified π-π interactions between PAH and, the Synergi polar-RP column. Using acetonitrile mobile phase, the retention behaviour became even more C_{18} like, and the Synergi polar-RP column essentially lost any semblance of the performance anticipated for a phenyl type stationary phase. For example, elution order changes between the three and four-ring members of the linear homologue PAH series were observed in the acetonitrile mobile phases, but this was not apparent in the methanol system. Similar selectivity changes were observed on C_{18} columns, but were not apparent on other phenyl type surfaces.[6]

The polarities of the acetonitrile mobile phases required to give the same retention factors for each PAH were significantly higher than for the methanol separations for both columns. This demonstrated that the π-π interactions with the stationary phase were affected by competing π-π interactions from the acetonitrile mobile phases.

Hydrophobic surface contact was maximised in the methanol mobile phase. Addition of benzene to the methanol mobile phases reduced this retention parameter, but the reduction was more significant for the benzene spiked acetonitrile mobile phases especially for higher molecular weight PAHs. In effect, addition of the π-electron competitors essentially resulted in the surface mimicking a more densely packed stationary phase surface, where the slopes of the S values versus the ring number plot decreased further from unity.

Lastly, the selectivity of the Synergi polar-RP column in acetonitrile mobile phases was similar to the performance of C_{18} surfaces rather than other phenyl-type surfaces that we have tested. Therefore, if the full potential of the Synergi polar-RP column is to be realised, careful consideration must be paid to the choice of mobile phase, especially the elimination of π-π type competition between solute and mobile phase. In the case of the Cosmosil 5PBB column, the excessive retention of PAHs with the use of methanol was significantly decreased when using acetonitrile as the mobile phase due to the observed π-π competition. As the use of acetonitrile does not significantly decrease selectivity when using the Cosmosil 5PBB column, as was observed with the Synergi polar-RP stationary phase, the use of this organic modifier is suitable to reduce retention and keep resolution, however, selectivity is lost between structural isomers.

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