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THERMODYNAMIC COMPARISON OF MONOMERIC AND POLYMERIC C₁₈ BONDED PHASES USING AQUEOUS METHANOL AND ACETONITRILE MOBILE PHASES

APRYLL M. STALCUP*

*Department of Chemistry, Georgetown University, Washington, DC 20057 (U.S.A.); and *Organic Analytical Research Division, Center for Analytical Chemistry, National Bureau of Standards, Gaithersburg, MD 20899 (U.S.A.)*

DANIEL E. MARTIRE

Department of Chemistry, Georgetown University, Washington, DC 20057 (U.S.A.)

and

STEPHEN A. WISE

Organic Analytical Research Division, Center for Analytical Chemistry, National Bureau of Standards, Gaithersburg, MD 20899 (U.S.A.)

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SUMMARY

The results of a thermodynamic comparison of the retention of four polycyclic aromatic hydrocarbons on a monomeric and a polymeric reversed-phase column using methanol and acetonitrile are presented. The results are discussed in terms of enthalpic and relative entropic contributions to overall retention.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are often encountered in environmental samples and are of interest because of their mutagenic and carcinogenic activities. Reversed-phase high-performance liquid chromatography (RP-HPLC) with chemically bonded C₁₈ stationary phases has been widely used as a means of separating, identifying and quantifying individual PAHs.

The retention mechanism(s) responsible for the separation and observed selectivities for PAHs on these chemically bonded C₁₈ stationary phases has (have) yet to be defined. In general, several factors have been shown to have an effect on column selectivity, including temperature¹⁻⁵, phase type⁶⁻⁸, surface coverage⁹, bonded ligand chain length¹⁰, mobile phase composition^{4,6,11} and aqueous solubility¹². Attempts to correlate various molecular descriptors such as solute shape⁶, size^{13,14}, polarizability¹⁵, or planarity⁷ with retention have met with varying degrees of success. Efforts have largely been directed toward an understanding of mobile or stationary phase effects without simultaneously addressing stationary-mobile phase dynamics. The most complete theoretical treatment to date is that put forth by Martire and

Boehm¹⁶. Their treatment addresses the role of the mobile phase and incorporates the contribution of stationary phase variables in a rigorous description of solute retention and selectivity.

The results of a thermodynamic comparison of selected compounds on two different types of bonded C₁₈ phases and the effect of mobile phase composition on retention are presented in this paper. This investigation was undertaken because polymeric and monomeric bonded C₁₈ phases were reported as having differences in selectivity for PAHs^{7,8}. A thermodynamic study is important because it allows the calculation of thermodynamic solution properties. Column comparisons are, in general, complicated by the coupling of mobile phase and stationary phase contributions to solute retention and column selectivity. This thermodynamic study will allow a decoupling of these effects and thereby provide a better basis for column comparisons. To minimize error¹⁷, solute retention is expressed in terms of V_n , the solute net retention volume. The results are discussed in terms of enthalpic and relative entropic contributions to overall retention.

EXPERIMENTAL*

Chromatographic experiments were performed on a single piston, reciprocating HPLC pump interfaced with a data system. The column eluent was monitored with a fixed-wavelength absorbance detector set at 280 nm. The prepacked columns (25 cm × 4.6 mm I.D.) used were a monomeric C₁₈ Zorbax column (DuPont, Wilmington, DE, U.S.A.) and a polymeric C₁₈ Vydac 201 TP column (The Separations Group, Hesperia, CA, U.S.A.). The column was fitted with a water jacket that was connected to a Lauda K-2/R thermostatted water bath (Brinkman Instruments, Westbury, NY, U.S.A.). The columns were thermostatted at the desired temperatures ($\pm 0.1^\circ\text{C}$) for 1 h prior to analysis. The column jacket was extended to include the tubing between the injector and the column. One thermometer was placed inside the column jacket and another thermometer was placed in the water bath. The temperature was monitored at both sites. The solvent reservoir was at ambient temperature.

General procedure

The premixed mobile phase was prepared by transferring the appropriate volumes of HPLC-grade water and acetonitrile or methanol obtained from various sources to the solvent reservoir. The column was reequilibrated with each change in mobile phase composition by passing approximately 30 column volumes of eluent through the column prior to solute injection. The nominal flow-rate used throughout the study was 2 ml/min. The actual flow-rate was measured by collecting column eluent in a 10-ml graduated cylinder and recording the collection time. Retention times were determined by the data system.

Benzo[*a*]pyrene (BaP), which is planar, and the isomers phenanthro[3,4,*c*]-phenanthrene (PhPh) and tetrabenzonaphthalene (dibenzo[*g,p*]chrysene) (TBN),

* 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 Bureau of Standards nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

which are non-planar, were selected for this investigation on the basis of observations by Wise and Sander^{7,8} that the degree of solute planarity affected solute retention differently on monomeric *vs.* polymeric C₁₈ phases. The elution order of these three solutes has been observed to correlate with phase selectivity for PAH isomer separations. On polymeric phases, where PAH selectivity is highest, BaP elutes last. On monomeric phases, where PAH selectivity is lower, BaP elutes earlier, sometimes co-eluting with PhPh. Chrysene, which is also planar, was chosen to facilitate comparison with previous work. The solutes were injected as a mixture in the organic component of the mobile phase unless overlapping retention times precluded unambiguous determination of retention volumes. Coeluting solutes were reinjected individually. Solutes were injected in duplicate unless the retention times differing by more than 2% dictated additional injections.

The column void volume was determined by injecting a solution of uracil in the column eluent¹⁸. This produced a well-defined peak in all cases except for 100% acetonitrile.

The extra-column volume was determined by removing the column from the system and injecting a uracil solution.

RESULTS

The chromatographic capacity factor, k' , is defined as¹⁹

$$k' = \frac{V_r - V_m}{V_m} = \frac{V_n}{V_m} \quad (1)$$

where V_r is the solute retention volume, V_m is the volume of the mobile phase, and V_n is the solute net retention volume.

From thermodynamics, k' can also be expressed in terms of standard enthalpies and entropies of transfer from the mobile to the stationary phase

$$\ln k' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln \varphi \quad (2)$$

where φ is the phase ratio defined by V_s/V_m ; V_s is the stationary phase volume.

Alternatively, the solute retention can be expressed in terms of the solute net retention volume.

$$\ln V_n = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln V_s \quad (3)$$

The experimental data was subjected to a linear regression fit with temperature as the independent variable. The parameters for the temperature dependent regression eqn. 4,

$$\ln V_n = a_T + b_T/T \quad (4)$$

where T is the absolute temperature in Kelvin, are listed in Tables I and II. The results for chrysene on the polymeric C_{18} column using acetonitrile and methanol are representative of all four solutes and are illustrated in Fig. 1a and b.

The coefficient, b_T , in eqn. 4, can be related to the enthalpy change associated with transferring the solute from the mobile to the stationary phase (eqn. 3). The dependence of the enthalpy for chrysene on composition for both organic modifiers is shown in Fig. 2 on the polymeric column. On both columns for all solutes studied, the enthalpic contribution to retention is approximately independent of acetonitrile concentration but dependent on methanol concentration.

The constant obtained from the temperature dependent fit (eqn. 4) is related to the entropy of transfer of the solute from the mobile to the stationary phase and the

TABLE I

PARAMETERS FOR REGRESSION EQN. 4 USING ACETONITRILE ON POLYMERIC AND MONOMERIC BONDED PHASES

Solute	θ	Polymeric			Monomeric		
		a_T	$b_T (\times 1000)$	r^2	a_T	$b_T (\times 1000)$	r^2
Chrysene	0.05	-10.80	3.46	0.9993	-4.93	1.92	0.9974
	0.10	-10.25	3.41	0.9968	-4.65	1.96	0.9998
	0.15	-9.55	3.31	0.9980	-4.28	1.97	0.9994
	0.20	-9.28	3.34	0.9982	-3.83	1.94	0.9968
	0.25	-8.93	3.34	0.9988	-3.46	1.93	0.9996
	0.30	-8.66	3.36	0.9986	-3.09	1.93	0.9997*
	0.35	-8.24	3.35	0.9990	-2.65	1.91	0.9929
PhPh	0.05	-9.29	3.02	0.9973	-4.55	1.94	0.9973
	0.10	-9.15	3.13	0.9987	-4.43	2.05	0.9992
	0.15	-8.35	3.02	0.9987	-4.04	2.06	0.9997
	0.20	-8.05	3.06	0.9999	-3.46	2.01	0.9981
	0.25	-7.65	3.06	0.9982	-2.96	1.98	0.9984
	0.30	-7.18	3.05	0.9987	-2.43	1.95	0.9973*
	0.35	-6.94	3.11	0.9995	-1.82	1.90	0.9934
BaP	0.05	-12.32	4.14	0.9993	-5.61	2.29	0.9991
	0.10	-12.07	4.18	0.9971	-5.41	2.35	0.9999
	0.15	-11.46	4.11	0.9986	-5.07	2.37	0.9993
	0.20	-11.08	4.10	0.9986	-4.61	2.34	0.9971
	0.25	-10.88	4.15	0.9988	-4.20	2.32	0.9997
	0.30	-10.53	4.16	0.9984	-3.77	2.30	0.9999*
	0.35	-10.15	4.16	0.9987	-3.30	2.28	0.9947
TBN	0.05	-8.64	2.97	0.9926	-4.52	2.10	0.9982
	0.10	-8.40	3.05	0.9994	-4.42	2.21	0.9991
	0.15	-7.77	2.99	0.9985	-4.06	2.24	0.9995
	0.20	-7.42	3.02	0.9996	-3.43	2.18	0.9984
	0.25	-6.93	2.99	0.9952	-2.87	2.13	0.9978
	0.30	-6.44	2.97	0.9970	-2.30	2.09	0.9967*
	0.35	-6.13	3.01	0.9974	-1.75	2.06	0.9865*

* Data taken at four temperatures; all others taken at five temperatures.

TABLE II

PARAMETERS FOR THE REGRESSION EQN. 4 USING METHANOL ON MONOMERIC AND POLYMERIC BONDED PHASES

Solute	θ	Polymeric			Monomeric		
		a_T	$b_T (\times 1000)$	r^2	a_T	$b_T (\times 1000)$	r^2
Chrysene	0.00	-8.66	2.88	0.9944	-2.66	1.21	0.9834
	0.05	-9.60	3.32	0.9972	-3.20	1.54	0.9983
	0.10	-10.42	3.71	0.9936	-3.93	1.93	0.9982
	0.15	-10.26	3.84	0.9993	-3.90	2.09	0.9997
	0.20	-10.66	4.12	0.9994	-4.79	2.54	0.9995*
	0.25	-11.35	4.51	0.9997	-4.83	2.73	0.9990*
	0.30	-12.17	4.95	0.9999	-4.89	2.93	0.9997**
PhPh	0.00	-6.04	1.98	0.9894	-1.71	0.96	0.9705
	0.05	-7.39	2.58	0.9925	-2.30	1.34	0.9927
	0.10	-8.63	3.13	0.9914	-3.19	1.81	0.9989
	0.15	-8.57	3.33	0.9995	-3.12	2.00	0.9977
	0.20	-8.89	3.63	0.9995	-4.37	2.60	0.9999*
	0.25	-9.66	4.08	0.9992	-4.39	2.82	0.9988*
	0.30	-10.72	4.63	0.9993			
BaP	0.00	-10.11	3.54	0.9975	-3.25	1.54	0.9728
	0.05	-10.99	3.96	0.9974	-3.80	1.88	0.9998
	0.10	-12.08	4.44	0.9948	-4.59	2.30	0.9979
	0.15	-11.92	4.58	0.9992	-4.57	2.47	0.9995
	0.20	-12.37	4.89	0.9996	-5.39	2.91	0.9998*
	0.25	-13.05	5.28	0.9997	-5.53	3.13	0.9989*
	0.30	-13.97	5.76	0.9999*	-6.22	3.55	0.9999**
TBN	0.00	-5.66	2.07	0.9940	-1.82	1.25	0.9699
	0.05	-6.62	2.57	0.9889	-2.49	1.66	0.9939
	0.10	-7.96	3.16	0.9942	-3.42	2.15	0.9992
	0.15	-7.84	3.36	0.9979	-3.45	2.38	0.9973
	0.20	-8.31	3.71	0.9972	-4.83	3.04	0.9999*
	0.25	-9.06	4.16	0.9978			
	0.30	-11.66	5.23	0.9999**			

* Data taken at four temperatures; all others taken at five temperatures unless otherwise noted.

** Data taken at three temperatures; all others taken at five temperatures unless otherwise noted.

volume of the stationary phase. The dependence of this term on mobile phase composition for both modifiers is illustrated for chrysene on the polymeric column in Fig. 3. Similar trends are observed for the other solutes investigated on both columns.

The experimental data was also subjected to a global regression fit which combined composition and temperature dependence into one equation. The form of the global regression equation for acetonitrile is

$$\ln V_n = a + b/T + c\theta \quad (5)$$

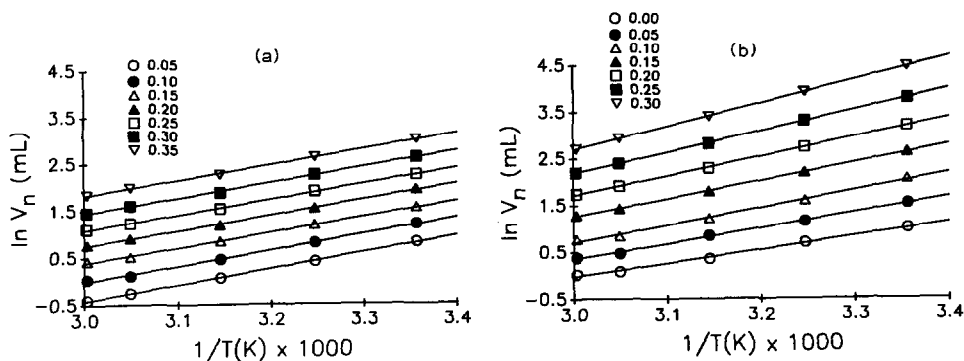


Fig. 1. Plots of $\ln V_n$ vs. $1/T(K) \times 1000$ for chrysenes using various volume fractions water in acetonitrile (a) and methanol (b) on the polymeric bonded phase (see Tables I and II).

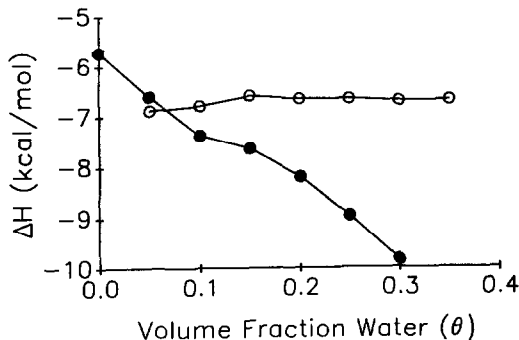


Fig. 2. Plots of enthalpy (kcal/mol) vs. volume fraction water in methanol (●) and acetonitrile (○) for chrysenes on the polymeric bonded phase.

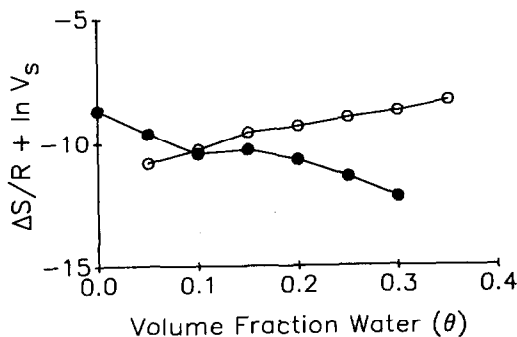


Fig. 3. Plots of $\Delta S/R + \ln V_s$ vs. volume fraction water in methanol (●) and acetonitrile (○) for chrysenes on the polymeric bonded phase.

where θ is the volume fraction water. This form is adopted because the enthalpic contribution to retention is virtually composition independent. For methanol, because of the apparent composition dependence of the enthalpic term, the form of the global regression equation adopted is

$$\ln V_n = a + b/T + c\theta + d\theta/T + e\theta^2/T \quad (6)$$

The decision to use this form was not arbitrary. Different equations were tried involving higher order compositional dependence; this form fits the theoretically derived results for the collapsed-chain limit in ref. 12 (eqn. 53) and gave the best fit.

The parameters for the acetonitrile global fit are listed in Table III. The difference in the b term, Δb , between columns for each solute is also included in Table III. The parameters for the methanol global fit are listed in Table IV.

TABLE III

PARAMETERS FOR THE REGRESSION EQN. 5 USING ACETONITRILE ON THE POLYMERIC AND MONOMERIC BONDED PHASES

<i>Solute</i>	<i>Parameter</i>	<i>Polymeric</i>	<i>Monomeric</i>
Chrysene	<i>a</i>	-10.85 (± 0.10)	-5.26 (± 0.10)
	<i>b</i> ($\times 1000$)	3.37 (± 0.03)	1.92 (± 0.03)
	<i>c</i>	7.33 (± 0.04)	7.37 (± 0.04)
	No. of pts.	35	35
	<i>r</i> ²	0.9991	0.9992
	Δb^*	1.45	
PhPh	<i>a</i>	-9.81 (± 0.11)	-5.05 (± 0.11)
	<i>b</i> ($\times 1000$)	3.07 (± 0.04)	1.96 (± 0.03)
	<i>c</i>	8.62 (± 0.05)	8.66 (± 0.04)
	No. of pts.	35	35
	<i>r</i> ²	0.9992	0.9992
	Δb^*	1.11	
BaP	<i>a</i>	-12.69 (± 0.01)	-6.02 (± 0.01)
	<i>b</i> (± 0.03)	4.14 (± 0.03)	2.30 (± 0.03)
	<i>c</i>	7.39 (± 0.04)	7.51 (± 0.04)
	No. of pts.	35	35
	<i>r</i> ²	0.9994	0.9993
	Δb^*	1.84	
TBN	<i>a</i>	-9.15 (± 0.01)	-5.06 (± 0.01)
	<i>b</i> (± 1000)	3.00 (± 0.04)	2.13 (± 0.04)
	<i>c</i>	8.73 (± 0.05)	8.82 (± 0.05)
	No. of pts.	35	34
	<i>r</i> ²	0.9990	0.9992
	Δb^*	0.87	

* Difference between columns in the b term.

TABLE IV

PARAMETERS FOR THE REGRESSION EQN. 6 USING METHANOL ON THE POLYMERIC AND MONOMERIC BONDED PHASES

<i>Solute</i>	<i>Parameter</i>	<i>Polymeric</i>	<i>Monomeric</i>
Chrysene	<i>a</i>	-8.89 (± 0.13)	-2.77 (± 0.08)
	<i>b</i> ($\times 1000$)	2.95 (± 0.08)	1.24 (± 0.04)
	<i>c</i>	-10.40 (± 0.74)	-8.53 (± 0.88)
	<i>d</i> ($\times 1000$)	6.03 (± 0.23)	5.80 (± 0.27)
	<i>e</i> ($\times 1000$)	1.60 (± 0.18)	0.95 (± 0.16)
	No. of pts.	37	32
	r^2	0.9991	0.9995
PhPh	<i>a</i>	-6.38 (± 0.12)	-1.72 (± 0.11)
	<i>b</i> ($\times 1000$)	2.08 (± 0.04)	0.97 (± 0.06)
	<i>c</i>	-13.93 (± 0.74)	-9.40 (± 0.82)
	<i>d</i> ($\times 1000$)	7.78 (± 0.23)	6.70 (± 0.25)
	<i>e</i> ($\times 1000$)	1.80 (± 0.18)	1.16 (± 0.15)
	No. of pts.	36	31
	r^2	0.9994	0.9997
BaP	<i>a</i>	-10.20 (± 0.11)	-3.54 (± 0.11)
	<i>b</i> ($\times 1000$)	3.56 (± 0.04)	1.64 (± 0.03)
	<i>c</i>	-11.82 (± 0.65)	-7.79 (± 1.00)
	<i>d</i> ($\times 1000$)	6.56 (± 0.20)	5.71 (± 0.31)
	<i>e</i> ($\times 1000$)	1.75 (± 0.14)	0.96 (± 0.18)
	No. of pts.	35	31
	r^2	0.9995	0.9996
TBN	<i>a</i>	-5.77 (± 0.14)	-1.94 (± 0.12)
	<i>b</i> ($\times 1000$)	2.11 (± 0.04)	1.28 (± 0.04)
	<i>c</i>	-13.89 (± 1.07)	-12.25 (± 1.15)
	<i>d</i> ($\times 1000$)	8.04 (0.32)	7.80 (± 0.35)
	<i>e</i> ($\times 1000$)	1.69 (± 0.22)	1.31 (± 0.30)
	No. of pts.	34	26
	r^2	0.9994	0.9995

DISCUSSION

The results illustrated in Figs. 1-3 suggest that the differences between methanol and acetonitrile must be related to fundamentally different retention mechanisms.

Mobile phase

In the case of methanol, some studies²⁰ indicate that methanol solution chemistry is dominated by hydrogen bonding and that a significant volume fraction of aqueous methanol is thereby inhibited from full participation in solute solvation. The extent of this association is composition and temperature interdependent. In contrast, acetonitrile is presumed to associate weakly with water through hydrogen bonding and is therefore more uniformly available for solute solvation within the temperature and composition range studied here. The upper critical solution temperature and critical

composition of acetonitrile–water mixtures are, respectively, 272 K and 38 mol percent acetonitrile²¹; however, it is reasonable to assume that acetonitrile enriched “micro-phases” persist at higher temperatures.

Stationary phase

Bulk solution experiments previously conducted^{22,23} and sorption isotherm data^{20,24} suggest that the methanol concentration in the stationary phase subtly increases with increasing volume fraction of methanol in the mobile phase. Additionally, there is some experimental evidence²⁵ which suggests that the use of hydroorganic solvents may introduce a competing retention mechanism. Sorption isotherm data for acetonitrile²⁶, however, indicates that the RP stationary phases are saturated with acetonitrile at fairly low-volume fractions of acetonitrile and remain fairly constant in composition in the region studied here.

As stated earlier, the enthalpic portion of $\ln V_n$ can be separated into mobile and stationary phase contributions. The methanol phase seems to be fairly straightforward, in principle. As the water content of the mobile phase increases, the non-polar solutes prefer the non-polar stationary phase to the increasingly hostile mobile phase environment and retention increases. The enthalpy of transfer also changes because both phases are changing with composition but not uniformly. The various regions in the methanol curve in Fig. 2 can be related to eqn. 6. According to eqn. 6, the temperature dependence is strongly coupled to the composition dependence. The temperature–composition interdependence of methanol–water hydrogen-bonding complicates the mobile phase–solute interactions and hampers the decoupling of mobile–bonded phase effects.

The acetonitrile case is, however, different. At first glance, one might interpret the compositional independence of the enthalpic contribution to $\ln V_n$ to be the result of compensatory bonded phase–solute interactions in response to a changing mobile phase. In light of the sorption isothermal studies, however, an alternative model based on microphase formation^{21,27,28} might be invoked. In this model, the non-polar solute in the mobile phase experiences an acetonitrile-enriched environment and a stationary phase essentially unaffected by changes in mobile phase composition. The net result is a more or less constant enthalpic contribution to retention despite differences in mobile phase composition. Similar results have been reported for several solutes by Hanai *et al.*¹³. Standard enthalpies of transfer were determined for a large number of solutes using two different compositions of aqueous acetonitrile. The solutes studied included assorted PAHs, alkylbenzenes, halobenzenes, alkanols and alkanes. The reported values for the enthalpies of transfer are fairly close for the two compositions used for most of the solutes. The exceptions are the alkanols. The difference in enthalpies for the alkanols at the two compositions used appears to decrease with increasing solute alkyl chain length. These results are consistent with the model presented here. The slight deviation from a constant value at high organic compositions may be due to experimental error because of the increasing importance of V_m in the calculation of V_n at lower retention volumes.

A qualitative comparison of the intercepts of the van 't Hoff plots for acetonitrile and methanol might be useful at this point. The intercept may be interpreted as

$$\text{Intercept} = \frac{S_s - S_m}{R} + \ln V_s \quad (7)$$

where S_s and S_m are the entropies associated with the stationary and mobile phases, respectively.

In the case of methanol, the relative entropy of transfer of the solute becomes more negative as the solute becomes preferentially retained in the stationary phase by being driven out of the increasingly hostile mobile phase environment with increasing water content. Further collapse of the bonded phase in response to the increase in volume fraction water may also contribute to this trend.

In contrast to the methanol case, the relative entropic contribution to retention for acetonitrile exhibits the opposite trend (see Fig. 3). As the mobile phase becomes depleted in acetonitrile, the availability of the acetonitrile-enriched microphases declines. The solute mobile phase entropy decreases while the solute stationary phase entropy and the stationary phase volume remains approximately constant. The net result is that the relative entropy of transfer becomes less negative with increasing water content.

A further consequence of this interpretation is that the differences in retention volumes on a particular column for a given solute at constant temperature but different volume fractions of acetonitrile must be the result of differences in the mobile phase entropy.

Using a simple thermodynamic argument, it can be shown that the change in $\ln V_n$ with respect to acetonitrile composition should be independent of the column. According to our model, the only composition dependent term in eqn. 3 is $\Delta S/R$ and the only composition dependent term from eqn. 5 is $c\theta$. Evaluating eqns. 3 and 5 at compositions θ_1 and θ_2 yields

$$c(\theta_1 - \theta_2) = \Delta(\Delta S)/R = \frac{[(S_s - S_{\theta_1}) - (S_s - S_{\theta_2})]}{R} \quad (8)$$

Because the stationary phase is apparently saturated with acetonitrile within our composition range, the entropy contribution due to the stationary phase drops out and we are left with

$$c = \frac{S_{\theta_2} - S_{\theta_1}}{R(\theta_1 - \theta_2)} \quad (9)$$

which is independent of column. This interpretation is supported by the close correlation of the coefficient associated with the mobile phase composition, c , for the two columns. (See Table III.) To test this interpretation further, the acetonitrile data for each solute on each column was normalized by extrapolating a value for $\ln V_n$ at $\theta = 0$ at each temperature, $\ln V_{n,0}$, and subtracting that value from each $\ln V_n$ at that temperature. According to eqn. 10

$$\ln V_n - \ln V_{n,0} = c\theta \quad (10)$$

the results should be independent of column. The normalized data for two columns used were combined and analyzed according to eqn. 10. The parameters for this fit are presented in Table V. The data indicates that the composition coefficient, c , seems to

TABLE V

PARAMETERS FOR THE NORMALIZED FIT (SEE EQN. 10) USING THE COMBINED ACETONITRILE DATA ON THE POLYMERIC AND MONOMERIC BONDED PHASES

Solute	<i>c</i>	<i>r</i> ²	No. of pts.
Chrysene	7.35 (±0.03)	0.9987	70
PhPh	8.64 (±0.03)	0.9989	70
BaP	7.45 (±0.03)	0.9989	70
TBN	8.77 (±0.04)	0.9985	69

correlate with solute size. (e.g., *c* for TBN ≈ PhPh > BaP ≈ chrysene) (see Tables III and V).

As stated earlier, in the case of acetonitrile, the temperature dependent term in eqn. 3 reflects the enthalpic contribution to retention. It represents the statistical average of all the different solute-stationary phase and solute-mobile phase interactions that the solute experiences as it traverses the column. As the solute progresses through the column, it interacts with a heterogeneous surface²⁹. The extent of the solute-stationary phase interaction is dependent not only on the solute molecular weight (size) and shape or geometry (contact area) but also on bonded ligand density. Higher ligand density results in higher enthalpic contributions to retention. Solute size also influences enthalpic contribution to retention but differences in solute geometry alter accessibility of molecular subunits and modify solute-stationary phase interactions. Long, planar solutes present, on average, a larger contact area for interaction with the stationary phase than more compact, non-planar solutes. This results in a larger enthalpic contribution to retention for long, planar solutes than would be predicted strictly on the basis of molecular size or Van der Waals volume. Also, elongated, planar solutes gain more in the enthalpic contribution to retention relative to more compact solutes in going from a monomeric column (low ligand density) to a polymeric column (high ligand density). From the data (see Table III), it is observed that the differences in the temperature dependent term between columns, Δb , are larger for the more planar, extended solutes (Δb : BaP > chrysene > PhPh > TBN). The fact that Δb is smaller for TBN than for PhPh may be attributed to the fact that the smooth, helical arrangement of the molecular subunits in PhPh allow it to interact more efficiently with the bonded phase and gain more in the relative enthalpic contribution to retention in going from a monomeric to a polymeric bonded phase than TBN, which has a twist about the center of symmetry. Alternatively, PhPh has a lower enthalpic contribution to retention relative to TBN on a phase with low ligand density because its configuration incorporates unoccupied space internally which does not interact with the bonded phase (PhPh has a "hole" in it).

When using acetonitrile on a particular column, eqn. 5 can be applied and the selectivity between solutes 1 and 2, $\ln \alpha_{12}$, can be expressed as¹⁹

$$\ln \alpha_{12} = \ln k_1/k_2 = a_1 - a_2 + (b_1 - b_2)/T + (c_1 - c_2) \theta \quad (11)$$

Resolution of solutes of similar size (and/or shape) appears to be insensitive to changes in mobile phase composition, when using acetonitrile (e.g., BaP-chrysene; TBN-

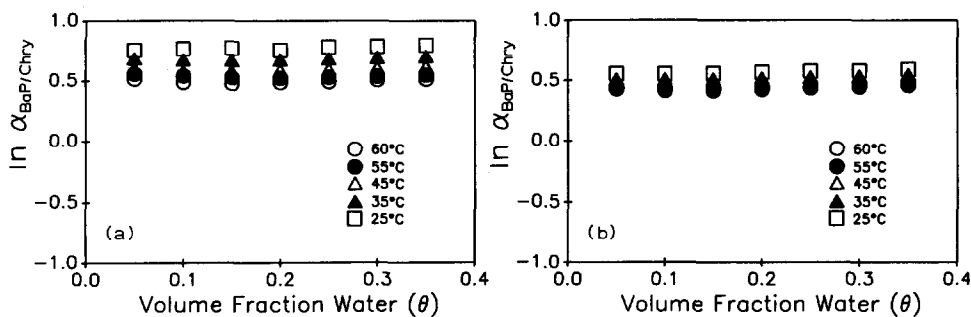


Fig. 4. Plots of the selectivity factor, $\ln \alpha_{12}$, for BaP and chrysene vs. volume fraction water in acetonitrile at various temperatures on the polymeric (a) and monomeric (b) bonded phases.

PhPh, see Figs. 4 and 5). In other words, changing the volume fraction of water, when using acetonitrile in the region studied here, will probably not enhance the separation obtained for solutes of similar size and/or shape to any appreciable extent. The resolution obtained on the polymeric phase for BaP–chrysene is somewhat greater, overall, than that obtained on the monomeric phase. The separation of these two solutes on the polymeric phase is also more sensitive to changes in temperature, with better resolution at lower temperatures. This suggests that the enhanced separation obtained for this pair on the polymeric phase is essentially the result of enthalpic effects arising from the higher bonded ligand density of the polymeric phase. In contrast, the selectivity for the isomers, PhPh and TBN, appears to be fairly unaffected by changes in temperature, as well as composition, irrespective of column (see Fig. 5). The most dramatic differences in selectivity between columns is exhibited by the planar–non-planar pairs. The resolution of these solute pairs is both temperature and composition dependent. The composition dependence probably originates in the differences in solute size (as evidenced by the correlation of solute size with the composition dependent term, c). The selectivity dependence on temperature reflects the difference in enthalpic contribution to retention between the solute pair. This temperature dependence, for the planar–non-planar pairs, is more pronounced on the polymeric phase than the monomeric phase. Overall, the observed selectivity for TBN–BaP decreased on the polymeric relative to the monomeric phase. This results from the

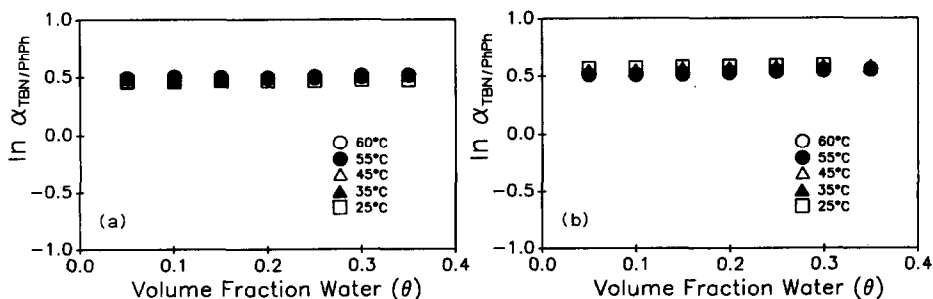


Fig. 5. Plots of the selectivity factor, $\ln \alpha_{12}$, for TBN and PhPh vs. volume fraction water in acetonitrile at various temperatures on the polymeric (a) and monomeric (b) bonded phases.

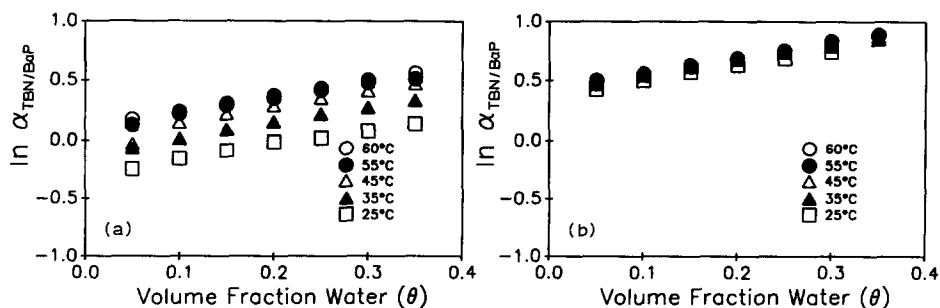


Fig. 6. Plots of the selectivity factor, $\ln \alpha_{12}$, for TBN and BaP vs. volume fraction water in acetonitrile at various temperatures on the polymeric (a) and monomeric (b) bonded phases.

enhanced enthalpic contribution to retention for BaP compared to TBN on the polymeric relative to the monomeric bonded phase. Note that the optimum resolution for this pair, on the polymeric phase, is achieved by increasing the temperature and volume fraction water (see Fig. 5). Essentially, this minimizes the effect of the enthalpic differences between the solutes while exploiting the difference in solute size. These conclusions are consistent with the "slot model" of Wise and co-workers^{6,7}, which is based on solute planarity and length-to-breadth ratios.

CONCLUSIONS

The retention behavior of four PAHs using methanol-water and acetonitrile-water mobile phases at different temperatures on a monomeric and a polymeric bonded phase is presented. A model is proposed which invokes the concept of "microphase" formation to explain the compositional independence of the enthalpic contribution to retention when using acetonitrile. The model is used to rationalize the close correlation of the composition dependent coefficient for each solute on both columns. The results are interpreted in terms of the source of possible differences in selectivity between monomeric and polymeric bonded phases for PAHs.

The retention data presented here supports several conclusions. The fact that the trends in the observed enthalpic and relative entropic contribution to retention with respect to mobile phase composition is similar on the two different columns leads to agreement with the conclusion of Wise and Sander⁷ that the differences between monomeric and polymeric bonded phases are essentially a matter of degree and not the result of some fundamentally different retention mechanisms. As stated in the Introduction, column comparisons are complicated by the coupling of mobile and stationary phase effects. Although the trends in the enthalpic and relative entropic contributions to retention, in the case of methanol, follow those anticipated from classical thermodynamics, the complex nature of methanol-water mixtures precludes any easy interpretation or decoupling of mobile-stationary phase effects. Although solute retention behavior is more difficult to predict in methanol-water mixtures, in practice this solvent system complements acetonitrile-water mobile phases by providing unique changes in column selectivity. This work suggests that acetonitrile may be the solvent of choice to use in column selectivity comparison studies for non-polar solutes.

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