Journal of Chromatography, 4610 (1989) 193–207
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1482

RETENTION MECHANISMS IN REVERSED-PHASE CHROMATOGRAPHY

STATIONARY PHASE BONDING DENSITY AND SOLUTE SELECTIVITY

KAREN B. SENTELL* and JOHN G. DORSEY*
Department of Chemistry, University of Florida, Gainesville, FL 32611 (U.S.A.)

SUMMARY

Chromatographic selectivity for small, non-polar solutes has been determined as a function of monomeric octadecyl stationary phase bonding density over the range 1.74–4.07 μmol/m^2. Phenyl or shape selectivity increases with increasing bonding density, whereas methylene selectivity remains approximately constant. These findings are in agreement with the mean field statistical thermodynamic theory of Dill, which predicts that increased stationary phase chain density should lead to increased anisotropic chain ordering and increased solute-shape selectivity. These studies provide further evidence that partitioning, not adsorption, is the dominant mode of retention for small, non-polar molecules in reversed-phase liquid chromatography.

INTRODUCTION

Chromatographic selectivity (α) is an important experimental probe in studies of the solute retention process. It reflects the difference between two solutes in the Gibbs free energy of transfer from the mobile phase to the stationary phase:

$$\alpha = k'_a/k'_b \text{ and } \ln \alpha = -\Delta(\Delta G)/RT$$

where k'_a and k'_b are capacity factors for solutes a and b, ΔG is the Gibbs free energy, R is the gas constant and T is the absolute temperature. Unlike differences in solute capacity factors, intercolumn selectivity differences cannot be due to different column phase ratios. If the same mobile phase composition is used when comparing different stationary phases, mobile phase contributions to the free energy of transfer are equivalent, cancelling each other in the selectivity ratio. In such a case, selectivity is indicative of differences in the different stationary phases[1]; this implies that very fundamental aspects of the stationary phase retention contribution can be studied via selectivity behavior.

The role of the stationary phase in reversed-phase liquid chromatographic (RPLC) selectivity has had much prior consideration. Many workers have examined

* Present address: Department of Chemistry, Cook Physical Science Building, University of Vermont, Burlington, VT 05405, U.S.A.

0021-9673/89/$03.50

the relationship between bonded-phase chain length and selectivity. Although some have reported little or no change in selectivity with stationary phase chain length[2,3], it has been reported by others that methylene selectivity increases as a function of chain length[4–7]. Others have observed increasing selectivity of benzene derivatives[8–10], styrene oligomers[11] and polynuclear aromatic hydrocarbons (PAHs)[12–15] with stationary phase chain length. The work of Hennion *et al.*[12] is especially significant, as the phases compared were prepared to have the same bonded-group surface coverage. Tchapla *et al.*[16] examined methylene selectivity for C_1, C_6, C_8, C_{14} and C_{18} monomeric bonded phases as a function of solute carbon number. They found that the selectivity continuously decreases up to a certain carbon number, at which a large step decrease in selectivity occurs. Their explanation of this phenomenon is that the stationary phase bonded chains solvate the alkyl chains of the solute molecule and, so long as the length of the solute alkyl chain is less than that of the bonded phase, increasing the number of methylene groups in the solute causes a constant stationary phase contribution to selectivity. Once the length of the solute alkyl chain exceeds that of the bonded phase, the remaining solute methylene groups no longer penetrate the chains; they undergo weaker dispersive interactions than those which penetrate, causing a sharp drop in selectivity. This behavior was not observed with the C_1 bonded phase, as solutes will not penetrate it. Lochmuller and Wilder[13] used a similar argument to explain increasing PAH selectivity as a function of bonded-chain length.

Correlations between selectivity and bonded-phase carbon loading have also been made[17–21]. However, early in the history of bonded phases Unger *et al.*[22] cautioned that carbon content alone is misleading because of differences in the surface area of the original silica, which result in different surface densities of the bonded alkyl groups. This concern makes the interpretation of many of the previous studies difficult. Engelhardt and Ahr[5] correlated selectivity for PAHs and phenylalkanes on monomeric and polymeric octadecyl phases with bonded-group surface coverage, noting that PAHs, being more "rigid", were especially affected by surface coverage. Tanaka *et al.*[23] observed increased hydrophobic selectivity as a function of monomeric octyl surface coverage. Hennion *et al.*[12] noted that the selectivity of their polymeric octadecyl phases increased up to 15% carbon, and subsequently leveled off; these results would also hold true for surface coverage, as the same silica substrate and silane reagent were used to produce all of these phases.

Comparison of the selectivity behavior of stationary phases prepared with different reagents, alkyl chain lengths and/or silica substrates adds these variables to an already complex situation and may explain the inconsistencies among conclusions drawn by different groups. Sander and Wise[24], Staroverov *et al.*[25], and Van den Driest and Ritchie[26] observed differences in selectivity as a function of silica substrate pore size and pretreatment which were especially marked for polymeric phases. Rather than solute size-exclusion effects, Sander and Wise attributed these differences to changes in the makeup of the bonded-phase structure as a consequence of these substrate parameters.

Antle and co-workers[27,28] stated that there are two types of reversed-phase (RP) column selectivity, solvophobic and chemical. Solvophobic selectivity arises from hydrophobic interactions between the solute molecules and the stationary phase. Tanaka *et al.*[23] and Jandera[29] noted from experimental evidence that hydrophobicity is the most important RP stationary phase selectivity parameter for non-polar solutes;

this has been confirmed by chemometric analyses, using cluster and principal components analysis[30] and factor analysis[31]. Chemical selectivity comes about from strong interactions (for example, hydrogen bonding or complexation) between the solute molecules and specific active sites, such as silanol groups or trace metal contaminants on the silica surface[27,29]; this effect is relatively unimportant for non-polar solutes.

A third type of selectivity, shape selectivity, can also be exhibited by chemically bonded phases. Some researchers[1,5,32–34] have examined selectivity for rigid molecules, such as PAHs, polyphenyls and cycloaliphatics, on bonded phases with various ligand structures. They found that these types of solutes were preferentially retained by alkylphenyl, alkylnaphthyl, alkylpyrenyl, cycloalkyl, cycloalkenyl and octadecyl stationary phases over other alkyl phases. They concluded that steric considerations were crucial in the retention of rigid or planar molecules. As octadecyl phases consist of lengthy alkyl chains bonded to the silica surface, the conformation of the bonded chains plays an important role in retention.

Wise and co-workers[24,35–38] and Van den Driest *et al.*[39] compared PAH selectivities on monomeric and polymeric octadecyl-bonded phases. Polymeric phases exhibit superior selectivity; moreover, selectivity increases as a function of polymeric surface coverage. Selectivities shown by monomeric phases are more like those of polymeric phases as the bonded-chain length increases; selectivities for polymeric phases become more "monomeric" with decreasing chain length[14]. Sander and Wise[14] explained these trends as being controlled by the overall thickness of the stationary phase.

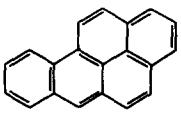
Martire and Boehm[40], using a liquid crystal model of the stationary phase, proposed the first statistical mechanical theory to address explicitly the effects of chain organization on solute retention and selectivity. More recently, Dill[41,42] proposed a lattice-interphase model of the bonded-phase surface and described the equilibrium partition coefficient for a solute from the chemical potentials of the solute in the mobile phase system and in the bonded-chain interphase. The molecular details of the retention process involve (i) the creation of a solute-sized cavity in the stationary phase, (ii) the transfer of the solute from the mobile to the stationary phase and (iii) the closing of the solute-sized cavity in the mobile phase. In this retention model, non-polar solute partitioning and selectivity will be strongly affected by the surface density of the bonded alkyl chains.

We are investigating experimentally the molecular mechanism of retention, as described by Dill[41,42]. He proposed that two driving forces dominate retention and selectivity: (i) the chemical differences of the contacts of the solute with surrounding molecular neighbors in the stationary and mobile phase and (ii) the partial ordering of the grafted stationary phase chains. This, at sufficiently high bonding density, leads to an entropic expulsion of solute from the stationary phase relative to that which would be expected in a simpler, amorphous oil–water partitioning process. At low densities, partitioning should increase linearly with the bonded-phase surface coverage as the surface becomes more fully covered by the hydrocarbon chains and therefore less polar. Partitioning should reach a maximum at the point at which neighbor interactions among chains become important. At higher surface densities, partitioning decreases owing to increasing entropic expulsion of solute by the grafted chains[41–43]. We have tested with an extensive database of almost 350 sets of experiments and

found, in agreement with theory, that the mobile phase contribution to retention can be described by the binary interaction constants of solutes with solvents[44]. We have also examined the effects of alkyl chain bonding density on the partitioning and retention of small, non-polar solutes[45], and have found that experimental partitioning behavior for non-polar solutes mirrors the predicted behavior, a maximum in partition coefficients being found at a stationary phase chain density of about 3.0 μmol/m^2. In this work we have tested this further by examining the effects of alkyl chain bonding density on selectivities of non-polar solutes.

EXPERIMENTAL

Monomeric, silica-bonded phases with surface coverages ranging from 1.74 to 4.07 μmol/m^2 were prepared as described previously[46] from 20–30-μm Davisil (W. R. Grace, Baltimore, MD, U.S.A.) with a pore diameter of 147 Å. The liquid chromatographic system used for the selectivity measurements has also been described previously[45]. Toluene (Eastman Organic Chemicals, Rochester, NY, U.S.A.), ethylbenzene (Fisher Scientific, Fair Lawn, NJ, U.S.A.), propylbenzene (Alfa Products, Danvers, MA, U.S.A.), butylbenzene (Eastman) and pentylbenzene (Alfa) standards were prepared in high-performance liquid chromatographic (HPLC)-grade methanol for methylene selectivity studies. Benzene (Mallinckrodt, Paris, KY, U.S.A.), biphenyl (Eastman), recrystallized three times from ethanol, and *p*-terphenyl (Sigma, St. Louis, MO, U.S.A.) standards in methanol comprised the phenyl selectivity test solutes. The Column Evaluation Test Mixture 1 (PAH) of the National Bureau of Standards (NBS, Gaithersburg, MD, U.S.A.) was used to measure the overall selectivity for PAHs; this mixture contains benzo[*a*]pyrene (BaP), 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN) and phenanthro[3,4-*c*]phenanthrene (PhPh). Their structures are shown in Fig. 1.



Benzo[a]pyrene (BaP)

Phenanthro [3,4-c] phenanthrene (PhPh)

1,2:3,4:5,6:7,8-Tetrabenzonaphthalene (TBN)

Fig. 1. Structures of the solutes in the NBS Column Evaluation Test Mixture No. 1 (PAH).

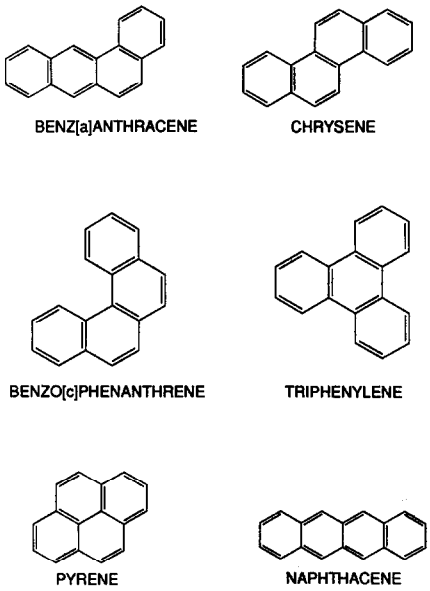


Fig. 2. Structures of the four-ring PAH test solutes.

PAH selectivity was also examined with the four-ring PAHs chrysene, benz[*a*]-anthracene, benzo[*c*]phenanthrene, naphthacene, triphenylene (all of mol.wt. 228.28) and pyrene (mol.wt. 202.26), shown in Fig. 2. The four-ring PAHs [Aldrich, Milwaukee, WI, U.S.A., except for naphthacene (Eastman)] were prepared in HPLC-grade acetonitrile. Methylene and phenyl selectivity studies were conducted at 35.0°C with methanol–water (55:45) and acetonitrile–water (85:15) as mobile phases. Owing to their limited methanol solubility, the four-ring PAHs were evaluated at 35.0°C with only acetonitrile–water (85:15) as the mobile phase. The NBS test mixture was also evaluated with acetonitrile–water (85:15), but at ambient temperature (25.0–29.0°C) so as to replicate better the conditions of Wise and Sander[36]. The mobile phase flow-rate was 1.5 ml/min in all instances; capacity factors were measured from triplicate chart-recorder tracings for each solute.

RESULTS AND DISCUSSION

The chromatographic selectivity was examined as a function of monomeric octadecyl stationary phase bonding density. When the same mobile-phase compositions are used to compare selectivities on different stationary phases, mobile phase contributions to selectivity are equivalent, and changes in selectivity are attributable to stationary phase contributions[1]. Although Wise and co-workers[24,35–37] have extensively examined the effect of polymeric alkyl bonding density on selectivity for PAHs, monomeric phases have not been exhaustively examined. Monomeric stationary phases, used here, can only result in monomeric layers on the silica surface, giving a relatively well characterized surface. For polymeric phases, surface density numbers should be viewed only as a rough indication of true chain density. As the degree of polymerization is almost never known, Sander and Wise[35] stated that "the

use of surface coverage values to calculate interchain distances is probably not justified for polymeric phases". Methylene and phenyl selectivities as a function of octadecyl bonding density for methanol–water (55:45) and acetonitrile–water (85:15) are listed in Tables I and II. As these selectivity values are calculated from the slopes of plots of ln k' *versus* homologue unit number for each stationary phase, the least-squares linear regression coefficients of correlation for each of these plots are included to verify linear behavior. Colin *et al.*[7] stated that a linear relationship exists between ln k' and the homologue unit number only for unit numbers above three to five. This number of units, termed the critical carbon number, results from the fact that the effect of an additional homologue unit should only become constant when it is sufficiently removed from the basic functional group. Thus, for homologues below the critical carbon number, the plot of ln k' *versus* homologue unit number is expected to exhibit curvature. However, this departure from linearity is generally small for RPLC systems, causing a very limited influence on the average slope of the plot[7]. This expected curvature was not found for either mobile phase system, as all of the correlation coefficients are greater than or equal to 0.991.

Methylene selectivity *versus* octadecyl bonding density for the homologous methylene series toluene, ethylbenzene, propylbenzene, butylbenzene and pentylbenzene is plotted in Fig. 3 for the methanol–water (55:45) system and in Fig. 4 for the acetonitrile–water (85:15) system. The average methylene selectivity value $\pm$ one standard deviation for the methanol–water mobile phase is 1.96 $\pm$ 0.03 and that for the acetonitrile–water system is 1.34 $\pm$ 0.07. Using methanol–water (55:45) as the mobile phase and octadecyl columns, Colin *et al.*[7] and Karger *et al.*[47] reported methylene selectivity values of 2.14 and 2.0, respectively. For octadecyl columns and acetonitrile–water (85:15), Colin *et al.*[7], Karger *et al.*[47] and Krstulovic *et al.*[6] reported values of 1.40, 1.3 and 1.4, respectively; our reported methylene selectivity values are

TABLE I

METHYLENE AND PHENYL SELECTIVITIES AT 35.0°C AS A FUNCTION OF OCTADECYL BONDING DENSITY FOR METHANOL–WATER (55:45) AS THE MOBILE PHASE

C_{18} bonding density (μmol/m^2)	*Methylene selectivity*	*Methylene correlation coefficient**	*Phenyl selectivity*	*Phenyl correlation coefficient***
1.74	1.92	0.9993	7.27	0.9997
1.98	1.96	0.9997	7.05	1.0000
2.07	1.92	0.9994	7.38	0.9996
2.09	1.97	0.9999	7.20	1.0000
2.75	1.94	0.9993	7.61	0.9999
3.06	1.99	0.9996	7.83	0.9999
3.24	1.97	0.9995	7.94	0.9999
3.34	1.97	0.9994	8.13	0.9997
3.56	1.93	0.9994	7.96	0.9998
3.60	1.96	0.9997	8.17	0.9997
4.07	2.00	0.9996	8.18	0.9994

* Correlation coefficient for the plot of ln k' *versus* carbon number; the slope of this line is ln(methylene selectivity).

** Correlation coefficient for the plot of ln k' *versus* phenyl number; the slope of this line is ln(phenyl selectivity).

TABLE II

METHYLENE AND PHENYL SELECTIVITIES AT 35.0°C AS A FUNCTION OF OCTADECYL BONDING DENSITY FOR ACETONITRILE–WATER (85:15) AS THE MOBILE PHASE

C_{18} bonding density ($\mu mol/m^2$)	*Methylene selectivity*	*Methylene correlation coefficient**	*Phenyl selectivity*	*Phenyl correlation coefficient**
1.74	1.29	0.9991	1.90	0.9996
1.98	1.28	0.9993	1.92	0.9994
2.07	1.21	0.9935	1.94	1.0000
2.09	1.31	0.9994	1.95	0.9999
2.75	1.53	0.9906	2.00	0.9995
2.84	1.35	0.9983	2.04	0.9999
3.06	1.34	0.9988	2.04	0.9997
3.15	1.34	0.9974	2.01	0.9995
3.24	1.35	0.9990	2.03	0.9992
3.34	1.36	0.9943	2.02	0.9992
3.56	1.35	0.9995	2.03	0.9995
3.60	1.36	0.9999	2.11	0.9997
4.07	1.36	0.9960	2.12	0.9971

* See Table I

comparable to these values for both mobile phase systems. It is not surprising that methylene selectivities are approximately constant in either system; methylene selectivity is a type of solvophobic selectivity, due solely to non-specific hydrophobic interactions between the solute molecules and the stationary phase, and it is therefore unaffected by the greater chain ordering resulting from increasing octadecyl bonding density. This observation is supported by the work of Lochmuller and Wilder[13], who found that methylene selectivities for small solutes on octadecyl columns compare favorably with liquid–liquid partition selectivities. It has also been predicted that solute-methylene selectivities should be unaffected by the molecular organization of the interphase[41].

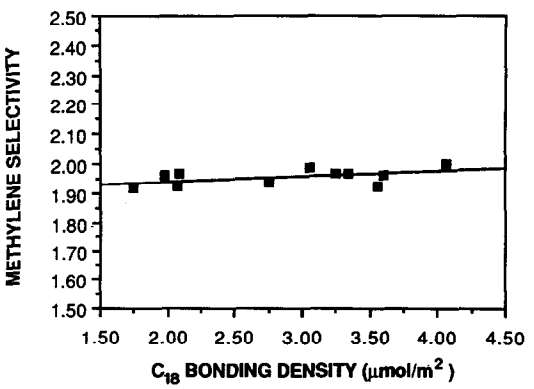


Fig. 3. Methylene selectivity *versus* octadecyl bonding density at 35.0°C for methanol–water (55:45) as the mobile phase.

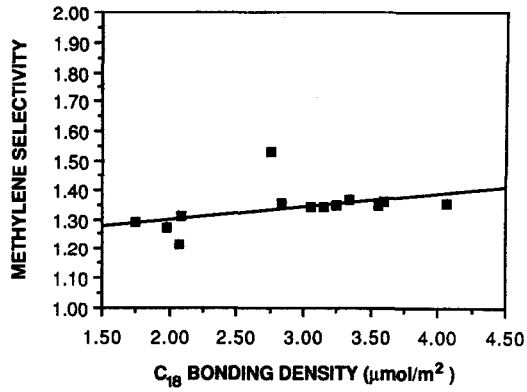


Fig. 4. Methylene selectivity *versus* octadecyl bonding density at 35.0°C for acetonitrile–water (85:15) as the mobile phase.

The relationship between phenyl selectivity and bonding density for the phenyl homologous series benzene, biphenyl and *p*-terphenyl is shown in Figs. 5 and 6 for methanol–water (55:45) and acetonitrile–water (85:15), respectively. Phenyl selectivity increases with increasing octadecyl bonding density in an approximately linear fashion with least-squares linear regression slopes of 0.508 and 0.0871 and correlation coefficients (r) of 0.955 and 0.941 for the methanol–water and acetonitrile–water systems, respectively. This correlation between phenyl selectivity and octadecyl bonding density can be attributed to shape selectivity. Martire and Boehm[40] predicted that solute selectivity should decrease as a function of solute shape in the order rod-like > planar > chain-like. This behavior was also observed by Lochmuller *et al.*[1], and this effect has been explained in terms of increased ordering of the bonded RP chains[1,6,40].

Wise and co-workers[24,35–37] examined the PAH selectivity of monomeric and polymeric octadecyl phases with bonding density ranges of 1.8–3.2 and 2.7–7.3 μmol/m^2, respectively. Wise and Sander[36] found that for polymeric phases with high bonding densities (greater than about 5.1 μmol/m^2) non-planar solutes were eluted before planar solutes and that non-linear solutes were eluted before linear solutes, even

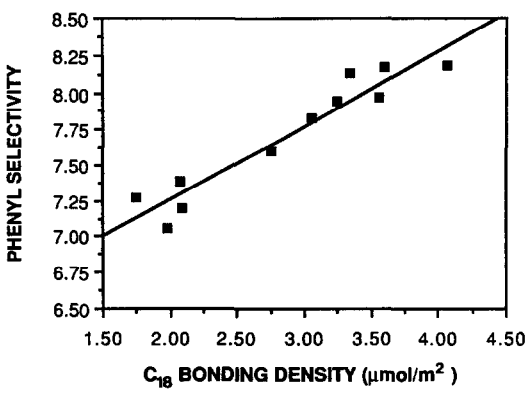


Fig. 5. Phenyl selectivity *versus* octadecyl bonding density at 35.0°C for methanol–water (55:45) as the mobile phase.

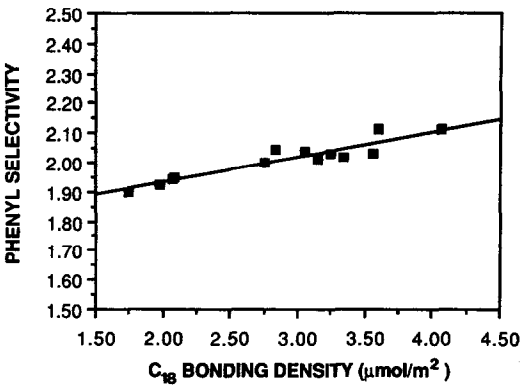


Fig. 6. Phenyl selectivity *versus* octadecyl bonding density at 35.0°C for acetonitrile–water (85:15) as the mobile phase.

if the solutes compared had similar molecular weights, overall shapes and molecular dimensions. Additionally, they found that the selectivity between planar–non-planar and linear–non-linear PAHs increases with increasing degree of non-planarity and non-linearity. Their "slot model" postulates that non-planar solutes have a greater "thickness", hindering penetration of the solute into the narrow slots between the bonded alkyl chains[36]. The situation is analogous for linear molecules, which would show greater retention than non-linear molecules. This also corresponds with Martire and Boehm's theory[40], which predicts that shape selectivity is greater for rigid-rod solutes than for globular solutes, especially when the stationary phase chains are fully extended or more rigid. Wise and Sander[36] argued that polymeric phases with higher alkyl densities are more extended and rigid than polymeric phases with low densities or monomeric phases.

The trend of greater phenyl selectivity with increasing octadecyl bonding density shown here is not surprising. The Dill interphase model[41,42] predicts that, as alkyl surface densities increase, the corresponding configurational constraints are also increased, creating a more rigid and ordered chain-packing structure. This anisotropy of the bonded chains gives rise to additional shape selectivity among solute molecules, as molecules which can most effectively align themselves with the chains are those which are most effectively retained. In this model, the driving force for retention is the creation of a solute-sized cavity in the stationary phase. As the bonding density and, consequently, chain ordering are increased, the free energy required for cavity formation also increases. It costs more free energy to insert solute substructures that are parallel to the silica–bonded chain interface than for substructures that align themselves with the chains and normal to the interface. Therefore, selectivity for linear and planar molecules will increase with increasing alkyl bonding density, as predicted by this theory and as shown by the results of our experiments.

It is interesting to compare the slope of the acetonitrile–water (85:15) phenyl selectivity plot (0.0871) with that for methanol–water (55:45) (0.508). One explanation of this disparity is the structural difference between the solvation layers of the bonded phase in the two very different mobile phase systems. The acetonitrile–water (85:15) solvation layer is relatively robust; at any of the bonded-phase alkyl densities the

stationary phase surface will be well solvated and the chains ordered. This means that the relative retention will only be affected to a small extent by changes in bonding density; chain ordering will increase little with increased packing constraints, as the chains are already relatively ordered. In methanol–water (55:45), the chains are not as well solvated and are rather disordered. Thus, shape selectivity will be affected by bonding density to a much greater extent in the methanol–water system; this is exhibited by the larger slope of the phenyl selectivity plot.

Sorption isotherm data[48] indicate that reversed-phase stationary phases become saturated with acetonitrile at fairly low volume fractions of acetonitrile and therefore these stationary phases maintain a relatively constant acetonitrile composition over the entire mobile phase composition range. Stalcup *et al.*[49] noted a relative insensitivity in PAH net retention volumes as a function of mobile phase composition in acetonitrile–water mobile phases. They explained this with a microphase formation model. In this model, non-polar solutes experience an acetonitrile-rich mobile-phase environment and a stationary phase environment that is essentially unaffected by changes in mobile phase composition. In contrast, Stalcup *et al.*[49] observed that PAH retention in methanol–water mobile phase systems increases with increasing mobile phase water content. For methanol–water mobile phases, sorption isotherm data[50] indicate that the methanol concentration in the stationary phase increases slightly with increasing amount of methanol in the mobile phase. The smaller slope of our acetonitrile–water selectivity plot is in agreement with the observations and conclusions of Stalcup *et al.*[49].

Sander and Wise[35] devised a simple, empirical, chromatographic test to gauge the relative monomeric or polymeric nature of a bonded phase. They found that the elution order of a three-component PAH test mixture of PhPh, TBN and BaP at ambient temperature with acetonitrile–water (85:15) is dependent on the type of stationary phase and surface coverage. For monomeric phases (bonding densities up to about 3.2 μmol/m^2), the elution order is BaP $\leqslant$ PhPh $<$ TBN; for oligomeric phases (bonding densities of 3.3 to about 4.2 μmol/m^2) the elution order is PhPh $<$ BaP $<$ TBN. Polymeric phases (bonding density $>$ 4.3 μmol/m^2) give the elution order PhPh $<$ TBN $<$ BaP. Each type of phase also results in a different narrow range of values for TBN–BaP selectivity. By examining the elution order of the compounds in the test mixture, the PAH selectivity of any RP column can be quickly predicted.

The selectivity behavior of the PAH test mixture of Sander and Wise on our monomeric columns, compiled in Table III, further confirms that shape selectivity increases with increasing alkyl bonding density. For bonding densities of 1.74–3.56 μmol/m^2 the TBN–BaP selectivity is about 1.7 and the elution order is BaP = PhPh $<$ TBN. At 3.60 and 4.07 μmol/m^2, the elution order changes to PhPh $<$ BaP $<$ TBN and the TBN–BaP selectivities are 1.56 and 1.63, respectively. The planar BaP molecule is now retained longer than the helical PhPh. Although this was classified as "oligomeric"-type behavior by Sander and Wise[35], this stationary phase was prepared from the monochlorosilane, as opposed to the trichlorosilane reagent used by Sander and Wise to prepare the oligomeric bonded phases. The oligomeric bonded phases are actually polymeric-type phases the bonding density (or "thickness") of which has been controlled by sequential polymerization.

The selectivities for the four-ring PAHs were also correlated with stationary-phase bonding density. The selectivity for every possible pairing of these compounds

TABLE III

TETRABUTYLNAPHTHALENE (TBN)–BENZO[*a*]PYRENE (BaP) SELECTIVITY AS A FUNCTION OF OCTADECYL BONDING DENSITY FOR ACETONITRILE–WATER (85:15) AS THE MOBILE PHASE

C_{18} bonding density ($\mu mol/m^2$)	*TBN–BaP selectivity**	*Stationary phase behavior***
1.74	1.68	Monomeric
1.98	1.68	Monomeric
2.07	1.72	Monomeric
2.09	1.70	Monomeric
2.75	1.73	Monomeric
2.84	1.72	Monomeric
3.06	1.75	Monomeric
3.15	1.73	Monomeric
3.24	1.72	Monomeric
3.34	1.70	Monomeric
3.56	1.69	Monomeric
3.60	1.56	Oligomeric
4.07	1.63	Oligomeric

* Ratio of k'_{TBN} to k'_{BaP}.

** Stationary phase characterization based on the classification system of Sander and Wise[35]. If the elution order is BaP $\leqslant$ PhPh < TBN, the stationary phase is considered to be monomeric; an elution order of PhPh < BaP < TBN is considered to be due to an oligomeric stationary phase.

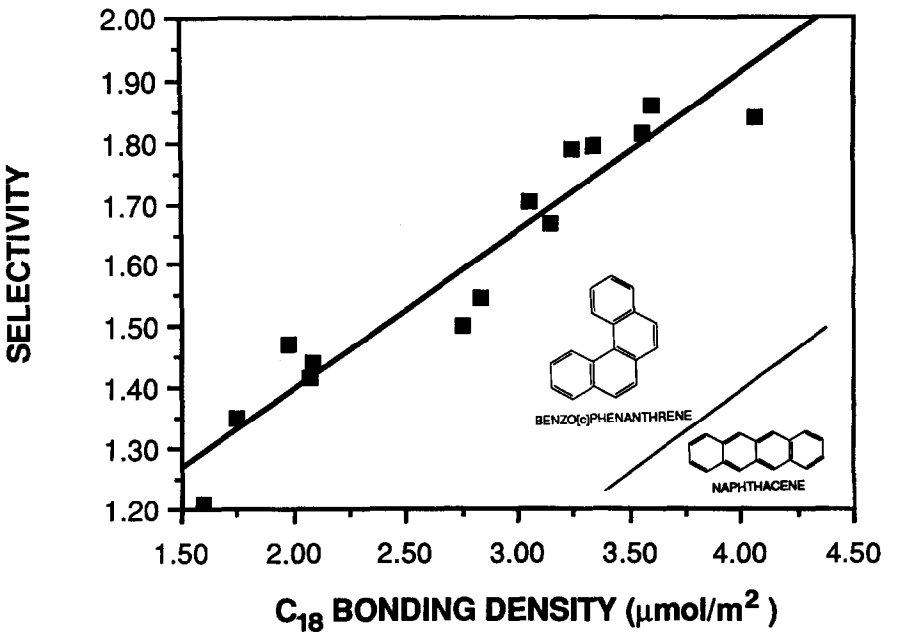


Fig. 7. Benzo[*c*]phenanthrene–naphthacene selectivity *versus* octadecyl bonding density at 35.0°C for acetonitrile–water (85:15) as the mobile phase.

TABLE IV

FOUR-RING PAH SELECTIVITIES AT 35.0°C AS A FUNCTION OF OCTADECYL BONDING DENSITY FOR ACETONITRILE–WATER (85:15) AS THE MOBILE PHASE

Bonding density (μmol/m^2)	*Benz[a]anthracene–naphthacene*	*Chrysene–naphthacene*	*Benzo[c]phenanthrene–naphthacene*
1.60	1.21	1.16	1.21
1.74	1.37	1.37	1.35
1.98	1.46	1.52	1.47
2.07	1.44	1.41	1.42
2.09	1.44	1.43	1.44
2.75	1.51	1.48	1.50
2.84	1.61	1.58	1.55
3.06	1.76	1.70	1.70
3.15	1.70	1.71	1.67
3.24	1.80	1.81	1.79
3.34	1.89	1.86	1.79
3.56	1.86	1.89	1.81
3.60	1.93	1.92	1.86
4.07	1.92	1.92	1.84
	Benzo[c]phenanthrene–triphenylene	*Triphenylene–pyrene*	*Benz[a]anthracene–chrysene*
1.60	1.02	1.11	1.05
1.74	1.00	1.13	1.00
1.98	1.06	1.14	0.96
2.07	1.03	1.16	1.03
2.09	1.04	1.12	1.01
2.75	1.06	1.13	1.02
2.84	1.06	1.11	1.02
3.06	1.08	1.08	1.03
3.15	1.07	1.10	1.00
3.24	1.11	1.09	1.00
3.34	1.06	1.11	1.01
3.56	1.07	1.11	0.99
3.60	1.08	1.11	1.00
4.07	1.08	1.09	1.00

on each of the monomeric octadecyl columns is compiled in Table IV. Selectivity was also plotted *versus* octadecyl bonding density, as shown for benzo[*c*]phenanthrene and naphthacene in Fig. 7. For most of the pairs, except triphenylene–pyrene and benz[*a*]anthracene–chrysene, a distinct correlation between selectivity and bonding density was observed. This is the same sort of behavior which Wise and co-workers[24,35–37] have reported for polymeric bonded phases. Linear behavior (coefficients of correlation $\geqslant 0.95$) was found for all the PAHs when paired with naphthacene; correlation coefficients $\geqslant 0.80$ were obtained for the benz[*a*]-anthracene–triphenylene, chrysene–triphenylene, benz[*a*]anthracene–pyrene and benzo[*c*]phenanthrene–triphenylene solute pairs. Except for naphthacene, in all

Triphenylene–naphthacene	*Pyrene–naphthacene*	*Benz[a]anthracene–triphenylene*	*Chrysene–triphenylene*	*Benz[a]anthracene–pyrene*
1.19	1.07	1.02	0.97	1.13
1.35	1.19	1.01	1.01	1.15
1.39	1.22	1.05	1.09	1.20
1.38	1.19	1.05	1.02	1.21
1.38	1.23	1.04	1.03	1.17
1.42	1.26	1.06	1.04	1.20
1.47	1.32	1.10	1.07	1.22
1.58	1.46	1.12	1.08	1.21
1.56	1.42	1.09	1.09	1.19
1.61	1.48	1.12	1.13	1.22
1.70	1.53	1.11	1.10	1.24
1.69	1.53	1.10	1.11	1.22
1.73	1.56	1.12	1.11	1.24
1.70	1.57	1.13	1.13	1.23

Benz[a]anthracene–benzo[c]phenanthrene	*Chrysene–benzo[c]phenanthrene*	*Chrysene–pyrene*	*Benzo[c]phenanthrene–pyrene*
1.00	0.95	1.08	1.13
1.01	1.01	1.15	1.13
0.99	1.03	1.25	1.20
1.02	0.99	1.18	1.19
1.00	0.99	1.16	1.17
1.00	0.98	1.18	1.20
1.04	1.02	1.19	1.17
1.03	1.00	1.17	1.17
1.02	1.02	1.20	1.17
1.01	1.01	1.23	1.21
1.05	1.04	1.22	1.17
1.03	1.04	1.24	1.19
1.04	1.03	1.23	1.19
1.04	1.04	1.22	1.18

instances in which a positive correlation between selectivity and bonding density was observed, the solute elution order corresponded with increasing length to breadth ratio (L/B), again concurring with the results of Wise and co-workers[35,37]. For the naphthacene solute the converse is true. For triphenylene–pyrene a negative correlation between selectivity and bonding density is exhibited and the solute elution order corresponds with decreasing L/B. Benz[*a*]anthracene and chrysene are poorly resolved and have a selectivity ratio of *ca.* 1 in every instance; this is not surprising, as this separation is notoriously difficult[37].

The fact that our monomeric phases exhibit the same PAH selectivity as the lower density polymeric phases of Sander and Wise is strong evidence that PAH and/or

shape selectivities are not a function of the degree of stationary phase polymerization or thickness but rather are a function of alkyl chain ordering. The trends in the observed enthalpic and relative entropic contributions to retention as a function of mobile phase composition reported by Stalcup *et al.*[49] also support this conclusion. Carbon-13 NMR studies of reversed-phase packings[51–53] are indicative that there is an increasing amount of bonded chain interaction with increasing alkyl surface density. The correlation of phenyl selectivity with alkyl bonding density further supports this conclusion. The overall results of our selectivity studies lend further credence to Dill's molecular mechanism of RPLC retention[41,42] and again indicate that partitioning is the dominant mode of RPLC retention for small, hydrophobic molecules.

CONCLUSIONS

Our studies significantly further our understanding of retention and selectivity processes in reversed-phase liquid chromatography. It is now clear that selectivity differences among different commercial columns are due not only to differences in the starting silica material, but also to differences in the chain density of the bonded alkyl phase. It is further clear that higher chain densities should lead to improved chromatographic selectivity, providing impetus for further studies of bonding reactions that can produce such high-density phases. It is also possible that this better understanding of solute selectivity will lead us closer to the development of a useful liquid chromatographic retention index system.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge many helpful discussions with Ken A. Dill and thank Lane C. Sander for providing samples of the NBS Column Evaluation Test Mixture. Support of this work by NIH GM33382 and support for K.B.S. from an ACS Analytical Division fellowship, sponsored by the Procter and Gamble Company, are also gratefully acknowledged.

REFERENCES

1 C. H. Lochmuller, M. L. Hunnicut and J. F. Mullaney, *J. Phys. Chem.*, 89 (1985) 5770.
2 H. Colin, A. M. Krstulovic, M.-F. Gonnord, G. Guiochon, Z. Yun and P. Jandera, *Chromatographia*, 17 (1983) 9.
3 W. R. Melander and Cs. Horváth, *Chromatographia*, 15 (1982) 86.
4 H. J. Isaaq, *J. Liq. Chromatogr.*, 4 (1981) 1917.
5 H. Engelhardt and G. Ahr, *Chromatographia*, 14 (1981) 227.
6 A. M. Krstulovic, H. Colin, A. Tchapla and G. Guiochon, *Chromatographia*, 17 (1983) 228.
7 H. Colin, G. Guiochon, Z. Yun, J. C. Diez-Masa and J. Jandera, *J. Chromatogr. Sci.*, 21 (1983) 179.
8 K. Jinno and K. Kawasaki, *Chromatographia*, 18 (1984) 103.
9 K. Jinno and K. Kawasaki, *Chromatographia*, 18 (1984) 499.
10 H. Hemetsberger, M. Kellermann and H. Ricken, *Chromatographia*, 10 (1977) 726.
11 J. J. Lewis, L. B. Rogers and R. E. Pauls, *J. Chromatogr.*, 299 (1984) 331.
12 M. C. Hennion, C. Picard and M. Caude, *J. Chromatogr.*, 166 (1978) 21.
13 C. H. Lochmuller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
14 L. C. Sander and S. A. Wise, *Anal. Chem.*, 59 (1987) 2309.
15 N. Tanaka, K. Sakagami and M. Araki, *J. Chromatogr.*, 199 (1980) 327.

16 A. Tchapla, H. Colin and G. Guiochon, *Anal. Chem.*, 56 (1984) 621.
17 B. Shaikh and J. E. Tomaszewski, *Chromatographia*, 17 (1983) 675.
18 A. L. Colsmjo and M. W. Ericsson, *J. Liq. Chromatogr.*, 9 (1986) 2825.
19 P. Spacek, M. Kubin, S. Vozka and B. Porsch, *J. Liq. Chromatogr.*, 3 (1980) 1465.
20 A. P. Goldberg, *Anal. Chem.*, 54 (1982) 342.
21 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 142 (1977) 213.
22 K. K. Unger, N. Becker and P. Roumeliotis, *J. Chromatogr.*, 125 (1976) 115.
23 N. Tanaka, H. Goodell and B. L. Karger, *J. Chromatogr.*, 158 (1978) 233.
24 L. C. Sander and S. A. Wise, *J. Chromatogr.*, 316 (1984) 163.
25 S. M. Staroverov, A. A. Serdan and G. V. Lisichkin, *J. Chromatogr.*, 364 (1986) 377.
26 P. J. van den Driest and H. J. Ritchie, *Chromatographia*, 24 (1987) 324.
27 P. E. Antle and L. R. Snyder, *LC, Liq. Chromatogr. HPLC Mag.*, 2 (1984) 840.
28 P. E. Antle, A. P. Goldberg and L. R. Snyder, *J. Chromatogr.*, 321 (1985) 1.
29 P. Jandera, *J. Chromatogr.*, 352 (1986) 91.
30 M. F. Delaney, A. N. Papas and M. J. Walters, *J. Chromatogr.*, 410 (1987) 31.
31 J. R. Chrétien, B. Walczak, L. Morin-Allory, M. Dreux and M. Lafosse, *J. Chromatogr.*, 371 (1986) 253.
32 K. Jinno, T. Nagoshi, N. Tanaka, M. Okamoto, J. C. Fetzer and W. R. Biggs, *J. Chromatogr.*, 392 (1987) 75.
33 N. Tanaka, Y. Tokuda, K. Iwaguchi and M. Araki, *J. Chromatogr.*, 239 (1982) 761.
34 H. Hemetsberger, P. Behrensmeyer, J. Henning and H. Ricken, *Chromatographia*, 12 (1979) 71.
35 L. C. Sander and S. A. Wise, *Anal. Chem.*, 56 (1984) 504.
36 S. A. Wise and L. C. Sander, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 248.
37 S. A. Wise and W. E. May, *Anal. Chem.*, 55 (1983) 1479.
38 S. A. Wise, W. J. Bonnett, F. R. Guenther and W. E. May, *J. Chromatogr. Sci.*, 19 (1981) 457.
39 P. J. van den Driest, H. J. Ritchie and S. Rose, *LC · GC, Liq. Chromatogr. Gas Chromatogr. Mag.*, 6 (1988) 124.
40 D. E. Martire and R. E. Boehm, *J. Phys. Chem.*, 87 (1983) 1045.
41 K. A. Dill, *J. Phys. Chem.*, 91 (1987) 1980.
42 K. A. Dill, J. Naghizadeh and J. A. Marqusee, *Annu. Rev. Phys. Chem.*, 39 (1988) 425.
43 J. G. Dorsey and K. A. Dill, *Chem. Rev.*, (1989) in press.
44 P. T. Ying, J. G. Dorsey and K. A. Dill, *Anal. Chem.*, submitted for publication.
45 K. B. Sentell and J. G. Dorsey, *Anal. Chem.*, submitted for publication.
46 K. B. Sentell, K. W. Barnes and J. G. Dorsey, *J. Chromatogr.*, 455 (1988) 95.
47 B. L. Karger, J. R. Gant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
48 P. L. Zhu, *Chromatographia*, 21 (1986) 229.
49 A. M. Stalcup, D. E. Martire and S. A. Wise, *J. Chromatogr.*, 442 (1988) 1.
50 R. P. W. Scott and C. F. Simpson, *Faraday Symp. Chem. Soc.*, 15 (1980) 69.
51 R. K. Gilpin and M. E. Gangoda, *Anal. Chem.*, 56 (1984) 1470.
52 P. Shah, L. B. Rogers and J. C. Fetzer, *J. Chromatogr.*, 388 (1987) 411.
53 E. Bayer, A. Paulus, B. Peters, G. Laupp, J. Reiners and K. Albert, *J. Chromatogr.*, 364 (1986) 25.