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Chromatographic shape selectivity with carbon dioxide–acetonitrile mobile phases

Effect of mobile phase composition and density

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Abstract

Trends in chromatographic shape selectivity with mobile phases consisting of mixtures of carbon dioxide and acetonitrile are investigated. Selectivity is evaluated as a function of mobile phase composition, temperature, and column bonding chemistry. SRM (standard reference material) 869a is used as a probe of shape selectivity, while the selectivity between triphenylene and *o*-terphenyl is used to investigate planarity selectivity. Four molecular mass 228 polyaromatic hydrocarbon isomers are used to investigate shape selectivity based on differences in length-to-breadth ratio. Shape selectivity trends as a function of temperature and column type are found to be similar to what is seen in reversed-phase liquid chromatography, while the trend seen as the amount of acetonitrile in the mobile phase increases is found to be different than in reversed-phase liquid chromatography. In addition, the effect of mobile phase density, i.e., solvent strength, on shape selectivity is investigated by examining shape selectivity as a function of density with neat carbon dioxide as the mobile phase.

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1. Introduction

The separation of shape isomers is a challenging problem in chromatography. Such isomers generally have similar hydrophobicities, as such, resolution of shape isomers must be from shape recognition rather than from differences in a bulk partition coefficient. In order to separate shape isomers, a chromato-

graphic system that has some form of shape discrimination capability must be utilized. Currently, the most popular method for separating shape isomers is reversed-phase liquid chromatography (RPLC) with a polymeric (e.g., prepared with di- or trichlorosilane) octadecyl (ODS) stationary phase [1].

In general, it is believed that shape selectivity arises from interactions in the stationary phase. It has been theoretically predicted [2,3] and experimentally shown [4,5] that at least one of the two phases in partition chromatography must be at least somewhat ordered for shape selectivity to be observed. Silica-

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bonded alkyl ligands have been shown to act more as an anisotropic interphase, rather than as a bulk liquid phase, in RPLC [6,7]. This is due to the fact that one end of the ligand is fixed to the silica surface while the other is free to move. Thus, an entropy gradient is established along the alkyl ligand. This ordering of the stationary phase ligands is thought to be the origin of chromatographic shape selectivity. It has been shown that the more ordered the stationary phase, the higher degree of shape selectivity is seen chromatographically [1]. In general, polymeric stationary phases, which are formed from polyfunctional silanes in the presence of water, show greater shape selectivity than monomeric phases (e.g., those formed with monofunctional silanes under anhydrous conditions). There are two reasons why polymeric phases are more shape selective: first, the polymer network is more ordered than the corresponding monomeric phase, and second, polymeric stationary phases usually have higher carbon loads than monomeric phases. Thus, the alkyl chains in a polymeric phase are closer together, and ligand–ligand cooperativity increases the order of the phase.

To a first approximation, the nature of the mobile phase should not have a great effect on shape selectivity. There is no intrinsic order in the mobile phase, as it is a bulk fluid. However, mobile phase variables such as composition and temperature can have an effect on the conformation of the stationary phase. It is thought that stationary phase chains change their conformation in response to the surrounding mobile phase [8]. With highly aqueous mobile phases, the stationary phase chains may collapse in on themselves to minimize the surface area of alkyl ligand exposed to water (i.e., a hydrophobic effect). In relatively non-polar mobile phases, the alkyl ligands may be more fully extended in a “brush” like conformation [8]. This change in the order of the alkyl ligands changes the shape selectivity of the system. Shape selectivity trends with hydroorganic mobile phases seem to correlate with these theories: it has been shown [9] that mobile phases with a higher organic content (and thus more fully extended and ordered stationary phase ligands) have better shape selectivity than mobile phases that are water rich.

There are two perspectives by which shape selectivity can be studied. These are: (1) how molecular

shape directly affects retention and selectivity, i.e., correlation of the retention factor k' (or its natural log) with a numeric descriptor of molecular shape, or (2) selectivity trends seen as a chromatographic variable, such as temperature, stationary phase type, or mobile phase composition is systematically changed. To date, nearly all work with shape selectivity has been in reversed-phase liquid chromatography with hydroorganic mobile phases and octadecyl stationary phases. Most initial work was focused on prediction of retention of shape isomers using descriptors of molecular shape. One of these is the length-to-breadth ratio (L/B), which was first introduced by Janini et al. [10]. A slightly different definition of L/B ratio was introduced by Wise et al. [11]. This definition of L/B ratio resulted in better correlation with chromatographic retention than the Janini definition. This correlation of retention with solute length gave rise to the empirical “slot model” for retention [12]. In summary, this model states that retention is due to penetration of solutes between alkyl chains, and long, narrow molecules (high L/B ratio) “fit” better than square shaped or bulky ones (low L/B ratio), and are thus retained more. This idea was presented more formally in Dill’s lattice model for retention [6,7].

A second molecular descriptor for shape selectivity was presented by Yan and Martire [3]. This descriptor, termed A_{\min} , is the minimum area of a side of a box enclosing the molecule. In general, smaller values of A_{\min} correspond to more “rod-like” molecules (as opposed to “block like”, which have larger values of A_{\min}). A linear relation between $\ln k'$ and A_{\min} was observed, with smaller values of A_{\min} resulting in larger retention values [4].

A third molecular descriptor that affects shape selectivity is the planarity of the solute molecule. A value referred to as the “dihedral angle of distortion” was identified by Garrigues et al. [13]. This value is a numeric descriptor related to the non-planarity of a molecule. Planar molecules have a dihedral angle of 0° , while non-planar molecules have non-zero values. This descriptor has been shown to be particularly useful for describing the retention of substituted isomers.

As stated, a second method for investigating shape selectivity is the examination of selectivity trends as

a chromatographic variable is changed. There are several variables that have been shown to be influential in the shape recognition ability of a chromatographic system, and chromatographic tests are often used to assess shape selectivity. The most common is standard reference material (SRM) 869a, developed by Sander and Wise at the National Institute of Standards and Technology (NIST) [14]. In this test, the selectivity of the tetrabenzonaphthalene/benzo[*a*]pyrene pair (abbreviated as $\alpha_{\text{TBN/BaP}}$) is used as a numeric value for shape selectivity. Lower values are taken to indicate better shape recognition. In general, values of less than 1.0 are seen for polymeric columns, while monomeric columns yield values over 1.7. It should be noted that the word “selectivity” is not used in the usual sense, where values of less than unity are not allowed. The elution order of TBN and BaP reverses based on the bonding chemistry of the stationary phase used, so it is possible that “selectivity” values— $\alpha_{\text{TBN/BaP}}$ —can be less than one. Values of $\alpha_{\text{TBN/BaP}}$ have been shown to correlate well with the ability (or inability) to separate more complex mixtures of polyaromatic hydrocarbons (PAHs).

A second test commonly used to evaluate shape selectivity is the selectivity of the triphenylene/*o*-terphenyl pair [15]. Although the two are not isomers, they do have the same *L/B* ratio. The primary difference in these two molecules is their planarity: triphenylene is planar while *o*-terphenyl is not. Based on the empirical slot model, the non-planar molecule should elute first because it is more difficult for it to “fit” in between the alkyl chains of the bonded phase. Thermodynamically, a larger cavity must be opened in the stationary phase chains for the non-planar compound, and this is entropically expensive, again arguing the non-planar molecule should elute first. Generally, the selectivity between the two molecules is enhanced when a polymeric stationary phase is used, as the average chain density is much greater than for a monomeric phase. The statistical mechanical theories [3,6–8] predict that it is the anisotropy of the grafted chains that gives rise to shape selectivity among solute molecules. In brief, molecules that can most effectively align with the grafted chains, normal to the interface, are those that are most effectively retained. It costs more free energy to insert each solute substructure that lies

parallel to the interface than each substructure that aligns with the chains normal to the interface; hence the shape selectivity. In agreement with this prediction, Lochmüller et al. have shown that molecules are retained in the order rods>disks>flexible chains [16].

The primary variable that has been studied with respect to shape selectivity is the morphology of the stationary phase. There are several stationary phase variables that can be examined: the bonding chemistry (monomeric vs. polymeric), the bonding density, and the alkyl chain length. As has been alluded to, stationary phases prepared with polymeric bonding chemistry exhibit enhanced shape selectivity. It is generally believed that these phases are more ordered and rigid than monomeric phases, and this is the origin of the enhanced shape selectivity. In addition to bonding chemistry, the bonding density of the stationary phase has been shown to have an effect on shape selectivity. In general, columns with a higher bonding density have better shape recognition characteristics than columns with lower bonding density. This is thought to be from stationary phase ordering induced from cooperativity between the alkyl ligands in higher bonding density phases. This effect was predicted theoretically by Dill [6] and confirmed experimentally by Sentell and Dorsey [17]. The length of the bonded alkyl ligand has also been shown to have an effect on shape selectivity. In general, longer alkyl chains have greater shape selectivity than shorter ligands [18].

It has been theoretically predicted that an increase in the solvation of the stationary phase should increase the order of the phase [19]. In theory, alkyl ligands that are well solvated will be in a more extended conformation, and thus more ordered. As a result of this increase in order of the phase, the shape selectivity should also increase. The effect of stationary phase solvation on shape selectivity has been investigated by Cole and Dorsey [20]. In their study, *n*-hexanol was added to the mobile phase to increase solvation of the stationary phase. It was believed that addition of hexanol would increase the order of the stationary phase, leading to enhanced shape selectivity. However, only slight changes in shape selectivity, as measured by SRM 869a and various isomer pairs, were observed. The magnitude of these changes was not nearly as great as expected. It should be noted

that selectivity enhancements for certain solutes were seen, most notably for estrogen diastereomers. However, this trend was attributed to changes in solvent selectivity due to the introduction of hexanol to the mobile phase, and not due to changes in the conformation of the stationary phase.

Temperature has been shown to have a significant effect on shape selectivity. Sander and Wise studied the effect of temperature on shape selectivity for both monomeric and polymeric columns, using SRM 869a [21]. They demonstrated that the shape selectivity characteristics of a given phase can be dramatically altered by changing the temperature—in fact, monomeric phases can be made to “act polymeric” at low temperature, and polymeric phases can be made to “act monomeric” at high temperature. Temperature effects on shape selectivity have also been studied by Sentell and Henderson [22]. They evaluated shape selectivity with both SRM 869a and various isomer pairs on both low and high bonding density monomeric columns. They also observed enhanced shape selectivity at lower temperatures, particularly on the higher bonding density column.

To date most investigations into shape selectivity have involved alkyl-bonded stationary phases with hydroorganic mobile phases. There have only been limited investigations of shape selectivity in supercritical fluid chromatography (SFC). Several publications by Jinno and co-workers have addressed planarity recognition characteristics in SFC systems. Their work has focused primarily on selectivity differences between sub- and supercritical mobile phases, and between different types of stationary phases [23,24]. Williams et al. [25] have also studied shape selectivity trends in supercritical fluid chromatography, with mobile phases consisting of up to 30% acetonitrile in carbon dioxide. They compared selectivity on monomeric vs. polymeric columns, and looked at the effect of temperature, pressure, and amount of organic modifier in the mobile phase. Their conclusions were drawn from results using SRM 869a as a test mixture, as well as more complex mixtures of PAHs. As in RPLC, the use of a polymeric stationary phase led to increased shape selectivity as compared to monomeric phases. With respect to mobile phase conditions, they observed that shape selectivity decreased with increasing temperature, as in RPLC. Pressure did not seem to

have much of an effect on selectivity. In addition, based on SRM 869a selectivity values, they concluded that addition of organic modifier increases the shape selectivity of the chromatographic system.

This investigation examines shape selectivity trends with mobile phases that are mixtures of acetonitrile and carbon dioxide. Unlike previous works, the composition of the mobile phase is varied over the entire possible concentration range, that is, from neat carbon dioxide to neat acetonitrile. This range of mobile phases encompasses the techniques of supercritical fluid chromatography, subcritical fluid chromatography, enhanced fluidity chromatography, and liquid chromatography. It should be noted that this transition from SFC to LC is continuous, and encompasses nearly the entire mobile phase range for unified chromatography [26]. For comparison to previous work, SRM 869a is used as a test mixture. In addition, the planarity recognition characteristics of these chromatographic systems are examined by the selectivity of the triphenylene/*o*-terphenyl pair. Shape selectivity based on length-to-breadth ratio is evaluated based on the selectivity seen with molecular mass 228 PAHs. At the temperatures and pressures utilized in this study, the mobile phases do not undergo any phase transitions as the composition is changed. For this reason, it is expected that the selectivity trends seen as the mobile phase goes from supercritical fluid to subcritical fluid to enhanced fluid to liquid will be continuous.

2. Experimental

2.1. Reagents

SFC-grade carbon dioxide was obtained from Air Products (Allentown, PA, USA). HPLC-grade acetonitrile was obtained from Fisher (Pittsburgh, PA, USA). Test solutes included benz[*a*]anthracene (BaA) (Sigma, St. Louis, MO, USA), benzo[*c*]phenanthrene (BcP), naphthacene (Nap), chrysene (Chr), triphenylene (Tri), *o*-terphenyl (*o*-Ter) (Aldrich, Milwaukee, WI, USA), and NIST SRM 869a, which consists of benzo[*a*]pyrene (BaP), phenanthro[3,4-*c*]phenanthrene (PhPh), and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN) (National Institute of Standards and Technology,

Gaithersburg, MD, USA). All test solutes were dissolved in acetonitrile.

2.2. Instrumentation

All experiments were performed on a Hewlett-Packard (Palo Alto, CA, USA) Model G1205A supercritical fluid chromatograph. Sample injection was with a Rheodyne (Rohnert Park, CA, USA) Model 7410 injector with a 200 nl sample volume. Analyte detection was with a Hewlett-Packard Model 1050 diode array detector. The detection wavelength was 254 nm. The flow-rate for all experiments was 1.0 ml/min at the pump head. System backpressure was maintained at 200 bar for all experiments with mixed mobile phases, and was set to an appropriate pressure to regulate mobile phase outlet density when neat CO₂ was used as the mobile phase. Mobile phases consisted of either neat carbon dioxide, or various volume fractions of acetonitrile in carbon dioxide, from 10 to 100% acetonitrile. The column temperature was also varied between 30 and 80 °C at each mobile phase composition with mixed mobile phases, and was set at 100 °C when neat CO₂ was used. Stationary phases used were a monomeric Ultrasphere C₁₈ phase, (5 μm particle diameter, 80 Å pores) (Beckman, Fullerton, CA, USA) and a laboratory-made polymeric C₁₈ phase, (3 μm particle diameter, 200 Å pores), kindly provided by Dr. Lane Sander of NIST. Both columns were 150×4.6 mm in size. Column void times were taken from an injection of pure acetonitrile, except in the case of 100% acetonitrile mobile phase, where an injection of pure methanol was used.

3. Results and discussion

3.1. Monomeric column

The first set of experiments was performed on a monomeric C₁₈ column. Shape selectivity was evaluated three ways: by using SRM 869a, by examining the selectivity of the triphenylene/*o*-terphenyl pair (planarity selectivity), and by examining the chrysene/benz[*a*]anthracene pair (*L/B* selectivity). The SRM 869a and planarity experiments were

performed at 30, 40, 60, and 80 °C, while the *L/B* selectivity experiments were performed at 30, 40, and 60 °C. The mobile phase was varied between acetonitrile–CO₂ (10:90) to 100% acetonitrile at each temperature.

In general, mobile phases containing higher concentrations of acetonitrile appear to be stronger eluents than mobile phases consisting primarily of carbon dioxide, at least with respect to retention of PAHs on ODS columns, which is indicative of a normal-phase retention process. It should be noted, though, that over the entire composition range all of the test solutes eluted with retention factors less than 10. Retention factors for the five- and six-ringed PAHs (the compounds in SRM 869a) varied from 0.90 (BaP with 100% acetonitrile mobile phase) to 9.9 (TBN with acetonitrile–CO₂, 10:90, mobile phase).

The results for the SRM 869a experiments are shown in Fig. 1a. In general, the value of $\alpha_{\text{TBN/BaP}}$ decreases with an increase in the amount of acetonitrile in the mobile phase. This should be indicative of an increase in shape selectivity as the organic content of the mobile phase increases. At first, this did not seem surprising, as this is the same trend seen in reversed-phase LC with acetonitrile–water mobile phases. It is interesting to note that for mobile phases with over 40% acetonitrile there is no significant change in the observed $\alpha_{\text{TBN/BaP}}$ value.

The effect of temperature on shape selectivity with these mobile phases is somewhat interesting (see Fig. 2). With mobile phases containing a large percentage of acetonitrile, there does not appear to be much significant change in shape selectivity as the temperature is varied from 30 to 80 °C. However, at lower modifier concentrations, there is a significant change in $\alpha_{\text{TBN/BaP}}$ as the temperature is changed. The direction of the change is not surprising—as temperature increases, $\alpha_{\text{TBN/BaP}}$ increases, just as it does in RPLC. What is interesting is the temperature range studied: in RPLC, temperature effects on shape selectivity are observed between 0 and 40 °C. Not much change is seen above 40 °C. However, when SFC mobile phases are used—that is, mobile phases with less than 30% modifier—changes are seen at higher temperature values. When the mobile phases being used are considered, the results are not as surprising as they may seem. The density of an SFC

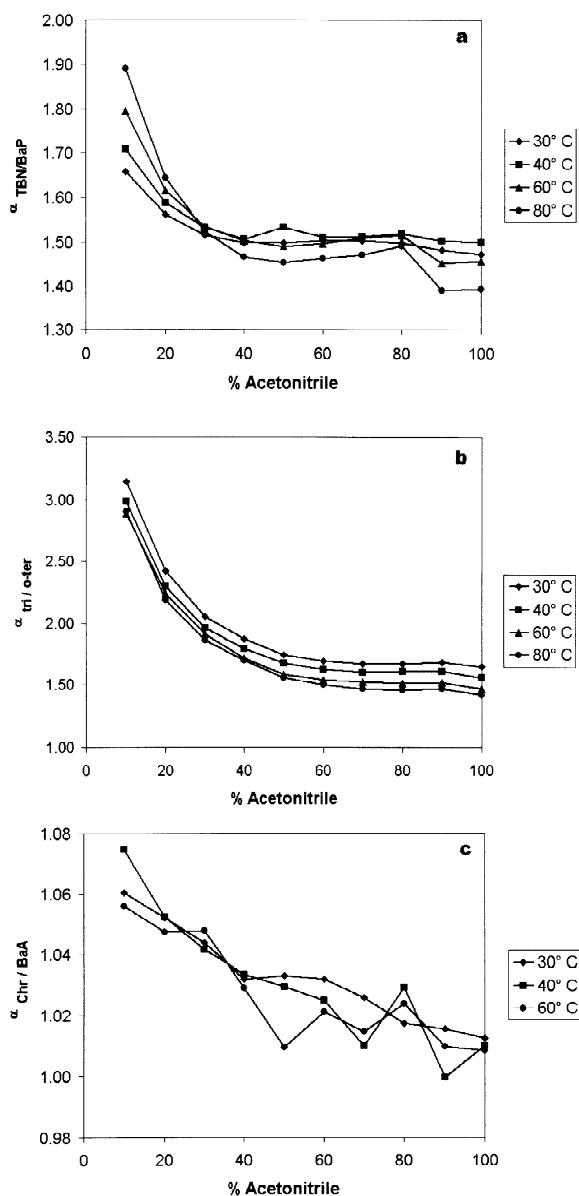


Fig. 1. Effect of mobile phase composition on shape selectivity with a monomeric column: (a) SRM 869a; (b) triphenylene/*o*-terphenyl; (c) chrysene/benz[*a*]anthracene. Temperatures used are noted in the figure legend. For all experiments, column outlet pressure is 200 bar, and flow-rate at the pump head is 1 ml/min.

mobile phase can change significantly with temperature, and this change in density changes the solvating power of the fluid. Changes in the properties of a CO₂-acetonitrile mixture with temperature are most

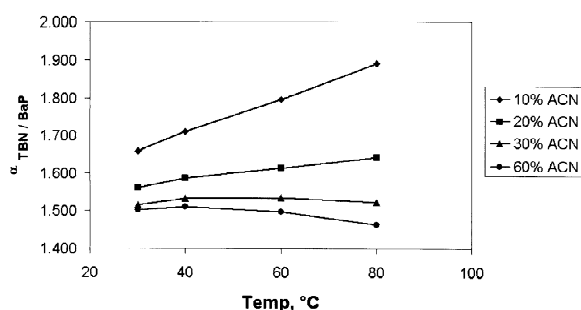


Fig. 2. Effect of temperature on shape selectivity with a monomeric column, as assessed by SRM 869a. Mobile phase compositions used are noted in the legend. Column outlet pressure is 200 bar, and flow-rate is 1 ml/min at the pump head.

noticeable at low modifier concentrations, and this accounts for the changes seen in shape selectivity at low modifier concentration.

The results of the planarity selectivity (triphenylene/*o*-terphenyl) experiments are shown in Fig. 1b. As with SRM 869a, the selectivity values level off at higher modifier concentrations—in this case, above 50% acetonitrile. However, the planarity experiments show that selectivity actually decreases as modifier is added—contradictory to what is inferred from the SRM 869a results. The best planarity selectivity occurs at low modifier concentrations, and planarity selectivity decreases as modifier is added to the mobile phase. This trend is seen at all temperatures studied. There are two possible explanations for this behavior. First, as previously stated, as the modifier amount in the mobile phase decreases, the mobile phase density decreases, making it a weaker eluent. The observed selectivity increase may simply be due to the fact that the mobile phase is weaker when lower modifier amounts are used. A second explanation is that the stationary phase properties change as modifier concentration changes. It has been shown [27,28] that significant amounts of carbon dioxide adsorb onto ODS stationary phases in SFC. This adsorption often exceeds monolayer coverage. Since carbon dioxide is an extremely non-polar solvent, it is expected to solvate the alkyl ligands well. This solvation should increase the order of the bonded phase and lead to enhanced shape selectivity at lower modifier values.

Shape selectivity trends were further explored by examining the selectivity between chrysene and

benz[*a*]anthracene. These two solutes are four-ringed PAH isomers of similar hydrophobicity but different *L/B* ratio. Thus, selectivity based on *L/B* ratio can be observed. Further, this pair is particularly difficult to separate in RPLC. The results of the *L/B* selectivity experiments are shown in Fig. 1c. As with the planarity selectivity experiment, lower modifier concentrations seem to produce the best shape selectivity. There is a noticeable trend downward as modifier content in the mobile phase increases. As in RPLC, this pair proved to be rather difficult to separate, with selectivities below 1.1. However, when modifier concentrations below 10% were employed, resolution of this pair on a monomeric column was possible. In fact, at 3% acetonitrile, and with a flow-rate of 3.0 ml/min, this pair was nearly baseline resolved in under 3 min (see Fig. 3). As previously stated, this pair is generally unresolvable by RPLC on monomeric stationary phases, so this separation represents a selectivity that is unique to SFC.

3.2. Polymeric column

The results of separating SRM 869a on a polymeric ODS column are shown in Fig. 4a. As expected, the values for $\alpha_{\text{TBN/BaP}}$ are all lower than 1, indicating selectivity characteristic of a polymeric stationary phase. Similar trends in selectivity were seen at each temperature studied as the mobile phase composition was varied. Between 10 and 40% acetonitrile in the mobile phase, the value of $\alpha_{\text{TBN/BaP}}$ decreased, as was seen with the monomeric column. However, between 40 and 100% acetonitrile, the value of $\alpha_{\text{TBN/BaP}}$ increased. This is in contrast to the “leveling off” seen in $\alpha_{\text{TBN/BaP}}$ values in the mobile phase range with the monomeric column. It is interesting to note that this increase was most significant at higher temperatures: at 60 °C, $\alpha_{\text{TBN/BaP}}$ values ranged from 0.86 to 1.00, or a range of 0.14 selectivity units, while at 30 °C the range was 0.63 to 0.68, or 0.05 selectivity units. At 40 °C, the

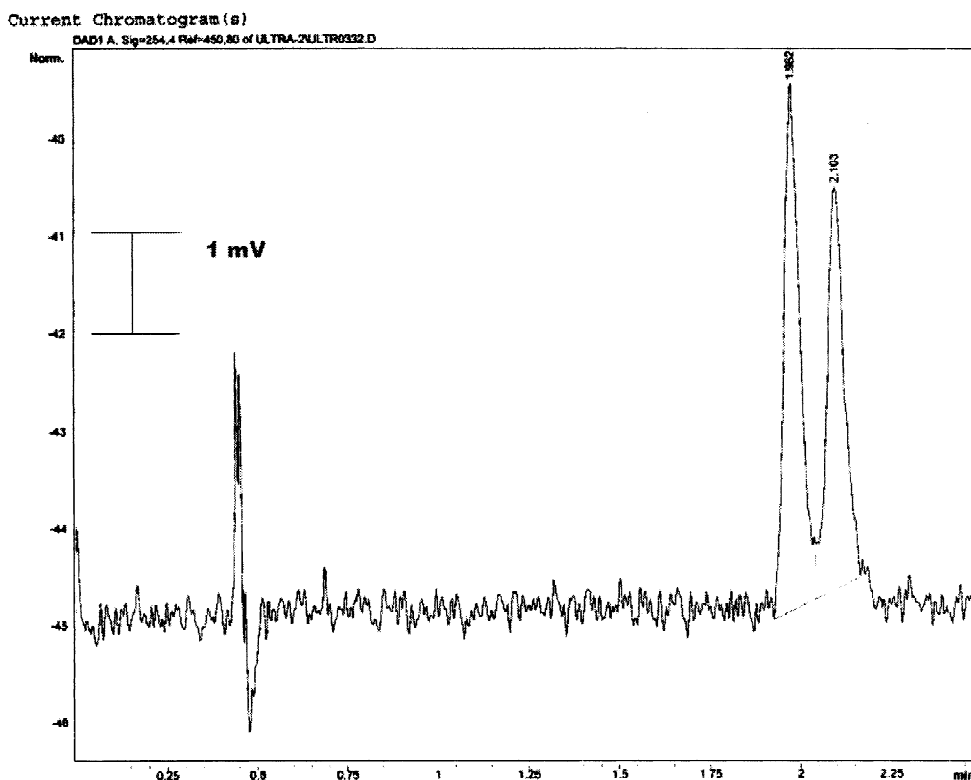


Fig. 3. Chromatogram of benz[*a*]anthracene (first peak) and chrysene (second peak) on a monomeric column. The mobile phase is 3% acetonitrile in CO₂ at 30 °C, with a column outlet pressure of 200 bar and flow-rate of 3.0 ml/min at the pump head.

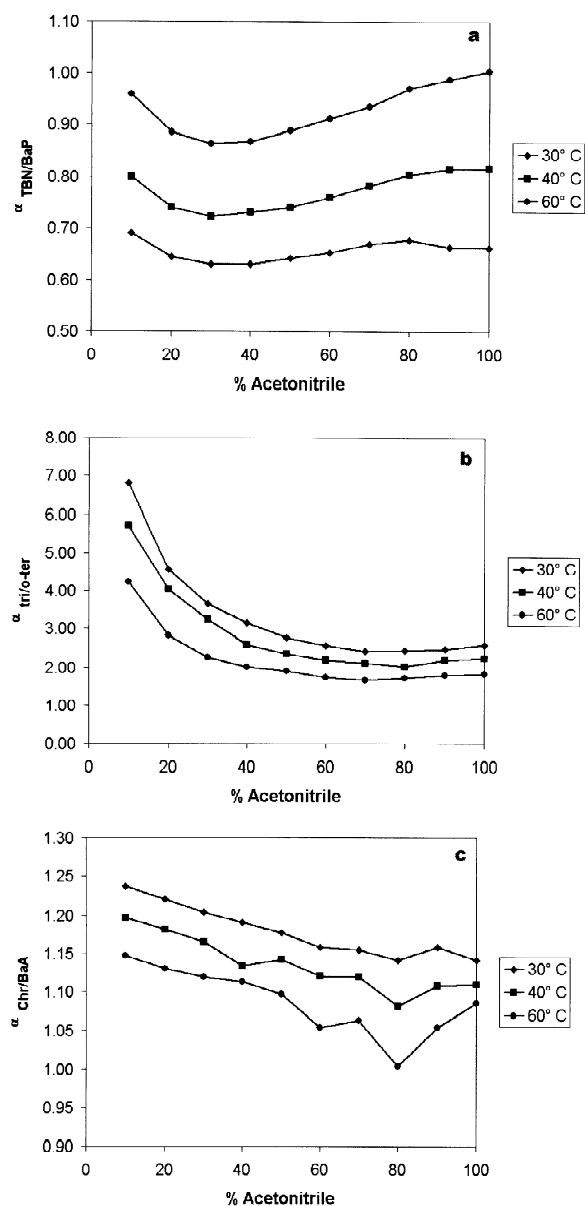


Fig. 4. Effect of mobile phase composition on shape selectivity with a polymeric column: (a) SRM 869a; (b) triphenylene/*o*-terphenyl; (c) chrysene/benz[*a*]anthracene. Temperatures used are noted in the figure legend. For all experiments, column outlet pressure is 200 bar, and flow-rate is 1 ml/min at the pump head.

other temperature studied, the value of $\alpha_{\text{TBN/BaP}}$ varied over a range of 0.09 selectivity units. This seems to indicate that changing the mobile phase composition has more of an effect on shape selectivity

at higher temperatures than at lower ones. This result is not necessarily surprising, because at higher temperatures the properties of the acetonitrile–carbon dioxide mixture are more sensitive to changes in mobile phase variables.

The results for the planarity selectivity experiments on the polymeric column are shown in Fig. 4b. In general, the planarity selectivity trends seen on the polymeric column are similar to trends seen with a monomeric stationary phase. In both cases, lower amounts of acetonitrile in the mobile phase produce better planarity selectivity. The most noticeable difference between the two columns is in the actual selectivity values—the planarity selectivity observed on the polymeric column is approximately twice that seen on the monomeric column.

The origins of the enhanced selectivity seen at lower modifier concentrations merit comment. It is interesting to note that the increased selectivity is nearly entirely a result of the increase in retention of triphenylene (see Fig. 5a). The retention of *o*-ter-

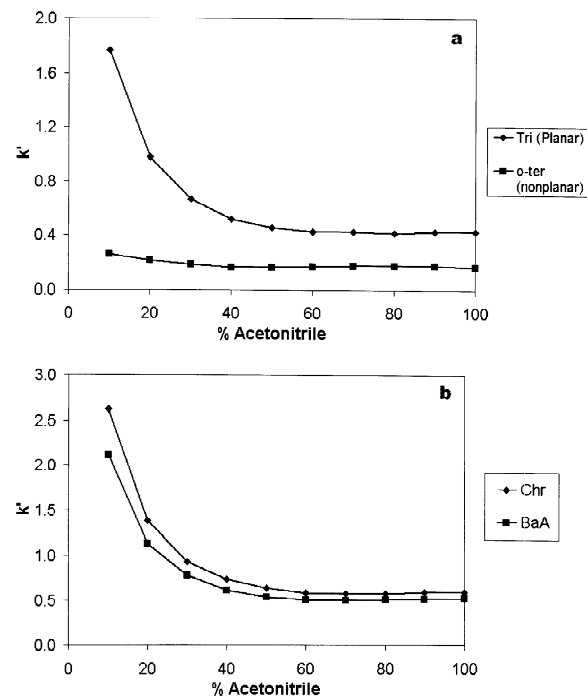


Fig. 5. Retention of (a) triphenylene and *o*-terphenyl and (b) benz[*a*]anthracene and chrysene on a polymeric column. For these experiments, the temperature is 30°C, the column outlet pressure is 200 bar, and the flow-rate at the pump head is 1 ml/min.

phenyl exhibits very little change as the mobile phase is changed, while the retention of triphenylene increases nearly fourfold. This suggests that the non-planar, and flexible, *o*-terphenyl is insensitive to changes in stationary phase morphology while the rigid, planar molecule triphenylene is sensitive to these changes.

Chrysene and benz[*a*]anthracene were also separated on the polymeric column in order to observe *L/B* selectivity changes as a function of mobile phase modifier concentration. The results are shown in Fig. 4c. As with the monomeric column, the highest selectivity between Chr and BaA was observed at the lowest modifier concentration. In addition, as expected, at a given modifier concentration, lower temperatures resulted in improved selectivity. It is interesting to compare the actual retention of the *L/B* isomers (shown in Fig. 5b) to the retention of the planarity selectivity pair. In contrast to the retention trends seen with triphenylene and *o*-terphenyl, both chrysene and benz[*a*]anthracene exhibit similar changes in retention as the mobile phase changes.

3.3. Comparison of selectivity trends

The selectivity trends observed with respect to stationary phase bonding chemistry are not unexpected. In general, polymeric bonding chemistry yields phases with enhanced shape selectivity in supercritical fluid chromatography as compared to monomeric phases. This is the same trend as is seen in RPLC with hydroorganic mobile phases. In addition, the trends seen as temperature is varied are similar to what would be expected from LC experiments, with lower temperatures providing better shape selectivity. However, the trends seen as mobile phase composition are varied are not as clear. Based on the results of SRM 869a experiments, shape selectivity should increase as the organic content of the mobile phase increases. However, the results of the planarity and *L/B* selectivity experiments suggest that this is not the case. For both triphenylene/*o*-terphenyl and chrysene/benz[*a*]anthracene, selectivity actually decreases as the organic content of the mobile phase increases.

A possible explanation of this result is based on the solvation of the stationary phase. As has been

previously stated, an increase in solvation of the stationary phase should lead to an increase in the order of the bonded alkyl chains [19], thereby increasing shape selectivity. In RPLC, this increased solvation can be achieved by either increasing the organic content of the mobile phase, or by adding additives (such as longer-chain alcohols) to the mobile phase. The aqueous part of the mobile phase, in RPLC, does not solvate the stationary phase chains to any great extent. However, in SFC, both components of the mobile phase have the ability to solvate the stationary phase, as compressed (subcritical or supercritical) carbon dioxide is an extremely non-polar solvent. In fact, it has been shown that carbon dioxide can easily exceed monolayer coverage on ODS, particularly when the temperature and pressure are close to the critical point [27]. The amount of adsorbed CO₂ has been shown to be an order of magnitude higher than adsorbed organic modifier [28]. One can then conclude that low-modifier content SFC mobile phases solvate stationary phase ligands very well—particularly at temperatures near CO₂'s critical temperature. This solvation of the stationary phase could be the origin of the enhanced shape selectivity seen at lower mobile phase organic content.

3.4. *L/B* Selectivity with neat CO₂ mobile phases

In order to examine the effect of mobile phase density on shape selectivity, experiments were carried out using neat CO₂ as a mobile phase. PAH isomers of molecular mass 228 (four-ring isomers) with differing *L/B* ratio, but similar hydrophobicity (see Fig. 6) were chromatographed, and the dependence of k' (or $\ln k'$) on *L/B* ratio was examined at different mobile phase densities, on both the monomeric and polymeric columns. Since increasing the mobile phase density in SFC is more or less equivalent to increasing the solvent strength by adding organic modifier in RPLC [31], these experiments should yield insight into the effect of both mobile phase density and solvent strength on shape selectivity.

A numeric description of shape selectivity on the basis of *L/B* ratio was determined by plotting $\ln k'$ of each solute (at a given mobile phase density) against the *L/B* ratio for that solute. These experi-

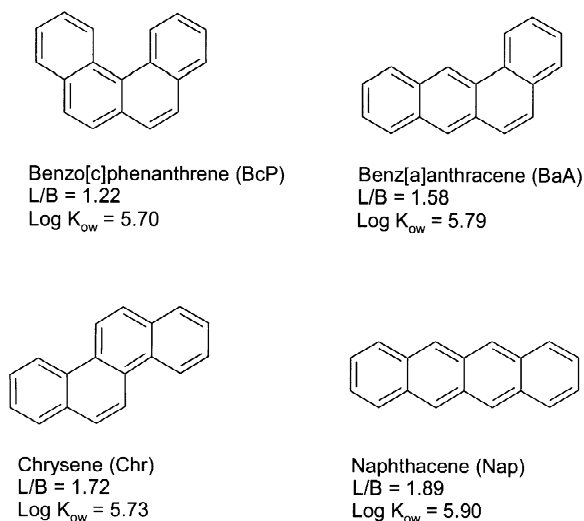


Fig. 6. Molecular mass 228 PAH isomers of varying L/B ratio, but similar octanol–water partition coefficients. Values of L/B ratio are from Ref. [11]; values of log K_{ow} (log of the octanol–water partition coefficient) are from Refs. [29] and [30].

ments were carried out at a temperature of 100 °C, and the mobile phase outlet density was regulated by applying an appropriate backpressure at the column outlet. It should be noted that although pressure is the variable set in the experiment, density is the true independent variable. It has been shown that temperature and density are the natural variables for description of retention in SFC [31], and that is why density is taken as an independent variable instead of pressure. The slope of the $\ln k'$ vs. L/B ratio plot was taken as the natural log of the “length-to-breadth” selectivity for a given density and stationary phase chemistry. This method is similar to finding homologous series selectivity values, but as L/B selectivity is not a discrete molecular unit these values cannot be used in a thermodynamic-type analysis as is commonly done with homologous series selectivity. However, the values are indicative, at least empirically, of the chromatographic system’s ability to separate isomeric solutes on the basis of L/B ratio.

The results of the L/B selectivity experiments are shown in Fig. 7a and b for the monomeric and polymeric columns, respectively. As expected, a linear relationship exists between $\ln k'$ and L/B ratio, on both the monomeric and polymeric columns. This

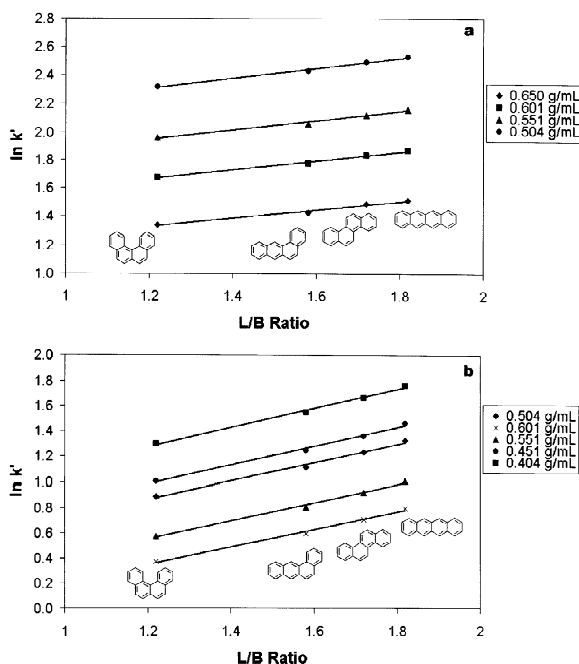


Fig. 7. Effect of solute L/B (length-to-breadth) ratio on retention on (a) monomeric and (b) polymeric columns. Neat CO_2 is the mobile phase, at 100 °C. The flow-rate is 1 ml/min, and the column outlet pressure is varied to set appropriate mobile phase densities.

is the same trend as is seen in RPLC with hydro-organic mobile phases. Since similar results are seen between SFC and RPLC, that is, with similar stationary phases but different mobile phases, it can be concluded that the mechanism for shape selectivity definitely is due to interactions in the stationary phase.

The value of the slope of the plots of $\ln k'$ vs. L/B ratio also merit comment. These values are shown in Table 1. It is interesting to look at both the trends seen as mobile phase density is changed, as well as the differences in slope between the two columns at common mobile phase densities. On both columns, as the mobile phase density increased, the slopes of the $\ln k'$ vs. L/B ratio lines decreased. This indicates that as the strength of the mobile phase is increased, the selectivity decreases, as one would expect. Stronger mobile phases reduce the interaction of solutes with the stationary phase, and since shape selectivity is a stationary phase phenomenon, it is reasonable to conclude that an increase in solvent

Table 1
Slope of $\ln k'$ vs. L/B ratio lines on both monomeric and polymeric columns

CO ₂ density (g/ml)	Monomeric slope	Polymeric slope
0.40	–	0.76
0.45	–	0.74
0.50	0.36	0.72
0.55	0.33	0.71
0.60	0.31	0.71
0.65	0.29	–

All experiments at 100 °C, using neat CO₂ as the mobile phase.

strength decreases shape selectivity. This is somewhat different from the result seen as solvent strength is increased in RPLC, where an increase in shape selectivity is seen as organic modifier is added to the mobile phase (i.e., as the mobile phase strength is increased).

A brief comment is also necessary on the comparison of the $\ln k'$ vs. L/B ratio slopes between the monomeric and polymeric columns at common outlet densities. The slope seen for the regression on the polymeric column is considerably larger than that seen on the monomeric column at the same mobile phase density. This reflects the enhanced shape selectivity seen with polymeric stationary phases, and underscores the idea that shape selective interactions arise in the stationary phase.

4. Conclusions

As was expected, the shape selectivity trends seen as the composition of the mobile phase was varied were continuous. There were not “breaks” as the mobile phase went from supercritical to subcritical to liquid. Also, as expected, the shape selectivity characteristics of the two types of bonded phases studied (monomeric and polymeric) were similar with carbon dioxide–acetonitrile mobile phases to what is observed in RPLC with hydroorganic mobile phases. Of most interest were the selectivity trends seen as the composition of the mobile phase was varied. As the amount of organic modifier in the mobile phase increased, the shape selectivity decreased—contradictory to what is seen with hydroorganic mobile phases. This could be due to the enhanced solvation of the stationary phase by carbon

dioxide as compared to acetonitrile. The effect of solute L/B ratio on shape selectivity was also investigated by utilizing neat carbon dioxide as the mobile phase, and examining the selectivity trends as the density of the carbon dioxide mobile phase was changed. The highest degree of shape selectivity was seen at lower mobile phase densities, which indicated that shape selectivity arises from interactions in the stationary phase.

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