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Review

Shape selectivity in reversed-phase liquid chromatography for the separation of planar and non-planar solutes

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ABSTRACT

Solute retention in reversed-phase liquid chromatography is the result of a variety of complex interactions between solute, mobile phase and stationary phase species. An understanding of the parameters that influence retention is useful in the development of separation methods with existing columns. Such knowledge is even more important for the design of new bonded stationary phases with engineered chromatographic properties. This review will examine some of the factors that affect retention and selectivity with alkyl-modified sorbents, particularly for the separation of solutes with well defined, rigid structure (*e.g.*, polycyclic aromatic hydrocarbons). The chromatographic discrimination of compounds on the basis of molecular structure, namely "shape selectivity", will be studied in terms of contributions from bonded phase morphology, and in terms of operational conditions. An emphasis is placed on practical choices that are available to control selectivity and optimize separations for isomers and related mixtures.

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1. INTRODUCTION

Solute retention in reversed-phase liquid chromatography (LC) is driven by a variety of competing interactions between the solute, mobile and stationary phase molecules. Such interactions may include the hydrophobic effect, dipole–dipole interactions, charge transfer interactions, size exclusion and ionic effects — in essence, the entire range of molecular interactions that can occur at liquid and solid interfaces. Adding to the complexity is the physical nature of modern sorbents used in LC. Bonded stationary phases commonly consist of chemically modified porous silica microparticles. The silica particles are modified with reactive silanes to produce surfaces with covalently bound monolayers 1–2.5 nm thick [1,2]. Constraints placed on the reaction restrict the density of this surface; phase loadings typically approach only about half of the density of the corresponding liquid silane. The pore network which permeates the particle ranges from about 8 to 30 nm in diameter, and the particles are 3–10 μm diameter. Thus, the dimensions of the bonded organic layer and the substrate pore diameter directly affect solute interaction at the molecular level, since both are similar to the molecular dimensions of the solute.

Solute retention with bonded stationary phases involves processes that are quite unlike liquid–liquid partitioning or liquid–solid adsorption. The development of a retention model based on partitioning or adsorption is appealing, yet the utility of such simple models may be limited. For example, solute retention in reversed-phase LC is commonly described in terms of the hydrophobic or solvophobic effect [3–5]. This theory relates retention to the reduction in hydrocarbonaceous surface area exposed to the polar mobile phase environment that occurs when the solute contacts the stationary phase. This explanation does not account for variations in retention that are observed between isomers, since many isomers have similar molecular properties (*e.g.*, polarity and contact area). LC sorbents are engineered materials. The selection of suitable substrate properties (particle size, pore size and surface area) and surface modification procedures (ligand and phase type) permits wide variations to be created in the retention properties of the resulting bonded stationary phase. An understanding of the processes that govern retention will permit prudent selection of these variables for the production of phases tailored for a specific application.

Solute shape plays an important role in the retention of non-polar compounds, particularly among isomers. Isomers with rigid, well defined structures such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), steroids and carotenoids can often be separated in reversed-phase LC on the basis of molecular shape. For example, the LC retention of PAH isomers has been correlated with a two-dimensional shape parameter designated “length-to-breadth ratio” [6], and with overall three-dimensional shape (solute planarity or non-planarity) [7–9]. Reversed-phase columns exhibit varying degrees of shape discrimination for these compounds. Selectivity differences among columns that are ostensibly the same (*e.g.*, C_{18} columns from different manufacturers) can be dramatic. An understanding of the origin of these selectivity differences, as well as knowledge of shape dependent retention trends, is useful in the selection of an appropriate reversed-phase column during method development.

Our studies of shape discrimination effects in liquid chromatography are an outgrowth of retention studies with PAHs carried out over the past 10 years. In an examination of the retention behavior of numerous PAHs, it was observed that the elution of certain PAHs changed markedly between various commercial C_{18} columns [10]. Other PAHs exhibited only slight retention variations among the columns examined. These early observations motivated much of our research on bonded phases with two primary goals: (1) the development of an understanding of the origins of retention differences among reversed-phase columns, and (2) increased knowledge of how solute shape affects retention.

In this paper we will review evidence detailing how chromatographic parameters affect shape selectivity. An empirical test is described for assessing the inherent shape discriminatory ability of an existing reversed-phase column. The effect of bonded phase parameters (*i.e.*, phase type, ligand length, phase loading and substrate properties) and operational conditions (temperature and mobile phase composition) on shape selectivity will be described in terms of this test and for the separation of PAH isomers. Efforts to characterize the morphology of phase structure by spectral means will be related to liquid crystalline-like properties of the phase. Finally, an empirical model of retention will be presented that unites these observations to explain shape selectivity effects in reversed-phase LC.

2. DISCUSSION

2.1. *Phase chemistry*

The retention of non-polar solutes in reversed-phase LC is affected by a variety of mobile and stationary phase parameters. More than any other single effect, the greatest changes in shape selectivity result from the type of bonding chemistry used in the preparation of the stationary phase. Most reversed-phase C_{18} columns are prepared by reaction of dimethylchlorooctadecylsilane with silica. The reaction, when carried out with an excess of the silane reagent, is limited by the number of available silanols at the silica surface. This type of phase is termed "monomeric", since only bonds between the silane reagent and the silica silanols are possible. In contrast, reaction of silica with octadecyltrichlorosilane in the presence of water results in bonds between silane reagent molecules as well as with the silica. This type of phase is termed "polymeric", and the extent of the reaction is controlled by the amount of water added, though the pore diameter of the silica also affects phase loading [11,12].

Selectivity differences between monomeric and polymeric C_{18} phases are clearly evident for the separation of PAHs. In 1979–1981 several studies [10,13–17] compared the differences in selectivity for commercial C_{18} columns from various manufacturers. In 1981 Wise *et al.* [6] compared the retention behavior of over 100 PAHs on a monomeric and a polymeric C_{18} column. In all of these studies it was found that only one particular stationary phase material, Vydac 201TP/HC-ODS, was successful in separating all 16 PAHs identified by the US Environmental Protection Agency as Priority Pollutants. Because of the unique selectivity of this particular material for the separation of PAHs, reversed-phase LC using this C_{18} column was specified in EPA Method 610 for the determination of PAHs in aqueous effluents [18]. However, none of these studies recognized the significance of the bonded phase synthesis on the selectivity. Only after a series of investigations in the early 1980s was the importance of the polymeric phase synthesis established [7,11,12,19]. Today, the enhanced selectivity of polymeric phase chemistry is widely recognized by analysts involved in the measurement of PAHs. However, these differences in phase selectivity are perhaps just as widely ignored outside of this specialty, since variations in retention behavior between monomeric and polymeric C_{18} phases are less dramatic for solutes without rigid structure (*i.e.*, molecules with free rotation about a single bond).

An example of the shape discrimination inherent to polymeric phases is shown in Fig. 1 for the separation of PAH isomers of molecular mass (M_r) 302. The same mobile phase gradient was used for both monomeric and polymeric C_{18} columns, yet baseline separation was achieved for the polymeric C_{18} column while almost no separation was possible with the monomeric phase [20]. The enhanced shape recognition ability illustrated in Fig. 1 is a general property observed for polymeric phases. Other phase parameters such as the extent of polymerization (*i.e.*, phase loading), ligand length, and substrate properties also affect shape selectivity, although monomeric phases never exhibit the degree of shape discrimination possible with polymeric phases.

The enhanced shape selectivity inherent to polymeric C_{18} phases is not related to silanol activity. To illustrate this point, a polymeric C_{18} phase was prepared on a wide pore silica using the typical synthesis procedure [11,21]. One portion of this phase was endcapped with trimethylchlorosilane (TMCS), one portion was endcapped with hexamethyldisilazane (HMDS), and the final portion was left unendcapped [22].

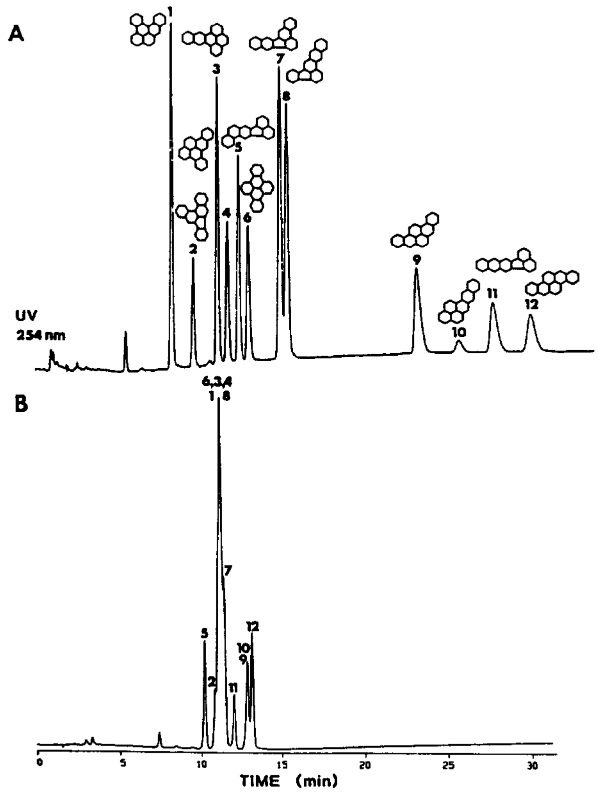

Fig. 1. Separation of 12 PAH isomers of M_r 302 on (A) polymeric C_{18} column and (B) monomeric C_{18} column. Mobile phase conditions: 90 to 100% acetonitrile in 10 min at 1.5 ml/min. From ref. 20.

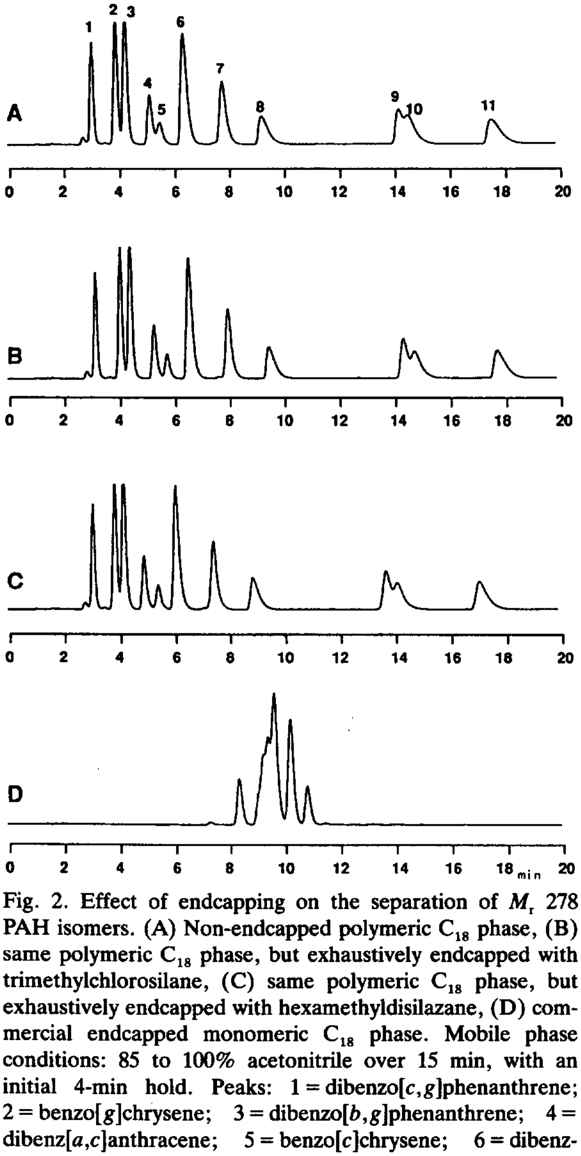

Fig. 2. Effect of endcapping on the separation of M_r 278 PAH isomers. (A) Non-endcapped polymeric C_{18} phase, (B) same polymeric C_{18} phase, but exhaustively endcapped with trimethylchlorosilane, (C) same polymeric C_{18} phase, but exhaustively endcapped with hexamethyldisilazane, (D) commercial endcapped monomeric C_{18} phase. Mobile phase conditions: 85 to 100% acetonitrile over 15 min, with an initial 4-min hold. Peaks: 1 = dibenzo[*c,g*]phenanthrene; 2 = benzo[*g*]chrysene; 3 = dibenzo[*b,g*]phenanthrene; 4 = dibenz[*a,c*]anthracene; 5 = benzo[*c*]chrysene; 6 = dibenz[*a,j*]anthracene; 7 = pentaphene, 8 = dibenz[*a,h*]anthracene; 9 = benzo[*a*]naphthacene; 10 = benzo[*b*]chrysene; 11 = picene; 12 = pentacene.

A separation of M_r 278 PAH isomers is shown in Fig. 2 for these three columns and for a commercial monomeric C_{18} column. Essentially no difference in the separation is observed among the three polymeric C_{18} phases regardless of endcapping. As expected, only limited resolution of the isomers was possible with the monomeric phase. Although silanol activity may have significance for the separation of polar analytes, this effect is not the source of the shape selectivity differences observed between monomeric and polymeric phases for PAHs.

2.2. *Shape selectivity test mixture*

Because column manufacturers are often reluctant to detail their column synthesis procedures, it is difficult to predict whether a given column will have monomeric- or polymeric-like selectivity. An empirical test was developed based on the retention of three PAHs to provide an indication of column selectivity [23–25]. This mixture was developed after studying the retention behavior of over 100 PAH standards on monomeric and polymeric C_{18} phases. Several of the compounds exhibited marked changes in relative retention for separations carried out on the two phase types and the relative retention of these particular compounds varied as the surface coverage or ligand density of the polymeric phase varied [19]. Based on these observations, three of these compounds were selected to make up the selectivity test mixture. Because the test was devised in this way, the mixture enables columns to be classified as having monomeric- or polymeric-like selectivity. The sensitivity of the test also facilitates a semi-quantitative evaluation of the degree of shape selectivity to be expected for the separation of more complex PAH mixtures.

The column selectivity test mixture, designed "Standard Reference Material (SRM) 869", contains benzo[*a*]pyrene (BaP), phenanthro[3,4-*c*]phenanthrene (PhPh), and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN)[a]. The elution order of these components depends on the phase type. With monomeric phases, the order is usually $\mathrm{BaP} \leqslant \mathrm{PhPh} < \mathrm{TBN}$ and with polymeric phases, $\mathrm{PhPh} < \mathrm{TBN} \leqslant \mathrm{BaP}$. By using the k' ratio of BaP and TBN to characterize phase selectivity, $\alpha_{\mathrm{TBN/BaP}} \leqslant 1$ for polymeric C_{18} phases and $\alpha_{\mathrm{TBN/BaP}} \geqslant 1.7$ for monomeric C_{18} phases. Columns exhibiting the elution order $\mathrm{PhPh} < \mathrm{BaP} < \mathrm{TBN}$ ($1 < \alpha_{\mathrm{TBN/BaP}} < 1.7$) are termed "intermediate", and often results with lightly loaded polymeric phases or with densely loaded monomeric phases. Among commercial C_{18} columns the entire range of $\alpha_{\mathrm{TBN/BaP}}$ values are observed, from about 0.6 to 2.2 [8]. It should be emphasized that significant variations in selectivity are possible within each of the classification groups. Experience indicates that these variations are greatest for polymeric phases, and changes in elution order have been observed among polymeric C_{18} columns with different phase loadings [8]. SRM 869 serves two purposes: column classification and selectivity assessment to aid method development, and phase characterization in quality control during the production of columns. Several column manufacturers currently use SRM 869 as part of their quality control criteria to assure that phase selectivity is reproducible from one column lot to another [26,27].

2.3. Phase synthesis

The extent of phase loading is limited by different reaction parameters for monomeric and polymeric phases. Monomeric surface modification reactions are ultimately limited by steric hindrance or shielding effects of the bonded ligands toward unreacted silica silanols. In contrast, polymeric reactions are limited by the quantity of water added during the modification. In a typical monomeric synthesis, a large excess of the monofunctional silane is dissolved in an aprotic solvent and is slurried with the silica [28–30]. Basic catalysts are sometimes added to remove the hydrochloric acid formed as a reaction byproduct, and thus increase phase loading [29,31]. Polymeric syntheses utilize an excess of a trifunctional silane, but a controlled quantity of water is added to initiate a limited polymerization [11,21,32]. Both reaction schemes usually involve reflux and washing steps. If reasonable care is taken to measure the quantities involved in the syntheses, excellent reproducibility is possible for both monomeric and polymeric phases, at least at research levels. For production quantities, reproducibility is a more significant concern and polymeric syntheses appear to exhibit more variability.

Substrate pore size is an important parameter in the production of polymeric C_{18} phases [12]. Phases prepared on narrow-pore substrates exhibit less of the retention behavior attributed to polymeric C_{18} phases than those prepared on wide-pore substrates. This distinction occurs for a pore diameter of approximately 15 nm; polymeric C_{18} phases prepared on silicas with pore diameters less than 15 nm have reduced shape recognition and exhibit retention characteristics similar to monomeric C_{18} phases. An example of this trend is illustrated in Fig. 3 for polymeric C_{18} phases prepared on Zorbax silicas with pore diameters ranging from 6 to 30 nm. The separations are of the 16 Priority Pollutant PAHs. Phases prepared with 6 and 10 nm pore diameter substrates exhibit monomeric-like selectivity,

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[a] SRM 869 is available through the Standard Reference Materials Program, NIST, Gaithersburg, MD 20899, USA.

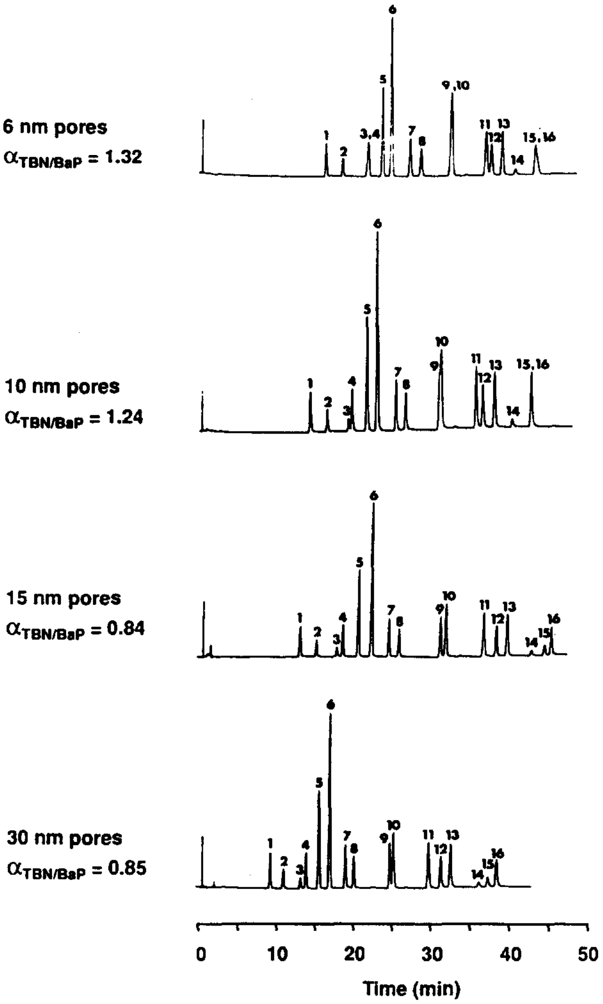


Fig. 3. Separation of the 16 Priority Pollutant PAHs (SRM 1647) on polymeric C_{18} phases prepared on substrates with varying pore diameter. Mobile phase conditions: 40 to 100% acetonitrile in 45 min at 2 ml/min. Peaks: 1 = naphthalene; 2 = acenaphthylene; 3 = acenaphthene; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = benz[*a*]anthracene; 10 = chrysene; 11 = benzo[*b*]fluoranthene; 12 = benzo[*k*]fluoranthene; 13 = benzo[*a*]pyrene; 14 = dibenz[*a*,*h*]anthracene; 15 = benzo[*ghi*]perylene; 16 = indeno[1,2,3-*cd*]pyrene. From ref. 12.

and with 15 and 30 nm substrates phases exhibit polymeric selectivity. Baseline resolution of the components was achieved only with the wide pore substrates.

A size-exclusion mechanism limiting phase synthesis has been advanced to explain the relationship between phase selectivity and pore size [12]. Because water is added directly to the reaction slurry during the synthesis of a polymeric phase, both the silane monomer and polymer coexist in the media. Surface modification results from competitive reaction of these species. Even a modest degree of polymerization (*e.g.*, 10 units; $M_r = 3500$) would result in relatively large reaction species. Modification of narrow-pore substrates would favor reaction with silane monomers, since access of the larger silane polymers within the pore network would be hindered due to size exclusion. In comparison, polymeric modification would be enhanced for wider-pore substrates since size-exclusion effects would be reduced. It should be noted that the selectivity differences illustrated in Fig. 3 are not due to size exclusion of the PAH analytes. Monomeric phases prepared on the same silica substrates exhibited differences in overall retention; however, no differences in column selectivity were observed among these phases.

2.4. Alkyl phase length and bonding density

The effect of alkyl chain length on retention has been studied in considerable detail by numerous research groups with conflicting reports [28,33–36]. The focus of most of these studies has been on absolute retention and methylene selectivity for alkyl homologues. Retention was reported to be related to carbon loading (resulting from different phase lengths) through linear [35,37] and non-linear expressions [33,38]. Other research indicated a limiting retention behavior at longer phase lengths [39–41], termed by Berendsen and De Galan [39] the "critical chain length". We investigated the relationship between phase length and column selectivity towards PAHs for a range of monomeric and polymeric alkyl phases [42]. SRM 869 was utilized as an indicator of shape selectivity, and $\alpha_{TBN/BaP}$ is plotted as a function of phase length in Fig. 4. The selectivity coefficient $\alpha_{TBN/BaP}$ decreases with increasing phase length for both monomeric and polymeric phases. Shorter alkyl phases such as octyl or dodecyl phases exhibited reduced shape selectivity, whereas long chain length phases were more selective toward solute

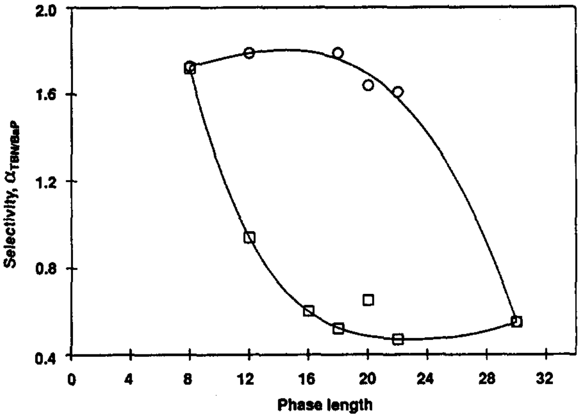


Fig. 4. Shape selectivity ($\alpha_{TBN/BaP}$, as assessed with "Column Selectivity Test Mixture", SRM 869) plotted as a function of bonded phase length for monomeric C_{18} column (○), and for polymeric C_{18} column (□). From ref. 42.

shape. For a given alkyl length, polymeric phases are more shape selectivity than monomeric phases. Because of these trends, better separations of PAH isomers are usually possible with long chain length (i.e., ≥18) polymeric phases.

Yet another parameter affecting shape selectivity is bonding density. Because monomeric syntheses are limited by the number of accessible silanol sites at the silica surface, monomeric C_{18} phases rarely exceed about 4 μmol/m^2 (more typically, *ca.* 3 μmol/m^2). Several unusual approaches have been reported for the production of dense monomeric phases including the use of silanes with special leaving groups [43], catalysts [29,31], and a novel method employing a low-temperature reaction driven by ultrasonic energy [31]. For polymeric phases, the surface coverage is controlled by the quantity of water added during the synthesis, and to a lesser extent by substrate pore diameter (see discussion above). Surface coverage values for polymeric phases commonly exceed 5 μmol/m^2, and in general polymeric C_{18} phases are about twice as dense as monomeric C_{18} phases [11]. Polymeric phase loading can be reduced to approach surface coverages comparable to monomeric phases by limiting the amount of water added during phase synthesis, and polymeric C_{18} phases so produced have retention and selectivity characteristics similar to monomeric C_{18} phases.

The extent of shape discrimination for polymeric C_{18} phases is directly related to phase density [7]. To illustrate this effect, four polymeric C_{18} columns were prepared with surface coverage values ranging from 2.7 to 5.1 μmol/m^2. A mixture of eleven M_r 278 PAH isomers

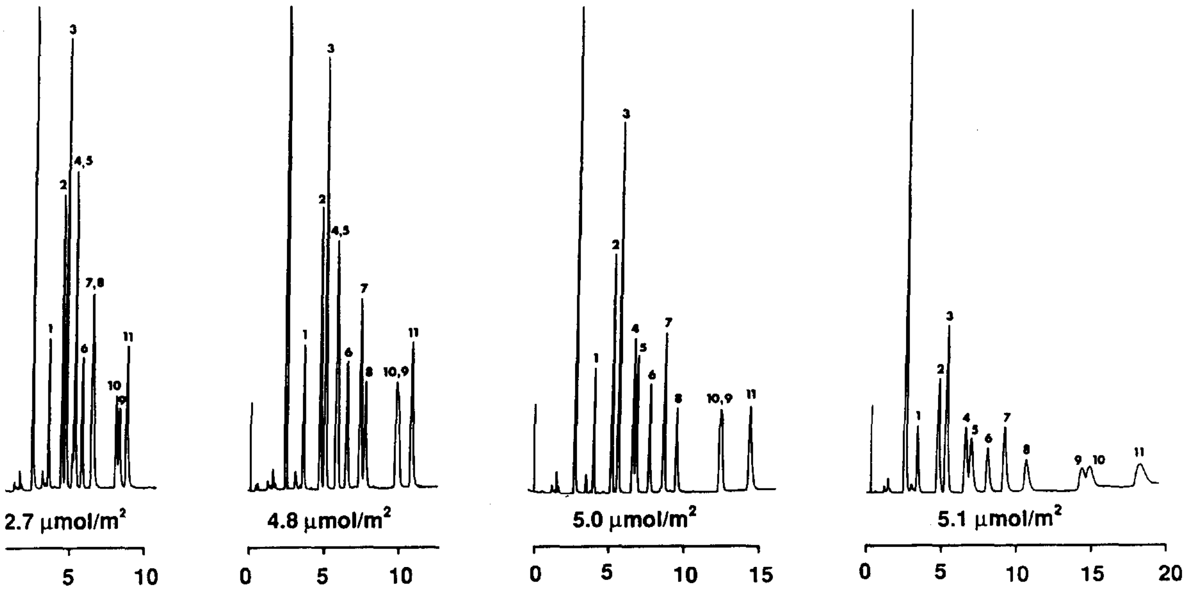


Fig. 5. Separation of 11 PAH isomers of M_r 278 on polymeric C_{18} columns with various phase loadings. For component identification, see Fig. 2. From ref. 7.

was separated on each of these columns, and the results are shown in Fig. 5. Better separations of the isomer mixture were achieved with more heavily loaded phases.

If all of the parameters described above are considered, phase selectivity toward solute shape can be maximized through the synthesis of a densely loaded, long-chain-length polymeric phase, prepared on a wide-pore substrate. Work is in progress to develop such a column optimized for the separation of carotenoid isomers.

3. EFFECT OF CHROMATOGRAPHIC CONDITIONS

3.1. *Subambient temperature effects*

One of the most under-utilized parameters for controlling column selectivity in liquid chromatography is column temperature. Dramatic changes in shape selectivity result for monomeric and polymeric C_{18} phases with changes in column temperature. When temperature is considered in method development, it is usually controlled either to improve retention time reproducibility, or to enhance column efficiency by operation at elevated temperatures. Little recognition has been given to the influence that temperature has on selectivity, especially at reduced column temperature. This may simply be a consequence of the lack of subambient temperature control with most commercial instrumentation; however, combination LC column heaters/coolers are now becoming available [44].

The effect of temperature on the separation of steroids was first reported by Sheikh and Touchstone [45,46]. They showed that better separations of steroids were possible by operation at subambient temperatures. We have observed that separations of PAH isomers on polymeric C_{18} columns are highly sensitive to variations in temperature. For example, the separation of chrysene and benz[*a*]anthracene is degraded by only a slight increase in column temperature. These observations prompted a systematic evaluation of the effect of temperature on column shape selectivity. Using SRM 869 to assess shape selectivity, $\alpha_{TBN/BaP}$ was determined at temperatures ranging from −20 to 80°C for both monomeric and polymeric C_{18} phases (see Fig. 6) [47]. The changes in selectivity with temperature are remarkable. Lower values of $\alpha_{TBN/BaP}$ represent increased shape discrimination, and from Fig. 6 it is apparent that shape selectivity increases fairly dramatically at low temperatures. This trend is observed for both monomeric and polymeric C_{18} columns, but at any given temperature, greater shape recognition (*i.e.*, lower $\alpha_{TBN/BaP}$ values) is possible with polymeric C_{18} phases compared with monomeric phases. The opposite trend occurs at elevated temperatures and shape discrimination for both types of columns is reduced. Thus polymeric C_{18} columns should not be operated above ambient temperature if shape discrimination mechanisms are important for the separation.

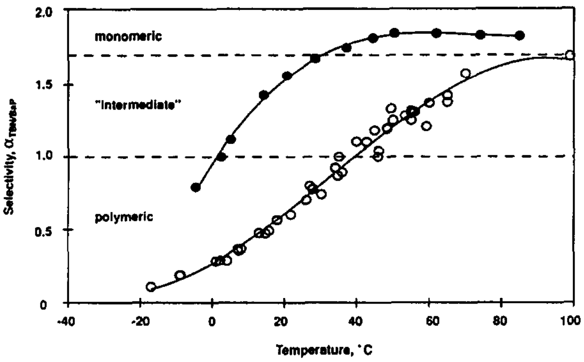


Fig. 6. Shape selectivity ($\alpha_{TNB/BaP}$) plotted as a function of temperature for monomeric (●) and polymeric (○) C_{18} columns. Enhanced shape discrimination is indicated by lower $\alpha_{TBN/BaP}$ values. From ref. 47.

The selectivity *vs.* temperature plots in Fig. 6 are rich in information and deserve further comment. Variations in selectivity are regular and continuous, and a "break" in column retention associated with phase transitions is not observed in the selectivity plots. Van 't Hoff plots of retention *vs.* inverse temperature do exhibit non-linearities; however, these changes are also gradual and probably should not be associated with a freezing–melting type of phase transition (see discussion below). The selectivity plot in Fig. 6 can be evaluated from a different perspective. For a range of selectivity values, either monomeric or polymeric columns can be utilized

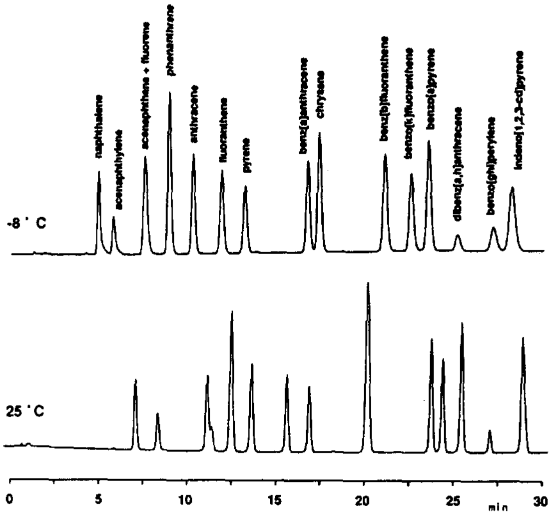


Fig. 7. Separation of the 16 Priority Pollutant PAHs on a monomeric C_{18} column at ambient and subambient conditions. Mobile phase conditions: (−8°C) acetonitrile–water (80:20) to acetonitrile–methyl *tert.*-butyl ether (80:20) over 20 min; (25°C) acetonitrile–water (50:50) to 100% acetonitrile over 30 min, 1.5 ml/min. From ref. 47.

at a specific value of $\alpha_{TBN/BaP}$ if column temperature is adjusted appropriately. This suggests that a monomeric C_{18} column could be used in place of a polymeric C_{18} column if the monomeric phase is cooled sufficiently. An example of this application is illustrated in Fig. 7 for the separation of the EPA Priority Pollutant PAHs. Both separations were carried out on a monomeric C_{18} column; however, baseline resolution of chrysene and benz[*a*]anthracene, and benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene were possible only at reduced column temperature. The overall separation at −8°C is very similar to separations possible with polymeric C_{18} phases operated at ambient temperature. It is further evident from Fig. 6 that the greatest enhancement of shape discrimination should be possible for polymeric C_{18} phases operated at low temperatures, and using this approach a separation of all six methylchrysene isomers was achieved (separation of 5- and 6-methylchrysene ordinarily is not possible by reversed-phase LC) [47].

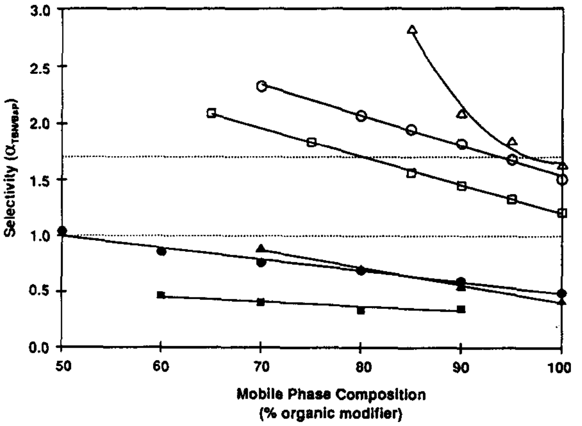


Fig. 8. Shape selectivity ($\alpha_{TBN/BaP}$) plotted as a function of mobile phase composition for monomeric (open symbols) and polymeric (closed symbols) C_{18} columns. Dotted lines indicate monomeric, polymeric and "intermediate" shape selectivity classifications (see text). △, ▲ = Methanol; ○, ● = acetonitrile; □, ■ = ethanol.

3.2. Mobile phase composition

The effect of mobile phase composition on retention in reversed-phase LC is well known, and this parameter remains the primary means through which retention is controlled. The dependence of column selectivity on the mobile phase environment has also been studied in detail for various solute classes. From a practical perspective, it is useful to employ different organic modifiers during the development of a separation method since selectivity is often dependent on mobile phase properties.

We examined the effect of mobile phase composition on shape discrimination for alkyl and aryl homologues [48]. Similar trends with composition were observed for monomeric and polymeric C_{18} phases. Alkylbenzene homologues were separated with different acetonitrile–water compositions, and methylene selectivity (defined by the slope of a plot of ln k' *vs.* homologue number) was plotted as a function of composition. As expected, methylene selectivity was observed to decrease with increasing mobile phase strength. Shape selectivity was assessed with SRM 869 using a variety of organic modifiers and mobile phase compositions. A plot of $\alpha_{TBN/BaP}$ *vs.* composition is shown in Fig. 8. Values for $\alpha_{TBN/BaP}$ decrease with increasing mobile phase strength, indicating enhancement of shape recognition at high organic compositions. These trends are fairly weak and are probably of little practical significance, especially since the decrease in overall retention with mobile phase strength is in opposition to any improvements in component separation. The variations in shape selectivity with mobile phase composition are small compared with the differences in shape selectivity observed between monomeric and polymeric C_{18} phases.

4. BONDED PHASE CHARACTERIZATION

Perhaps the most common technique for assessing bonded phase properties is chromatographic characterization. The use of test mixtures such as SRM 869 can provide a good indication of chromatographic retention behavior; however, this approach gives only indirect information about the morphology of the phase. Carbon analyses are widely utilized to provide an indication of phase loading. In conjunction with the surface area of the substrate, carbon analyses can be used to calculate the phase surface coverage in μmol/m^2 or groups/nm^2. Carbon analysis, however, cannot provide information about phase morphology, *i.e.*, the structure and arrangement of the molecules that constitute the phase. Phase morphology is fundamental to understanding the origins of retention and selectivity differences among various phases.

4.1. Small-angle neutron scattering

The technique of small-angle neutron scattering (SANS) employs low-energy neutrons to characterize detail with the range of 1–100 nm. Because the features of alkyl-modified porous silica fall within this range, SANS is potentially useful for characterizing bonded phase morphology. Neutrons are strongly scattered by the pore structure of unbonded silica [49,50]. Characterization of alkyl-modified silica is considerably more complex since scattering can result at the silica, bonded phase, and air interfaces [1,2]. In addition, scattering from the bonded phase is much weaker than scattering from the pore structure, since the volume fraction of the alkyl phase is much less than the volume fraction of the silica. Fortunately, interferences from the pore structure can be eliminated effectively by a process termed contrast variation [1,49]. In this approach the bonded silica is wetted with a liquid chosen to have the same scattering properties (*i.e.*, scattering length density) as the silica. This is readily achieved by appropriate mixtures of methanol and deuterated methanol, the proportions of which are determined from calculation or experiment. Scattering for a modified silica sample placed in a density matched solvent will occur at the bonded phase interface, since the scattering length density of the silica and the solvent are identical.

A summary of the SANS measurements carried out on various modified silicas is given in Table 1. It should be emphasized that the measurements were performed in the presence of a methanol–deuterated methanol environment, so the results are relevant to chromatographic systems. Two properties of the bonded phases were determined: bonded phase thickness and alkyl chain volume fraction [1]. This appears to be the only reported direct determination of bonded phase thickness. The monomeric C_{18} phase thickness was measured to be 1.7 nm, and the polymeric C_{18} phase 2.1 nm. For comparison, a fully extended C_{18} chain in the all-*trans* conformation is about 2.3 nm in length. These results are striking, since monomeric and polymeric phases have nearly the same thickness. The fact that both values are less than the value for the fully extended chain suggests that the individual chains are either angled or bent to account for the reduced thickness. It seems

TABLE 1

PHYSICAL PROPERTIES OF ALKYL-MODIFIED SILICA

Phase type	Thickness (nm)	Alkyl chain volume fraction
C_8 monomeric	1.0 ± 0.2	0.65 ± 0.15
C_{18} monomeric	1.7 ± 0.3	0.66 ± 0.15
C_{18} polymeric	2.1 ± 0.3	0.88 ± 0.1
C_{30} monomeric	2.5 ± 0.4	0.63 ± 0.15

unlikely that the dramatic differences in shape selectivity observed between monomeric and polymeric C_{18} phases can be accounted for by the small difference in measured thickness. Instead, the selectivity difference is probably due to differences in ligand density. The second property measured by SANS, and listed in Table 1, is alkyl chain volume fraction. This quantity is the fraction of space occupied by alkyl chains. The complementary volume fraction describes the space not occupied by alkyl chains, *i.e.*, the space between the chains within the phase thickness. The alkyl chain volume fraction for each of the monomeric phases is very similar, about 0.65. For the polymeric C_{18} phase, however, this volume fraction is significantly greater, *i.e.*, 0.88. This is consistent with the larger bulk phase loadings determined from polymeric phases compared with monomeric phases. If the phases have similar thicknesses, the only way to account for the higher loading is by increased ligand density.

4.2. Infrared spectroscopy

Additional information about phase morphology can be obtained with Fourier transform infrared (FT-IR) spectroscopy [51]. Measurement of infrared spectra for alkyl-modified silica is inherently difficult due to scattering and absorbance of the silica substrate. Careful background subtraction and signal averaging can help to minimize these interferences. Information about alkyl chain conformation can be obtained from an evaluation of absorption spectra within a narrow interval in the IR fingerprint region. R.G. Snyder [52] studied the spectra for long-chain normal hydrocarbons and assigned several transitions from 1400 to 1320 cm^{-1} to conformational defects in the alkyl chains (see Fig. 9). For example, the transition at 1354 cm^{-1} is due to tggt′ conformation (bend conformation) and the transition at 1367 cm^{-1} is due to gtg′ defect (kink conformation). These transitions occur in the spectra of alkyl-modified surfaces, and provide insight about alkyl conformation and order within bonded phases. At 44°C a significant degree of disorder exists within liquid octadecane, as is evidence by transitions at 1354 and 1367 cm^{-1}. Bonded phase spectra were measured for a variety of phases, and the C_{18}-modified silica exhibited spectra similar to the spectra for free liquid octadecane [51]. It is clear that the alkyl chains within the modified surface are not fully extended in the all-*trans* conformation, but instead have bends and kinks which reduce the thickness of the layer. This finding is consistent with SANS bonded phase thickness measurements.

More insight into phase morphology is provided by the measurement of bonded phase spectra at reduced temperatures. Spectra for a monomeric C_{22}-modified silica were collected at temperatures ranging from −30 to 47°C (Fig. 10). Transitions resulting from kink and bend conformations are clearly seen to decrease in intensity at reduced temperatures. The transition

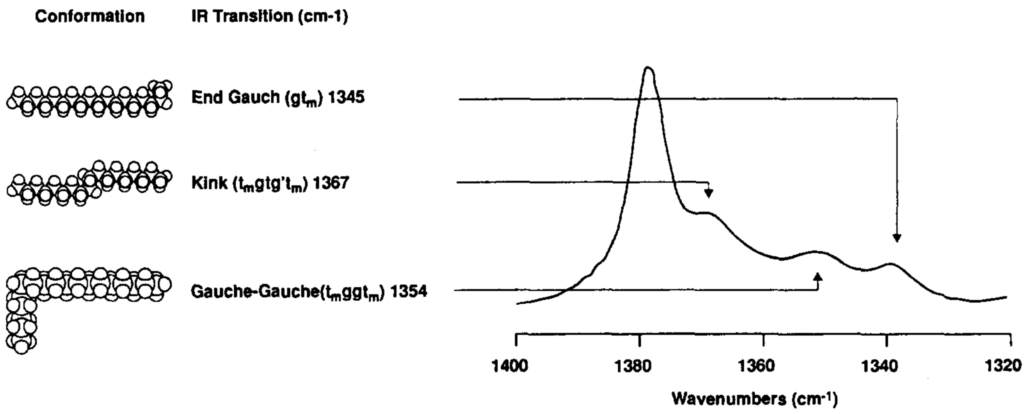


Fig. 9. Assignment of conformational defects to transitions in the infrared spectra for liquid octadecane at 44°C (see ref. 52).

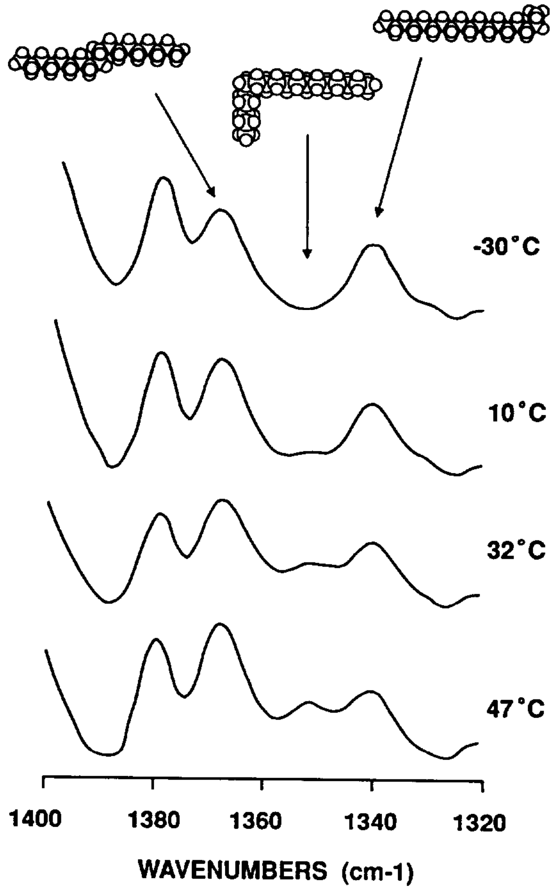


Fig. 10. FT-IR absorption spectra of a C_{22}-modified silica substrate recorded at various temperatures. From ref. 51.

at 1354 cm^{-1} (bend conformation) is completely absent at −30°C. Thus, alkyl chain order increases with decreasing temperature. The spectra in Fig. 10 show a complete lack of evidence for a phase transition of the type observed for hydrocarbon crystallization. The formation of a crystal lattice results in dramatic changes in the IR spectra of normal hydrocarbons, including peak splitting, band narrowing, and spectral shifts. These changes are not observed for covalently bonded alkanes. Instead, the continual changes in the spectra indicate the absence of a crystalline phase transition and gradual ligand ordering as conformation defects are reduced. The lack of evidence for a crystalline phase transition is consistent with the regular, continuous changes in selectivity that are observed with changes in temperature in chromatographic separations.

5. SOLUTE RETENTION AND SHAPE DISCRIMINATION

The realization that solute shape in some way affects retention may appear obvious, yet the details of shape effects are still not fully understood. Isomers provide excellent probes for studying shape recognition. Interpretation of retention behavior is simplified since many of the physical and chemical properties of the solutes are similar for a given isomer set. These similarities make separations difficult with columns that exhibit low shape selectivity, such as monomeric C_{18} columns. Better separations of isomers are usually possible with columns which exhibit enhanced shape recognition, such as polymeric C_{18} columns.

Among PAH isomers, the dimensions of the molecule are observed to be related to retention behavior. Long narrow molecules are retained longer than square molecules for the same molecular mass. Janini and co-workers [53–55] first described a relation between the shape of PAHs and retention for gas chromatographic separations carried out on liquid-crystalline stationary phases. They observed that among isomeric PAHs, retention increased with the ratio of the length to the width of the molecule. Radecki *et al.* [56] described a shape parameter defined as the ratio of the length to the breadth (*L/B*) of a box of minimum area drawn to enclose the atoms of a molecule. They also observed a general increase in solute retention expressed in terms of retention indices, with *L/B* for gas chromatographic separation of PAH isomers on a liquid-crystalline stationary phase. Wise *et al.* [6] first reported the effect of PAH isomer shape on retention in reversed-phase LC. They defined *L/B* as the ratio of length to breadth dimensions of a box drawn about the molecule such that the maximum *L/B* value is produced. In practice, this is accomplished by an iterative computer algorithm. Wise *et al.* observed a better correlation of retention with *L/B* values calculated in this way compared with the approach of Radecki *et al.*, especially for the separation of methyl-

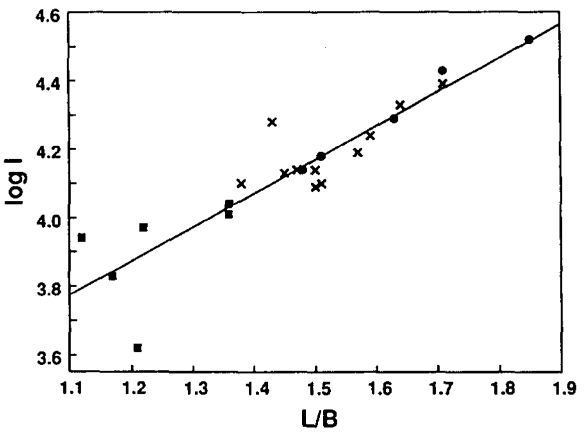


Fig. 11. Retention (expressed as log retention indices, *I* [6]) plotted as a function of *L/B* for various methyl-substituted PAH isomers. ■ = Benzo[c]phenanthrenes; × = benz[*a*]-anthracenes; ● = chrysenes.

substituted PAHs. A plot of retention *vs. L/B* is shown in Fig. 11 for methyl-substituted benzo[*c*]-phenanthrene, benz[*a*]anthracene and chrysene isomers, separated with a polymeric C_{18} column [6].

The three-dimensional nature of PAH molecules is sometimes overlooked, probably because of the planar structure implied by chemical representations. Certain PAH molecules have non-planar conformations that result from steric hindrance of proximate hydrogen and/or carbon atoms. The strain introduced creates a twist in the molecule that can be characterized by the dihedral angle between affected atoms. This parameter ("dihedral angle of distortion") was described by Garrigues and co-workers [9,57] for methyl-substituted PAHs.

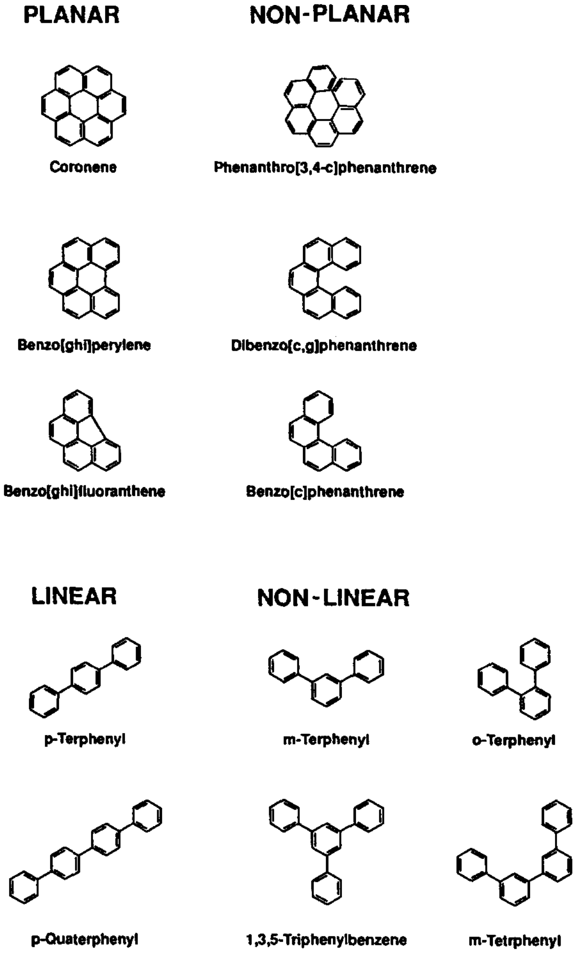


Fig. 12. Planar and non-planar PAH probes used in shape selectivity evaluation of various monomeric and polymeric C_{18} columns (see Table 2).

The effect of non-planarity on retention can be studied by selecting probe compounds with similar overall shape, but different planarity. Such probes need not be isomers; however, any molecular mass differences must also be considered in the evaluation of retention behavior. A set of planar and non-planar PAHs is shown in Fig. 12 and selectivity factors ($\alpha_{\text{planar/non-planar}}$) are listed in Table 2 [7]. In each case, the non-planar PAH is retained less than the corresponding planar PAH, for both monomeric and polymeric C_{18} columns, even though the molecular mass difference should be expected to contribute to the retention of the non-planar species. For example, phenanthro[3,4-*c*]phenanthrene (PhPh) has two additional carbon atoms compared with coronene, yet PhPh is less retained than coronene. The difference in shape recognition between monomeric and polymeric C_{18} columns is evident in Table 2. Selectivity factors are much larger with the polymeric phases compared to monomeric phases; in one instance (*p*-quaterphenyl and 1,3,5-triphenylbenzene) the comparison is 46 to 1.5!

The effect of solute non-planarity on retention has been observed for another class of compounds, namely methyl-substituted PAHs. Wise

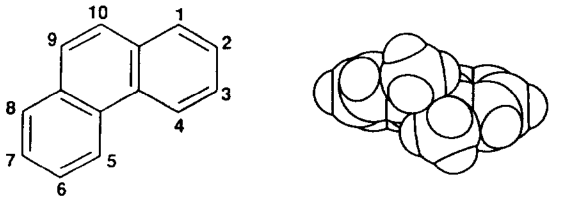
TABLE 2

PLANAR/NON-PLANAR SELECTIVITY COEFFICIENTS

Compound	Acetonitrile–water (85:15)				100% Acetonitrile			
	Monomeric	Polymeric			Monomeric	Polymeric		
		Low	Normal	Heavy		Low	Normal	Heavy
p-/*m*-Terphenyl	1.2	1.3	2.1	3.0	1.2	1.3	2.3	3.5
p-/*o*-Terphenyl	1.3	1.7	2.6	3.8	1.4	1.5	2.9	4.3
p-/*m*-Tetraphenyl	1.4				1.4	4.4	11.7	34.5
p-Quaterphenyl/ 1,3,5-triphenylbenzene	1.5				1.5	3.0	14.2	46.0
Benzo[*ghi*]fluoranthene/ benzo[*c*]phenanthrene	1.1	1.2	1.4	1.6	1.2	1.4	1.7	1.8
Benzo[*ghi*]perylene/ dibenzo[*c*,*g*]phenanthrene	1.8	2.8	4.5	4.1	2.1	3.3	7.8	7.5
Coronene/ phenanthro[3,4-*c*]phenanthrene	2.7	5.1	9.6		3.4	6.2	12.1	17.1

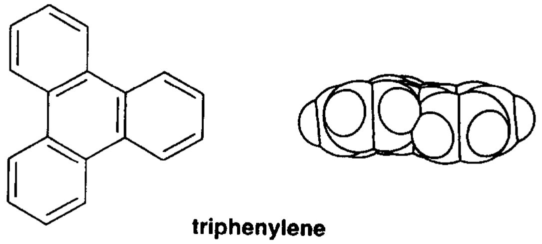
et al. [9] reported on the "anomalous retention behavior" of certain methyl-PAHs, which elute *before* the unsubstituted parent compound. Usually methyl substitution increases retention. Careful study of these compounds revealed that each early-eluting methyl-PAH was strained and twisted to conform to a non-planar shape. The conformations were determined by molecular modeling software, and a general trend was observed: substitution in the "bay region" of a PAH results in strain and non-planarity. A simple example is illustrated in Fig. 13. 4-Methylphenanthrene is non-planar, whereas 3-methylphenanthrene is planar. Interestingly enough, the addition of a ring (or in some cases, methyl substituents) at positions opposite the bay region (positions 9 and 10 with phenanthrene) can also lead to non-planarity even though no steric hindrance is apparent [58]. Thus, triphenylene is modeled by some molecular mechanics programs with slight non-planarity although each of the three bay regions are unsubstituted.

Within isomers sets, planarity and *L*/*B* must both be considered in evaluating retention. Unfortunately, an expression relating retention to *L*/*B* and the degree of planarity has not yet been developed. One difficulty in this process is the development of an appropriate molecular descriptor for non-planarity. The dihedral angle of distortion does provide an indication of non-planarity, but the value is dependent on the specific atoms selected to define the dihedral angle. Other approaches have included the use of shadow areas, minimum area, and various forms of molecular surface areas and volumes. One of the more promising approaches to the development of a descriptor which encompasses



phenanthrene (planar)

4,5-dimethylphenanthrene (non-planar)



triphenylene

Fig. 13. Effect of substitution on molecular structure for polycyclic aromatic hydrocarbons.

overall molecular shape has been advanced by Yan and Martire [59,60]. They describe retention as the linear combination of three terms: contact area, volume and "minimum area". Contact area and volume terms account for thermodynamic driving forces, whereas "minimum area" accounts for solute shape effects. This shape descriptor is defined by a three dimensional box drawn to enclose the structure of the molecule such that the contact area = length × breadth, and the thickness is specified by a constant (planar molecules) or by X-ray crystallographic data (for non-planar molecules). The minimum area parameter is then just the area of the end of this box, or breadth × thickness. Good correlations of certain retention data have been reported for the relation; however, other data sets have exhibited significant deviations from the predicted trends. Further study of the minimum area descriptor is in progress.

To help visualize the effect that *L/B* and solute planarity have on retention, we proposed an empirical model called the "Slot model [7]". Spaces between alkyl chains in the stationary phase are viewed as slots into which the solute molecules penetrate during retention. Non-planar molecules are hindered from penetration into narrow slots, and consequently have reduced interaction with the stationary phase (reduced retention). Planar molecules penetrate more easily into these slots, and exhibit increased retention. Similarly, long narrow molecules fit into existing slots more readily than square shaped molecules. The effect of phase density and ligand length on shape discrimination can be discussed in terms of this model. Densely loaded phases have less space between alkyl chains (narrower slots) and provide more differentiation of planarity than lightly loaded phases with widely spaced alkyl chains. The envisioned slots are also better defined with densely loaded phases, since the alkyl ligands are constrained to more extended, ordered conformations. As chain spacing increases and slot width becomes large compared to the dimensions of the solute, shape discrimination is minimized. Also, phase length can be viewed in terms of slot depth, and shape selectivity is observed to increase with alkyl chain length. Thus, the "Slot model" representation is consistent with retention trends for both planarity and *L/B*. The minimum area parameter developed by Yan and Martire [59,60] can be viewed as a mathematical description of the required slot dimensions.

In their investigation of shape selectivity of polymeric C_{18} phases, Wise *et al.* [6] suggested that this behavior was comparable to that observed for gas chromatography liquid-crystalline phases. A detailed comparison of the shape discrimination exhibited by polymeric C_{18} phases and liquid-crystalline phases utilized in gas chromatography was reported later by Wise *et al.* [61]. A series of PAH isomers were separated with monomeric and polymeric C_{18} phases by LC, and a comparison was made for separations carried out on 5% phenylmethylpolysiloxane and smectic liquid-crystalline phases by gas chromatography [61]. LC separations were carried out isocratically, and gas chromatographic separations isothermally. Changes in elution order and overall selectivity were observed among the various chromatographic systems, but the elution order for the LC separation on the polymeric C_{18} column was the same as for the gas chromatographic separation on the liquid-crystalline column. Similarly, the retention behavior of the monomeric C_{18} LC phase and the polysiloxane gas chromatography phase were comparable. This suggests that polymeric C_{18} phases may have a degree of anisotropy similar to liquid-crystalline phases, whereas monomeric C_{18} phases are less ordered.

The changes in retention behavior for C_{18} phases with temperature provide further evidence for phase ordering. FT-IR spectra indicate that covalently bonded alkanes become rod-like at reduced temperatures. These extended ligands are directed away from the silica surface, and the resulting surface has considerable order. We hypothesize that it is this order that provides the enhanced liquid crystalline-like selectivity observed at reduced temperatures. Likewise, the close proximity of alkyl chains in densely modified polymeric phases is thought to result in ligand extension and ordering. The smooth, continuous variation in selectivity with temperature argues against the existence of a melting–freezing type of phase transition. Rather, the reduction of conformational defects with decreasing temperature results in phase ordering and enhancement of shape discrimination.

6. CONCLUSIONS

Phase selectivity is a dynamic property of chromatographic systems that can be controlled to achieve separations. Perhaps the most effective way to alter selectivity is by selection of the appropriate phase type, since monomeric and polymeric C_{18} phases offer wide differences in selectivity toward solute shape. Operational parameters provide additional control over selectivity. Dramatic changes in selectivity occur with temperature. Shape discrimination is enhanced at low temperatures, such that polymeric-like selectivity can be mimicked by monomeric C_{18} phases. An understanding of how retention is affected by phase morphology and chromatographic conditions is fundamental to the method development process, and to the development of new phases tailored to specific separation problems.

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