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Chromatographic Evaluation of Porous Carbon-Clad Zirconia Microparticles

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The chemical vapor deposition of hydrocarbons on porous zirconia (ZrO₂) microparticles creates a reversed-phase support that is significantly different from conventional bonded reversed-phase supports. This carbon-overlaid zirconia support (C/ZrO₂) is stable to conditions of extreme pH (0–12) and temperature (80 °C). It differs greatly from conventional supports in its chromatographic selectivity; the separation of positional isomers is facile, and they are well resolved compared to the separation on ODS silica. The unique chromatographic nature of this material was studied in detail to better understand and find applications for this novel stationary phase. The results of loading capacity and chromatographic efficiency studies are also discussed.

Zirconia Chromatographic Supports. ZrO₂ is not a rare metal oxide; it has been used in material science for fashioning chemically and thermally robust components. Little use has been made of this material for high-performance liquid chromatography beyond reports from this laboratory.^{1–17} It is particularly well suited for use as a chromatographic support due to its extreme mechanical and chemical stability. Columns of uncoated porous ZrO₂ microparticles (5–8 μm) have been subjected to pressures of 9000 psi and aqueous solutions over the pH range 0–14 (up to 100 °C) without any ill effect.² Other chromatographic supports would suffer severe damage by such treatment. Another key property of ZrO₂ is its excellent thermal stability; its more refractory nature permits heating to high temperatures (700–1100 °C) with considerably less change in pore structure than either

silica or alumina. Changes in the pore structure of chromatographic supports, specifically the loss of surface area accompanying sintering, are generally undesirable.

Porous ZrO₂ with a light loading of cross-linked polybutadiene (PBD) can withstand conditions as extreme as pH 14 (aqueous) at 100 °C without any detectable loss of either the ZrO₂ support or polymeric network.³ Under the same conditions, alumina packing materials coated with PBD were substantially damaged. Although PBD–ZrO₂ is quite promising as a support material, it has one very significant limitation; a substantial amount of the ZrO₂ surface is exposed. Increasing the load of polymer does not give a large commensurate decrease in the solutes' accessibility to the ZrO₂ surface sites. As a result solutes containing Lewis base moieties (such as carboxylate, phosphate, sulfonate, etc.) strongly interact with the exposed ZrO₂ surface. This can cause extreme peak broadening, tailing, or even worse, irreversible binding of the solute to the stationary phase. In some cases, this situation can be remedied by adding phosphate, fluoride, or a carboxylate to the mobile phase, but this is not always desirable.¹⁷ Furthermore ion exchange sites, which can cause irreversible binding of charged species and certainly complicate the retention process, are still present on the surface.

The availability of ZrO₂ particles of high quality (small uniform particle size and well-defined, uniform pore structure) provides a unique opportunity to create and study chromatographic supports using high-temperature chemical modification. High-temperature modification is of interest for two reasons: (1) we expect that a reversed-phase chromatographic support with a rigid structure and adsorptive nature would have unique chromatographic properties in contrast to that of conventional bonded-phase supports which experience continual conformational change;^{18,19} (2) high-temperature chemical modification allows us to incrementally alter the surface chemistry by atomic units rather than molecular units (e.g., silane chemistry and polymer coating). Modifying surfaces on the atomic scale may increase the efficiency of particle coating, which in turn will "seal" the zirconia surface from access by solutes. Thus, it might create a more homogeneous surface by removing the possibility of solute interactions with the metal oxide matrix.²⁰ These types of interactions are most often detrimental to the chromatographic efficiency of the support, much like the residual hydroxyl groups and metal (impurity) sites on silica supports.^{21,22} Methods of chemically masking the small amount of remaining ZrO₂ surface with strong, hard Lewis bases such as fluoride or phosphate have also been developed in this laboratory.^{7–10}

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Table 1. Characteristics of Packing Materials

| ID | C/ZrO ₂ | particle size ^a | av pore diam ^b | surface area ^c | % carbon ^d (w/w) | carbon ^e ($\mu\text{mol m}^{-2}$) | % coverage ^f | thickness ^g (monolayers) |
|----|--------------------|----------------------------|---------------------------|---------------------------|-----------------------------|--|-------------------------|-------------------------------------|
| A | heptane (Hp) | 8 | 230 | 28.0 | 1.3 | 39 | 61 | 1.5 |
| B | isooctane (Io) | 8 | 230 | 27.6 | 1.9 | 57 | 89 | 2.2 |
| C | 1-butanol (B) | 8 | 230 | 27.5 | 1.9 | 58 | 90 | 2.2 |
| D | cyclopentane (Cp) | 8 | 230 | 28.4 | 1.6 | 47 | 94 | 1.8 |
| E | toluene (T) | 8 | 113 | 39.0 | 3.4 | 73 | 90 | 2.8 |
| F | 1,7-octadiene (Od) | 8 | 230 | 26.3 | 5.0 | 158 | 96 | 6.0 |
| G | Hypercarb | 7 | 250 | | 100 | | | |
| H | Vydac C-18 | 10 | 300 | 100 | 9.0 | | | |
| I | ODS-Hypersil | 5 | 110 | 250 | 10.0 | | | |

^a Particle size (in μm) by Coulter counter. ^b Average pore diameter (in \AA) by Hg porosimetry (before coating). ^c Surface area (in m^2/g^{-1}) by nitrogen BET (before coating). ^d Percent carbon by elemental analysis. ^e Calculated as (% carbon) $(1 \times 10^6)/(100)(\text{surface area})(12.011)$. ^f Using phenylphosphonate and calculated as $100[1 - \{\mu\text{mol of adsorbed phosphate}/(3.6 \mu\text{mol of Zr}^{4+}/\text{m}^2)(\text{surface area})(\text{mass of packing})\}]$. ^g Calculated on the basis of surface area occupied by a carbon atom using the length of C-C bond in graphite as 1.42 \AA . Calculated as $[N_A\pi(1.42 \times 10^{-10})^2(\text{result from e})/1 \times 10^6]$.

In previous work,¹ the development and characterization of a reversed-phase chromatographic support made by the chemical vapor deposition (CVD) of toluene on porous ZrO₂ microspheres was described. That method produced a mechanically and chemically stable chromatographic support that combined the advantages of conventional inorganic supports (i.e., mechanical stability, well-controlled pore structure, and high surface area) with those of carbon-based supports (i.e., chemical stability and unique chromatographic selectivity). The use of carbon as a stationary phase in HPLC has been well documented and is still being studied by a variety of research groups.²³⁻²⁶ We now report on the expansion of the CVD process to encompass additional carbon vapor sources.

This work focuses on the quality of carbon-overlaid zirconia (C/ZrO₂) chromatographic supports obtained by the chemical vapor deposition of various hydrocarbons on porous ZrO₂ particles. A variety of C/ZrO₂ supports, made from the same base porous ZrO₂ particles but differing only in the source of carbon for the CVD reaction, are studied. The chromatographic nature of the carbon-coated materials is examined in detail in an attempt to explain the unique chromatographic character of these and other carbon reversed-phase supports.

EXPERIMENTAL SECTION

Reagents. The chemicals used in this study were obtained from the following suppliers: toluene, cyclopentane, hexane (Omnisolve grade), and heptane (reagent grade) were obtained from EM Science, Cherry Hill, NJ; 1,7-octadiene (99%) and all solutes were obtained from Aldrich Chemical Co. Inc., Milwaukee, WI; 1-butanol (reagent grade), acetonitrile (ACN), and tetrahydrofuran (THF) were HPLC grade and obtained from Fisher Scientific, Fairlawn, NJ; isooctane was obtained from Burdick and Jackson, Muskegon, MI; and 2-propanol (Chromar grade) was obtained from Mallinckrodt, Paris, KY. The unstabilized THF was tested for peroxides before use. Water for the HPLC mobile phase was purified by passing house deionized water through a Barnstead/Thermolyne (Dubuque, IA) Nanopure water purification system with an "Organic-Free" final cartridge followed by a 0.2 μm particle filter. Phenylphosphonic acid for the determination of the relative amount of unblocked ZrO₂ was obtained from Pfaltz & Bauer, Waterbury, CT.

Chromatographic Support Preparation. The CVD process utilizes a tube furnace in which volatile organic compounds are

passed over the porous ZrO₂ particles at an elevated temperature ($\sim 700^\circ\text{C}$) and reduced pressure (~ 5 to 10 Torr).²⁷⁻²⁹ The reduced pressure is maintained using a vacuum pump while the carbon source is slowly introduced. This procedure creates a uniform carbon coating on the porous particles in which it is possible to attain greater than 97% coverage of the available ZrO₂ surface (see below) while still retaining the porous structure of the particles. After completion of CVD, the carbon-coated particles were rinsed with THF or heptane to remove soluble pyrolysis products. The material was packed into a column and used as a reversed-phase support.

For purposes of comparison three commercially available chromatographic support materials were also examined. Two of these supports, Vydac C-18 (Sep/a/ra/tions Group, Hesperia, CA) and ODS-Hypersil (Shandon Scientific Ltd., Runcorn, Cheshire, U.K.), are silica-based chemically bonded octadecyl reversed-phase materials. Hypercarb, a commercially available porous graphitic-carbon- (PGC-) based support, was obtained from Keystone Scientific Inc., Bellefonte, PA. This PGC support is made by the silica gel template method of Knox and Kaur.³⁰⁻³² The identity and physical characteristics of the particles used in this study are given in Table 1.

Column Packing. Column blanks were cut and polished to 5 or 15 cm lengths from 0.25 in. o.d., 0.46 cm i.d. precision bore 316 stainless steel tubing (Supelco). Parker-Hannifin, 316 stainless steel column end fittings were used with 2 μm stainless steel frits (Supelco). The columns were packed using an upward stirred slurry technique. For a 15 \times 0.46 cm column blank, ~ 8 g of particles was slurried in 25 mL of 90/10 2-propanol/hexane, and this mixture was forced into the column using pure 2-propanol at 5500–6000 psi by a Haskel pneumatic pump.

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Chromatographic Studies. All studies were conducted on a Hewlett-Packard (Palo Alto, CA) 1090 high-performance liquid chromatograph with a DR5 solvent delivery system and a filter photometric detector. Reported chromatographic parameters were averages of at least triplicate determinations of each solute and detection was at 254 or 210 nm. Absorbance data from the filter photometric detector were digitized, integrated, and plotted with a Hewlett-Packard 3393A integrator that controlled the liquid chromatograph through an HP-IL interface loop. Digitized data were stored on a Hewlett-Packard 9153A disk drive connected to the HP-IL interface loop. Column dead time was measured from both solvent mismatch and deuterium oxide peaks. Uracil and sodium nitrate cannot be used as dead volume markers because they are either slightly retained on the carbon support or interact with residual ZrO_2 , resulting in broadened peaks. Column efficiencies were calculated from the retention times and peak widths reported by the integrator, which assumes a Gaussian band.

RESULTS AND DISCUSSION

Carbon Coverage and Deposition Rate. All of the carbon-coated ZrO_2 supports, except the 1-butanol material, were made by depositing for 45 min at the same temperature and pressure. The 1-butanol material was made using two 45 min depositions since the coating proceeded slowly under the experimental conditions. It is possible to measure the chemical vapor deposition efficiency as a function of carbon source by measuring the amount of carbon on the particles and the coverage of the carbon layer with respect to available zirconia surface sites. Given the deposition efficiency for a certain type of carbon source and how well the carbon "seals" the surface, one can estimate the time needed to cover the surface to an appropriate extent (typically greater than 98%). Table 1 gives both the percentage carbon on the particles and percentage of the particle covered by carbon as measured by the phenylphosphonic acid breakthrough method.¹ Unsaturated hydrocarbons deposit on the particle at a much higher rate than do saturated hydrocarbons. It appears that the unsaturated hydrocarbons are more reactive and create a carbon surface susceptible to further deposition, that is, formation of multilayers. On the other hand, the less reactive saturated hydrocarbons produce a thin surface layer of carbon in the same time. At a given carbon load, the saturated hydrocarbons appear to be more efficient at completely coating the ZrO_2 surface. It is conceivable that the lower reactivity of the saturated hydrocarbons in combination with the porous nature of the ZrO_2 substrate increases the probability of molecules diffusing into the porous particle and decomposing inside the pores. Alternatively, the reaction of saturated hydrocarbons with the hot ZrO_2 may be self-quenching as the carbon coats the ZrO_2 surface.

Chromatographic Characterization. The type of chromatographic support obtained by the vapor deposition process is highly dependent on the hydrocarbon used. In particular, when a saturated hydrocarbon is used as the carbon source, the chromatographic efficiency and loading capacity of the support material are much greater than those of supports made by CVD of unsaturated hydrocarbons. Figure 1 shows chromatograms of ethylbenzene, butyl phenyl ether, propiophenone, and nitrobenzene on columns packed with both heptane- and toluene-derived ZrO_2 supports under identical conditions. Table 2 gives the capacity factors and reduced plate heights for these peaks. Despite the fact that nitrobenzene and propiophenone are less

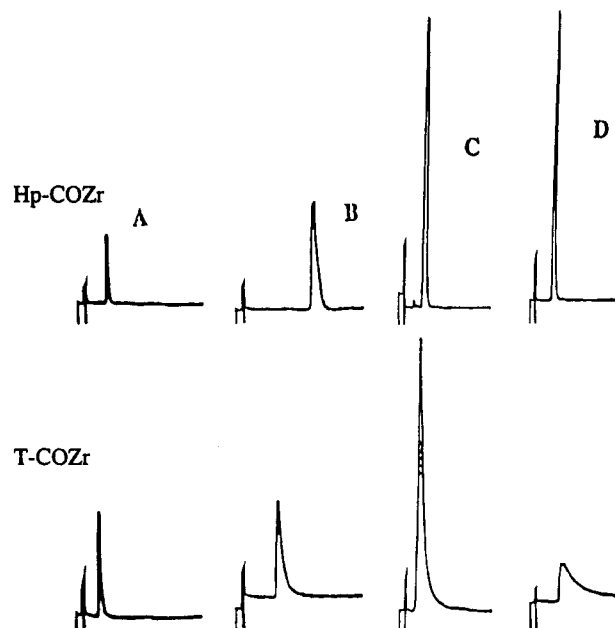


Figure 1. Chromatographic peak shape comparison of ethylbenzene (A), butyl phenyl ether (B), propiophenone (C), and nitrobenzene (D) on Hp-C/ ZrO_2 and T-C/ ZrO_2 listed in Table 2.

Table 2. Column Performance Parameters for Selected Solutes on Carbon-Overlaid Zirconia^a

| solute | capacity factor | | reduced plate height | |
|--------------------|----------------------|---------------------|----------------------|---------------------|
| | Hp-C/ ZrO_2 | T-C/ ZrO_2 | Hp-C/ ZrO_2 | T-C/ ZrO_2 |
| ethylbenzene | 3.3 | 2.4 | 4.5 | 11 |
| butyl phenyl ether | 10.0 | 5.1 | 7.4 | 28 |
| propiophenone | 3.8 | 2.5 | 7.6 | 120 |
| nitrobenzene | 3.6 | 5.5 | 5.3 | 194 |

^a 40/60 THF/water; flow rate 1 mL min⁻¹, 254 nm detection.

retained than butyl phenyl ether, their reduced plate heights are much worse. On the basis of these data, it is evident that large differences do exist between these two support materials and the heptane-based phase is clearly chromatographically preferable to the toluene-based packing material.

Logarithm of Capacity Factor vs Homolog Number.

The reversed-phase nature of the chromatographic supports was demonstrated by examining the slopes of plots of the logarithm of the capacity factor against the number of methyl and methylene carbons for a homologous series. Both the slope and the intercept of the resulting line are a measure of the hydrophobicity (reversed-phase retentivity) of the packing material. The plots have a positive slope and are rather linear, indicating reversed-phase type retention; however, slight curvature is noted at low homolog number on the carbon columns but not on the silica C-18 columns. This effect is attributed to the difference in retention processes between the C-18 column and the carbon columns. We postulate that the retention mechanism is exclusively an adsorption process on the rigid carbon surface. Support for this interpretation comes from carbon's superior chromatographic selectivity for isomers over that of conventional C-18 supports.⁵ On the conventional C-18 columns, the retention mechanism is much more a "partition-like" process involving a dynamic surface that is not as sensitive to solute shape. Thus, the curvature at low homolog number is due to the fact that the alkyl chain of the solute becomes smaller

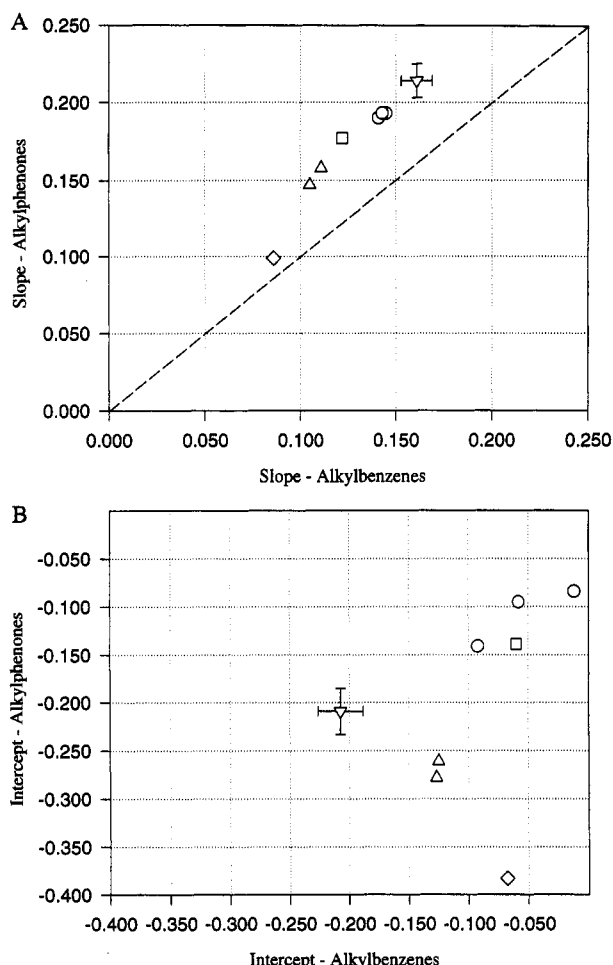


Figure 2. Analysis of the alkylbenzene and alkylphenone homolog series on columns listed in Table 1: (A) slope analysis; (B) intercept analysis. Conditions: 50/50 THF/water, flow rate 1 mL min⁻¹, 40 °C, and 254 nm detection. Columns: (○) saturated C/ZrO₂, (□) Cp-C/ZrO₂, (△) unsaturated C/ZrO₂, (▽) Hypercarb, and (◇) Vydac C-18.

and the relative contribution of the benzene ring to retention becomes more significant.

Figure 2A shows a comparison between slopes of the homolog study on various carbonaceous phases listed in Table 1. Error bars are shown only when larger than the symbols and represent a single standard deviation. The slope (free energy) of the log k' vs homolog number line is independent of the relative amounts of carbon; therefore, the observed effects must be attributed to interactions of the solutes with the stationary phase when mobile-phase conditions are held constant. It can be seen that the C/ZrO₂ supports categorize themselves according to the vapor source; an octadecylsilane phase and Hypercarb are included for reference. That is, the magnitude of the slope for the materials made by CVD using heptane, isooctane, and 1-butanol are quite similar; the same can be stated for toluene and 1,7-octadiene. The positions of the carbon supports relative to the one-to-one line indicate that the chromatographic retention is quite different from that in the octadecylsilane phase. It is generally accepted that homologous series of solutes yield almost the same slope of log k' vs homolog number when analyzed on conventional bonded RPLC phases; consequently, they would be scattered around the one-to-one line.³³ The retention differences must originate in the

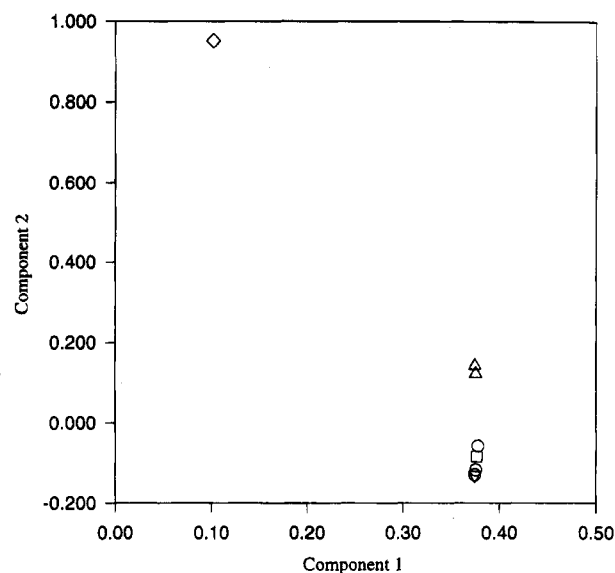


Figure 3. Eigenvector projections from principal component analysis of the homolog series retention data. Components 1 and 2 include 99.91% of the variance in the data matrix. Columns: (○) saturated C/ZrO₂, (□) Cp-C/ZrO₂, (△) unsaturated C/ZrO₂, (▽) Hypercarb, and (◇) Vydac C-18.

stationary phase and its physicochemical properties. The fact that the C-18 column lies in close proximity to the one-to-one line and an energy difference of greater than 100 cal mol⁻¹ exists when moving from the silane phase to Hypercarb suggests that the above effects are nontrivial. An intercept-intercept plot, shown in Figure 2B, was constructed to check for additional relationships. Since the intercept also reflects the relative carbon loading, it is not surprising that we see materials with higher weight percent carbon grouped together. As stated earlier, unsaturated materials deposited carbon at a higher rate than did saturated materials. A slope-intercept plot is not shown because no meaningful relationships were found and there is difficulty in interpreting such plots because of statistical artifacts that can lead to unreliable suppositions.^{34,35}

The categorization of the carbonaceous phases by the data, shown in Figure 2, is not explicit; therefore, we employed chemometrics in an effort to further evaluate data patterns.^{36,37} Principal component analysis, performed on the normalized and mean-centered matrix containing the logarithm of the retention data across all the columns used, found that two abstract components incorporated 88.33 and 11.58% of the variance, respectively. Figure 3 shows the resulting eigenvector projection; it unambiguously reveals three distinct clusters. First, the octadecylsilane phase is far removed from any of the carbon materials and illustrates the dramatic differences one can expect between carbon and bonded-phase silanes. Second, the eigenvector projection reveals differences between carbon supports prepared from unsaturated and saturated vapor sources. There is a small degree of scatter within these latter two clusters, but the behavior of a methylene unit is expected to be similar within a group.

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The mobile-phase conditions for this study are striking given the strength of tetrahydrofuran as a modifier for reversed-phase chromatography. In the 50/50 tetrahydrofuran/water solvent system, both the alkylphenones and alkylbenzenes are highly retained on carbon surfaces; however, much lower retentivity and smaller slopes are noted for the conventional C-18 silica support under the same conditions. The carbon supports are on average much more retentive than are the conventional supports, especially for the more polar solutes, that is, the alkylphenones. These results are consistent with the findings of Tanaka.³⁸

The alkylphenones are more retained on the carbon supports than are the alkylbenzenes of the same homolog number. To our knowledge, all C-18 bonded silica shows the opposite behavior with respect to the above solutes; the more polar (hydrophilic) alkylphenones are always much less retained than the alkylbenzenes. This effect is a clear sign of the unique nature of retention on carbon-based supports. The selectivity between solute classes (i.e., the selectivity between nitrobenzene and toluene) on the carbon support is very different from conventional reversed-phase supports. Thus, separations that are difficult on C-18 columns may be much simpler on carbon columns, and it is expected that there will be instances where the reverse will be true. The retention properties are so different even though both are fundamentally reversed-phase supports that it cannot be assumed that a separation that fails on a conventional bonded phase will fail on a carbon surface and vice versa.

Sample Loading Capacity Study. The linear loading capacity of a chromatographic support is extremely important. A material with low loading capacity will have poor chromatographic efficiency, and the capacity factor will depend on the amount of sample injected.³⁹ Several reports have indicated that carbon packing materials have very low loading capacity.^{1,40–42} For this reason it is important to evaluate the loading capacity of the carbon-based supports.

Three compounds, based on their differing behavior on the carbon supports, were selected as test solutes. Each solute was injected over a slightly different concentration range due to differences in their molar absorbances at 254 nm. Figure 4 shows the resultant plots of solute capacity factor vs the logarithm of the amount of solute injected on each column. The loading capacities cover close to 3 orders of magnitude and illustrate some important differences in these materials. Once again the supports appear to fall into separate classes. The packing materials derived from heptane, iso-octane, cyclopentane, and 1-butanol have good loading capacities for all solutes examined with a slight rolloff in capacity factors at high sample loading. A slight rolloff is not surprising; the amount of sample is large for a column of these dimensions. On the other hand, the columns packed with toluene- and 1,7-octadiene-derived materials have reasonable loading capacities for both butyl phenyl ether and butylbenzene, but there is no region where the capacity factor of nitrobenzene does not change with the amount of nitrobenzene injected. At low loading, the capacity factor for nitrobenzene is much larger than that for the other two solutes. If there were a linear loading region for

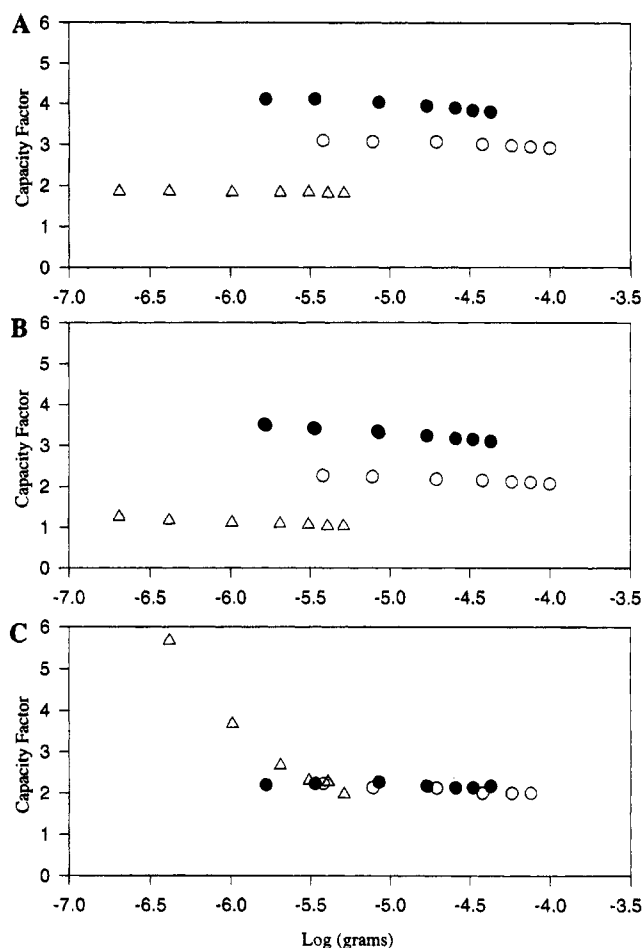


Figure 4. Capacity factor vs mass of butylbenzene (○), butyl phenyl ether (●), and nitrobenzene (△) injected on Hp-C/ZrO₂ (A), Hypercarb (B), and T-C/ZrO₂ (C). Conditions: 50/50 THF/water, flow rate 1 mL min⁻¹, and 254 nm detection.

nitrobenzene on this class of stationary phases, then nitrobenzene should be much more retained than both butyl phenyl ether and butylbenzene. However, the elution order on the phases made with saturated hydrocarbons is nitrobenzene followed by butylbenzene and finally butyl phenyl ether. The loading capacity for Hypercarb columns is very good for all solutes examined and the solute elution order follows that of the C/ZrO₂-saturated carbon supports. These results coupled with the peak shapes shown in Figure 1 and that nitrobenzene does not interact appreciably with residual zirconia sites suggest nonuniform adsorption sites on the carbon supports made from unsaturated organic vapors and a homogeneity of sites in the other materials.

Organic Modifier Concentration Effects. The effect of organic modifier concentration on retention was studied and compared to the results obtained on a conventional column. Typically the logarithm of the capacity factor is linearly related to the percentage of organic modifier in the mobile phase on a bonded reversed-phase column.^{43,44} This linear relationship is often limited to the region of 30–70% organic modifier concentration.⁴⁵ Cp-C/ZrO₂ and T-C/ZrO₂ columns, Hypercarb column, and

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ODS-Hypersil were chosen for this study. Acetonitrile and tetrahydrofuran were used as organic modifiers over the range of 30–100%. Linear regions on all phases were found in the range 30–70%, but more curvature was noted on the carbon phases compared to the ODS-Hypersil.

About 10 years ago, Horvath and co-workers⁴⁶ introduced the concept of the retention modulus, μ , as a means of comparing the similarity of the energetics of retention on a variety of related but differing bonded phases. They defined the modulus as follows:

$$\mu_{i-I-II} = \left(\frac{k_{i-I}}{k_{i-II}} \right) = \frac{\phi_I}{\phi_{II}} \left(\exp \left[\frac{\Delta G_{i-I}^\circ - \Delta G_{i-II}^\circ}{RT} \right] \right) \quad (1)$$

[where i is a particular solute, I and II denote the columns, and ϕ is the phase ratios. A value of the retention modulus greater than 1 implies greater free energy of retention, phase ratio, or both in column I. If the moduli are not constant for all analytes, one can make a simplifying assumption that the ratio ϕ_I/ϕ_{II} is constant and the differences in the moduli arise only from differences in the free energies for solute interaction with the stationary phase. This assumption is not necessarily valid when modulus values for solutes are compared across a range of mobile-phase compositions; therefore, one cannot separate the chemical contribution from the physical contribution to retention.

Since the retention modulus reflects changes in both phase ratio and free energies, interpretation of the results may be compromised by phase ratio effects if the phase ratio term between columns is not constant. The phase ratio effect can be removed by dividing the capacity factor of solute i on column I by the sum of the capacity factors of all solutes on column I at a particular mobile-phase composition. The same can be done for the capacity factor of solute i on column II, where column II is the ODS material. We define a normalized retention modulus, μ^* , so that it will be related to only chemical phenomena and not the phase ratio:

$$\mu_i^* = \frac{(k'_i / \sum k'_i)}{(k'_{i_{\text{ODS}}} / \sum k'_{i_{\text{ODS}}})} = \frac{(\phi_I K_{i_I} / \phi_I \sum K_{i_I})}{(\phi_{\text{ODS}} K_{i_{\text{ODS}}} / \phi_{\text{ODS}} \sum K_{i_{\text{ODS}}})} \quad (2)$$

$$\mu_i^* = \frac{K_{i_I}}{K_{i_{\text{ODS}}}} \left[\frac{\sum K_{i_{\text{ODS}}}}{\sum K_{i_I}} \right] = \left(\exp \left[\frac{\Delta G_{i_{\text{ODS}}}^\circ - \Delta G_{i_I}^\circ}{RT} \right] \right) \left[\frac{\sum K_{i_{\text{ODS}}}}{\sum K_{i_I}} \right] \quad (3)$$

where ϕ_I and ϕ_{ODS} are the respective phase ratios, k' is the capacity factor, and K is the partition coefficient. Moduli values across a range of mobile-phase compositions can be compared.

Acetonitrile. Figure 5 shows the normalized retention modulus, μ^* , as a function of mobile-phase composition for all the carbon columns. Error bars reflect one standard deviation and are shown when larger than the symbols used in the plots. From the plots

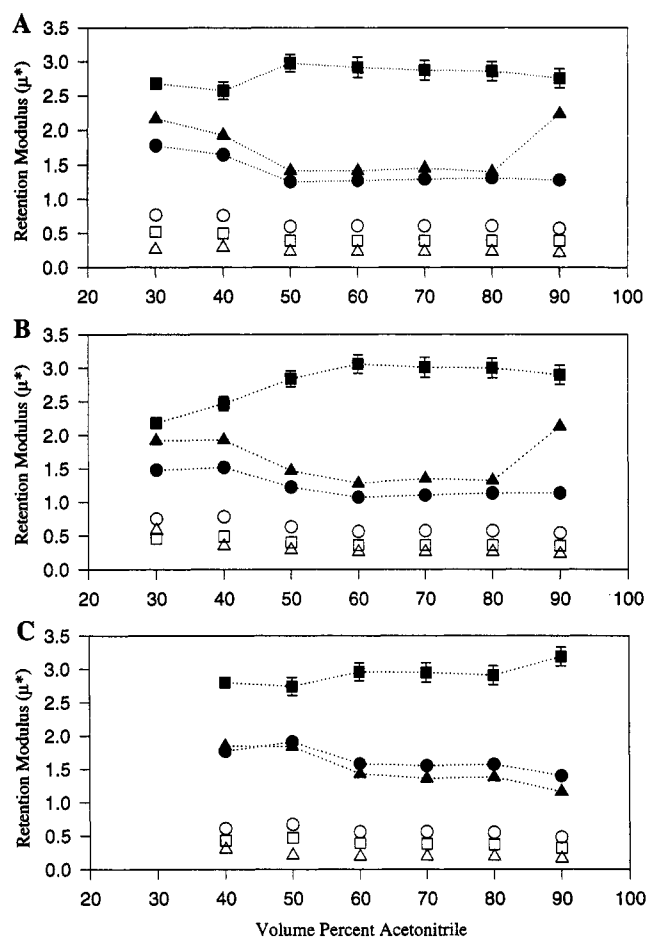


Figure 5. Normalized retention modulus (μ^*) vs. volume percent acetonitrile in the mobile phase for biphenyl (■), butyrophenone (●), butylbenzene (Δ), iodobenzene (○), nitrobenzene (▲), and o-xylene (□) relative to Hypersil-ODS on Cp-C/ZrO₂ (A), T-C/ZrO₂ (B), and Hypercarb (C). Conditions: flow rate 1 mL min⁻¹, 254 nm detection, and temperature 40 °C.

it is apparent that the carbon columns exhibit similar retention behavior; the order of the solutes in each plot is constant over the entire range of composition. The values of the moduli for butylbenzene, o-xylene, and iodobenzene are virtually constant; however, those for butyrophenone, nitrobenzene, and biphenyl change with mobile-phase composition. These more polar and polarizable solutes are quite sensitive to changes in the mobile-phase composition, but the contribution to retention that the modulus measures is only that associated with the stationary phase. Therefore, the results clearly show that the acetonitrile induces variations in the solute interactions with the stationary phase.

Tetrahydrofuran. Figure 6 shows the normalized retention modulus, μ^* , as a function of mobile-phase composition for all the carbon columns. In tetrahydrofuran, the moduli display much more variation between solutes and mobile-phase composition. Cross-over between solutes occurs both at low and high percent tetrahydrofuran, but it is not seen with acetonitrile. Even the moduli for butylbenzene, o-xylene, and iodobenzene show changes as a function of tetrahydrofuran composition. Again, these variations must take place through solvent modifications of the stationary phase and are greater in tetrahydrofuran compared to acetonitrile. Therefore, tetrahydrofuran is a much stronger stationary-phase modifier than is acetonitrile. The mobile phase

(45) Snyder, L. R.; Dolan, J. W.; Gant, J. R. *J. Chromatogr.* **1979**, *165*, 3–30.
(46) Horvath, Cs.; Melander, W. R.; Huang, J.-X.; Stout, R. W.; DeStefano, J. J. *Chromatographia* **1985**, *20*, 641–651.

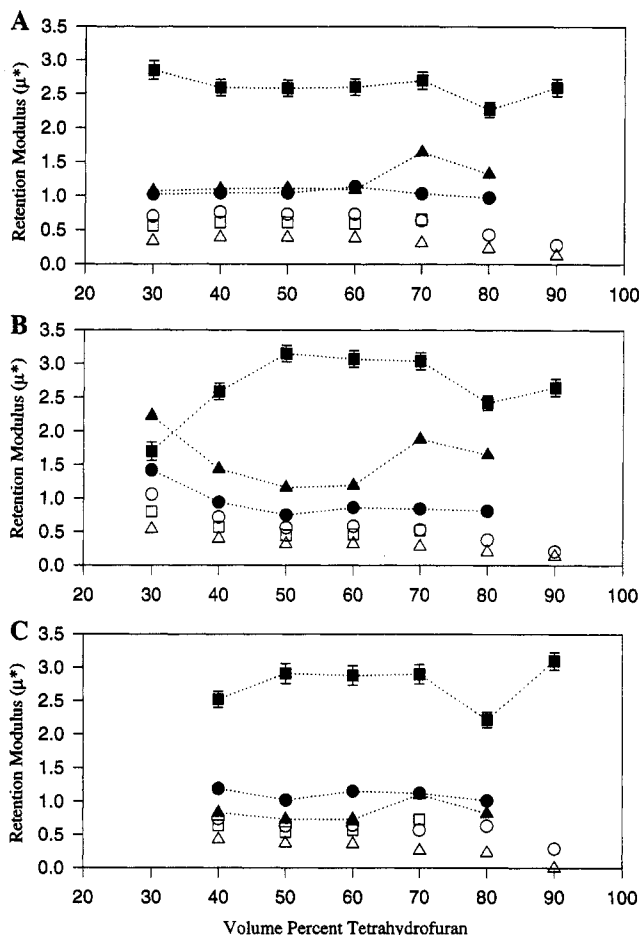


Figure 6. Normalized retention modulus (μ^*) vs volume percent tetrahydrofuran in the mobile phase for biphenyl (■), butyrophenone (●), butylbenzene (Δ), iodobenzene (○), nitrobenzene (▲), and *o*-xylene (□) relative to Hypersil-ODS on Cp-C/ZrO₂ (A), T-C/ZrO₂ (B), and Hypercarb (C). Conditions are the same as Figure 5.

participates in retention through sorption on the carbon phases, which changes their physicochemical properties.

Flow Rate Study. The chromatographic efficiencies of the supports were explored by measuring the plate heights of four different solutes over a reasonable range of mobile phase flow rates. These test solutes were chosen to represent a wide variety of functional groups, capacity factors, and chromatographic efficiencies. It must be pointed out that it is difficult to measure the efficiency of a chromatographic support on short columns, but the small amounts of packing material available severely limited the column dimensions. Even under these circumstances, the large differences present in the efficiencies of the supports facilitated comparisons and in some cases the supports demonstrated rather good efficiencies.

Figure 7 shows the representative results obtained from flow rate studies on the carbonaceous columns. Although there is significant scatter in the data, the carbon materials again fall into two distinct classes. Those phases based on saturated carbon compounds, shown in part A, demonstrate respectable, but certainly not excellent, chromatographic efficiencies (for columns of these dimensions and particle size) for all of the solutes examined. Also note that the rate of increase in reduced plate height at larger linear velocity is quite small. Hence, the packing materials exhibit good mass transfer characteristics. On the other hand, the supports in part B, based on toluene and 1,7-octadiene,

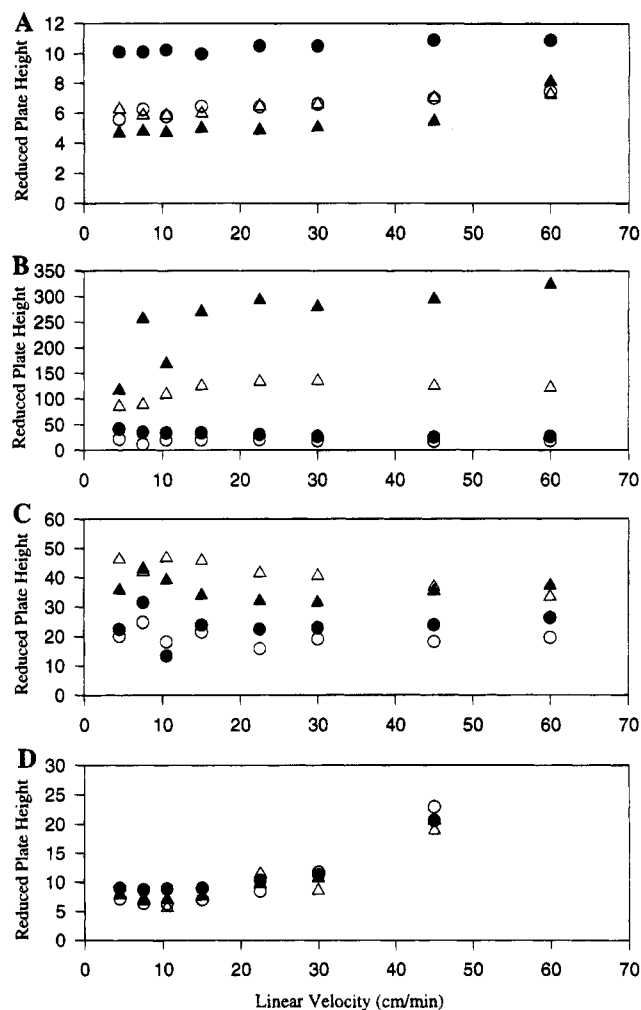


Figure 7. Reduced plate height vs linear velocity for butylbenzene (○), butyl phenyl ether (●), valerophenone (Δ), and nitrobenzene (▲) on Hp-C/ZrO₂ (A), T-C/ZrO₂ (B), Hypercarb (C), and Hypercarb using 65/35 ACN/water mobile phase (D). Conditions: 50/50 THF/water, and 254 nm detection.

show extremely poor chromatographic efficiencies for both nitrobenzene and valerophenone. The efficiencies for butylbenzene and butyl phenyl ether are considerably better. Poor packing as a cause of the low chromatographic efficiency can be ruled out by the much smaller values of reduced plate heights for the latter two solutes. Unequivocally, this second group of supports is chemically different. The nitrobenzene and alkylphenones most likely interact very strongly with a small number of sites on this carbon surface. This possibility is supported by the loading studies, where we noted that the material is easily overloaded for nitrobenzene but not for butylbenzene or butyl phenyl ether. The reduced plate heights for valerophenone and nitrobenzene increase very rapidly at higher flow rates, which suggest slow desorption from high-energy sites on the stationary phase. This hypothesis is further confirmed by our finding in previous work¹ that indicates high-temperature hydrogen treatment of the toluene-derived packing served to improve its chromatographic efficiency. These high-energy sites may be due to oxidation of the carbon surface, defect sites, or both.

The Hypercarb support, shown in parts C and D, had very low chromatographic efficiency in tetrahydrofuran/water mobile phases. This effect is puzzling, and preliminary investigations demonstrated that this column has reasonable chromatographic

efficiency in acetonitrile/water mobile phases. For that reason the flow rate study was repeated using an acetonitrile/water mobile phase; the column took an extraordinarily long time to equilibrate (~400 column volumes) before regaining its initial chromatographic efficiency. The data, taken after full column equilibration using 65/35 acetonitrile/water mobile phase, are given in Figure 7D. Here the minimum reduced plate heights are more similar to those of the saturated C/ZrO₂ columns. However, the reduced plate height increases dramatically as the flow rate is increased; this is in stark contrast to the behavior observed on the saturated C/ZrO₂ columns. The mechanism of the process on the Hypercarb eludes us, but since our main interest is the C/ZrO₂ materials we decided not to pursue the behavior of Hypercarb at this time. However a significant structural difference between these materials is that Hypercarb is considered to be crystalline (graphite) whereas the CVD carbons are expected to be amorphous. The C/ZrO₂ columns do not exhibit the strong dependence of efficiency upon change in mobile-phase organic modifier, although there are some modest changes. The changes in efficiency with type of modifier are interpreted as being due to differences in the amount of sorbed mobile phase on the carbon surface. A uniform layer of sorbed solvent would tend to create a more homogeneous chromatographic surface.

PTH Amino Acid Separations. The utility of the C/ZrO₂ supports was explored by attempting the separation of a mixture of amino acids and comparing that separation to those obtained on a conventional bonded-phase support. Chromatographic separation of phenylthiohydantoin-derivatized amino acids (PTH amino acids) is used extensively in the identification and sequencing of amino acids resulting from the Edman degradation of proteins. Typical separation protocols call for conventional bonded reversed-phase supports.⁴⁷ Difficulties with this method include the following: low retention; peak broadening (interaction with residual silanols); coelution and short column lifetime due to the use of trifluoroacetic acid in the mobile phase. The use of C/ZrO₂ columns might avoid the coelution, low-retention problems. It will certainly solve the column lifetime problem. The use of carbon columns might not provide a universal fix, but its value would have to be tested on a case-by-case basis.

A set of 15 PTH amino acids were separated on B-C/ZrO₂ and ODS-Hypersil supports using an acetonitrile/water (0.1% trifluoroacetic acid) gradient. The separations on both columns started at the same composition, but the gradient on the carbon column terminated at 75% acetonitrile. The corresponding gradient on the ODS-Hypersil column ran to only 65% acetonitrile; this was done to ensure elution of compounds from the more retentive carbon column. It should be noted that the conventional ODS column was substantially more efficient than the 1-butanol-derived carbon phase. The chromatograms that were obtained for the separation of PTH amino acids on the ODS-Hypersil and C/ZrO₂ supports are displayed in parts A and B of Figure 8, respectively.

As seen from the chromatograms, the elution order is not the same on both columns. PTH-histidine and PTH-arginine did not elute on the ODS-Hypersil support, but elute early on the B-C/ZrO₂ support. We believe that the side-chain amino groups on these compounds cause these solutes to be strongly bound to

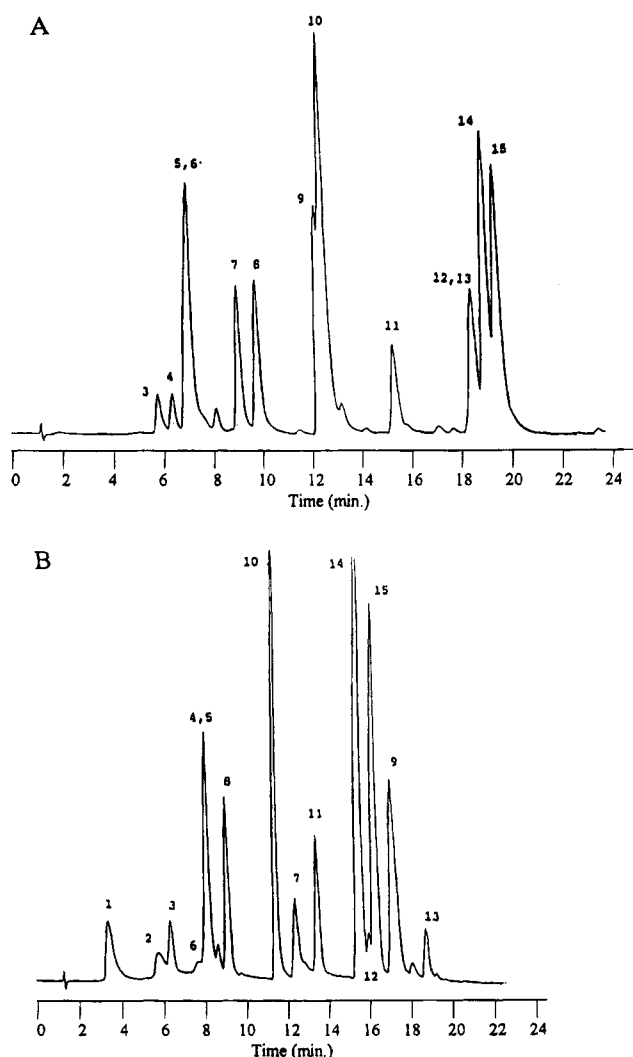


Figure 8. Gradient separation of PTH amino acids. Conditions: acetonitrile/water (0.1% trifluoroacetic acid), flow rate 0.3 mL min⁻¹, 40 °C, 100 × 2.1 mm column, and UV detection at 254 nm. (A) Hypersil-ODS, 5 μm particles. Gradient: 0–2 min 20% ACN, 2–25 min gradient to 65% ACN, 25–35 min hold at 65% ACN. (B) C/ZrO₂, 8 μm particles. Gradient: 0–2 min 20% ACN, 2–25 min gradient to 75% ACN, 25–35 min hold at 75% ACN. Peaks: (1) His, (2) Arg, (3) Ser, (4) Thr, (5) Gln, (6) Gly, (7) Glu, (8) Ala, (9) Tyr, (10) Aba, (11) Val, (12) Ile, (13) Phe, (14) Leu, and (15) Nle.

residual silanol sites on the ODS-Hypersil support. This problem could be solved with the addition of TEA to the mobile phase. Additionally, these two species are protonated in this mobile phase, which would explain the early elution of these compounds on the carbon column.

CONCLUSIONS

The C/ZrO₂ and Hypercarb supports offer unique retention mechanisms. That is, not only are these supports hydrophobic, but they also retain solutes through electronic (π - π) interactions. This result is very important for the prediction of solute retention on carbon supports; solvophobic theory⁴⁸ is not adequate for the prediction of elution order.

The use of saturated hydrocarbons for the CVD of carbon on the surface of ZrO₂ results in a more efficient chromatographic support than when unsaturated hydrocarbons are used. This is

(47) Qureshi, G. A. In *HPLC of Proteins, Peptides and Polynucleotides: Contemporary Topics and Applications*; Hearn, M. T. W., Ed.; VCH: New York, 1991; Chapter 19.

(48) Horvath, Cs.; Melander, W. R.; Molnar, I. *J. Chromatogr.* **1976**, *125*, 129–156.

attributed to the improved energetic homogeneity of the carbon surface. These C/ZrO₂ supports offer a mechanically robust alternative to the more delicate Hypercarb support. Synthesis of the C/ZrO₂ support is less costly, and better control over particle characteristics (surface area, pore size, particle size) than the Hypercarb materials can be achieved.

Both the C/ZrO₂ and the Hypercarb supports have demonstrated reasonable chromatographic efficiency and loading capacity for a variety of solutes. The extremely retentive and selective nature of the carbon support makes it an excellent candidate for consideration when conventional chemically bonded reversed-phase supports fail—specifically the separation of structural isomers. This is true when a modest number of solutes are to be separated; however, as Giddings has pointed out, in complex multicomponent mixtures there is really no alternative to the use of columns with large numbers of plates and concomitant high peak capacity.⁴⁹ The chromatographer must use caution when first utilizing carbon supports and realize that although these

supports generally behave as reversed-phase materials they have many unique chromatographic characteristics. Therefore, simple translations of chromatographic methods from conventional supports to carbon supports may be difficult.

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(49) Giddings, J. C. In *Gas Chromatography-1964*; Goldup, A., Ed.; The Institute of Petroleum: London, 1965.

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