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Evaluation of a zirconia-based carbon–polymer composite reversed-phase chromatographic support

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ABSTRACT

Three zirconia-based composite reversed-phase high-performance liquid chromatography supports with exceptional alkaline stability are described. The first support was prepared by chemical vapor deposition of carbon onto a porous zirconia substrate; the second by hydrogen treatment of the carbon coated zirconia particle at elevated temperature and the third by deposition and cross-linking of a hydrophobic polymer layer over the carbon-coated zirconia particle. The alkaline stability, and chromatographic selectivity, efficiency and loading capacity of these packing materials were examined and compared. Hydrogen treatment of the carbon particles served to remove or modify high-energy adsorption sites on the carbon surface. The polymer coating on the carbon particles increased the chromatographic efficiency and loading capacity over that of the bare carbon supports. These materials appear to be indefinitely stable at 80°C in a mobile phase of methanol–water (50:50) buffered at pH 12.

INTRODUCTION

Chemically bonded porous silica is undoubtedly the most common support matrix for reversed-phase high-performance liquid chromatography (HPLC) [1,2]. Silica has many favorable characteristics that are responsible for its widespread use. These favorable characteristics include mechanical stability, ease of modification of the surface chemistry, chemical stability and rigidity in a wide variety of solvent systems. In addition, silica is available as small spherical particles with well controlled pore structure, uniform particle diameter, high surface area and large pore volume. However, it is also well known that silica-based supports have some shortcomings which can severely limit the separations that can be performed using such supports [3–9]. These disadvantages include: the high solubility of silica in aqueous environments (especially at high temperature or high pH), which may result in the loss of column bed integrity, the effect of certain types of residual silanols which result in poor efficiency for basic compounds thereby complicating chromatographic analysis, and the chemical instability of silane bonding chemistry used to modify the silica surface which results in gradual changes in retention, selectivity and peak shape. There have

been reports on conventional silica supports being used at relatively high pH values (pH = 9.2) [10,11]. However, dissolution of the silica support occurred as noted by formation of a void at the head of the column, and such work was carried out with little water in the mobile phase. The combination of water and alkali is responsible for the instability of silica, and most reversed-phase separations are carried out with an appreciable amount of water in the mobile phase.

In reversed-phase preparative liquid chromatography instability of the support and the bonded ligand network also leads to contamination of product with dissolved silica and/or ligand [12,13]. This has serious repercussions on the use of silica stationary phases for the purification of materials for the pharmaceutical and biotechnology industries. The narrow pH window in which silica can be used (pH 2–8) often impedes the optimization of separation conditions. For example, in reversed-phase chromatography of amines it may well be preferable to do the separation at pH 10, 11 or even 12 where aliphatic amines are unprotonated. Even at neutral pH silica dissolves to a considerable extent, and elevated temperature further complicates the problem. Thus one is not able to operate columns at high temperatures where the chromatographic efficiency is often higher [14]. Reversed-phase chromatography of proteins for analytic purposes is most preferably carried out at 50 to 60°C to facilitate mass transfer of these bulky molecules [15]. Higher temperatures may also be useful for the separation of biomolecules or molecules which undergo slow isomeric conversion at room temperature [16]. Slow isomerization processes can broaden chromatographic peaks or give rise to several peaks for a single species [17]. By raising the temperature the rate of isomeric conversion is increased and the peaks narrow.

Alumina has greater alkaline stability than silica [18]. However, studies in this laboratory have shown that alumina dissolves to a considerable extent in acidic (pH < 3) and basic environments (pH > 12) [19].

Polymeric supports have superior chemical stability, but unfortunately they swell and shrink with changes in type of mobile phase organic modifier and concentration. Thus the pore structure and column backpressures vary in gradient elution.

Previous studies of porous zirconia in this laboratory have shown that this support is completely stable over the pH range 0–14. Porous zirconia with a light loading of cross-linked polybutadiene can withstand conditions as extreme as pH 14 (aqueous) at 100°C (350 column volumes) without any detectable loss of either the zirconia support or polymeric network. This material is indefinitely stable over the whole aqueous pH range (0–14).

High temperatures and extremely alkaline or acidic conditions are necessary to sterilize columns for use in the purification of pharmaceutical products or to clean columns after they have become fouled by many injections of crude samples (*e.g.* crude feedstock from a bioreactor or biological fluids). Obviously a chromatographic support which can withstand many cycles of high temperature combined with extreme alkaline (or acidic) conditions would be of considerable utility to the pharmaceutical and biotechnology industries.

For our purposes (discussed below) zirconia also has another very important characteristic. That is, due to its more refractory nature, zirconia can be heated to high temperatures (*i.e.* > 500°C) with 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 in general undesirable.

In this study we will describe the modification of the surface chemistry of the zirconia particles using chemical vapor deposition (CVD) to create a thin layer of carbon on the porous beads.

Carbon-based supports are not uncommon for gas and liquid chromatography. Reviews of carbon based supports for HPLC have appeared in the literature [20–22]. Many different methods have been utilized in attempts to form porous carbonaceous supports that are chemically homogeneous and can withstand the mechanical stress experienced by the packing material in liquid chromatography. Some of the more notable methods include:

(1) Carbonaceous polymer infiltration of porous silica particles (acting as a template) followed by firing at approximately 1000°C (thereby converting the polymer to an amorphous carbon) followed by an alkaline treatment to remove silica (leaving a porous amorphous carbon) and finally high temperature firing at 2500–3000°C to graphitize fully the remaining porous particle [23].

(2) Deposition of a pyrocarbon layer on porous silica by passing organic vapors at a pressure between 1 and 25 atmospheres over particles heated to 400–700°C [24,25].

(3) Deposition of a pyrocarbon layer on carbon black particles heated to 600–800°C with the intention of hardening the particles so that they can withstand the mechanical stress involved in liquid chromatography [26].

(4) Electrochemically reducing poly(tetrafluoroethylene) in a lithium amalgam *in vacuo* to produce a porous carbonaceous particle followed by further heating to 2800°C to graphitize the particles [27].

(5) Deposition of a pyrocarbon on a porous silica particle which had been infiltrated with a nickel(II) nitrate solution and then reducing the nickel(II) with hydrogen at high temperatures. The nickel acts as a catalyst which allows the pyrocarbon to be deposited under much more mild conditions (400°C) [28].

Although many of the above materials can withstand the stress of column packing and function as a reversed-phase chromatographic support, they also had significant limitations. In many cases the carbon surfaces were extremely chemically heterogeneous, thereby leading to low chromatographic efficiency and loading capacity. Most notably these materials showed extreme variations in column efficiency from solute to solute, and oxidation of the carbon surface which caused changes in the chromatographic properties with time, especially when harsh mobile phases are used [29]. The residual exposed silica on the pyrocarbon coated silica supports caused two problems: first there are the usual effects of residual silanols, and second the exposed silica support is susceptible to dissolution at high pH.

In this work a CVD process is carried out by passing organic vapors over the particles at elevated temperatures. These elevated temperatures are necessary for making uniform carbonaceous coatings. As will be shown the resulting CVD carbon-coated material is useful as a reversed-phase support for liquid chromatography and it is much more retentive than typical C₁₈ columns, especially for polar solutes (*i.e.* nitroaromatics, phenols, ketones, aldehydes).

The carbon layer serves a second purpose in that it acts to block the zirconia surface from interactions with solutes. Zirconia, just as alumina and silica, can interact quite strongly with certain types of functional groups. These strong interactions have deleterious effects on the peak shape and sample recovery similar to those observed for amines on silica. In the case of zirconia these functional groups are oxyanions (*e.g.*

phosphate, carboxylic acids, sulfates, nitrates). However, in our studies we are able to render the zirconia beads inert towards this troublesome interaction by depositing the thin carbon film on the surface.

The carbon-coated zirconia beads described in this work are suitable for reversed-phase chromatography. In our studies, we have also modified the surface chemistry of the particle by cross-linking a polymeric network on the particle. These subsequent modifications result in a support which does not have exposed "catalytic" inorganic support, but does have the mechanical and chemical stability of the zirconia support. The chromatographic character and chemical stability of the carbon-clad zirconia, as well as those of polymer-coated carbon-clad zirconia, were investigated in this work.

EXPERIMENTAL

Reagents

Toluene for CVD was Omnisolve grade from EM Science (Cherry Hill, NJ, U.S.A.). Polybutadiene (45% vinyl, 55% *cis*- and *trans*-1,4; average molecular mass 4500) was obtained from Aldrich (Milwaukee, WI, U.S.A.). Dicumyl peroxide was obtained from Pfaltz & Bauer (Waterbury, CT, U.S.A.). The solvents used in preparation of the polymer coated particles were obtained from the following manufacturers: hexane (Omnisolve grade, EM Science), tetrahydrofuran (Fisher Scientific, Fairlawn, NJ, U.S.A.), dichloromethane (HPLC grade, Burdick and Jackson Labs., Muskegon, MI, U.S.A.). Isopropanol was Chromar grade from Mallinckrodt (Paris, KY, U.S.A.). All solutes for the chromatographic studies were obtained from Aldrich. Water for the HPLC mobile phase in this study was deionized water that was further purified by passing it through a Barnstead/Thermolyne (Dubuque, IA, U.S.A.) Nanopure water purification system with an "Organic-Free" final cartridge followed by a 0.2- μm particle filter. Tetrahydrofuran for the mobile phase was HPLC grade and was obtained from Fisher Scientific. Benzenephosphonic acid (phenylphosphonate), for the determination of the relative amount of unblocked zirconia, was obtained from Pfaltz & Bauer. Phosphoric acid was ACS grade and was obtained from Fisher Scientific.

Method of making particles

Porous zirconia support. The porous carbon-coated zirconia supports for this study were prepared in the Ceramic Technology Center of the 3M Company (St. Paul, MN, U.S.A.) by a proprietary CVD process. The preparation of these particles is described elsewhere [30].

Toluene was used as the carbon source for the preparation of these CVD coatings. By this process it is possible to prepare particles in which more than 97% of the available zirconia surface is covered with carbon.

In some cases carbon-coated particles were further treated with hydrogen at elevated temperatures in an attempt to hydrogenate the surface in the hope of removing high-energy adsorption sites, thereby creating a more energetically homogeneous surface. This material will be referred to as "hydrogen-treated CVD toluene".

After the CVD process was completed the carbon-coated particles were rinsed

with tetrahydrofuran or heptane to remove any soluble pyrolysis products. The material was then packed into a column and used as a reversed-phase support.

Polymeric coating of the particle. Once the particles had been coated by the CVD process, the chromatographic character of the particle (reversed phase, ion exchange, etc.) can be further controlled by coating the particle with a selected pre-polymer. Polymeric coating was done in a two step process. First a pre-polymer was deposited on the particle surface. Second the pre-polymer was immobilized by a cross linking reaction. This process creates a permanent polymeric layer on the particle surface.

The pre-polymer was deposited by solvent removal as follows. The particles were dried for 8–10 h in a vacuum oven at 70–100°C prior to coating. A 1% (w/v) solution of pre-polymer was made in hexane. Pre-polymer solution was then added to the particles. The amount of pre-polymer solution used was typically 0.6 mg pre-polymer per square meter of total surface area of the particles to be coated. This gives a thin but uniform pre-polymer coating on the particle. Next the volume of hexane in the pre-polymer solution was adjusted to 5 ml of solvent per gram of packing material. The solution was then sonicated briefly (5 min) while a vacuum was pulled on the reaction flask in order to ensure that the pores were completely wet with pre-polymer solution. The reaction vial was then capped and shaken gently for 4–8 h to allow the pre-polymer to diffuse completely into the porous material. The chemical cross-linking agent (dicumyl peroxide) was then added to the solution and the solution was agitated for an additional 1–4 h. The solution was then placed in a rotary evaporator and the solvent was removed over a 2–4 h period with very gentle heating (30–40°C) while periodically pulling a vacuum on the flask. A round-bottomed flask with a side baffle, to mix the particles, was used when removing the solvent. Mixing of the particle bed assists in creating a uniform pre-polymer layer on the surface of the bead. Before the cross-linking reaction was carried out the polymer-coated particles were heated to 120°C for 1 h to allow the polymer to “flow” on the surface to insure a uniform coating of polymer. Immediately after this step the cross linking-reaction was brought about by chemical free radical initiation. The particles with the pre-polymer and dicumyl peroxide on the surface were heated to 160°C to initiate the cross-linking reaction. The cross-linking reaction was carried out for 4 h under an inert gas (nitrogen or helium) or under vacuum to prevent oxidation of the polymer. The particles were then allowed to cool while stored under vacuum or inert gas. After cooling, the particles were rinsed (100 ml per gram of particles) with each of the following solvents hexane, dichloromethane, tetrahydrofuran, and finally hexane. This procedure removes the residual unpolymerized pre-polymer from the particles which would otherwise bleed off the column during chromatography.

The physical characteristics of the particles used in this study are given in Table I.

Column packing

Column blanks were cut and polished to the desired length from 0.64 cm O.D., 0.46 cm I.D. Precision Bore 316 stainless-steel tubing (Supelco, Bellefonte, PA, U.S.A.). Parker-Hannifin, 316 stainless-steel column end fittings were used with 2- μ m stainless-steel or titanium frits (Supelco). The columns were packed using an upward slurry technique [31]. For a 0.46 \times 15 cm column blank approximately 8 g of particles were slurried in 25 ml of isopropanol–hexane (90:10) and this mixture was forced into

TABLE I
PHYSICAL CHARACTERISTICS OF PACKING MATERIALS

SA = Surface area by BET nitrogen adsorption; APD = average pore diameter by mercury porosimetry; d_p = average particle diameter by Coulter counter.

Material	SA (m ² /g)	APD (Å)	d_p (μm)	Porosity (%)	Carbon (%)
Bare zirconia	56.5	90	7	44	<0.3
CVD toluene	39.0	113	7	38	3.4
Hydrogen-treated CVD toluene	47.2	—	7	—	—
Polymer-coated CVD toluene	—	100	7	—	5.3

the column using pure isopropanol at 5500 to 6000 p.s.i. pressure by a Haskel pneumatic pump.

Well-packed columns are critical to the chemical characterization and stability studies of the zirconia support. One of the key parameters to getting well-packed columns with the upward slurry technique is to have a stable suspension of the particles in an appropriate solvent. The high density of zirconia (approximately 2.5 times that of silica) and the extremely hydrophobic surface make the production of well-packed columns of CVD carbon-clad zirconia particles somewhat difficult. The high density causes the particles to settle quickly out of solution. The hydrophobic surface causes the particles to agglomerate and settle even faster. We have developed a method that reproducibility gives well-packed carbon-clad zirconia columns. This method involves using a very non-polar slurry solvent (hexane or tetrahydrofuran) to suspend the packing material and a miscible solvent of higher viscosity (isopropanol or methanol) to displace the slurry into the column. In our method the non-polar slurry solvent prevents particle agglomeration and the highly viscous solvent packs the column tightly without excessively high flow-rates. Very high flow-rates cause the column to heat up and consume large amounts of solvent. Zirconia columns have been packed at pressures between 5000 and 9000 p.s.i., the higher pressures giving better packed columns.

Chromatographic studies

All studies were conducted on a Hewlett-Packard (Palo Alto, CA, U.S.A.) 1090 high-performance liquid chromatograph with a DR5 solvent delivery system and a filter photometric detector. Detection of all solutes was at 254 nm. The chromatograph was equipped with an extended pH range kit which included a Tefzel® injection valve rotor seal, Kel-F® ball check valve cartridges and high-pH inlet filters. Absorbance data from the filter photometric detector were digitized, integrated and plotted with a Hewlett-Packard 3393A integrator, which controlled the liquid chromatograph through an HP-IL interface loop. Digitized data were stored on a Hewlett-Packard 9153A Winchester disk drive connected to the HP-IL interface loop.

Column dead time was measured from both solvent mismatch and deuterium oxide peaks. Uracil cannot be used as a dead volume marker since it is slightly retained on the carbon support and sodium nitrate cannot be used because in some cases it

interacts with residual zirconia thereby resulting in broadened and slightly retained peaks. Column efficiencies were calculated from the retention time and peak width reported by the integrator as follows:

$$N = 5.545 \cdot (t_r/w_{1/2})^2 \quad (1)$$

$$h = L/(N \cdot d_p) \quad (2)$$

where N is the number of theoretical plates, t_r is the retention time of the solute in minutes, $w_{1/2}$ is the width of peak at half height in minutes, h is the reduced plate height, L is the length of the column (in meters), and d_p is the particle diameter (in meters).

A 0.46×5 cm column was packed with $10 \mu\text{m}$ Vydac[®] C₁₈ (Separations Group, Hesperia, CA, U.S.A.) silica support using an upward slurry technique. This column was used to represent a conventional chemically bonded reversed-phase support and compared the supports synthesized and studied in this report.

Determination of exposed zirconia

Zirconia surfaces have an extremely strong affinity for both inorganic and organic orthophosphate [32]. As discussed above, carbon coated on silica does not completely cover all of the silica surface, and we expected this to be the case for the zirconia particles. In order to assess the extent of coverage of carbon on zirconia we made use of the fact that orthophosphate quantitatively adsorbs on zirconia. Using this interaction we can quantify the amount of accessible zirconia by monitoring the removal of phosphate from a solution in contact with zirconia.

The amount of exposed zirconia has been determined in two ways. In the early stages of this work the determination was carried out by a static adsorption method as follows. About 0.1 g of packing material weighed out to the nearest hundredth of a milligram was placed in a clean 30-ml polyethylene bottle. A 2–10 mM standard solution of phosphate was made from phosphoric acid and doubly deionized water. An exact amount of phosphate solution (10 or 20 ml) was then added to the polyethylene bottle and a vacuum was pulled while sonicating to completely wet the pores. Then the solution was agitated every 30 min for the next 6–8 h. The solution was then allowed to stand for 20–24 hours. The phosphate solution was removed from the polyethylene bottle with a 10-ml glass syringe and passed through a $0.2\text{-}\mu\text{m}$ filter to remove any remaining zirconia particles. The filtered solutions and blanks (standard phosphate solutions) were analyzed for phosphorous using inductively coupled plasma spectroscopy. The amount of phosphorous that adsorbed to the packing material was determined by the difference between phosphate concentration in the standard solutions and the sample solutions. Total surface area covered by carbon was determined by comparing phosphate adsorption results for carbon-coated supports with those of adsorption results for bare zirconia particles.

A more refined method involved observing the breakthrough of an UV-active organophosphate compound (phenylphosphate) on a chromatographic column. This method minimized errors caused by incomplete wetting of the pores (water does not wet the extremely hydrophobic carbon surface very well) since a hydro-organic mobile phase is used, does not require much sample handling, and has much quicker turn

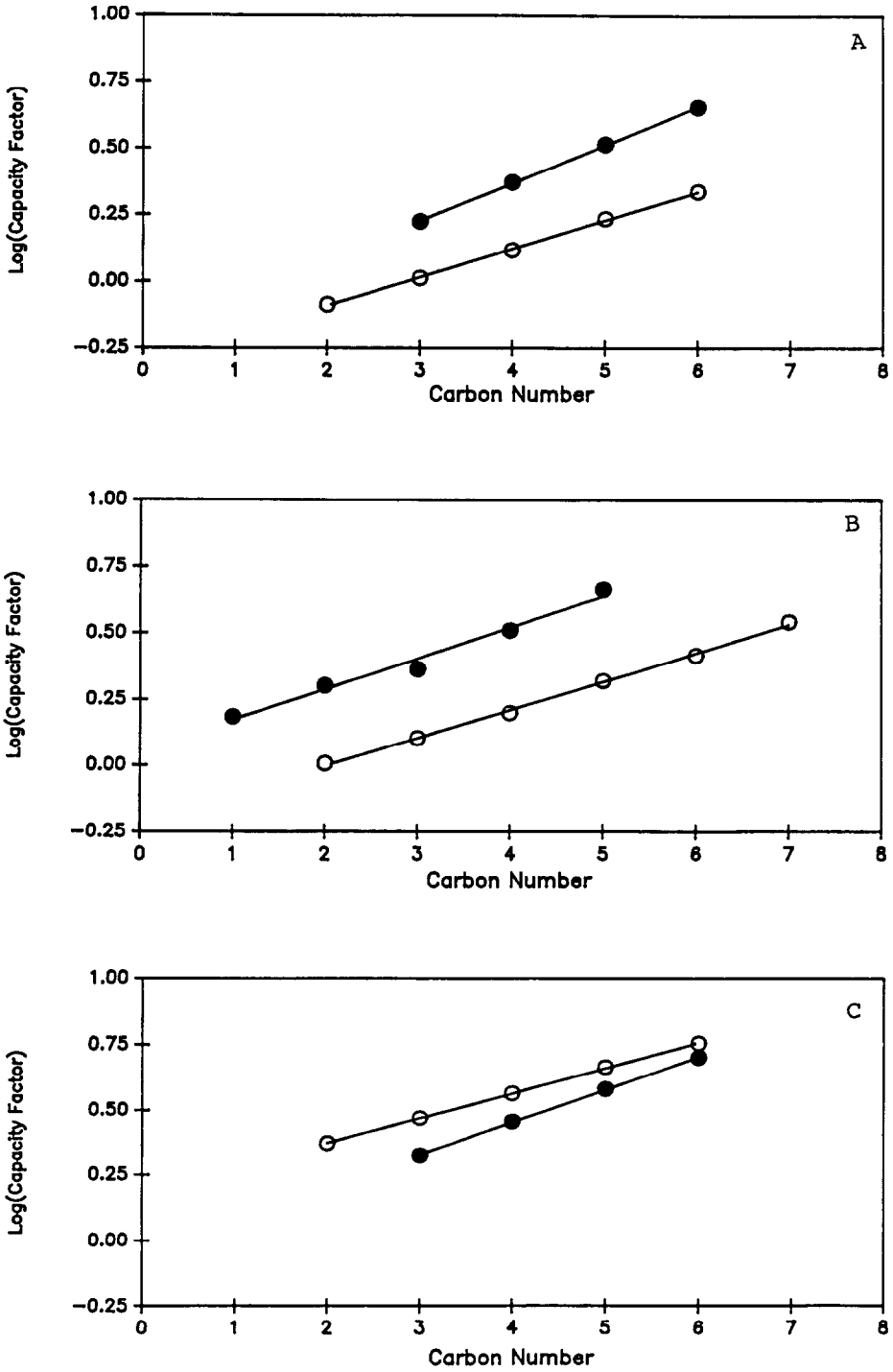


Fig. 1.

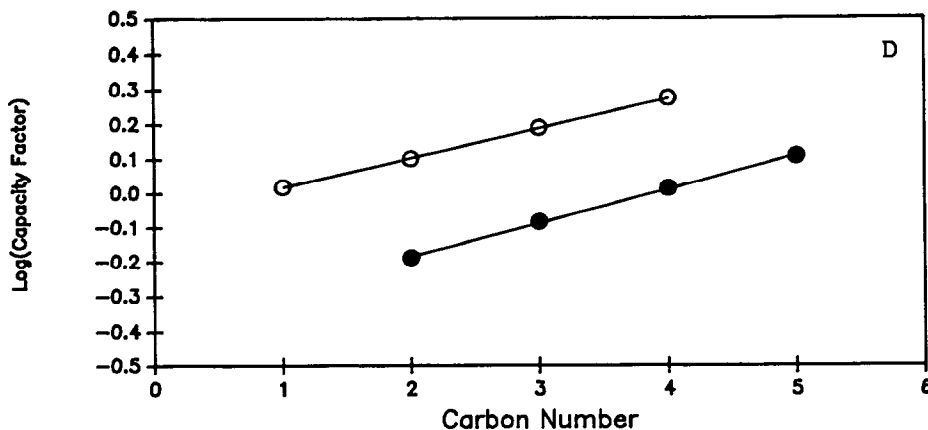


Fig. 1. Logarithm of capacity factor *versus* homologue number for alkylbenzenes (○) and alkylphenones (●). (A) CVD toluene column at 35°C in tetrahydrofuran–water (70:30). (B) Hydrogen-treated CVD toluene at 35°C in tetrahydrofuran–water (70:30). (C) Polybutadiene-coated CVD toluene at 35°C in tetrahydrofuran–water (50:50). (D) Vydac C₁₈ at 35°C in tetrahydrofuran–water (50:50).

around time. The dead volume of the carbon clad-zirconia column in methanol–water (50:50) was determined as above and a standard solution of phenylphosphate in the same mobile phase was passed through the column at a fixed flow-rate between 0.2 and 0.5 ml/min. The breakthrough curve can be observed via the absorbance of the column effluent at 254 nm on a calibrated strip chart recorder. Because the flow-rate is critical to the breakthrough study, it was calibrated both volumetrically and gravimetrically. By measuring the mass of the packing in the column, and knowing the surface area (measured by BET) the amount of phosphate adsorbed per square meter can be estimated. From previous work with bare zirconia we know that 3.6 μmol of phenylphosphate adsorb per square meter of zirconia. Thus the amount of exposed zirconia can be computed. These two methods give very similar results.

RESULTS AND DISCUSSION

Retention characteristics

The reversed-phase nature of the three types of packing material studied in this work was confirmed by examining the dependence of capacity factor on homologue number for a series of alkylbenzenes and alkylphenones. These results are plotted for the CVD toluene, hydrogen-treated CVD toluene and polybutadiene-coated CVD toluene materials in Fig. 1. The slopes of the lines and intercepts for these plots are given in Table II. Solute retention invariably decreased upon increasing the concentration of organic modifier in the mobile phase. Fig. 2a and 2b show chromatograms of the alkylphenones separated on CVD toluene and polybutadiene-coated CVD toluene, respectively.

These plots show some very interesting trends and characteristics of these packing materials, some of which contrast remarkably with the properties of conventional bonded phase supports. Fig. 1a shows that the alkylphenone solutes are

TABLE II
SLOPES AND INTERCEPTS FOR LOG_{10} (CAPACITY FACTOR) *vs.* HOMOLOGUE NUMBER

Packing material	Alkylbenzenes		Alkylphenones	
	Slope	Intercept	Slope	Intercept
CVD toluene	0.107(0.003) ^a	-0.305(0.012)	0.143(0.002)	-0.204(0.008)
Hydrogen-treated CVD toluene	0.107(0.002)	-0.217(0.012)	0.116(0.009)	-0.055(0.031)
Polybutadiene-coated CVD toluene	0.0964(0.001)	0.179(0.004)	0.126(0.002)	-0.051(0.009)
Vydac C ₁₈	0.0856(0.001)	-0.068(0.002)	0.0988(0.002)	-0.383(0.007)

^a Standard deviation of coefficient.

much more retained than are the alkylbenzene solutes on the CVD toluene column in tetrahydrofuran-water (70:30). This was also the case regardless of the type of mobile phase modifier (methanol, acetonitrile or tetrahydrofuran) at all concentrations of the modifier. This figure also shows that the selectivity (relative retention) of a methylene group in an alkylphenone is greater than the selectivity of a methylene group in an alkylbenzene. That is, the slope of the line for the alkylphenone homologous series is greater than that of the alkylbenzene homologous series.

For purposes of comparison the same study was carried out on a Vydac C₁₈ column. Fig. 1d shows a plot of the logarithm of the capacity factor *versus* the carbon number of the alkylphenone and alkylbenzene homologous series in tetrahydrofuran-water (50:50). Comparing Fig. 1a and Fig. 1d we see that conventional chemically

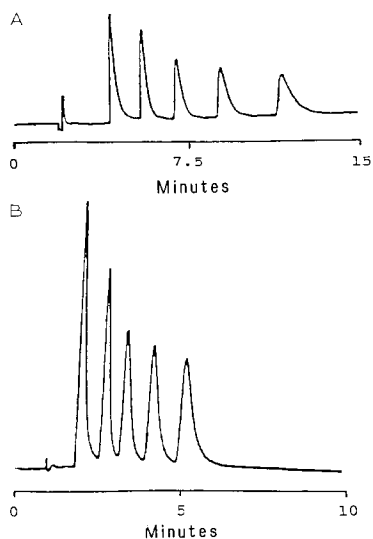


Fig. 2. Separation of alkylphenone homologous series. (A) On CVD toluene column (12.5 × 0.45 cm); conditions: tetrahydrofuran-water (70:30), 0.5 ml/min, 40°C, UV detection at 254 nm. (B) On polybutadiene-coated CVD toluene column (12.5 × 0.46 cm); conditions: tetrahydrofuran-water (50:50), 1 ml/min, 40°C, UV detection at 254 nm.

bonded reversed-phase supports are much less retentive than are the CVD toluene supports, and the alkylbenzenes are retained much more than are the alkylphenones on the C₁₈ column for a given homologue. Another difference between CVD toluene and conventional bonded reversed-phase supports is that for a conventional phase the selectivity for a methylene group in an alkylphenone is very similar to that for a methylene group in an alkylbenzene. This elution sequence is remarkable and important. Obviously the alkylphenones interact with water much more strongly than do the alkylbenzenes due to the presence of the dipolar and strongly hydrogen bond basic carbonyl group in the alkylphenone. Consequently we infer that the solute-adsorbent interaction in the case of the carbon surface is certainly much stronger and possibly more selective than in the case of a conventional bonded phase. As will be shown these strong interactions have some deleterious consequences.

When the CVD toluene support was hydrogen-treated, the chromatographic properties were significantly altered. Fig. 1b shows a similar plot for the hydrogen-treated CVD toluene material with the same mobile phase as used in Fig. 1a. This plot shows that the absolute retentions of the alkylphenones are still greater than those of the alkylbenzenes. The elution sequence did not change from that on the CVD toluene material. It is also evident that, while the slope of the logarithm of the capacity factor *versus* homologue number for the alkylbenzenes did not change relative to that in Fig. 1a, the slope for the alkylphenones decreased significantly and is now quite similar to the slope for the alkylbenzenes. This suggests that at least some of the strong adsorption sites on the carbon surface have been modified by the hydrogen treatment. This issue will be discussed in more detail below.

Fig. 1c shows a plot of the logarithm of the capacity factor of the alkylphenones and alkylbenzenes *versus* homologue number on the polybutadiene-coated CVD toluene material in tetrahydrofuran-water (50:50) mobile phase (note: this material was not hydrogen treated). Since the polybutadiene-coated material, as is a conventional bonded reversed phase, is much less retentive than either of the materials discussed above, the concentration of tetrahydrofuran in the mobile phase had to be decreased from that used in Figs. 1a and 1b in order to make the capacity factors similar to those on the CVD toluene and hydrogen-treated CVD toluene materials. This plot shows that there are some striking changes upon coating the carbon-clad particles with polymer. First, note that the retention of the alkylphenones has decreased below that of the alkylbenzene series at the same homologue number. The lower retention of an alkylphenone relative to a homologous alkylbenzene is typical of the behavior observed with conventional bonded reversed-phase supports. Thus the polybutadiene-coated CVD toluene is chemically much more analogous to a bonded reversed-phase support. In addition the slope of the logarithm of the capacity factor *versus* homologue number for both series is now lower than on either of the bare CVD toluene supports despite the fact that the mobile phase contains more water. The selectivity for a methylene group increases in reversed-phase chromatography when the organic modifier concentration in the mobile phase is decreased. Clearly the polymer coating has an enormous impact on the chromatographic characteristics of the carbon clad support. Certainly the solute-adsorbent interactions are significantly weakened.

Loading studies

The loading capacity of a chromatographic support is important for both analytical- and preparative-scale chromatography. As a column is overloaded (that is as an excessive amount of sample is loaded on the column) capacity factors and column efficiency both decrease.

In preparative chromatography one generally wants to load as much sample as possible on to the column (to increase throughput) without sacrificing band spacing or efficiency. In analytical-scale separations the peaks are identified by retention time. If the retention times change with sample size peak identification becomes more difficult. Column efficiency in analytical separations is also very important since it is desirable to have a large "peak capacity" and good resolution so that many different species can be separated. This is only possible for a column with high efficiency. In this work loading studies were carried out by injecting different amounts of solute on the column and monitoring the retention time.

The open circles in Fig. 3a show a plot of the capacity factor of butyl phenyl ether

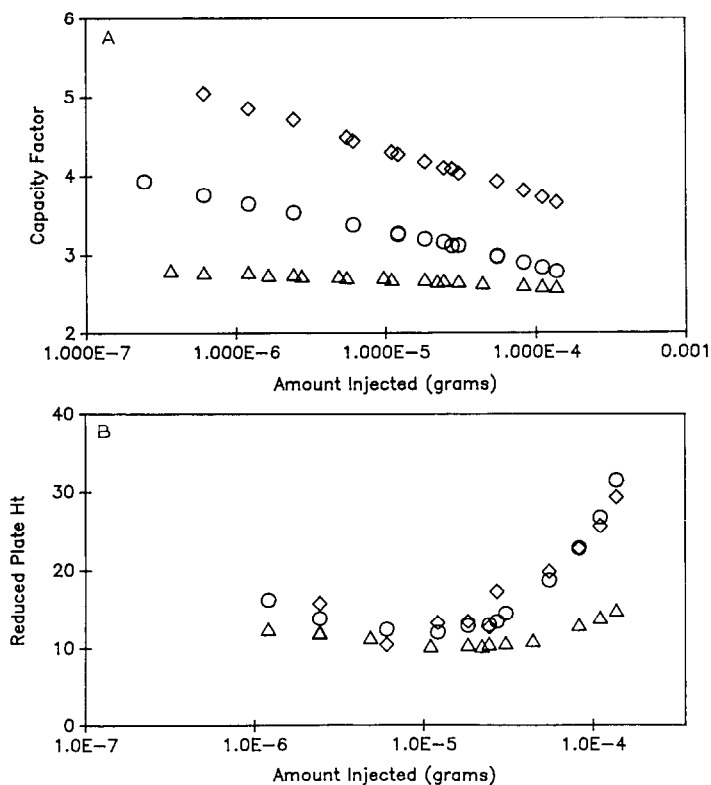


Fig. 3. Loading study results. (A) Capacity factor for butyl phenyl ether *versus* amount of solute loaded. (○) CVD toluene in tetrahydrofuran-water (60:40); (△) hydrogen-treated CVD toluene in tetrahydrofuran-water (60:40); (◇) polybutadiene-coated CVD toluene in tetrahydrofuran-water (50:50). (B) Reduced plate height for butyl phenyl ether *versus* amount of solute loaded. (○) CVD toluene in tetrahydrofuran-water (60:40); (△) hydrogen treated CVD toluene in tetrahydrofuran-water (60:40); (◇) polybutadiene-coated CVD toluene in tetrahydrofuran-water (50:50).

versus the logarithm of the amount of butyl phenyl ether injected onto a column packed with CVD toluene support. This plot shows that the carbon-clad zirconia has a rather poor loading capacity relative to a conventional bonded phase column. Over the entire concentration range examined the capacity factor decreased upon increasing the sample size. Essentially it was not possible to inject a sufficiently small sample, within our detection capabilities, so that the solute concentration was within the linear range of the adsorption isotherm.

The open diamonds in Fig. 3a show the capacity factor of butyl phenyl ether *versus* the logarithm of the amount of butyl phenyl ether injected onto a column packed with hydrogen-treated CVD toluene support using the same conditions as for the CVD toluene support. This support also has poor loading characteristics similar to that of the non-hydrogen-treated material. There does not appear to be an achievable low solute concentration at which the adsorption isotherm is linear for this solute.

These results are typical of those obtained for other types of solutes (phenyl-alcohols, alkylbenzenes, alkylphenones, nitrobenzene) tested on these two types of stationary phases. While this behavior has been reported for proteins [33], we are not aware that it has been reported for small solutes. This behavior suggests that not only does the carbon surface interact quite strongly with the solute but also that it is extremely heterogeneous and is comprised of many different types of sites.

The loading capacity on the polymer-coated CVD toluene was also examined. However, in order to make the capacity factors comparable to those on the other materials, a lower concentration of organic modifier (50% tetrahydrofuran) was used. The loading study results are shown as the open triangles in Fig. 3a. The loading capacity for this material is much greater than that of either of the carbon-clad zirconias. This plot shows that the capacity factor remains relatively constant over the entire solute concentration range examined. The limited solubility of butyl phenyl ether in the mobile phase prevented the examination of the effect of higher solute loading.

The reduced plate height was also monitored as a function of the amount of butyl phenyl ether injected on the three columns. This data is shown in Fig. 3b where the open circles, the open diamonds and the open triangles represent the CVD toluene, the hydrogen-treated CVD toluene and the polymer-coated CVD toluene support, respectively. This plot shows that the reduced plate height increases substantially as more sample is loaded on either the CVD toluene or the hydrogen-treated CVD toluene support. On the other hand, the reduced plate height on the polybutadiene-coated CVD toluene support is not greatly effected by the sample loading.

Thus the loading capacities of both of the carbon-clad supports are much lower than those of either the conventional bonded phases or the polymer-coated carbon supports. This severely limits the utility of these materials for general reversed-phase chromatography. On the other hand, the polybutadiene-coated CVD toluene support clearly demonstrates loading characteristics far superior to those of the uncoated support, and more typical of the bonded reversed-phase supports. These characteristics are obtained at the expense of the unique selectivity of the carbon phases relative to conventional supports.

Flow-rate studies

Carbon-based supports are known to have low efficiency [34], consequently

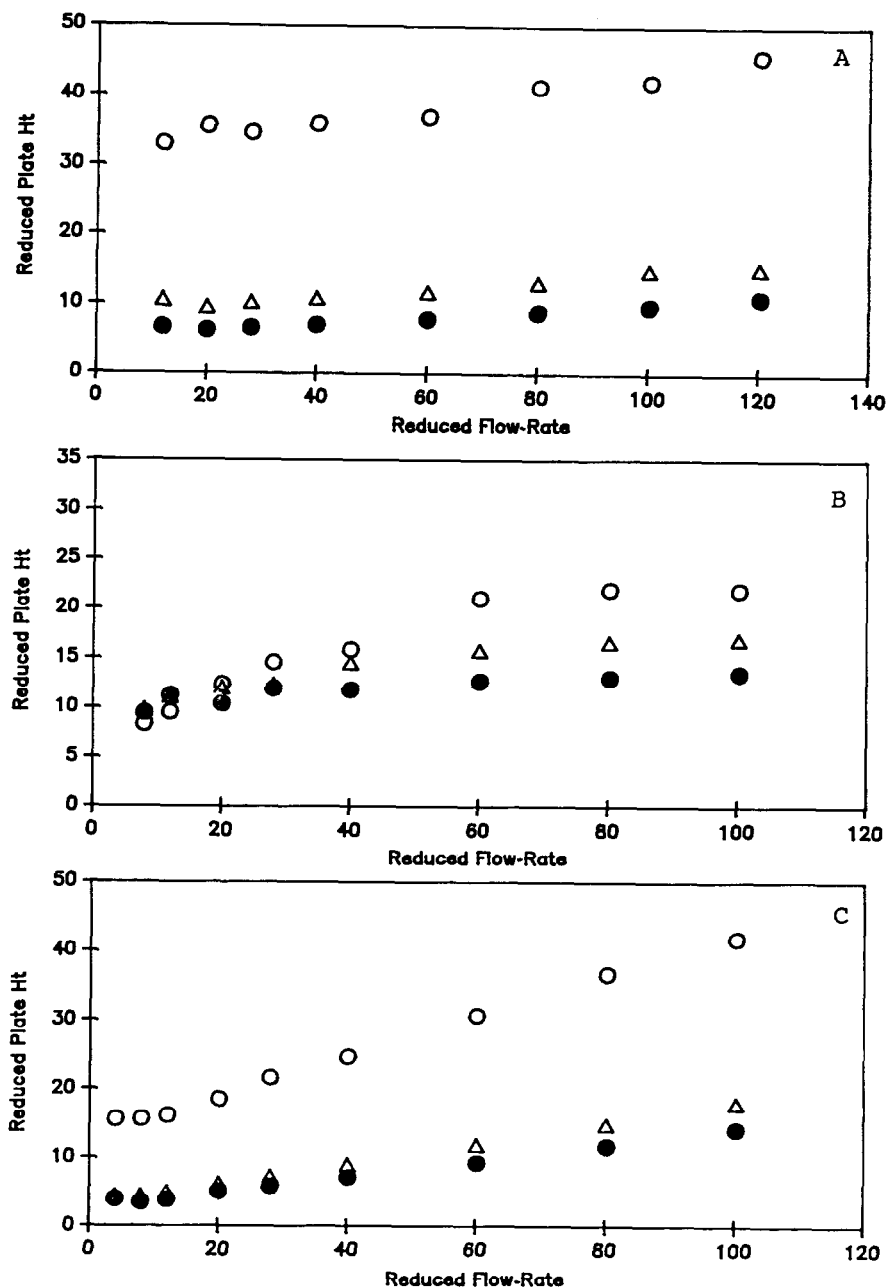


Fig. 4. Flow-rate study results. Reduced plate height for butyrophenone (○), butyl phenyl ether (△) and propylbenzene (●) vs. reduced flow-rate. (A) CVD toluene column in tetrahydrofuran-water (60:40); (B) hydrogen-treated CVD toluene in tetrahydrofuran-water (60:40); (C) polybutadiene-coated CVD toluene in tetrahydrofuran-water (50:50).

flow-rate studies were carried out on all three materials to determine the efficiency of the packing material and the quality of the column packing procedures. The solutes chosen for this study were propylbenzene, butyl phenyl ether and butyrophenone. The flow-rate studies were carried out in tetrahydrofuran–water mobile phases at 40°C. For a good chromatographic support in a well-packed column at a reduced linear velocity of 10–20 the reduced plate height reaches an optimum minimum value of 2–3 [35]. A chromatographic material which has been well packed and has good mass transfer characteristics will not show a large increase in reduced plate height as the flow-rate is increased.

Flow-rate data for the three test solutes on the CVD toluene support using a mobile phase of tetrahydrofuran–water (60:40) are shown in Fig. 4a. This plot shows that the reduced plate height increases rather slowly as the flow-rate increases, indicating that the column has good mass transfer characteristics. However, the minimum reduced plate height for butyrophenone ($h = 35$, $k' = 3.6$) is extremely large, for butyl phenyl ether ($h = 10$, $k' = 3.3$) and propylbenzene ($h = 6$, $k' = 2.1$) these minimum values, although within the range of what has been reported for carbon-based chromatographic supports, are well above the optimum values of 2–3 commonly obtained with conventional bonded phases. A chromatogram of butyrophenone eluting from this column at a flow-rate of 0.7 ml/min (reduced flow-rate of 28) is shown in Fig. 5a. The asymmetry of this peak at half height is 2.2. This rather large value also suggests that the column is severely overloaded.

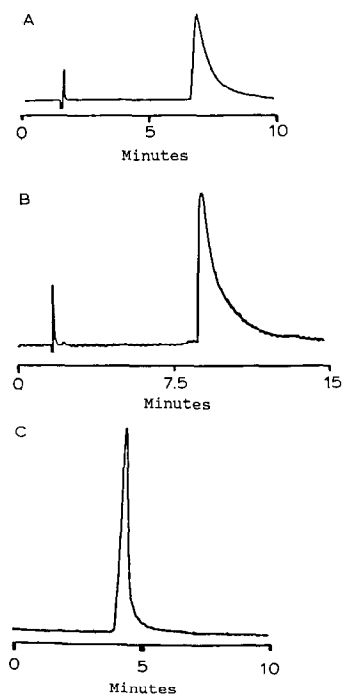


Fig. 5. Chromatograms of butyrophenone (conditions from flow-rate study) (A) CVD toluene column; (B) hydrogen-treated CVD toluene column; (C) polybutadiene-coated CVD toluene column.

The flow-rate study on the hydrogen-treated CVD toluene support in tetrahydrofuran–water (60:40) shows significant differences from the non-treated support (Fig. 4b). Most importantly the reduced plate height for butyrophenone has dropped drastically from 35 on the non-treated support to 10 ($k' = 4.3$) on the hydrogen-treated support. This corroborates our hypothesis that the hydrogen treatment of the carbon surface results in a more chemically homogeneous surface. Fig. 5b shows the peak for butyrophenone eluting from this column at a flow-rate of 0.7 ml/min. The asymmetry of this peak at half height is 1.8. This peak is more symmetrical than that in Fig. 5a, but the peak shape suggests a non-linear isotherm.

The results of a flow-rate study of the polybutadiene-coated CVD toluene support in tetrahydrofuran–water (50:50) are shown in Fig. 4c. The efficiency of this material is higher than either of the bare carbon-clad supports. The minimum reduced plate heights are much lower for this material than those of the CVD toluene support. Propylbenzene, butyl phenyl ether and butyrophenone have minimum reduced plate heights of 3.5, 4.4 and 15.5, respectively, and capacity factors of 2.4, 2.7 and 1.8, respectively. The values for propylbenzene and butyl phenyl ether are close to the theoretically optimum of 2–3. Fig. 5c shows the chromatogram of butyrophenone eluting from this column at a flow-rate of 0.7 ml/min. The asymmetry of this peak at half height is 1.2. This peak is much more symmetrical than the other two peaks as a result of the enhanced loading capacity of this material due to the polymer coating. We postulate that the high value of the minimum reduced plate height for butyrophenone, although much improved relative to the bare CVD toluene support, is due to residual interactions of this solute with bare carbon. If this is the case, then the efficiency for alkylphenones could be further improved by using hydrogen-treated CVD toluene as the basis for the polymer coating, since butyrophenone does not interact detrimentally with the hydrogen-treated material.

To determine whether residual exposed high-energy sites on the carbon may cause poor chromatographic efficiency for butyrophenone we examined the chromatographic efficiency at a very large loading with an alkylphenone. If interactions with

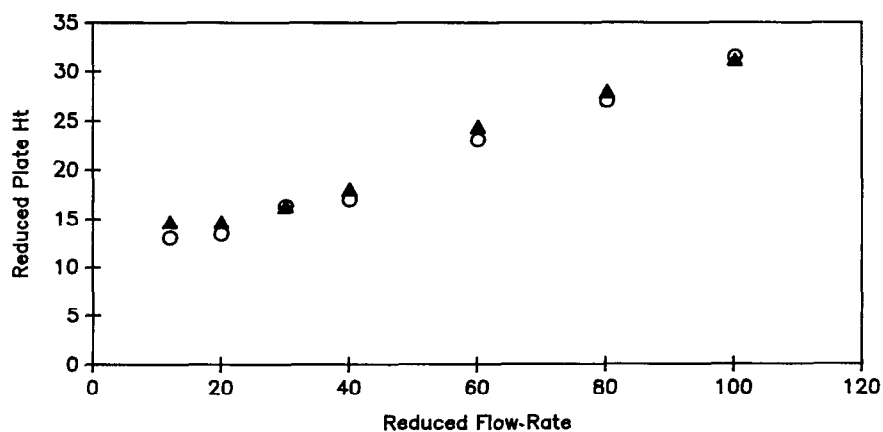


Fig. 6. Flow-rate study results using large sample loading of alkylphenone in tetrahydrofuran–water (60:40) on polybutadiene-coated CVD toluene support. (○) Butyrophenone; (▲) valerophenone.

residual bare carbon were responsible for the poor efficiency of these solutes, we hypothesize that, upon injection of a large amount of sample, the small number of residual sites would be saturated by the solute, and most of the retention would be due to interactions with the polymer. Thus the efficiency of the column should improve for injections of higher concentration of solute. The data for this study are given in Fig. 6. The minimum reduced plate height decreased to 13 and 14.5 for butyrophenone and valerophenone, respectively. In addition, the increase in the reduced plate height as the flow-rate increase is less than that for the more dilute solution. Although this decrease in reduced plate height is only marginal, the typical effect of increasing the sample loading is to increase the reduced plate height. Thus it is possible that residual high-energy sites on the carbon surface are the source of this problem. Hydrogen treatment of the carbon surface prior to polymeric coating may improve the efficiency for polar solutes.

It should also be noted that the rate of increase in the plate height as a function of flow-rate is much greater for the polymer-coated support than for the bare carbon support. We interpret this as the effect of restricted mass transfer in and out of the polymeric layer and possible impedance to the mass transfer into and out of some of the pores.

The results from the three types of studies (logarithm of the capacity factor *versus* homologue number, loading and flow-rate studies) are all internally consistent. The CVD toluene support is very retentive, but has a very heterogeneous surface as reflected by the low chromatographic loading capacity and low chromatographic efficiency for some solutes (alkylphenones and nitroaromatics).

The hydrogen treatment of the CVD toluene support serves to increase the homogeneity of the carbon surface as reflected in the increase in chromatographic efficiency for the more polar solutes (alkylphenones) and the change in the slope of the logarithm of the capacity factor *versus* homologue number for the alkylphenone series is more similar to the slope of the alkylbenzene series. We hypothesize that the hydrogen treatment moderates some of the high-energy adsorption sites on the carbon surface.

The use of the polybutadiene coating on the CVD toluene support acts to decrease retention. We assume that a substantial fraction of the adsorption sites on the carbon surface becomes deactivated by deposition of the polymeric layer. To some extent the mechanism of retention may change from a pure "adsorption-like" process to a more "partition-like" process when a polymer coating is used. This is reflected by the decrease in the slope of logarithm of the capacity factor *versus* carbon number for both the alkylbenzene and alkylphenone series, and the change in elution order for the alkylbenzene and alkylphenone series relative to that observed on a conventional bonded phase column. The chromatographic efficiency and sample loading capacity increase to a considerable extent upon polymer coating and are quite similar to those observed on conventional bonded reversed-phase supports.

Stability study

The ability of these packing materials to withstand aqueous mobile phases at high temperature and high pH was tested by flushing the columns with a mobile phase comprised of methanol-water (50:50) which was buffered at pH 12 while both the column and the mobile phase flowing into the column were heated to 80°C. This is

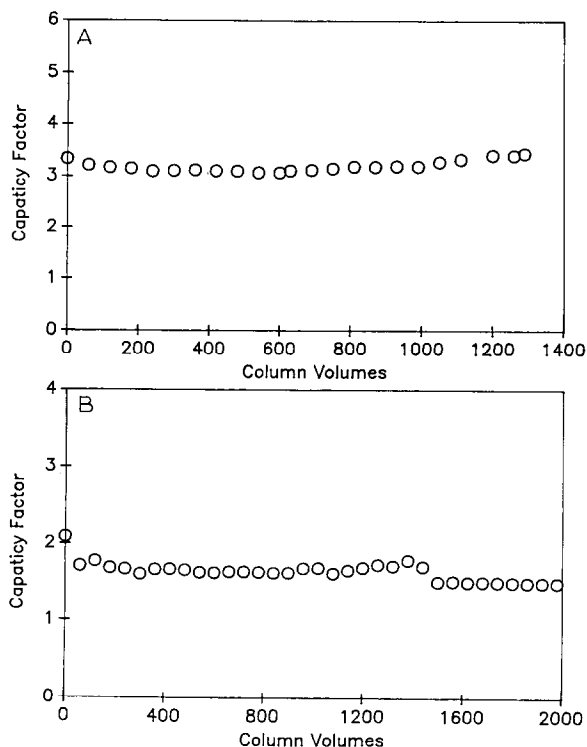


Fig. 7. Stability study results. Capacity factor of benzene vs. column volumes of methanol-water (50:50) pH 12 (at 80°C) pumped through the column. (A) CVD toluene support; (B) polybutadiene-coated CVD toluene support.

a fairly stringent test of the pH stability since a silica column would completely dissolve and an alumina column would be substantially damaged under these conditions [36]. The volume of mobile phase passed through the column, and not the time of contact, is the principal factor in damaging a column [36]. For this reason the stability results are shown as plots of the capacity factor *versus* column volumes flushed through the column. The capacity factor of a test solute was determined repeatedly during this treatment to see if the retentivity of the column was altered. Loss of retention suggests that either carbon cladding or the polymer network was being lost from the particle, or that the chromatographic surface was aging under these extreme test conditions. After the stability study was completed the packing materials were analyzed for carbon.

The stability of the CVD toluene material was examined at a flow-rate of 0.5 ml/min. This study lasted 60 h during which 2 l of the alkaline mobile phase were passed through the column. Benzene was used as the test solute since it is reasonably well retained under these conditions. Fig. 7a is a plot of capacity factor against the number of column volumes of mobile phase passed through the column. This figure shows a very slight initial decrease in capacity factor followed by a very slight increase. Although the initial loss of capacity factor may be due to loss of carbon from the surface, it is very small and may be due to aging of the carbon surface since the rate of

TABLE III
CARBON ANALYSIS RESULTS FOR CVD TOLUENE SUPPORT

Source of sample	Carbon (%)
Column head	3.3
Column middle	3.3
Column tail	3.4
Fresh packing	3.4

decrease falls off. If carbon were to be lost from the surface we expect that the capacity factor would decrease to zero rather than level off at a high value since benzene is not retained on bare zirconia in this mobile phase. The initial decrease in capacity factor may also be due to warming of the column to 80°C.

Samples of the above column packing material along with virgin packing material were submitted for carbon analysis (Table III). The carbon analysis results show that the CVD carbon is stable on the surface of the particle under the conditions of the stability study. The amount of carbon on the surface before and after the stability study is not measurably different.

The stability of the polybutadiene-coated CVD toluene was examined at a flow-rate of 1.0 ml/min. A plot of capacity factor vs. column volumes passed through the column is given in Fig. 7b. The plot shows an initial small drop in capacity factor which is typical for all polybutadiene coated materials when first exposed to harsh base conditions [19], followed by leveling off of the capacity factor at longer times. The scatter in the capacity factor between 1000 and 1440 column volumes was due to instrumental problems caused by the extremely corrosive conditions used in this study. The study was not repeated in entirety due to the stress these conditions place on the equipment but rather the stability study was continued following instrument repair.

Upon completion of the stability study the polybutadiene-coated CVD toluene column packing was submitted for carbon analysis along with fresh packing material. These results are given in Table IV. The amount of carbon on the particles did not change due to the harsh chemical conditions of this study. Thus there is no loss of polymeric network or CVD carbon cladding during the stability study. We hypothesize that the slight loss in retention at the very beginning of the stability study is due to aging of the polymer under these extreme chemical conditions.

Overall, these two materials demonstrate remarkable pH stability. As mentioned

TABLE IV
CARBON ANALYSIS RESULTS FOR POLYBUTADIENE-COATED CVD TOLUENE SUPPORT

Source of sample	Carbon (%)
Column head	5.3
Column middle	5.2
Column tail	5.3
Fresh packing	5.3

TABLE V
PHOSPHATE STUDY RESULTS

Material	Coverage (%)
CVD toluene	89.5
Hydrogen-treated CVD toluene	89.3
New CVD toluene	97.7

above, under these conditions silica bonded reversed-phase columns would be completely destroyed, and alumina supports would be seriously compromised. The carbon supports have also been exposed to 5 *M* nitric acid solutions for short periods (100 to 200 column volumes) without notable changes in chromatographic performance.

Determination of exposed zirconia

The purpose of these studies was to determine the fraction of the surface covered by carbon as a function of the CVD conditions. In early work we were able to achieve 85% coverage of the surface by CVD. To date samples in which more than 97% of the surface of the zirconia substrate was covered with carbon have been prepared. We believe that nearly 100% coverage can be achieved by making some additional modifications to the CVD conditions. Phosphate adsorption data for different carbon-clad supports is given in Table V.

It is also possible to use the high-energy phosphate interaction to block the remaining (if any) bare zirconia sites in the same fashion as cyclam or triethylamine are used with bonded phase silica columns to block the residual silanol sites [37–39].

CONCLUSIONS

In conclusion, the CVD carbon-clad zirconia particles behave as a reversed-phase support which shows strong retention of non-polar solutes and has superior alkaline stability compared to silica- and alumina-based reversed-phase supports (especially bonded phase supports). This support also has enhanced retention and unique selectivity with respect to bonded phase or polymer-coated reversed-phase supports. However, even though this material has reasonable chromatographic efficiency for some solutes (alkylbenzenes, alkyl phenyl ethers, phenyl alcohols) there are other solutes which show low efficiencies (alkylphenones, nitroaromatics). Hydrogen treatment of the carbon particles improves the efficiency somewhat, but the fact remains that both the hydrogen-treated and untreated support materials have low loading capacities. This characteristic makes the CVD carbon-clad zirconia less than ideal as a support for reversed-phase liquid chromatography. However, the highly retentive nature and selective character of the bare carbon supports make them good candidates for special purpose separations for solutes that are not well retained or well separated on bonded reversed-phase supports. These supports should also be very useful for preconcentrating solutes from dilute solutions (*i.e.* for solid phase extractions) prior to gas chromatographic or liquid chromatographic separations since

the bare carbon supports are very retentive, especially for the more polar solutes (nitroaromatics, phenols, ketones, alcohols, etc.). This material may also be suitable for other types of chromatographic techniques (such as gas chromatography and supercritical fluid chromatography). In this case the hydrogen-treated CVD support will be the material of choice. The hydrogen-treated material will also be a good base material for supporting a polymer coating since high-energy adsorption sites have been removed or modified by the hydrogen treatment.

Polybutadiene coating of carbon clad-zirconia improves the performance in several ways and its retention characteristics are similar to those of bonded phase supports. Polymer coating increases the efficiency and loading capacity and decreases the retentivity of the support. However, these advantages come at the cost of some loss in selectivity. In addition these materials have excellent chemical and thermal stability. Even with the polybutadiene-coated material we have not achieved reduced plate height values as low as those reported for bonded-phase HPLC. We believe that the poor behavior of alkylphenone solutes is due to interactions of these solutes with the residual high-energy sites on the carbon surface of the particles. It may be possible to improve the efficiency for alkylphenone solutes in two ways; a more uniform layer of polybutadiene may be formed so as to coat the particle more completely leaving no accessible carbon and/or by using the hydrogen treated carbon-clad zirconia as a base for the polymer coating since this material has better efficiency for alkylphenones than does the untreated material.

In subsequent communications we will report on the chromatography on supports with higher levels of carbon coverage, the effect of using different carbon sources, and a more detailed characterization of the chromatographic support.

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