Evaluation of the stability and selectivity for various adjustable stationary phases using zirconium oxide supports in high-performance liquid chromatography

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Abstract

The selectivity and retention properties of a zirconia stationary phase were reversibly altered using various ligands containing Lewis base functional groups. A simple loading procedure allowed a variety of ligands to be attached to the zirconia surface via Lewis interactions. The resulting stationary phases were shown to be stable and produced different selectivity and retention properties from the native zirconia material. The metal oxide adsorbent was converted to a diol-type stationary phase using glucose-6-phosphate for use under normal-phase conditions. Reversed-phase supports were produced by loading either octyl- or octadecylphosphonic acid onto the native zirconia support. The properties of these new phases were then compared to commercially available bonded silica analogs. Ligands bound to the surface in this manner were effectively removed and the native zirconia was regenerated using a dilute base wash procedure. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Preparative separations using various chromatographic media are a useful means for producing high-purity materials for pharmaceutical analysis [1,2]. Reference standards of active drug substance, impurities, enantiomers and even clinical supplies may be readily prepared in high yield and purity via preparative separations. In addition, preparative separations can also be useful for obtaining significant quantities of unknown impurities for further characterization when various hyphenated techniques fail to produce adequate information. Yet, despite the advantages of preparative separations, the high cost of preparative phases is a significant hindrance. A collection of preparative columns, which is necessary to ensure the scale-up of a variety of separations, can require a significant financial investment.

Under ideal circumstances, the desired separations could all be tailored to a single type of stationary phase, thus limiting the number of required preparative phases. In practice, however, this is not often the case and the separation scientist must rely on a number of various stationary phases to perform the desired separations. This is especially true when

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chiral stationary phases are required for chiral separations.

Earlier studies have shown that some types of stationary phases can show markedly different selectivity, depending upon the mobile phase components. Dynamically coated silica stationary phases have shown utility for a variety of separations. Ghaemi and Wall [3,4] used silica, dynamically coated with surfactants, as stationary phases for reversed-phase chromatography. Pawlowska et al. [5,6] demonstrated the enantiomeric separations of benzoin, hexobarbital, and glutethimide, etc., using a permethylated β-cyclodextrin (TM-β-CD) dynamically coated silica column. The main drawbacks to this approach are the stability and reproducibility of the system and system peaks that can interfere with detection of the components of the sample.

Other work has shown that metal oxides, such as zirconia, show different normal-phase selectivities depending upon the loading of a Lewis base onto the Lewis acid sites present on the metal oxide surface [7–9]. Washing a zirconia support with an acetate buffer, for example, results in different selectivity toward neutral analytes than a phosphate buffer washed support. These Lewis base-modified supports are relatively stable and do not produce system peaks observed for dynamically coated silica supports.

Our goal is to use the Lewis interactions between the zirconia stationary phase and selected Lewis base ligands to produce a stable, but reversible, means for attaching a ligand to the stationary phase surface in order to change the selectivity of the zirconia support. In this manner, we aim to take advantage of the desirable physical characteristics of the zirconia (mechanical and chemical stability) while allowing a wide range of ligands to be attached to the surface [10]. Previous studies have shown that various Lewis bases can be readily removed from the zirconia surface by flushing the stationary phase with dilute sodium hydroxide solution [11].

Experimentally, a native zirconia stationary phase can be flushed with a dilute sodium hydroxide solution to remove any Lewis bases acquired by the stationary phase during manufacture or processing. Next, a dilute solution of a desired ligand, containing a Lewis base functional group as an anchor, is flushed through the column to attach the desired ligand to the stationary phase via Lewis interactions.

The column is then flushed with mobile phase to remove any excess ligand. Separations are then performed as desired. After the desired separations are completed, the native zirconia can be regenerated by flushing with dilute sodium hydroxide in preparation for the next application. This technique would allow the separation scientist the opportunity to achieve a wide variety of separations using a single preparative phase whose selectivity is dictated by the choice of the Lewis base ligand.

In this study, we investigate this loading/re-loading mechanism and evaluate the stability and selectivity of the stationary phases produced using a variety of ligands. Glucose-6-phosphate will be used to produce a stationary phase similar in selectivity to a diol-type column for applications under normal-phase conditions. Octyl- and octadecylphosphonic acids will be used to produce hydrophobic reversed-phase supports. The resulting stationary phases will be compared to commercially available silica-based analogs of the desired stationary phases.

2. Experimental

2.1. Equipment

The high-performance liquid chromatography (HPLC) systems used in this study consisted of a Hewlett-Packard HP1100 HPLC system (Wilmington, DE, USA) and a Dionex DX-500 ion chromatography system (Sunnyvale, CA, USA). The HP 1100 was outfitted with an autosampler and a thermostated column compartment. Detection of analytes was at 210 or 220 nm and the data was processed using Hewlett-Packard ChemStation software. The Dionex DX-500 system was used for washing the column with dilute sodium hydroxide solutions and was outfitted with metal-free components.

2.2. Chemicals

All solutes used in this study (Table 1) were obtained from Aldrich (Milwaukee, WI, USA) and were reagent grade or better. Absolute ethanol was obtained from Aaper Alcohol and Chemical (Shelbyville, KY, USA). Water was obtained using a

Table 1
LSER parameters for probe analytes [14–16]

Analyte	$V/100$	π^*	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	R_2
Acetophenone	1.0139	1.01	0.00	0.48	0.818
Anisole	0.9160	0.75	0.00	0.29	0.708
Benzene	0.7164	0.52	0.00	0.14	0.610
Benzonitrile	0.8711	1.11	0.00	0.33	0.742
Benzophenone	1.4808	1.50	0.00	0.50	1.447
Benzyl alcohol	0.9160	0.87	0.33	0.56	0.803
Butyl benzene	1.2800	0.51	0.00	0.15	0.600
p-Chlorophenol	0.8975	1.08	0.67	0.20	0.915
Ethyl benzene	0.9982	0.51	0.00	0.15	0.613
Methyl benzoate	1.0726	0.85	0.00	0.46	0.733
Naphthalene	1.0854	0.92	0.00	0.20	1.340
Nitrobenzene	0.8906	1.11	0.00	0.28	0.871
p-Nitrotoluene	1.0315	1.11	0.00	0.28	0.870
Phenol	0.7751	0.89	0.60	0.30	0.805
3-Phenyl propanol	1.1978	0.90	0.30	0.67	0.821
n-Propyl benzene	1.1391	0.50	0.00	0.15	0.604
Toluene	0.8573	0.52	0.00	0.14	0.601
p-Xylene	0.9982	0.52	0.00	0.16	0.613

Milli-Q water system (Millipore, Milford, MA, USA) with a distilled water feed. Acetonitrile, isopropanol, hexane and tetrahydrofuran were HPLC grade obtained from EM Science (Gibbstown, NJ, USA). Glucose-6-phosophate (disodium salt) was obtained from Sigma (St. Louis, MO, USA). *n*-Octylphosphonic acid (98%) and *n*-octadecylphosphonic acid (98%) were obtained from Alfa Aesar (Ward Hill, MA, USA). Sodium hydroxide (50%) was obtained from EM Science. This concentration of sodium hydroxide (diluted to 0.1 *M* prior to use) is preferred for the washing step due to its low carbonate content since carbonate readily adsorbs onto the zirconia surface via Lewis interactions [12].

2.3. Columns

The ZirChrom PHASE columns used in this study (150×4.6 mm, 3 μm particles) were obtained from ZirChrom Separations (Anoka, MN, USA). A LiChrosorb 5 Diol column (250×4.6 mm, 5 μm particles) was obtained from Phenomenex (Torrance, CA, USA). A Zorbax C$_8$ column (150×4.6 mm, 5 μm particles) and a Zorbax XDB-C$_{18}$ column (150× 4.6 mm, 5 μm particles) were obtained from Hewlett-Packard.

2.4. Chromatographic conditions

Solute solutions were made in ethanol at a final concentration of 0.5 mg/ml. Normal-phase separations were performed using 100% hexane as mobile phase. The column temperature was 25 °C with a flow-rate of 1 ml/min. Reversed-phase separations were performed using a water–acetonitrile (70:30, v/v) mobile phase. The column temperature was 25 °C with a flow-rate of 1 ml/min. Injection volume was 5 μl, and solvent front (ethanol) was used to measure t_0 in both normal-phase and reversed-phase conditions.

2.5. Column preparation

2.5.1. Native zirconia
Native zirconia phases (Zirchrom PHASE) were washed with 50 ml 0.1 *M* sodium hydroxide solution at 0.5 ml/min at 25 °C using a metal-free HPLC system to remove any extraneous Lewis base contaminants from manufacture. The column was then equilibrated with mobile phase at 0.5 ml/min for 100 min at 25 °C prior to beginning each study.

2.5.2. Preparation of Lewis base attached zirconia
Lewis base ligands were loaded onto the base washed zirconia as follows.

(1) Glucose-6-phosphate: A 10 m*M* solution of glucose-6-phosphate in water was pumped onto the column at 0.5 ml/min for 400 min at 25 °C. The column was then flushed with isopropanol at 0.5 ml/min for 100 min at 25 °C. The column was then equilibrated with mobile phase (100% hexane) at 0.5 ml/min for 100 min at 25 °C prior to beginning each study.

(2) Octylphosphonic acid: Octylphosphonic acid was loaded onto the column by pumping a 10 m*M* solution of octylphosphonic acid in water–acetonitrile (50:50) onto the column at 0.5 ml/min for 400 min at 25°C. The column was then flushed with water–acetonitrile (70:30) at 0.5 ml/min for 100 min at 25 °C prior to beginning each study.

(3) Octadecylphosphonic acid: A 10 m*M* solution of octadecylhosphonic acid in tetrahydrofuran was pumped onto the column at 0.5 ml/min for 400 min at 25 °C. The column was then flushed with water–

acetonitrile (70:30) at 0.5 ml/min for 100 min at 25 °C prior to beginning each study.

2.5.3. Removal of attached Lewis ligands

Glucose-6-phosphate and octylphosphonic acid were removed from the zirconia stationary phase by washing with 50 ml 0.1 *M* sodium hydroxide solution at 0.5 ml/min at 25 °C using a metal-free HPLC system. The regenerated zirconia was tested using the same solute set. Similar retention properties were observed comparing to the retention properties of the original, unmodified native zirconia. The regenerated zirconia was subsequently used for other Lewis ligand loading studies, e.g., octadecylphosphonic acid.

Due to the hydrophobicity of octadecylphosphonic acid, the typical 0.1 *M* sodium hydroxide solution was not effective for removing the loaded C_{18}-phosphate moiety. Experimentation indicated that this limitation was readily overcome by using a mixture of tetrahydrofuran and 0.1 *M* sodium hydroxide (50:50, v/v) to regenerate the zirconia column.

2.6. Linear solvation energy relationship (LSER) analysis

The properties of the ligand substituted zirconia columns were quantitatively compared to their bonded silica analogs using LSERs [13]. In these comparisons, the solvatochromic parameters for the probe analytes were related to the log of their retention factor using the following equation:

$$\log k' = SP_0 + \nu V_x + rR_2 + s\pi^* + a\sum \alpha_2^H + b\sum \beta_2^H$$
(1)

where SP_0 is the regression intercept [13], V_x is McGowan's characteristic volume, R_2 is the excess molar refraction, π^* is the dipolarity/polarizability, $\sum \alpha_2^H$ is the solute hydrogen bond donating ability and $\sum \beta_2^H$ is the solute hydrogen bond accepting ability. The coefficients ν, r, s, a and b represent the differences between the solute–stationary phase interaction and the solute–mobile phase interaction. The solvatochromic parameters of the analytes used in this study are given in Table 1.

3. Results and discussion

3.1. Normal-phase separations

As a reference, the chromatographic properties of the native zirconia column used in this study were examined under normal-phase conditions prior to any surface modification. Like other metal oxides, namely alumina, the chromatographic properties using normal-phase conditions are very dependent upon the amount of water present at the adsorbent surface. During these evaluations, no attempt was made to activate the stationary phase by removing adsorbed water. Therefore, all data reported here presumably represents a water-saturated surface equilibrated to the water content of the mobile phase.

3.1.1. Retention and selectivity

The retention factors for a variety of neutral analytes obtained on the native zirconia column are given in Table 2. The data showed that neutral analytes were weakly retained on the native zirconia. Analytes with hydrogen bonding accepting functional groups (carbonyl, cyanyl and nitro groups) were more retained than the neutral analytes. Hydrogen bond donor analytes (benzyl alcohol, phenol and *p*-chlorophenol) were not eluted using pure hexane as the mobile phase due to their strong interactions with the stationary phase and the poor eluotropic strength of hexane towards these types of interactions.

After loading glucose-6-phosphate (G-6-P), the selectivity of the newly formed stationary phase did not change considerably, but the retention was generally higher for the eluted analytes (Table 2). The same solutes were analyzed using a LiChrosorb 5 Diol column under identical chromatographic conditions (Table 2). Similar retention properties were observed for both stationary phases.

Examination of the peak profile of selected solutes (Fig. 1) shows that both the native zirconia and G-6-P loaded zirconia produced efficient, symmetric peaks for non-hydrogen bond donor analytes. The peak shape on the diol column was sharp, but tailed, presumably due to a void which may have formed in this particular column. For both the native zirconia and G-6-P loaded zirconia, the overall selectivity was nearly the same as a conventional silica-based

Table 2
Retention factors obtained under normal-phase conditions for native zirconia, glucose-6-phosphate loaded zirconia and diol silica

Analyte	k'		
	Native zirconia	G-6-P loaded zirconia	Diol silica
Acetophenone	1.53	2.21	1.67
Anisole	0.16	0.26	0.25
Benzene	0.05	0.11	0.04
Benzonitrile	1.05	1.47	1.46
Benzophenone	1.58	2.32	1.77
Benzyl alcohol	eno	eno	eno
Butyl benzene	0.02	0.11	0.01
p-Chlorophenol	eno	eno	eno
Ethyl benzene	0.03	0.05	0.02
Methyl benzoate	0.79	1.00	0.83
Naphthalene	0.11	0.16	0.20
Nitrobenzene	0.47	0.79	0.82
p-Nitrotoluene	0.79	0.89	0.80
Phenol	eno	eno	eno
3-Phenyl propanol	eno	eno	eno
n-Propyl benzene	0.04	0.05	0.02
Toluene	0.04	0.05	0.04
p-Xylene	0.04	0.05	0.03

eno indicates that elution was not observed. Elution conditions: 100% hexane at 1 ml/min at 25 °C.

diol column, but the retention was slightly higher using the G-6-P loaded zirconia.

3.1.2. LSER comparison of phases

Regression of the retention factors for each of the analytes in Table 2 against their solvatochromic parameters (Table 1) using Eq. (1) allows a quantitative comparison of the G-6-P zirconia and silica diol phases. The regression coefficients given in Table 3 showed that the only statistically significant difference between the two phases was the interactions of the analytes with the stationary phases via dipolarity/ polarizability interactions. Inclusion of other solvatochromic fit parameters, shown in Eq. (1), did not improve the quality of the fits. All other analyte–stationary phase interactions were quite similar using this limited solute set, although the elution of hydrogen bond donor solutes was not observed using either stationary phase under these conditions.

Addition of a polar modifier to the mobile phase would promote elution of the hydrogen bond donor solutes analytes at the expense of near-zero retention factors for the other analytes. The exclusion of the important hydrogen bonding analytes from the regression prevented a rigorous LSER analysis of the phases, but since the same limited set of analytes were used for both phases, a crude comparison between the phases is possible.

3.1.3. Stability of the G-6-P zirconia

The stability of the phase is essential to the use of a modified support, especially for preparative separations. Changes in retention require adjustment of the elution conditions and degradation of the stationary phase results in decomposition products accumulating with the desired product. Fig. 2 shows the retention stability for benzophenone on G-6-P loaded zirconia. After an initial shift in retention, presumably due to equilibration of the water content of the support, the retention factors obtained using G-6-P modified zirconia were stable up to 2500 column volumes. This indicates that the Lewis base ligands are securely anchored to the Lewis acid sites on the zirconia surface. The duration of stability, which is far in excess of that is normally required for preparative separation projects, indicated the long-term stability of the support for analytical or process-scale applications.

3.2. Reversed-phase separations

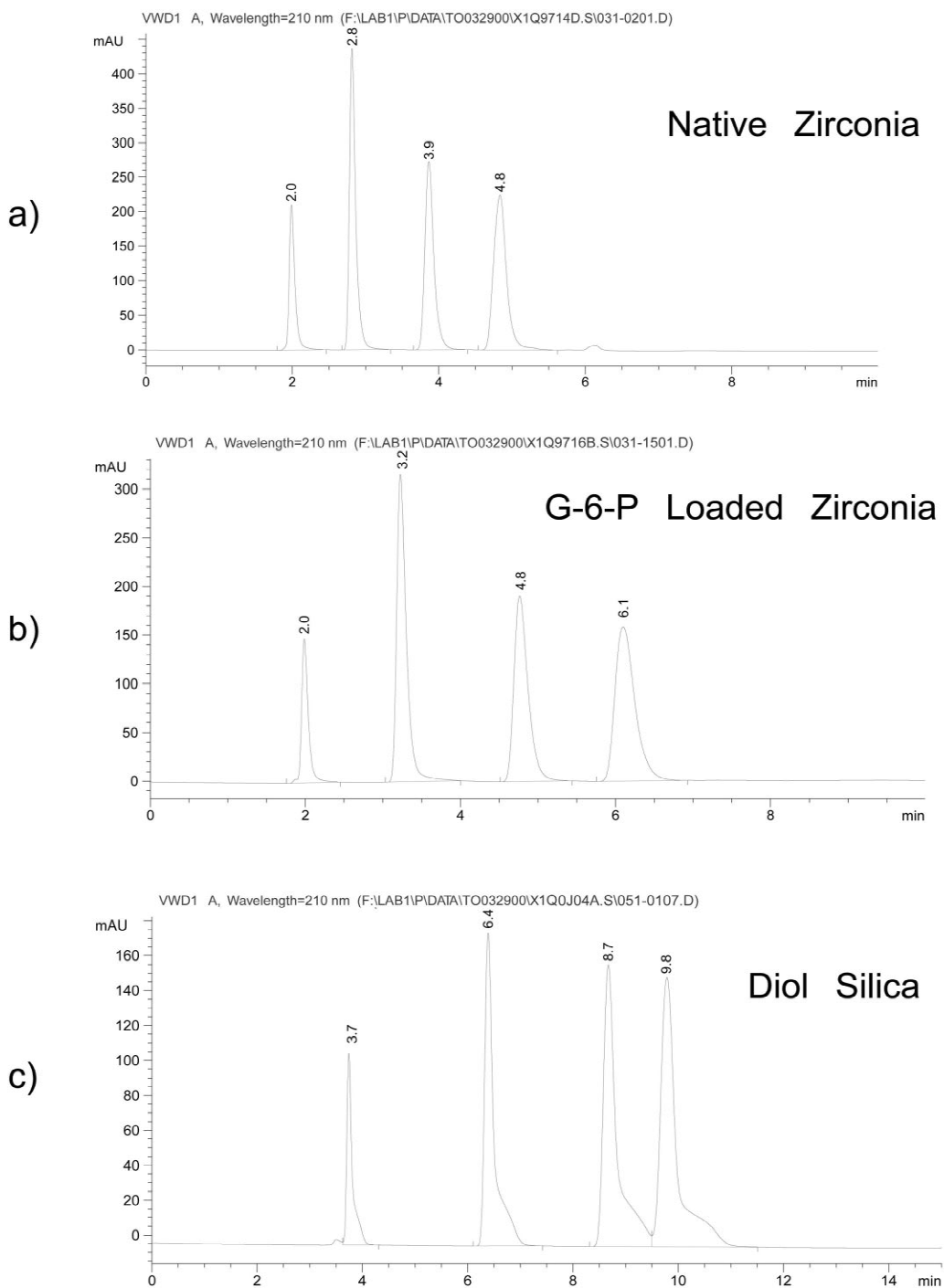The retention properties of the native zirconia

Fig. 1. Comparison of peak profiles for: (a) native zirconia, (b) G-6-P loaded zirconia and, (c) diol silica. Analytes (in order of elution) are: benzene, nitrobenzene, benzonitrile and benzophenone. Mobile phase consisted of hexane at a flow-rate of 1.0 ml/min at 25 °C. Detection was at 210 nm.

Table 3
LSER comparison of G-6-P zirconia and silica diol

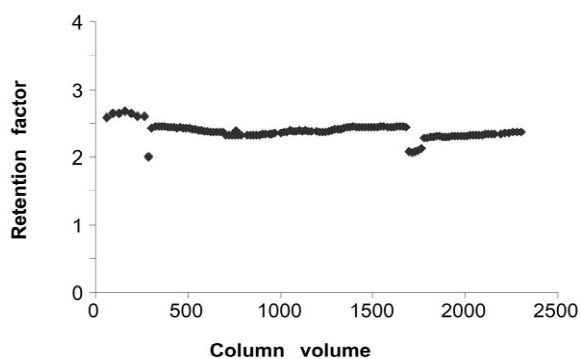|	G-6-P zirconia	Diol silica
SP_0	−1.28±0.17	−1.47±0.20
B	3.06±0.37	3.02±0.45
V	−1.09±0.20	−1.41±0.20
S	1.20±0.16	1.66±0.19
r^2	0.991	0.991

Fig. 2. Retention stability of benzophenone on G-6-P loaded zirconia. Mobile phase consisted of hexane at a flow-rate of 1.0 ml/min at 25 °C. Detection was 210 nm.

under reversed-phase conditions are given in Table 4. Using a water–acetonitrile (70:30, v/v) mobile phase, the analytes were not retained. This is not surprising since the native zirconia surface is not expected to show any hydrophobic characteristics.

3.2.1. Retention and selectivity of octylphosphonic acid modified zirconia

When octylphosphonic acid was loaded onto the native zirconia, the selectivity and retention properties of the stationary phase changed considerably. The newly formed zirconia stationary phase (C_8-Z) exhibited reasonable separations for hydrophobic analytes (Table 4), which were not retained using native zirconia. Retention of all the analytes increased significantly with non-polar, hydrophobic analytes being most retained. The retention factor of benzophenone is 2.6 on C_8-Z, compared to 0.0 on the native zirconia support. This indicates that the phosphate moiety of octylphosphonic acid was anchored to the zirconia surface by the ligand exchange interaction. As a result, C_8-Z displayed retention characteristics of a bonded octyl-silica stationary phase due to the hydrophobic interactions between the octyl alkyl chain and the non-polar solutes.

Table 4
Retention factors obtained under reversed-phase conditions for native zirconia, *n*-octylphosphonic acid loaded zirconia and octyl silica

Analyte	k'		
	Native zirconia	C_8 loaded zirconia	C_8 silica
Acetophenone	0.00	0.72	5.56
Anisole	0.01	1.13	12.71
Benzene	0.03	1.10	13.04
Benzonitrile	0.00	0.82	6.65
Benzophenone	0.00	2.65	41.29
Benzyl alcohol	0.01	0.36	1.59
Butyl benzene	0.00	4.70	281.3
p-Chlorophenol	0.01	1.58	8.85
Ethyl benzene	0.03	2.56	57.16
Methyl benzoate	0.00	1.14	11.29
Naphthalene	0.01	2.81	53.86
Nitrobenzene	0.01	1.07	10.24
p-Nitrotoluene	0.02	1.65	20.18
Phenol	0.03	0.52	2.63
3-Phenyl propanol	0.01	0.89	4.74
n-Propyl benzene	0.03	3.54	122.53
Toluene	0.02	1.66	26.24
p-Xylene	0.02	2.48	55.67

Elution conditions: water–acetonitrile (70:30) at 1 ml/min at 25 °C.

The same set of analytes was chromatographed on a Zorbax C_8 column for comparison. The retention factors of the analytes are given in Table 4. The retention factors were generally much higher on the bonded octyl-silica stationary phase, especially for the non-polar analytes, than on the octylphosphonic acid loaded zirconia. For example, the capacity factor of toluene was 26.2 on Zorbax C_8 compared to 1.66 on the C_8-Z. It is likely that the ligand loading density on the C_8-Z is far lower than that on the Zorbax C_8 column. Studies have shown that the retention factors are related to bonding density of the alkyl chain on the silica surface [17]. At low bonding densities, non-polar solute partitioning and retention will increase linearly with increasing alkyl chain bonding density [18]. Since the maximum coverage of alkyl ligands on the zirconia surface is limited by the surface density of the Lewis acid sites, the final alkyl ligand density is likely to be lower than that possible for the direct silylation of surface hydroxyls on the silica surface. Meanwhile, the surface area of the zirconia support in the experiment is 21 m^2/g, which is much lower than that of the Zorbax silica support (300 m^2/g). Although the packing density of zirconia is about 3–4 times higher than that of silica [19], a 21 m^2/g zirconia has a surface area equivalent to 63–84 m^2/g silica. Due to the difference in surface area, the stationary phase volume of the C_8-Z (V_s) would be lower than that of the Zorbax C_8 column [20]. This could also contribute to the lower retention factors observed on the C_8-Z comparing to the Zorbax C_8.

Examination of the peak profile shows that the Zorbax C_8 produces very symmetric peaks for most of the solutes, the asymmetry factors are in the range of 0.9 to 1.1. On the C_8-Zirconia, the solutes exhibit tailing peaks, the asymmetry factors are from 1.1 to 1.4. This may also be due to the lower ligand density on the zirconia surface.

3.2.2. LSER comparison of phases

An LSER comparison was made between the octylphosphonic acid-modified zirconia and the Zorbax C_8 phases. Table 5 shows that both phases showed strong dependences on the same solvatochromic parameters. Solute hydrogen bond accepting ability, characteristic volume and diplarity/polarizability were similar in influence between the phases,

Table 5
LSER comparison of octylphosphonic acid loaded zirconia and Zorbax C_8

	C_8 loaded zirconia	Zorbax C_8
SP_0	-0.71 ± 0.09	-0.20 ± 0.14
B	-1.49 ± 0.11	-2.74 ± 0.18
V	1.34 ± 0.09	2.57 ± 0.14
S	-0.05 ± 0.06	-0.39 ± 0.10
r^2	0.981	0.976

but differed in relative magnitude. This may be a consequence of the difference in ligand density postulated based on the overall retention properties of the two phases and the lower surface area of the zirconia phase. It should be noted that inclusion of additional LSER parameters (from Eq. (1)) did not improve the quality of the regression fits of the data.

3.2.3. Stability of the octylphosphonic acid modified zirconia

The stability of the C_8-Z phase is shown in Fig. 3. Under these conditions, the retention for benzophenone decreased gradually until approximately 1500 column volumes of mobile phase had passed through the column, after which, the retention remained relatively constant. This is likely due to leaching of some octylphosphonic acid which had been adsorbed to the ligated octylphosphonic acid via hydrophobic interactions. During the stability evaluation, this weakly bound material is washed off by the water–acetonitrile (70:30, v/v) mobile phase.

3.2.4. Reversible loading
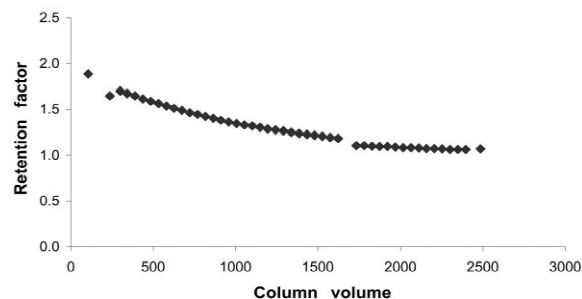
The Lewis base (octylphosphonic acid) on the

Fig. 3. Retention stability of *p*-nitrotoluene on octylphosphonic acid loaded zirconia. Mobile phase was water–acetonitrile (70:30) with a flow-rate of 1.0 ml/min at 25 °C. Detection was at 220 nm.

C_8-Z stationary phase was washed off with dilute sodium hydroxide solution. The retention properties of the regenerated native zirconia phase were again evaluated under the same reversed-phase conditions used prior to loading the octylphosphonic acid (Table 6). The retention factors were very similar to those on the native zirconia obtained previously (Table 5). This data indicated that the base washing procedure effectively removed the Lewis base ligands attached to the zirconia surface.

3.2.5. Retention and selectivity of octadecylphosphonic acid modified zirconia

Retention of the neutral analytes increased dramatically when the regenerated zirconia column was loaded with octadecylphosphonic acid (Table 6). The retention factor of benzophenone is 7.0 on the C_{18}-Z, 2.6 on the C_8-Z and 0.0 on the native zirconia stationary phase (Fig. 4). This further demonstrates that the alkylphosphonic acids attached to the zirconia surface. The neutral solutes interacted strongly with the octadecyl chains on the modified stationary phase (C_{18}-Z) and showed much longer retention factors than observed using the octyl ligand (Table 6). The retention factors of the analytes on the

Zorbax C_{18} column under the same mobile phase conditions are also given in Table 6. The neutral analytes are retained much longer than on the octadecyl-ligated zirconia. Again, we contribute this lower retention on C_{18}-Z phase to the lower alkyl chain loading density on the zirconia surface and the lower surface area of the zirconia support.

The peak shapes on the Zorbax C_{18} are very symmetric, the asymmetry factors are in the range of 0.9 to 1.1. However, on the C_{18}-zirconia, the solutes show severe tailing peaks, the asymmetry factors are from 1.4 to 1.8. Again, this may be due to the lower ligand loading density on the C_{18}-Z.

3.2.6. Stability of the octadecylphosphonic acid modified zirconia

The retention stability of the octadecylphosphonic acid-loaded zirconia is shown in Fig. 5. Retention of benzophenone was relatively constant to over 2500 column volumes. This indicates that the octadecyl phosphonic ligands are securely attached to the Lewis acid sites on the zirconia surface. The initial loss of hydrophobic ally adsorbed excess octadecyl ligands was not observed since the loading solution

Table 6
Retention factors obtained under reversed-phase conditions for regenerated zirconia, *n*-octadecylphosphonic acid loaded zirconia and octadecyl silica

Analyte	k'		
	Regenerated zirconia	C_{18} loaded zirconia	C_{18} silica
Acetophenone	0.02	0.45	5.13
Anisole	0.03	1.35	13.40
Benzene	0.03	1.95	14.80
Benzonitrile	0.03	1.15	5.92
Benzophenone	0.10	7.02	44.67
Benzyl alcohol	0.03	0.46	1.33
Butyl benzene	0.48	eno	eno
p-Chlorophenol	0.14	2.61	6.87
Ethyl benzene	0.12	7.79	71.78
Methyl benzoate	0.04	1.99	11.47
Naphthalene	0.12	9.31	70.27
Nitrobenzene	0.04	1.78	9.67
p-Nitrotoluene	0.05	3.54	20.87
Phenol	0.07	0.69	2.11
3-Phenyl propanol	0.03	1.46	4.25
n-Propyl benzene	0.23	15.45	170.33
Toluene	0.06	4.03	33.33
p-Xylene	0.11	7.98	76.07

eno indicates that elution was not observed. Elution conditions: water–acetonitrile (70:30) at 1 ml/min at 25 °C.

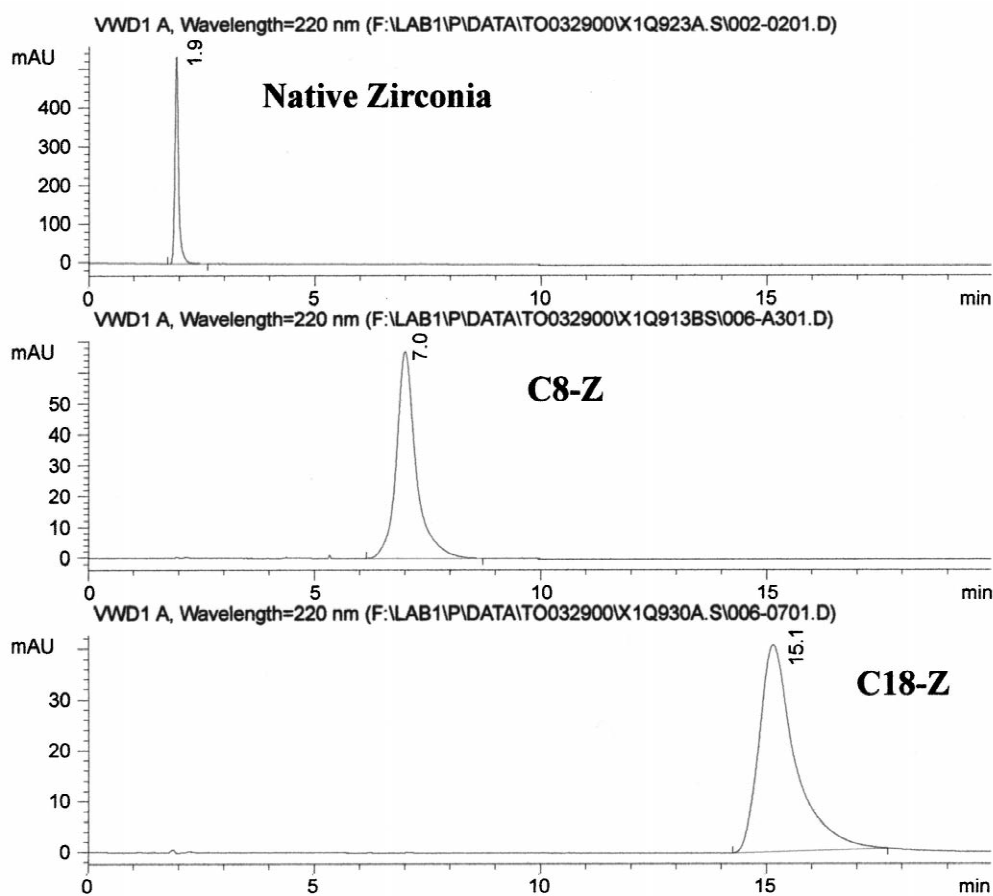Fig. 4. Chromatogram of benzenephenone on the native zirconia, C_8-Z and C_{18}-Z stationary phases.

contained a good solvent (tetrahydrofuran) to prevent this inadvertent process. The newly modified C_{18}-Z stationary phase showed long term stability in the
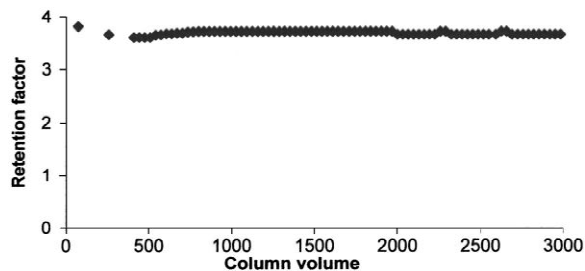

Fig. 5. Retention stability of _p_-nitrotoluene on octa-decylphosphonic acid loaded zirconia. Mobile phase was water–acetonitrile (70:30) with a flow-rate of 1.0 ml/min at 25 °C. Detection was at 220 nm.

reversed-phase conditions indicating suitability for preparative and/or process scale separation use.

3.2.7. LSER comparison with Zorbax C_{18}

The octadecyl ligated zirconia support was compared to the Zorbax C_{18} phase using the LSER technique applied to the earlier phases. Table 7

Table 7
LSER comparison of octadecylphosphonic acid loaded zirconia and Zorbax C_{18}

	C_{18} loaded zirconia	Zorbax C_{18}
SP_0	-0.92 ± 0.12	-0.27 ± 0.18
B	-2.26 ± 0.16	-3.01 ± 0.24
V	2.30 ± 0.14	2.87 ± 0.18
S	-0.280 ± 0.09	-0.54 ± 0.13
r^2	0.985	0.968

shows that the results are very similar to those obtained for the octyl ligated zirconia support. Solute hydrogen bond accepting ability, characteristic volume and dipolarity/polarizability all are significant factors governing retention although the magnitude of the coefficients for the octadecyl ligated zirconia are smaller in magnitude than those obtained using the Zorbax C_{18} support. Again, the differences in alkyl ligand density at the support surface and the surface area of the stationary phases are likely the source of this difference. Inclusion of additional LSER fit parameters failed to improve the quality of the fits.

4. Conclusion

A variety of Lewis base ligands were loaded onto the Lewis acid sites of the zirconia surface. The modified stationary phases showed different selectivity and retention properties compared to the native support. Under normal-phase conditions, analytes were more retained on the glucose-6-phosphate phase compared to the native zirconia. The selectivity of the glucose-6-phosphate loaded zirconia was similar to a conventional silica-based diol column, although retention was slightly higher.

Under reversed-phase conditions, octylphosphonic acid and octadecylphosphonic acid were loaded on the native zirconia to form hydrophobic stationary phases. The selectivity and retention properties of the modified stationary phases were much different from the native zirconia phase. Compared to conventional silica-based C_8 and C_{18} columns, the retention factors of the modified zirconia phases were relatively low due to the lower loading density of the alkyl ligands on the zirconia surface and the lower surface area of the zirconia support.

These Lewis base loaded zirconia stationary phases were stable up to 2500 column volumes of mobile phase. The loading process was demonstrated to be reversible and renewable. The technique dem-

onstrated here offers chromatographer the opportunity to achieve different selectivities on a single type of stationary phase. This has the potential of dramatically lowering the cost of various types of columns required for analytical and preparative applications. Future work will be focused on improving the ligand coverage on zirconia surface by different loading process, as well as investigating the applications of other Lewis base ligands, particularly in the area of chiral separations.

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