

A Comparative Study of the Chromatographic Selectivity of Polystyrene-Coated Zirconia and Related Reversed-Phase Materials

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The chromatographic selectivities of polystyrene-coated zirconia (PS-ZrO₂) have been investigated in detail by means of homologue series retention studies and studies of a variety of nonpolar aromatic positional isomers. This material has been compared to polybutadiene-coated zirconia, octadecyl- and phenyl-bonded silica, and polymer-based reversed phases. In addition, the separations of a wide variety of compound classes on PS-ZrO₂ were compared with those on other aromatic and aliphatic RPLC phases. The PS-ZrO₂ material exhibits substantial differences in selectivity toward nonpolar and polar analytes as compared to other phases, especially with respect to polybutadiene-coated zirconia (PBD-ZrO₂). On PS-ZrO₂, polynuclear aromatic hydrocarbons (PAHs) are retained much more than are alkylbenzenes, whereas on PBD-ZrO₂ the opposite is observed. In addition, PS-ZrO₂ has much greater selectivity than does PBD-ZrO₂ for the separation of structural isomers which differ in the position of phenyl groups. In general, even though the carbon content of PS-ZrO₂ is much lower than that of conventional reversed-phase materials, such as octadecyl-bonded silica (C18-SiO₂), polar analytes exhibit sufficient retention and differential selectivity on PS-ZrO₂ that resolution comparable to that on C18-SiO₂ can be obtained but with substantially improved analysis times.

There has been a great deal of interest in polymer-coated metal oxide supports for HPLC^{1–12} because such materials can overcome a number of problems in using silica-based materials at extreme pHs and high temperatures.^{13–19} Zirconia, due to its superb

chemical and thermal stability, is one of the major alternatives to silica.^{5,7,11,20–22} Numerous studies have shown that zirconia-based materials can be used over a pH range from 0 to 14^{11,22} and that separations on PBD-ZrO₂ at 200 °C can be achieved in seconds.^{20,21}

Previously we reported on the synthesis and evaluation of a novel aromatic polymer-coated zirconia HPLC support.¹¹ The actual polymer coated on the zirconia was a copolymer of chloromethylstyrene and vinylmethylsilane. For simplicity, we refer to this material as polystyrene-coated zirconia (PS-ZrO₂). Our prior physical characterization of PS-ZrO₂ indicated that the coated zirconia maintains the well-defined pore structure of the bare material and exhibits good mass transfer characteristics. Furthermore, the new material is stable at extreme pHs (pH = 1–13) and at temperatures as high as 160 °C. On the basis of the study of linear solvation energy relationships (LSERs) on PS-ZrO₂ and other aromatic and aliphatic reversed-phase materials,²³ we showed that PS-ZrO₂ has substantially different retention characteristics when compared with PBD-ZrO₂ and is more similar to polystyrene-divinylbenzene type phases (e.g., PRP-1) in terms of molecular polarizability than it is to aliphatic or even aromatic bonded phases.

In chromatographic method development, it is very common practice to optimize resolution. Resolution is controlled by the selectivity factor (α), plate number (N), and retention factor (k'), and among these parameters, the selectivity factor has the most significant effect on resolution.²⁴ As a result, resolution can be optimized by tuning the selectivity by changing the eluent type, the stationary-phase type, the eluent composition, and sometimes the temperature. In fact, eluent and stationary-phase types are the two most effective variables for modulating the selectivity.²⁵ Even

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Table 1. The Physical Properties of Different Stationary Phases

column	dimension	particle size (μm)	surface area (m^2/g)	pore size, (\AA)	carbon content (%)
PRP-1	$15 \times 0.46 \text{ cm}$	10	415	80	<i>a</i>
PS-ZrO ₂	$10 \times 0.46 \text{ cm}^b$	2.5	30	200	2
PBD-ZrO ₂	$15 \times 0.46 \text{ cm}$	3	30	300	3
phenyl-SiO ₂	$15 \times 0.46 \text{ cm}$	5	180	80	5
C18-SiO ₂	$5 \times 0.46 \text{ cm}$	5	180	80	10

^a Not suitable for comparison. ^b A 5-cm column packed in this laboratory was used in the separation of steroids.

though the eluent composition is easy to adjust, it has a smaller effect on the *effective* chromatographic selectivity than does the type of eluent and stationary phase, that is, changing the composition of eluent usually produces fewer changes in band spacing than does changing the type of mobile or stationary phase.

The purpose of the present study is to further investigate differences in the effective chromatographic selectivity between PS-ZrO₂ and related RPLC phases using a wide variety of compounds, such as aromatic isomers, polynuclear aromatic hydrocarbons (PAHs), triazine herbicides, phenolic compounds, and steroids. Although PS-ZrO₂ is much less hydrophobic than either PBD-ZrO₂ or C18-SiO₂ when examined in terms of the free energy of retention of a methylene group,¹¹ the unique differences in the effective selectivity on PS-ZrO₂ are the major points of this work. High selectivity is important and useful per se in that a more selective phase does not need high carbon loading to achieve a specified resolution and thus a shorter analysis time can result.

EXPERIMENTAL SECTION

Reagents. The chemicals, reagent-grade or better, used in this study were obtained from Aldrich (Milwaukee, WI), unless otherwise specified. Acetonitrile (ACN), 2-propanol (IPA) and tetrahydrofuran (THF) were HPLC grade and obtained from Mallinckrodt (Paris, KY), while HPLC water was produced by treatment of house deionized water through use of a Barnstead/Thermolyne (Dubuque, IA) Nanopure water purification system with an organic-free final cartridge followed by a 0.2- μm particle filter. PAHs were donated by the National Institute of Standards and Technology (NIST) (Gaithersburg, MD). Triazine herbicides were purchased from Supelco (Bellefonte, PA), while steroids were from Calbiochem (San Diego, CA).

Analytical Columns. The physical properties of five types of reversed-phase materials examined in this work are summarized in Table 1. The sources of the columns are as follows. A Zorbax SB-Phenyl column was a gift from Hewlett-Packard (Wilmington, DE) while a PBD-ZrO₂ column was donated by ZirChrom Separation (Anoka, MN). A Hamilton PRP-1 column was purchased from Chrom Tech (Apply Valley, MN). A Zorbax SB-C18 column was packed in this laboratory by an upward stirred slurry method, while a PS-ZrO₂ column was packed by Alltech (Deerfield, IL) using the polychloromethylstyrene-vinylmethylsilane coated zirconia particles made in this laboratory by the method described previously.²³ The dimensions of all columns used are also given in Table 1.

Chromatographic Conditions. A Hewlett-Packard 1100 liquid chromatograph equipped with a vacuum degasser, a quaternary

pump, an autosampler, a thermostated-column compartment, a variable-wavelength UV detector, and a computer-based HP ChemStation were used to carry out the chromatographic measurements. All chromatographic experiments were made in duplicate at 30 °C at a flow rate of 1 mL/min with UV detection at 254 nm unless otherwise noted. The dead time was determined using acetone for PS-ZrO₂ and PBD-ZrO₂ and uracil for PRP-1, Phenyl-SiO₂, and C18-SiO₂. The injection volume was 1–2 μL with a concentration of 1–2 mg/mL of the analyte dissolved in pure acetonitrile.

RESULTS AND DISCUSSION

Hydrophobic Selectivity. In RPLC, the hydrophobic selectivity can be characterized by the slope of the linear relationship between the logarithm of retention factor (*k'*) and the number of methylene groups (*n*_{CH₂}) for a homologue series of compounds,²⁶ as indicated by the Martin equation

$$\log k' = A + Bn_{\text{CH}_2} \quad (1)$$

Here *A* is the intercept, or the absolute retention of the first compound of the homologue series, and varies with terminal functionality of the series. *B* is related to the free energy of transfer per methylene group ($\Delta G_{\text{CH}_2}^\circ = -2.3RTB$) from the mobile phase to the stationary phase and should be and is often, but not invariably, found to be independent of the homologue series in question.²⁶

Five homologue series (three aromatic and two aliphatic) were studied on five different stationary phases in two different mobile-phase compositions. Most but not all plots of log *k'* vs *n*_{CH₂} were linear with the correlation coefficients (*R*²) better than 0.99. Nonlinearity was observed for the alkyl acetates and nitro alkanes on PBD-ZrO₂ and PS-ZrO₂ in 50/50 acetonitrile/water. This is probably due to the very low retention of solutes with *n*_{CH₂} below 3 in this eluent. As the water content in the eluent was increased from 50 to 70% (v/v), we did observe better correlation coefficients. It has been reported, for some homologue series, that log *k'* vs *n*_{CH₂} plots are nonlinear for *n*_{CH₂} values below 3–5 due to the effect of a functional group of the series.²⁶

Effect of the stationary phase on $\Delta G_{\text{CH}_2}^\circ$. Table 2 summarizes the $\Delta G_{\text{CH}_2}^\circ$ values for these homologue series in 50/50 acetonitrile/water. We observe in Figure 1 that for a given homologue series, the $\Delta G_{\text{CH}_2}^\circ$ value varies with the type of stationary phase. The average $\Delta G_{\text{CH}_2}^\circ$ value of the five homologues becomes more positive in the order of C18-SiO₂ \approx PRP-1 > PBD-ZrO₂ > PS-ZrO₂ > Phenyl-SiO₂. This indicates a decrease in hydrophobicity in the above order for these phases. Given the fact that the $\Delta G_{\text{CH}_2}^\circ$ values from the gas phase to bulk hexadecane and toluene are very similar (–685 cal/mol and –697 cal/mol, respectively²⁷), the $\Delta G_{\text{CH}_2}^\circ$ values for the aliphatic phases are predicted by a simple partition model to be close to those for the aromatic phases; but we observe quite different values. If we only compare inorganic oxide based stationary phases (see Figure 1), for all homologue series studied, the aliphatic phases always have a greater value of $\Delta G_{\text{CH}_2}^\circ$ than do the aromatic phases. This trend

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Table 2. Summary of the $\Delta G_{\text{CH}_2}^\circ$ Values (cal/mol) for Five Homologue Series on Five Stationary Phases^a

homologue series	C18-SiO ₂	PRP-1	PBD-ZrO ₂	PS-ZrO ₂	phenyl-SiO ₂	average	median
alkyl phenones ^b	-322 ± 7	-340 ± 18	-326 ± 17	-242 ± 17	-215 ± 7	-289	-322
alkyl acetates ^c	-321 ± 6	-316 ± 3	-328 ^g	-253 ^g	-228 ± 3	-289	-316
nitro alkanes ^d	-307 ± 1	-312 ± 1	-298 ^g	-229 ^g	-223 ± 3	-274	-298
alkyl benzenes ^e	-311 ± 4	-294 ± 4	-287 ± 3	-201 ± 1	-197 ± 1	-258	-287
alkyl anilines ^f	-303 ± 3	-293 ± 1	-248 ± 4	-205 ± 3	-189 ± 1	-248	-248
median	-309	-303	-293	-223	-206		
average	-313	-311	-297	-226	-210		
av deviation ^h	7	14	24	18	14		
range	19	47	80	42	39		

^a Based on the retention data in 50/50 acetonitrile/water at 30 °C and calculated by $\Delta G_{\text{CH}_2}^\circ = -2.3RTB$. R is the gas constant in cal mol⁻¹ K⁻¹. T is the temperature in K. B is the slope in eq 1. ^b The n_{CH_2} values are 1–4. ^c The n_{CH_2} values are 1–5. ^d The n_{CH_2} values are 1–3, 5, 6. ^e The n_{CH_2} values are 0–4. ^f The n_{CH_2} values are 0–3. ^g As a result of the nonlinearity of the whole data set, only the last two solutes in the homologue were used and, hence, the standard error is not available. ^h The average of deviations from the mean value.

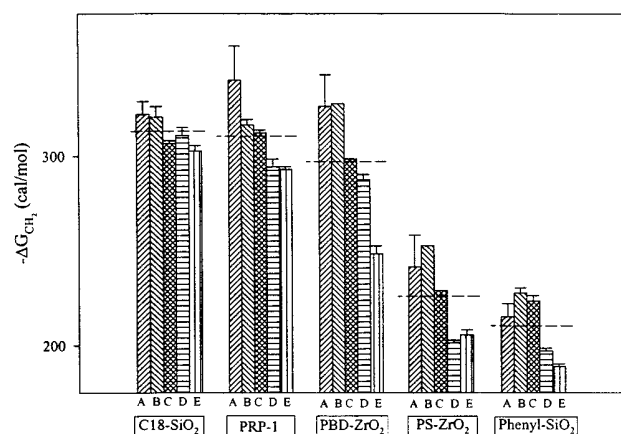


Figure 1. Comparison of the free energy of transfer per methylene group for different homologue series on a given stationary phase. The dashed line denotes the average $\Delta G_{\text{CH}_2}^\circ$ from all homologue series. Conditions: 50/50 acetonitrile/water. (A) Alkyl phenones; (B) Alkyl acetates; (C) Nitro alkanes; (D) Alkyl benzenes; (E) Alkyl anilines.

agrees with our previous LSER studies of these phases.²³ The LSER model^{28–30} is given below:

$$\log K = \log K_0 + v V_2 + s \pi_2^* + a \Sigma \alpha_2^H + b \Sigma \beta_2^H + r R_2^2$$

Here $\log K_0$ is the regression intercept, V_2 , π_2^* , $\Sigma \alpha_2^H$, $\Sigma \beta_2^H$, and R_2 are solute descriptors corresponding to the solute's size, dipolarity/polarizability, hydrogen-bond (HB) acidity, HB basicity, and excess molar refraction, respectively. The fitting coefficients v , s , a , b , and r reflect the complementary properties of the mobile and stationary phases. In general, for a homologue series, only the solute's size ($V_2/100$) increases substantially as the number of methylene groups increases. As a result, $\Delta G_{\text{CH}_2}^\circ$ of a homologue series is closely related to the v coefficient of the mobile-stationary pair. In our previous study²³ of the retention characteristics of different stationary phases, smaller v coefficients were observed for the aromatic phases (PS-ZrO₂ and Phenyl-SiO₂) as compared to those for the aliphatic phases (PBD-ZrO₂ and C18-

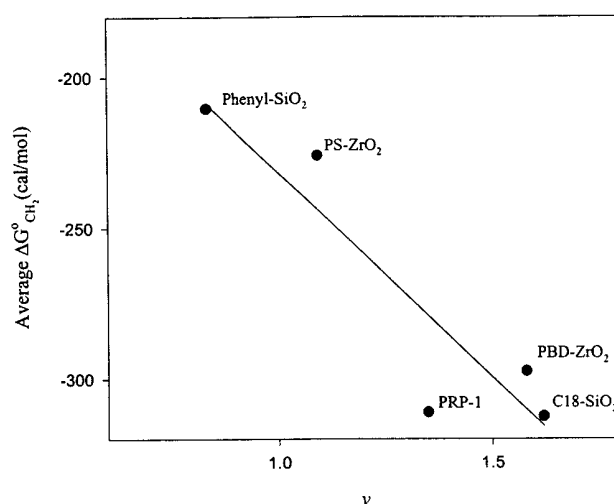


Figure 2. A plot of average $\Delta G_{\text{CH}_2}^\circ$ vs the LSER coefficient (v) for five stationary phases studied in 50/50 acetonitrile/water. The solid line is the least-squares line of all data points.

SiO₂). A correlation between the average $\Delta G_{\text{CH}_2}^\circ$ value and the v coefficient for the stationary phases studied is shown in Figure 2. Given that the miscibility of toluene and acetonitrile is much higher than that of hexane and acetonitrile, we rationalize that the less negative $\Delta G_{\text{CH}_2}^\circ$ and smaller v coefficients of the two aromatic phases result from relatively high sorption of acetonitrile from the mobile phase onto the stationary phase.²³

Finally, if we compare the PRP-1 phase with all the other phases, for all homologue series we find that the $\Delta G_{\text{CH}_2}^\circ$ of PRP-1 is comparable to that of C18-SiO₂ and, thus, is more negative than other aromatic phases. This result is also consistent with the v coefficient of the PRP-1 in the LSER studies²³ (see Figure 2). Although we suspect that the PRP-1 phase acts through a more adsorption-like retention process, we cannot fully explain its retention behavior at this point.

Effect of the functionality of the homologue series on $\Delta G_{\text{CH}_2}^\circ$. Tchaplal and co-workers²⁶ found that the terminal functionality of a homologue series has only a minor effect on $\Delta G_{\text{CH}_2}^\circ$. However, we observe some systematic trends in Figure 1. On all stationary phases studied except C18-SiO₂, the $\Delta G_{\text{CH}_2}^\circ$ value of each homologue is somewhat dependent on the functional group which defines the series. As seen in Table 2, the average deviation of the $\Delta G_{\text{CH}_2}^\circ$ value of all homologue series for C18-SiO₂ is only

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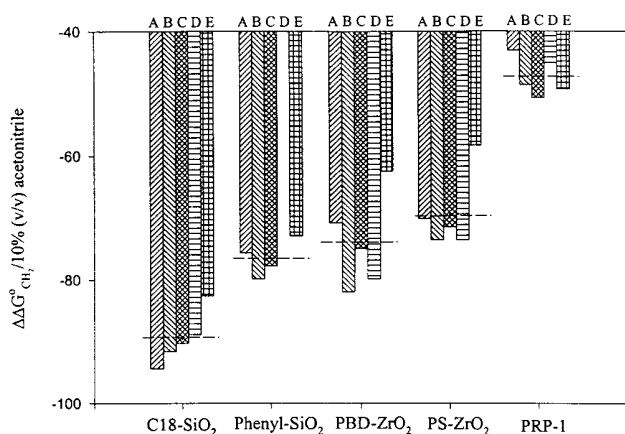


Figure 3. Comparison of the change in $\Delta\Delta G_{CH_2}^\circ$ per 10% (v/v) change in acetonitrile concentration for different homologue series on a given stationary phase. $\Delta\Delta G_{CH_2}^\circ$ for PRP-1 was obtained from $\Delta G_{CH_2}^\circ$ in 70% and 50% acetonitrile while $\Delta\Delta G_{CH_2}^\circ$ for the other three phases was from $\Delta G_{CH_2}^\circ$ in 50% and 30% acetonitrile. The dashed line denotes the average value from all homologue series. (A) Alkyl benzenes; (B) Nitro alkanes; (C) Alkyl acetates; (D) Alkyl anilines; (E) Alkyl phenones.

about 7 cal/mol, which is only half that for other phases. Furthermore, both the average and median $\Delta G_{CH_2}^\circ$ values for a given homologue series become more positive in the following order: alkyl phenones < alkyl acetates < nitro alkanes < alkyl benzenes < alkyl anilines. Interestingly, for all phases, the $\Delta G_{CH_2}^\circ$ values of the individual homologue series follow approximately the same order within the error range. Therefore, we conclude that the differences in the $\Delta G_{CH_2}^\circ$ values of the various homologue series are not random. These differences result from the unique functionality of each given homologue series. The behavior of the alkylanilines on the PBD-ZrO₂ phase is also quite distinctive. At this point we have no explanation for this departure from Martin's generalization that a methylene group makes a constant contribution to the free energy of retention independent of the defining functional group.

Effect of the Organic Modifier Concentration. In Figure 3 we show the change in $\Delta\Delta G_{CH_2}^\circ$ per 10% (v/v) change in the acetonitrile concentration ($\Delta\Delta G_{CH_2}^\circ$) for the different homologue series on a given stationary phase. Here we also see that, as expected, both the stationary phase type and the homologue type affect the $\Delta\Delta G_{CH_2}^\circ$ values. For C18-SiO₂, the $\Delta\Delta G_{CH_2}^\circ$ value becomes more positive in the following order: alkyl benzenes < nitro alkanes < alkyl acetates < alkyl anilines < alkyl phenones; and the order for phenyl-SiO₂ is approximately the same. The interesting fact is that the orders for both PBD-ZrO₂ and PS-ZrO₂ are rather similar. On the other hand, the order for PRP-1 is very different from all the other phases. This is probably due to the fact that the inherent chemical properties of the base materials differ in the presence of mobile phase.

As shown in Figure 4, the effect of mobile phase composition on $\Delta G_{CH_2}^\circ$ is closely correlated to its effect on the v coefficient for all stationary phases studied except for PS-ZrO₂, which is the point that deviates the most from the least-squares line. Here, the change in the v coefficient was calculated from data given elsewhere.²³ On the basis of a number of LSER studies,^{23,25,31} the v coefficient of a C18-SiO₂ phase is similar to that of an octyl silica

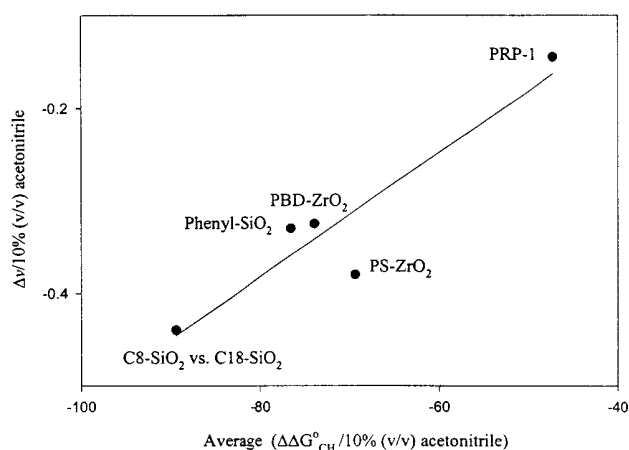


Figure 4. Plot of the change in the v coefficient vs the change in $\Delta\Delta G_{CH_2}^\circ$ upon a 10% (v/v) change in mobile-phase composition for different stationary phases. The change in the v coefficient of C8-SiO₂ was used to correlate the $\Delta\Delta G_{CH_2}^\circ$ value of C18-SiO₂. The solid line is the least-squares line through all data points. $\Delta\Delta G_{CH_2}^\circ$ is averaged over five homologue series.

bonded phase (C8-SiO₂) and, thus, the change in the v coefficient of C8-SiO₂ was used to correlate the $\Delta\Delta G_{CH_2}^\circ$ value of C18-SiO₂. It is interesting to note that the mobile-phase composition has the largest effect on $\Delta G_{CH_2}^\circ$ and the v coefficient for C18-SiO₂ but has the smallest effect for PRP-1. Clearly the retention process on PRP-1 in the mobile phases studied is quite different from that on the other stationary phases.

Unique Differences in the Selectivity between Aromatic and Aliphatic Stationary Phases. The major difference between an aromatic and an aliphatic stationary phase is that the aromatic phase affords the possibility of strong $\pi-\pi$ (or enhanced polarizability) interactions, in addition to hydrophobic interactions, with the analytes. To study this, we selected a set of alkylbenzenes (ethylbenzene, butylbenzene, hexylbenzene, and octylbenzene), a set of polyphenyls (biphenyl, *p*-terphenyl, and *p*-quaterphenyl), and a set of linear PAHs (naphthalene, anthracene, naphthacene, and dibenz[a,h]anthracene). The selectivity factor (α) for each analyte on each phase studied was calculated as the ratio of its retention factor to that of benzene on the same column. Table 3 clearly shows significant differences in the selectivities of PS-ZrO₂ and PBD-ZrO₂ for these compounds. For the alkylbenzenes, the ratios of selectivity factors for PBD-ZrO₂ and PS-ZrO₂ all exceed unity; on the other hand, for the PAHs, the ratios are equal to or less than unity. On PS-ZrO₂, the PAHs are much more retained than are the alkylbenzenes due to strong $\pi-\pi$ interactions between the phenyl groups of the analytes and the phenyl groups of the PS-ZrO₂. Furthermore, the alkylbenzenes elute from the PBD-ZrO₂ phase much later than they do from the PS-ZrO₂ phase because of the stronger hydrophobic interactions between the alkyl groups of the solutes and the alkyl backbone of the PBD-ZrO₂ phase as compared with the PS-ZrO₂ phase. As a result, we observe dramatic changes in the retention order of alkylbenzenes and PAHs on PBD-ZrO₂ and PS-ZrO₂.

Differences in the selectivities of these compounds on C18-SiO₂ and phenyl-SiO₂ are not as large as those seen for PBD-ZrO₂ and PS-ZrO₂. As shown in Table 3, for the PAHs and

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Table 3. Comparison of the Selectivity of Alkylbenzenes and Polyaromatic Hydrocarbons on the Aromatic and Aliphatic Phases Studied^a

solutes	$V_2/100^b$	$\alpha_{PS-ZrO_2}^c$	$\alpha_{PBD-ZrO_2}^c$	$\frac{\alpha_{PBD-ZrO_2}}{\alpha_{PS-ZrO_2}}^c$	$\alpha_{Phenyl-SiO_2}^d$	$\alpha_{C18-SiO_2}^d$	$\frac{\alpha_{C18-SiO_2}}{\alpha_{Phenyl-SiO_2}}^d$
ethylbenzene	0.998	1.9	2.5	1.3	1.5	2.1	1.4
butylbenzene	1.280	3.8	6.7	1.8	2.3	4.6	2.0
hexylbenzene	1.562	7.1	17.7	2.5	3.5	10.4	3.0
octylbenzene	1.844	13.3	47.1	3.5	5.4	24.2	4.5
naphthalene	1.085	2.7	2.6	1.0	1.5	1.8	1.2
anthracene	1.454	8.0	7.2	0.9	2.4	3.7	1.5
naphthacene	1.823	25.8	19.9	0.8	3.9	8.1	2.1
dibenz[a,h]anthracene	2.192	62.5	39.8	0.6	5.6	13.4	2.4
biphenyl	1.324	4.1	4.0	1.0	1.9	2.6	1.3
<i>p</i> -terphenyl	1.932	16.5	15.7	1.0	3.7	6.9	1.9
<i>p</i> -quarterphenyl	2.540 ^e	67.8	60.3	0.9	7.6	18.7	2.5

^a The selectivity factor (α) is calculated as $K_{\text{solute}}/K_{\text{benzene}}$. ^b The ($V_2/100$) values were obtained from ref 32. ^c Based on the retention data in 50/50 acetonitrile/water. ^d Based on the retention data in 70/30 acetonitrile/water. ^e Estimated from biphenyl and *p*-terphenyl using McGowan's method.³³

Table 4. Summary of the Slope of log k' vs the Solute's Molecular Size ($V_2/100$) for Alkylbenzenes, PAHs, and Polyphenyls for Different Stationary Phases^{a,c}

	PS-ZrO ₂	PBD-ZrO ₂	phenyl-SiO ₂	C18-SiO ₂
alkylbenzenes	0.992 ± 0.009	1.504 ± 0.003	0.658 ± 0.001	1.261 ± 0.010
PAHs	1.26 ± 0.04	1.06 ± 0.07	0.52 ± 0.02	0.79 ± 0.05
polyphenyls	1.005 ± 0.003	0.967 ± 0.003	0.488 ± 0.009	0.711 ± 0.003
correlation coeff. (R^2) ^b	0.1624	0.9995	0.9853	1.00

^a Based on the available $V_2/100$ values shown in Table 3. ^b Correlation coefficient was obtained from the slopes on one phase vs those on C18-SiO₂. ^c Mobile phases were as in Table 3.

polyphenyls, the ratios of the selectivity factor of C18-SiO₂ and that of phenyl-SiO₂ are all larger than unity. Therefore, only the band spacings and not the elution orders on C18-SiO₂ differ from those on Phenyl-SiO₂. Note that we obtained our data for the silica phases in 70/30 acetonitrile/water because retention of the analytes in this eluent is comparable to that on the zirconia phases in 50/50 acetonitrile/water. We suspect that the PAHs and polyphenyls are in better contact with the aromatic moieties in PS-ZrO₂ than in Phenyl-SiO₂ and thus π - π interactions are stronger.

We compare the dependence of log k' on solute molecular size ($V_2/100$)^{32,33} for alkylbenzenes, PAHs, and polyphenyls in Table 4. There are clearly some very interesting phenomena. For PS-ZrO₂, the PAHs show a much greater dependence of log k' on molecular size than do alkylbenzenes and polyphenyls, while the latter two show similar dependencies. We suspect that the π electrons of the polyphenyls are not continuously conjugated and thus the π - π interactions of polyphenyls with PS-ZrO₂ are not as strong as those of PAHs with PS-ZrO₂. On the other hand, for the other three phases, the dependence decreases in the same order: alkylbenzenes < PAHs < polyphenyls. We further calculated the correlation coefficients of the slopes of one phase versus those of C18-SiO₂. As shown in Table 4, both phenyl-SiO₂ and PBD-ZrO₂ correlate rather well with C18-SiO₂ (R^2 is 0.9853 and 0.9995, respectively) while PS-ZrO₂ correlates poorly with C18-SiO₂. In addition to the solute's molecular size, other molecular properties, such as aromaticity, might contribute to the retention of PAHs and polyphenyls on PS-ZrO₂.

Table 5. Summary of Selectivity Factors of Isomers on Different Stationary Phases^a

phase	phenyltoluenes		terphenyls		stilbenes	terpinenes ^b
	<i>m</i> / <i>o</i>	<i>p</i> / <i>m</i>	<i>m</i> / <i>o</i>	<i>p</i> / <i>m</i>	trans-/cis-	γ -/ α -
PRP-1	1.26	1.00	2.13	1.09	1.13	1.08
PS-ZrO ₂	1.17	1.00	1.70	1.06	1.22	1.07
PBD-ZrO ₂	1.03	1.00	1.46	1.07	1.09	1.00
phenyl-SiO ₂	1.03	1.00	1.13	1.07	1.00	1.00
C18-SiO ₂	1.05	1.00	1.23	1.09	1.03	1.13

^a Based on the retention data in 50/50 acetonitrile/water. ^b UV detection at 210 nm.

Isomer Selectivity. Most reversed-phase materials, such as monomeric C18-SiO₂ phases, are not commonly used for the separation of nonpolar structural isomers. For example, ethylbenzene and xylene isomers are essentially inseparable on the aromatic or aliphatic stationary phases studied here. Because there are substantial differences in the selectivity of PAHs on these phases, we chose a series of phenyl-substituted isomers (see Figure 5), to examine the isomeric selectivity on aliphatic and aromatic phases. Terpinene isomers were also used to assess the effect of conjugation on retention. The selectivity factors of these isomers on the different phases are given in Table 5. We see a slight improvement in the selectivity of the phenyltoluene isomers compared with that of the inseparable xylene isomers. Although the para and meta isomers coelute on all phases, the ortho and meta isomers are well separated on both PRP-1 and PS-ZrO₂. Furthermore, the separation of the terphenyl isomers is even better: the three isomers are separable on all phases. Interestingly, the selectivity of the para/meta pair is rather similar for all

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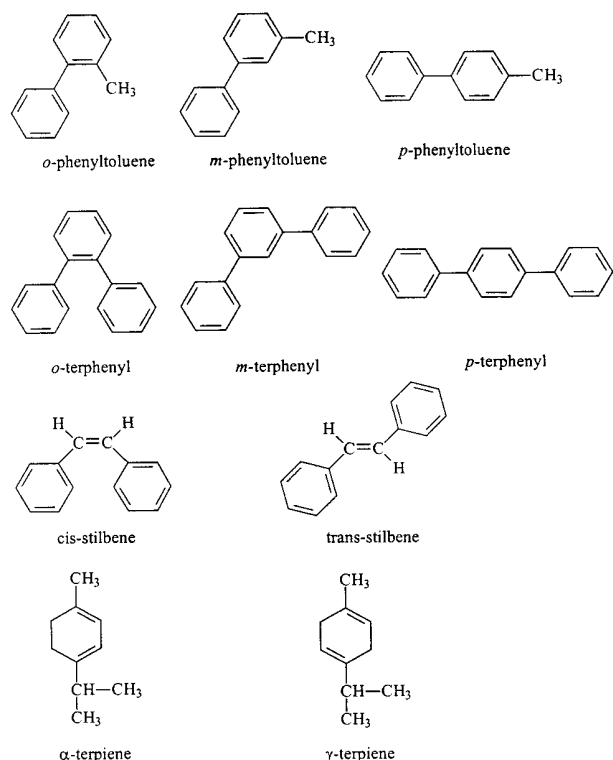


Figure 5. Structures of selected isomers.

phases, while the selectivity of the ortho/meta pair is quite different. The PRP-1 and PS-ZrO₂ phases, in particular, show much greater selectivity than do the other phases. In the case of the stilbenes and terpinenes, the silica phases cannot compete with PRP-1, PS-ZrO₂, and PBD-ZrO₂ for resolving these isomers. It is not at all clear why both types of zirconia-based phases are superior to both types of silica-based phases for these separations.

In summary, PS-ZrO₂ is very comparable to PRP-1 in terms of the selectivity of the isomers studied here, probably due to the fact that the polystyrene deposited on PS-ZrO₂ is chemically similar to the PRP-1 phase. This is consistent with our earlier LSER study,²³ where we showed that PS-ZrO₂ is more similar to PRP-1 in terms of molecular polarizability (the rR_2 term in eq 2). It is surprising that the phenyl-SiO₂ phase does not show any advantages over C18-SiO₂ for the separation of these isomers and is not as selective as PS-ZrO₂ and PRP-1.

General Separations. It is impossible to say that one stationary phase is generally superior to another in terms of selectivity. Only on a case by case basis can we say that one stationary phase is more selective than another. From the discussions above, we have shown that PS-ZrO₂ has different selectivities in comparison with other commercially available RPLC phases. Here we explored applications of PS-ZrO₂ to a wider variety of compounds. We chose some important environmental and pharmaceutical samples to demonstrate the robustness and effectiveness of PS-ZrO₂ for practical-method development for tuning selectivity, optimizing resolution, and reducing analysis time.

Triazine Herbicides. Triazines are a class of herbicides which serve a vitally important role in agriculture. While triazines are effective in killing weeds and grasses, they cannot be metabolized and detoxified by crop plants. Consequently, the use of these

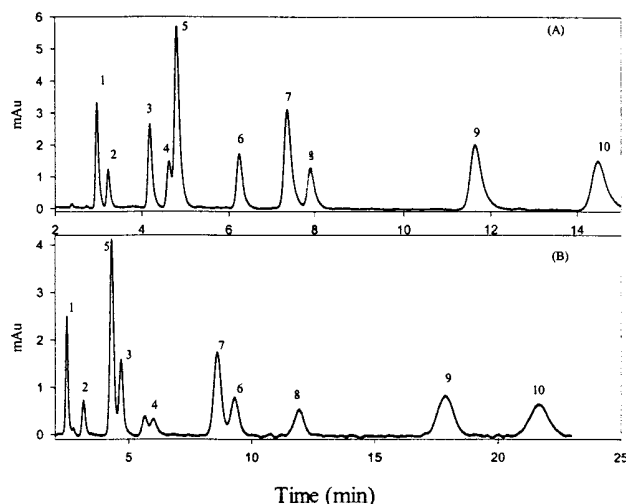


Figure 6. Chromatograms of separation of triazine mixture on (A) PS-ZrO₂ (10 × 0.46 cm) and (B) C18-SiO₂ (5 × 0.46 cm). Conditions: 30/70 acetonitrile/water, 30 °C, 1 mL/min, UV detection 254 nm. Compounds: 1. Simazine; 2. Atraton; 3. Atrazine; 4. Prometon; 5. Simetryn; 6. Propazine; 7. Ametryn; 8. Terbutylzine; 9. Prometryn; 10. Terbutryn.

species is an environmentally important issue as is their detection and analysis in soil, water, plants, and animals. We studied differences in the selectivity of 10 triazines on different stationary phases in 30/70 acetonitrile/water. Figure 6 shows chromatograms of these triazines on PS-ZrO₂ and C18-SiO₂. Even though the carbon content of PS-ZrO₂ is only about 2% and is much less than that of C18-SiO₂ (10%), the triazines are sufficiently retained and show unique selectivity on PS-ZrO₂. They are well separated on PS-ZrO₂ with resolution comparable to but with much shorter analysis time than the separation on C18-SiO₂. A detailed comparison of the retention order of a set of triazines on different stationary phases is presented in Figure 7. Two pairs of analytes change their elution position when PRP-1 is replaced with PS-ZrO₂. Surprisingly, there are only slight changes in the retention but no significant changes in the elution order when the column is switched from PS-ZrO₂ to PBD-ZrO₂; however, the analytes are relatively more retained on PS-ZrO₂ than on PBD-ZrO₂. We also observe considerable differences in the selectivity of triazines on Phenyl-SiO₂ and C18-SiO₂; furthermore, C18-SiO₂ seems to be more selective than is Phenyl-SiO₂ with this eluent. To rationalize differences in selectivity, we compare the retention of the same "family" of triazines, that is, those having the same polar group (–OH, –SCH₃, or –Cl), but different numbers of methyl groups, on the different stationary phases. We found that the log *K'* of a compound in a triazine family correlates linearly with the number of methyl groups, and, on a given stationary phase, the slope of the linear function is similar for the different functional groups.³⁴ However, on different stationary phases, the change in log *K'* per methyl group is slightly different and increases in the order of PRP-1 ≈ PS-ZrO₂ < Phenyl-SiO₂ ≈ PBD-ZrO₂ < C18-SiO₂. This order is not quite the same as the order of hydrophobicity of these phases, Phenyl-SiO₂ < PS-ZrO₂ < PBD-ZrO₂ < PRP-1 ≈ C18-SiO₂, based on the study of homologue series discussed above.

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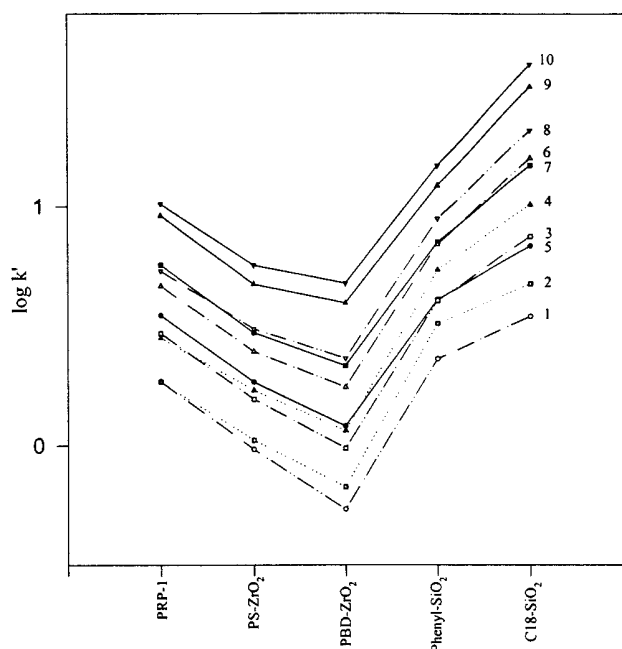


Figure 7. Comparison of the $\log K'$ of triazine compounds on five stationary phases in 30/70 acetonitrile/water. The number corresponds to the name of the compound in Figure 6.

Phenolic Compounds. Phenolic compounds represent a major class of contaminants released from industrial processes, water treatment, and agricultural pesticides. These highly toxic compounds have drawn great environmental attention. The separations of eleven phenolic compounds on PS-ZrO₂ and C18-SiO₂ are shown in Figure 8, where we observe different resolution of these

compounds on the two phases. We do not show chromatograms for PBD-ZrO₂ and Phenyl-SiO₂ because their elution orders are very similar to those on PS-ZrO₂ and C18-SiO₂, respectively. We examined the relationship between $\log K'$ of the chlorinated phenols and the number of chloro groups and the relationship between $\log K'$ of methylated phenols and the number of methyl groups, at substituted positions 2, 4, and 6. We found that the differences in the selectivity on these phases are mainly due to the differences in the chemical nature of stationary phases and the functionality of the substituents.³⁴

Steroids. Steroids are complex polycyclic molecules found in all plants and animals. The most important function steroids serve is as hormones. Accordingly, synthetic steroids have very vital medicinal purposes in the treatment of many diseases, such as psoriasis, rheumatoid arthritis, and asthma, and as anti-inflammatories. In the pharmaceutical industry, the analysis of steroids is one of the major steps in the development of new drugs. Many steroids have either carbonyl groups or hydroxyl groups, which can function as Lewis bases and can interact with Lewis acid sites exposed on the surface of the zirconia-based materials. However, as shown in Figure 9, the addition of a small amount of strong, hard Lewis bases, such as 20 mM ammonium fluoride buffer, results in relatively good resolution of these selected steroids on a short PS-ZrO₂ column (5 cm). Interestingly, on a PBD-ZrO₂ column that is three times longer (15 cm), a rather poor separation of these compounds was obtained with a slightly different elution order. Separation of these steroids on a C18-SiO₂ column in 50/50 acetonitrile/water is slightly better than that on PS-ZrO₂, and the elution sequence is similar to that on PBD-ZrO₂. However, phenyl-SiO₂ cannot resolve some of the steroids (solutes 3–6 coelute), and the elution order for solutes 1, 2, and 7 is similar to that on other phases.

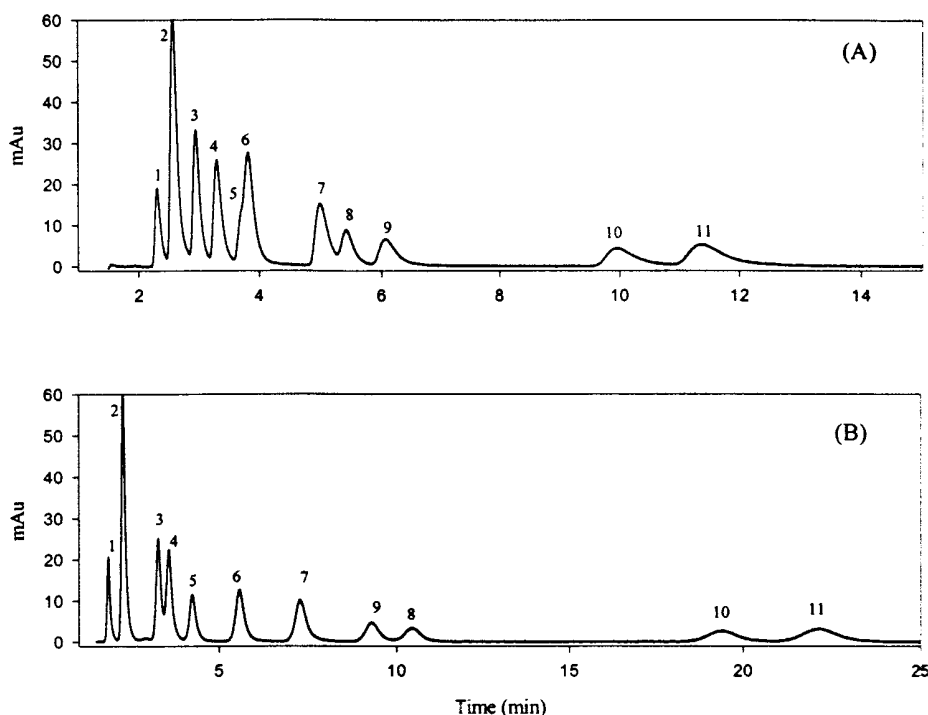


Figure 8. Chromatograms of separation of eleven phenolic compounds on (A) PS-ZrO₂ (10 × 0.46 cm) and (B) C18-SiO₂ (5 × 0.46 cm). Conditions: 30/70 acetonitrile/0.1% TFA (pH = 2.1), 30 °C, 0.8 mL/min. Analytes: 1, phenol; 2, *p*-fluorophenol; 3, *o*-cresol; 4, *o*-chlorophenol; 5, *p*-chlorophenol; 6, 2,4-dimethylphenol; 7, 3-methyl-4-chlorophenol; 8, 2,4,5-trimethylphenol; 9, 2,4-dichlorophenol; 10, 2,4,6-trichlorophenol; 11, 2,4,5-trichlorophenol.

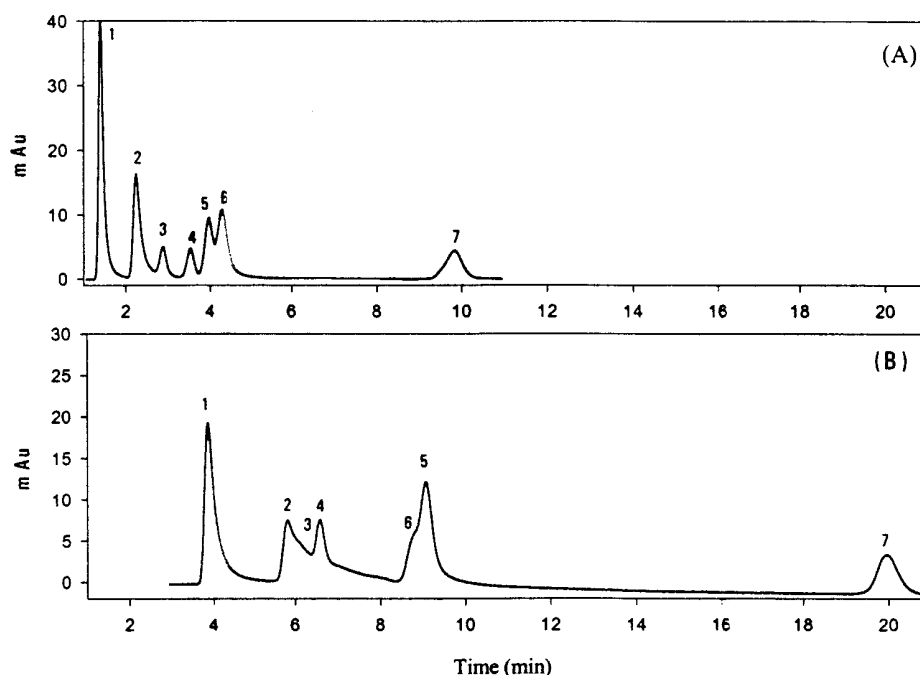


Figure 9. Chromatograms of separation of seven steroids on (A) PS-ZrO₂ and (B) PBD-ZrO₂. Conditions: 30/70 acetonitrile/20 mM NH₄F. PS-ZrO₂: 5 × 0.46 cm, 1 mL/min. PBD-ZrO₂: 15 × 0.46 cm, 0.8 mL/min. Solutes: 1, cortisone; 2, corticosterone; 3, cortisone acetate; 4, 11-ketoprogesterone; 5, 17 α -hydroprogesterone; 6, deoxycorticosterone; 7, progesterone.

CONCLUSION

We have shown that PS-ZrO₂ is substantially different from PBD-ZrO₂ and other RPLC phases on the basis of both the conceptual LSER model²³ but also on the basis of results of a number of practical separations of a variety of environmental and pharmaceutical samples. We believe that the retention characteristics by which the PS-ZrO₂ acts to resolve different analytes must involve a direct interaction between the polar or polarizable groups on the analyte and the phenyl groups on the PS-ZrO₂ surface. At comparable carbon contents on PS-ZrO₂ and PBD-ZrO₂, PS-ZrO₂ is less hydrophobic than PBD-ZrO₂, but nonetheless, PAHs and polar solutes are more retained on PS-ZrO₂. Consequently, PS-ZrO₂ shows rather different selectivities than does PBD-ZrO₂ or C18-SiO₂ for many types of compounds including steroids. Another advantage of using PS-ZrO₂ is its effectiveness in terms of selectivity and analysis time. In several examples we have found that we could achieve comparable or better resolution in much shorter time with a PS-ZrO₂ phase as compared with silica-bonded

phases, which have higher carbon contents. The interesting selectivities shown by PS-ZrO₂ make it an excellent candidate for difficult separation when conventional silica-bonded phases or PBD-ZrO₂ based phases fail.

In conclusion, PS-ZrO₂ does indeed exhibit different selectivities than PBD-ZrO₂ or C18-SiO₂ toward a variety of analytes, and it is a unique, selective stationary phase which can provide effective separations for many environmental pollutants and drug samples.

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