Chemometric characterization of Lewis base-modified zirconia for normal phase chromatography

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Abstract

Microporous zirconia was evaluated for its chromatographic properties as a normal phase support. Since previous studies in aqueous media had indicated a strong effect of eluent Lewis base on chromatographic properties, a number of Lewis base pretreatments of the support were evaluated as to their effect on normal phase selectivities. The retention characteristics of over thirty well characterized probe solutes were determined on eight different "acid"- or "base"-washed zirconia supports. These results were compared with those obtained for a silica column. Chemometric methodology was used to characterize the similarities and differences between the "acid"- and "base"-washed supports. The lack of chromatographic reproducibility previously observed under normal-phase conditions on zirconia appears to be a result of the lack of specific descriptions regarding the "acid" or "base" pretreatment.

1. Introduction

Early in the development of porous microparticulate zirconium oxide as a stationary phase for high-performance liquid chromatography, Rigney et al. [1,2] showed that this material demonstrates remarkable chemical stability. Zirconia does not dissolve to any significant extent in very basic or acidic solutions, thus allowing a wide variety of pH values to be used for separations. In addition, columns which have been used for bioseparations may be chemically sterilized by treatment with hot caustic without degradation of the support material.

Subsequent experiments have shown that the adsorptive properties of this material may be significantly altered by dynamically treating the support with Lewis bases [3-10]. Lewis bases show varying degrees of interaction with the Lewis acid sites present on the zirconia surface depending upon structure, charge, etc. Hence, they show varying degrees of eluotropic strength towards other solute Lewis bases which compete for the same Lewis acid sites [6,7,9]. Although some interactions may be quite strong, washing the support with 0.1 M sodium hydroxide brings back to the original adsorptive properties of the material [3,9]. This recycling can be carried out numerous times with the main limitation being the stability of the stainless steel column and pumping hardware in caustic. Since the adsorptive properties of this material are readily man-

ipulated and regeneration is rapid and reproducible, the support is an ideal candidate for use as a "custom" adsorbent for normal-phase chromatography.

Selectivity in normal-phase chromatography is presently dominated by the composition of the mobile phase [11]. The adsorption strength of silica or alumina is usually modified by addition of water or other polar additives. Retention is then optimized by adjusting the eluotropic strength of the eluent. The shape selectivity, which is the dominant advantage in normal-phase chromatography, is more or less constant for a given adsorbent. Some differences are observed between acidic, neutral and basic alumina, but these differences are not well characterized [11].

The object of this investigation is the development of novel adsorptive phases using zirconium oxide as an underlying support. Adsorption of various Lewis bases should affect the net adsorptive interactions towards solutes through the masking of Lewis acid sites and production of secondary interactions between adsorbed Lewis bases and the solutes. These customized phases could be used for various separations requiring unique selectivities. After the separations are performed, the support could be stripped of the Lewis base modifier and the zirconia, modified with a different Lewis base, used for another separation. This would give the separation scientist a spectrum of selectivities with only one column. To facilitate the rational characterization of a number of Lewis base-modified phases, a combination of solvatochromic theory and principal component analysis was used for cluster analysis. Principal components analysis allows effective data reduction and visualization of results.

2. Experimental

2.1. Chemicals

All solutes used in this study were reagent grade or better and were obtained from commercial sources. Sodium fluoride, ammonium hydroxide (30% solution), sodium hydroxide (50%

solution), phosphoric, sulfuric, boric, hydrochloric and formic acids were analytical-reagent grade reagents obtained from Mallinckrodt (Paris, KY, USA). Isopropanol, dichloromethane and hexane were ChromAR-grade solvents also from Mallinckrodt. HPLC-grade water was obtained from Baxter Scientific (McGraw Park, IL, USA).

2.2. Chromatographic systems

Chromatography was performed on two separate systems. Retention studies were performed on a Hewlett-Packard (Avondale, PA, USA) Model 1090M liquid chromatograph with a DR5 ternary solvent-delivery system and a diode array detector. Data were processed using a Hewlett-Packard 9000/Series 300 computer outfitted with ChemStation software. Corrosive solutions were pumped using a Dionex (Sunnyvale, CA, USA) Model 300 liquid chromatograph.

2.3. Chromatographic supports

The porous microparticulate zirconium oxide particles used in this study were obtained from the Ceramic Technology Center at 3M and were described in detail earlier [1–3,9]. The particles had a nominal diameter of 5.3 μm ± 1.3 μm, an average pore diameter of 308 Å by mercury porosimetry and an average BET surface area of 32.5 m^2/g. The particles were initially pretreated in order to remove as many of the manufacturing impurities as possible and provide a consistent starting point, as described in detail earlier [3,9].

Columns were prepared in 50 mm × 4.6 mm column blanks fitted with 1/4-in. Parker end fittings (1 in. = 2.54 cm). Titanium screens with 2 μm mesh were used instead of frits to minimize any potential metal ion contamination from the frits. Columns were packed by the upward slurry technique using isopropanol as the solvent. Packing pressure was 4500 p.s.i. (1 p.s.i. = 6894.76 Pa). Following the packing procedure, all columns were flushed with water to displace all the packing solvent prior to further treatment. The silica column was packed with 5 μm Spherisorb silica (50 mm × 4.6 mm) and was obtained from Phenomenex (Torrance, CA, USA).

Lewis base modification was accomplished by flushing 50 ml of 0.1 M sodium hydroxide through the columns at 1.0 ml/min (25°C). For each of the different phases, this was followed by 50 ml of 0.1 M ammonium hydroxide, 0.1 M hydrochloric acid, 0.1 M sulfuric acid, 0.1 M phosphoric acid, 0.1 M sodium fluoride with 0.05 M hydrochloric acid, 0.1 M boric acid or 0.1 M formic acid. This was immediately followed by a flush with 50 ml HPLC-grade isopropanol.

2.4. Stability studies

The stability of each of the Lewis base-modified supports was assessed by monitoring the retention of benzyl alcohol at 45°C. The eluent [1% (v/v) isopropanol in hexane] was pumped at 0.75 ml/min at 45°C and 10-μl volumes of 1000 ppm (w/w) benzyl alcohol in dichloromethane were made each hour following a 1000 column equilibration period. Stability was then assessed for a period of 4000 column volumes. A commercially prepared silica column was also tested for comparison. The capacity factors were calculated based on peak maxima.

2.5. Retention studies

The retention characteristics of the Lewis base-modified phases were probed using a wide variety of solutes. Injections of 10 μl of each solute (1000 ppm in dichloromethane) were made and elution monitored at 254 nm.

2.6. Multivariate data analysis

The inherent similarity of the Lewis base-modified zirconia phases and solutes was investigated using principal components analysis. The use of principal components analysis for exploratory data analysis is well known [12]. The data were analyzed using the singular value decomposition (SVD) approach to principal components analysis [13]. All data transformations and calculations were made using built-in routines of MATLAB 4.0 (MathWorks, Natick, MA, USA, 1993). The principal component axes that define the variance in both the Lewis base pretreatment (chemical modification) and the variance in the probe solutes were calculated using log transformed k' data.

3. Results and discussion

3.1. Column stability

Benzyl alcohol was chosen as a probe of stationary phase stability since its retention is sensitive to changes in hydrogen bonding. Loss of adsorbed water or chemisorbed Lewis base would affect the amount and strength of hydrogen bonding sites, thus affecting the resulting capacity factor. The results of these stability studies are given in Table 1.

A commercially available silica column was

Table 1
Stability of Lewis base-modified phases

Treatment	n	k'		h	
		Average	R.S.D. (%)	Average	R.S.D. (%)
NaOH	53	2.67	1.3	25.5	4.0
NH$_4$OH	53	5.18	1.3	12.7	18.8
HCl	53	1.85	1.9	9.2	9.2
H$_2$SO$_4$	52	1.02	4.9	6.0	5.4
H$_3$PO$_4$	51	1.05	4.0	13.0	5.5
H$_3$BO$_3$	52	7.50	3.2	176.9	12.7
HCOOH	51	6.19	1.6	87.9	3.2
NaF/HCl	51	1.88	2.4	12.3	1.5
Silica	53	1.20	4.4	3.0	2.7

used, as received from the vendor, as a reference system. The equilibration of silica columns in normal-phase chromatography is known to be quite slow [11]. Therefore, to compare stability rather than equilibration periods, the columns were pre-equilibrated with 1000 column volumes of eluent. Stability was then assessed with the assumption that most of the equilibration had been completed.

The Lewis base-modified zirconia phases showed a reasonable degree of stability compared to the silica phase. A slow change in capacity factor was evident in the data, but may be attributed to the last stages of water content equilibrium. Despite this effect, the standard deviations for the modified zirconia phases showed remarkable stability.

3.2. Retention of test solutes

A wide variety of solutes was used to further assess the changes incurred by pretreatment of the zirconia particles. Table 2 lists the solutes and their solvatochromic parameters which will be useful in rationalizing the changes in retention between the phases [15,16]. The terms are described as: π^* = solute dipolarity/polarizability, α = hydrogen bond acidity and β = hydrogen bond basicity.

The capacity factors for the test solutes in a hexane eluent containing 1% (v/v) chloroform are shown in Table 3. In general, the Lewis base-modified zirconia phases show similar selectivity to silica. However, a number of solutes deviate markedly from this generalization.

Weakly retained solutes, on silica and modified zirconia, tend to have low π^*, α and β values. These include aromatic solutes with aliphatic and halogen substituents. Little selectivity or retention is shown for any of these solutes despite the relatively weak eluent.

On silica, solutes with larger solvatochromic parameters (α and β) tend to have higher capacity factors. This trend is also observed among the modified zirconia supports, although the different phases show quite different selectivities towards these solutes.

It is evident from the data of Tables 2 and 3

Table 2
Solvatochromic parameters for probe solutes

Solute	Number	π^*	α	β
Acetone	26	0.38	0.01	0.50
Acetophenone	16	0.90	0.00	0.51
Aniline	12	0.73	0.26	0.38
Anisole	13	0.73	0.00	0.26
Benzaldehyde	19	0.92	0.00	0.42
Benzene	32	0.59	0.00	0.14
Benzonitrile	2,33	0.90	0.00	0.42
Benzyl alcohol	15	0.99	0.39	0.42
Benzyl cyanide	6	0.75	0.00	0.48
Bromobenzene	18	0.79	0.00	0.06
2-Butanone	29	0.39	0.00	0.48
n-Butylbenzene	17	0.49	0.00	0.12
tert.-Butylbenzene	7	0.42	0.00	0.12
Chlorobenzene	4	0.71	0.00	0.07
m-Cresol	20	0.68	0.58	0.24
o-Dichlorobenzene	3	0.80	0.00	0.03
p-Dichlorobenzene	5	0.70	0.00	0.03
Ethylbenzene	8	0.53	0.00	0.15
Iodobenzene	21	0.81	0.00	0.05
Methyl benzoate	28	0.76	0.00	0.39
Nitrobenzene	22	1.01	0.00	0.30
Nitromethane	27	0.85	0.12	0.25
o-Nitrotoluene	10	0.90	0.00	0.25
Phenol	25	0.72	0.60	0.22
n-Propylbenzene	9	0.51	0.00	0.12
Pyridine	31	0.87	0.00	0.62
Styrene	24	0.55	0.00	0.18
Toluene	23	0.55	0.00	0.14
m-Xylene	30	0.51	0.00	0.17
o-Xylene	14	0.51	0.00	0.17
p-Xylene	11	0.51	0.00	0.17

that most solutes with large α and β values are not eluted under these mild elution conditions. Of the solutes which do elute, significant differences in selectivity are observed. For example, sodium hydroxide-washed zirconia shows small capacity factors for benzaldehyde, nitromethane and pyridine while the other phases show very large capacity factors under identical conditions. Even ammonium hydroxide-washed zirconia shows significantly different retention patterns despite both being "base-washed" zirconia (Fig. 1). Other less dramatic differences in retention are observed for benzyl cyanide.

In order to better understand the inherent differences and similarities between the probe solutes and Lewis base modified zirconia phases

Table 3
k' for 1% (v/v) chloroform in hexane

Solute	Silica	NaOH	HCl	NH$_4$OH	H$_3$PO$_4$	H$_2$SO$_4$	NaF	H$_3$BO$_3$	HCOOH
Acetone	0.85	0.15	0.19	0.10	0.15	0.34	0.39	0.03	0.05
Acetophenone	0.30	0.04	0.15	0.08	0.08	0.12	0.12	0.01	0.03
Aniline	0.48	0.37	4.79	0.75	>30	>30	5.05	1.01	1.56
Anisole	0.04	0.03	0.07	0.06	0.03	0.04	0.01	0.00	0.01
Benzaldehyde	0.19	0.12	0.18	0.20	0.09	0.18	0.15	0.07	0.11
Benzene	0.01	0.07	0.09	0.10	0.05	0.05	0.01	0.00	0.01
Benzonitrile	0.13	0.07	0.09	0.09	0.05	0.09	0.10	0.01	0.02
Benzyl alcohol	1.10	3.00	1.92	5.27	0.99	1.27	1.95	8.15	6.99
Benzyl cyanide	0.17	0.09	0.10	0.08	0.05	0.13	0.14	3.05	0.02
Bromobenzene	0.00	0.04	0.08	0.08	0.03	0.05	0.01	0.00	0.01
2-Butanone	0.53	0.12	0.17	0.11	0.11	0.16	3.13	0.02	0.03
n-Butylbenzene	0.00	0.04	0.06	0.09	0.03	0.05	0.01	0.00	0.01
tert.-Butylbenzene	0.00	0.04	0.07	0.08	0.02	0.05	0.00	0.00	0.00
Chlorobenzene	0.00	0.05	0.05	0.06	0.04	0.08	0.01	0.01	0.01
m-Cresol	0.64	>30	7.94	>30	0.51	0.50	1.86	>30	>30
o-Dichlorobenzene	0.00	0.04	0.07	0.07	0.04	0.06	0.01	0.00	0.01
p-Dichlorobenzene	0.00	0.04	0.07	0.10	0.03	0.07	0.01	0.00	0.01
Ethyl benzene	0.00	0.04	0.06	0.07	0.02	0.05	0.01	0.00	0.00
Iodobenzene	0.00	0.05	0.07	0.08	0.03	0.05	0.01	0.00	0.01
Methyl benzoate	0.17	0.08	0.10	0.07	0.06	0.05	0.08	0.00	0.02
Nitrobenzene	0.02	0.06	0.08	0.11	0.04	0.09	0.00	0.00	0.00
Nitromethane	0.12	>30	0.09	>30	0.06	0.07	0.14	>30	>30
o-Nitrotoluene	3.01	0.04	0.08	0.09	0.03	0.08	2.91	0.00	0.00
Phenol	0.69	>30	8.85	>30	0.56	0.50	2.27	>30	>30
n-Propylbenzene	0.00	0.05	0.08	0.09	0.03	0.05	0.00	0.00	0.00
Pyridine	6.70	0.62	23.33	1.79	>30	>30	5.57	2.88	4.82
Styrene	0.00	0.04	0.07	0.12	0.04	0.04	0.00	0.00	0.00
Toluene	0.00	0.06	0.08	0.06	0.04	0.04	0.01	0.00	0.01
m-Xylene	0.00	0.05	0.07	0.11	0.03	0.04	0.01	0.00	0.01
o-Xylene	0.00	0.05	0.08	0.07	0.02	0.04	0.01	0.00	0.01
p-Xylene	0.00	0.04	0.08	0.08	0.03	0.06	0.01	0.00	0.00

with respect to silica, a multivariate approach to data analysis was taken.

3.3. Principal components analysis

The data from Table 3 were evaluated by principal component analysis (PCA) using the singular value decomposition method. The singular value decomposition allows simultaneous principal component analysis of both the solute (column) space and the chemical modification (row) space.

In PCA of a matrix R (n samples $\times m$ responses), the eigenvectors of the variance–covariance matrix (RR^T) are computed. The eigenvectors or principal components axes define the weight each response contributes to account for the maximum variance between the samples.

The singular value decomposition states that any matrix R can be decomposed into three matrices U, S and V (Eq. 1):

$$R = USV^T \qquad (1)$$

where the columns of U are the eigenvectors of RR^T, that is the principal component axes describing the variance in the solute (column) space for these data. The columns of V are the eigenvectors of R^TR. In other words, the columns of V define the principal component axes of R^T which describe the variance in the chemical modification (row) space of these data. The main diagonal elements of S are the square roots of the eigenvalues of both RR^T and R^TR.

It is known from solvatochromic theory that there is an approximate linear relationship between ln k' and the solvatochromic parameters π^*, α and β. This intrinsic linear relationship to
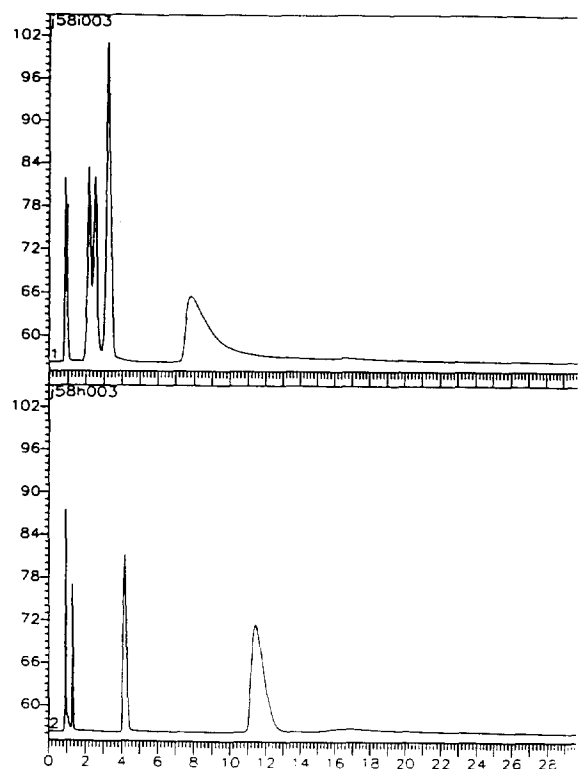
Fig. 1. Chromatograms show the difference between "base"-washed zirconias. (Top) Sodium hydroxide-washed zirconia; (bottom) ammonium hydroxide-washed zirconia. x-axis: Time in min; y-axis: absorbance at xxx nm.

ing differences between the chemical modifications and conversely which chemical modifications are useful in resolving the solutes.

Two principal component axes were used for characterization of these data. The reasons for using only two eigenvectors stem from the precision of the data analyzed. Over 97% of the variance is described in the first two eigenvectors of the log transformed data. From Table 1, the precision of these retention data is good to about 3–4%. Given the small incremental contribution to the description of variance by additional eigenvectors, and the precision of the measurements, it is reasonable to expect that most chemical information should be evident in the first two principal components.

3.4. Solute role in chemical modification variance

The contributions of the solutes to describe differences between the chemical modifications will be shown. The easiest manner to display these data is to plot the solutes scores in the chemical modification space. The solutes scores are plotted in the space defined by the chemical modification principal component axes 1 and 2 (Fig. 2). The number labels in the graph correspond to the solute number in Table 2 (solute 1 is the system blank). Solutes with positive scores are positively correlated to the variance defined by the principal component and those with negative scores are negatively correlated. It is important to note that both positive and negative scores are equally important in the description of the variance.

Many observations can be made from Fig. 2. Immediately, one can see a cluster of solutes that are only weakly retained on all the chemically modified zirconia phases. This tightly grouped cluster contains about half of all the solutes. It is noteworthy that these solutes have π^* values that are representative of the range covered by all solutes, however, every point in the cluster has low α and β parameters. The second cluster is not quite as tight as the first but is well defined. These solutes uniformly have high β

the ln k' data is supported when the eigenvalues are compared for the k' and ln k' matrices. The first eigenvector describes 72% of the variance when the k' data is analyzed using PCA, whereas, the first eigenvector of the log transformed data describes 92% of the variance in the data. In addition, the ln k' data show better resolution between the both solutes and the chemical modifications so a more meaningful interpretation of the data is possible. For these reasons the remainder of the discussion and all conclusions will be based on the PCA of the ln k' data.

It is possible to do a form of cluster analysis using the principal component axes and coordinants (scores) to investigate the inherent similarities between solutes and chemical modifications. The same plots can be used to visually determine which solutes are important in defin-
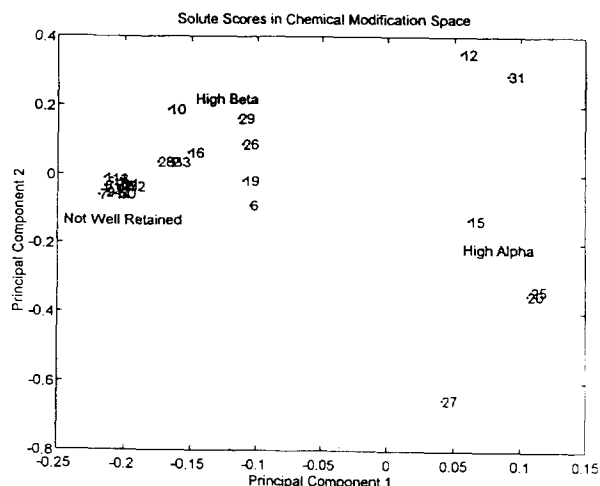
Fig. 2. Solutes plotted in Lewis base modification principal component space (principal component 1 versus 2). PCA performed on ln k' data from Lewis base-modified zirconia only. Clusters are evident for those solutes which are not well retained, those with high β values and those with high α values.

3.5. Chemical modification role in solute variance

To characterize the inherent similarities and differences between the Lewis base-modified zirconia and bare silica, the chemical modification scores were plotted on the solute space principal component axes. In this section, the (PCA) will compare and contrast the selectivities of the Lewis base-modified zirconia for the probe solutes. The data for silica are included in this analysis to be used as a comparator. The chemically modified zirconia and silica scores are shown for solute space principal component 1 versus 2 in Fig. 3. The chemically modified zirconia points are labeled with both a number and the identity of the Lewis base modification. There are four clusters that can be seen in the plot of principal component 1 versus 2. These clusters can be categorized as strong bases, strong acids, weak acids (with high Lewis basicity), and silica.

Interestingly, the sodium fluoride-modified zirconia is very similar to bare silica. There is almost no difference in the scores of these materials on principal component 1 which ac-

parameters with the exception of o-nitrotoluene (solute 10). The third cluster is composed of all solutes that have high α parameters. Each of these analytes has a positive score in principal component 1, whereas all other solutes have negative scores. The one anomaly in this cluster is pyridine (solute 31) which has a very high β parameter but a low α. Pyridine is well known as a very good Lewis base. Parameters that define Lewis basicity (e.g. β and π^*) for some Lewis bases may be different for pyridine. These observations point to the current lack of a Lewis basicity scale, but also to the possibility of establishing such a scale. These observation show that the differences between the Lewis base-modified zirconia are best displayed by test solutes with high α or β parameters. These are solutes with high Lewis base character.

Solutes 2 and 33 both represent benzonitrile. This solute was run first and last as a check of reproducibility of the chromatographic phases. Fig. 2 shows that the scores for the replicates are almost identical which supports the claim of stability and reproducibility.
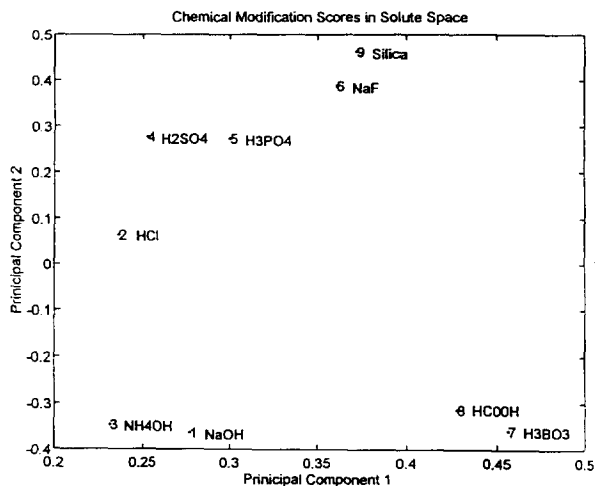

Fig. 3. Lewis base-modified zirconia phases and bare silica phase plotted in solute principal component space (principal component 1 versus 2). The differences between "acid" or "base" treatments are illustrated.

counts for over 90% of the variance in the data. In addition, these phases are very close in the principal component 2 space. The practical implication of this is that from a first-order approximation, there is little difference in the selectivity of silica and NaF-pretreated zirconia.

The "strong base" and "weak acid"-modified zirconia (sodium hydroxide, ammonium hydroxide, formic acid and boric acid) show solute retention characteristics which are opposite to silica in principal component 2. The important observation between these two phases is that there is a significant difference between "strong base"-modified zirconias. Caution must be taken by the analyst to specifically state which base wash is required.

The distribution of the acids on this plot is even more pronounced than for the bases. There is a large and clear separation between the strong and weak acids. The significant difference between sulfuric and phosphoric acids on principal component axis 1 and hydrochloric and sulfuric acid on principal component axis 2 emphasizes that the nature of the "acid" wash must be clearly defined or perceived reproducibility problems will arise.

4. Conclusions

Care should be taken when preparing "acid"- or "base"-washed zirconia for normal-phase applications. Changes in selectivity and efficiency result from interactions between Lewis bases in the wash solution and the zirconia Lewis acid sites. Any pretreatment of an adsorbent, prior to use under normal-phase conditions, should be explained and reproduced in detail.

Once modified, however, the zirconia supports prove to be quite stable and useful for obtaining a variety of selectivities with only one column. Hydrogen bonding interactions are quite pronounced for the Lewis base-modified zirconia, the extent of which differs greatly among the various Lewis bases used to modify the zirconia.

Dipolarity/polarizability appears to be a much weaker interaction under the conditions employed in these studies. Similar results can be expected for alumina adsorbents as well, since the Lewis acid properties of alumina are comparable to that of zirconia [10].

PCA was shown to be a powerful tool for the characterization of diverse chromatographic phases. Qualitative PCA, in combination with solvatochromic theory, suggests that a quantitative Lewis basicity scale may be able to be established.

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