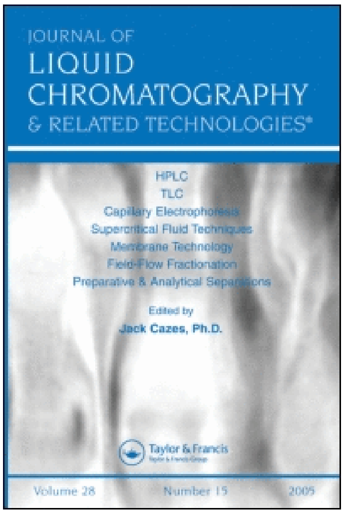

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Hu, Yu-Ling , Feng, Yu-Qi and Da, Shi-Lu(2001) 'CHROMATOGRAPHIC EVALUATION OF ALKYLPHOSPHONIC ACID-MODIFIED CERIA-ZIRCONIA IN REVERSED-PHASE HPLC', Journal of Liquid Chromatography & Related Technologies, 24: 7, 957 — 971

To link to this Article: DOI: 10.1081/JLC-100103422

URL: http://dx.doi.org/10.1081/JLC-100103422

J. LIQ. CHROM. & REL. TECHNOL., 24(7), 957–971 (2001)

CHROMATOGRAPHIC EVALUATION OF ALKYLPHOSPHONIC ACID-MODIFIED CERIA-ZIRCONIA IN REVERSED-PHASE HPLC

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ABSTRACT

Porous ceria-zirconia sperules were surface modified with alkylphosphonic acid to create reversed phase material. The chromatographic properties of the ceria-zirconia-based stationary phase were evaluated in detail with neutral, basic, and acidic compounds as probes, over a wide range of mobile phase composition and pH. Polycyclic aromatic hydrocarbons (PAHs) were well separated and their elution order were in accordance with those reported on the silica-based reversed-phase column.

Typical reversed-phase chromatographic behavior was also observed with basic solutes at pH10.0, whereas dual mode retention mechanism was adopted to elucidate retention characteristics of some of the basic solutes at pH4.0. Acidic compounds exhibited strong interaction with the alkylphosphonic acid modified ceria-zirconia (PA-CeO_2-ZrO_2) with methanol-water mixture as mobile phases, but these interaction were reduced to different

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extents in the presence of competing agents, such as acetate, sulfate, and oxalate. o-hydroxybenzoic acid irreversibly adsorbed on the PA-CeO_2-ZrO_2 stationary phase, even in the presence of oxalate, which may be explained by the formation of chelate ring.

INTRODUCTION

Recently, there has been an increasing interest in the use of porous zirconia as an alternative support to silica for high performance liquid chromatography.[1] This material has many properties of excellent support, including desirable mechanical strength of silica, but with improved chemical stability. Excellent success has been achieved in preparation, characterization, and evaluation of zirconia in the last few years. However, the development of zirconia as a chromatographic support still fall far behind that of silica. Despite its attractive potential, zirconia has drawbacks that inhibit its wide application.

The specific surface of zirconia is relatively low compared to that of silica.[1] A considerable number of pore constrictions for zirconia exist depending on different preparation methods.[2] The surface of zirconia is chemically heterogeneous. Solutes containing Lewis-base moieties strongly interact with the exposed zirconia surface, leading to peak broadening, tailing, or even worse, irreversibly adsorbing the solutes to the stationary phase.[3] Based on the limitation mentioned above, an enormous amount of work has been done to improve physical properties of zirconia.

Shalliker and Douglas[4,5] have improved the specific surface area and specific pore volume of zirconia to some extent by a sodium chloride impregnation technique. The same group has prepared spherical silica-zirconia composites by coating zirconia microspheres with silica.[6,7] Their results illustrated that addition of silica to zirconia prevented the formation of monoclinic zirconia.

Silica-zirconia composites have also been prepared by Kaneko et al[8-10] by a coprecipitation method. The new materials exhibit different properties that are not observed in their separate component. Although, the work is very interesting, they did not carry it on comprehensively. Our special interest is to improve the pore structure and surface properties of zirconia by addition of other metal oxides. In recent years, we have prepared and evaluated silica-zirconia, magnesia-zirconia, alumina-zirconia, and ceria-zirconia composites in sequence.[11-13]

Physical characterization of ceria-zirconia illustrating higher specific surface area, larger specific pore volume, and better pore connectivity were obtained with ceria-zirconia rather than with zirconia. Chromatographic evaluation of the bare ceria-zirconia material in normal phase mode suggested that the stationary phase was superior for separation of polar and basic compounds.

Reversed-phase liquid chromatography is still the most common mode of HPLC due to its versatility for separation of various solutes. Therefore, we are interested in chemically modifying the surface of ceria-zirconia, turning it into a reversed-phase material. There are three major methods for modification of metal oxide. The first is bonding the metal oxide with alkylsilicane groups, a method that is commonly adopted for modification of silica. Octadecyl-zirconia[14-16] and octadecyl-titania[17-19] stationary phases have been prepared following this procedure.

Although certain success has been obtained with this method, the instability of M-O-Si (i.e., Zr-O-Si, Ti-O-Si, Al-O-Si) compared with Si-O-Si, limited its wide application.[20] Carr and his coworkers, devoted a large amount of time to coating zirconia with polymers, such as polybutadiene[21-23] and polystyrene.[24] The polymer-coated zirconia was stable over a wide range of pH, and the materials have effectively been used to separate low-molecule solutes, peptides, and proteins. However, chromatographic efficiency of polymer-coated material depends largely on the coating procedure. Inhomogeneous aggregation of polymer into the pores of support will decrease column efficiency significantly.[25]

As zirconia has a great affinity towards Lewis bases, methods have been developed for modification of zirconia by adsorption of Lewis bases on its surface. Based on this method, Carr et al have modified zirconia with fluoride,[26] phosphate,[27] and ethylenediamine-N,N'-tetramethylphosphonic acid (EDTPA)[28] to create cation-exchange stationary phases. Magnesia-zirconia-based reversed phase has been recently prepared by adsorption of Lewis base in our laboratory.[29-32] The stationary phase exhibits ideal properties required in liquid chromatography.

In this study, the ceria-zirconia-based alkylphosphonic-acid-modified reversed phase has been prepared and evaluated with various compounds as probes. Results show the new material exhibited reversed-phase characteristics analogous to other hydrophobic material. However, ion-exchange interaction and ligand-exchange interaction also contributed to the retention of basic and acidic solutes in certain cases.

EXPERIMENTAL

Chemicals

Reagent grade methanol, acetate, Tris, sodium chloride was purchased from Shanghai Reagent Company. All samples were obtained from commercial sources and were of reagent grade or better. Alkylphosphonic acid was synthesized in our laboratory as reported previously.[29,30]

Preparation of Alkylphosphonic Acid-Modified Ceria-Zirconia

Ceria-zirconia composite was prepared according to the modified procedures of our previous method.[11] Briefly, zirconyl chloride and cerium nitrate were dissolved in distilled water and the mixture was then poured into light petroleum containing Span 80 and Tween 85 with stirring, at the rate of 1800 rpm. After 10 min, the pH was adjusted to approximately 10 by slowly introducing $NH_{3.}$ The reaction mixture was stirred for at least 48 h. Then the hydrogel particles of the mixed oxides obtained were subjected to a multi-stage washing procedure. After a classification procedure, the collection of particles was heated at 120°C for 2 h in order to turn the hydrogels to xerogels. Thereafter, the xerogels were calcinated at 600°C for 1 h to remove the organic residues.

The ceria-zirconia composite was slurry-packed into a 15 cm × 4.6 mm stainless-steel column. 10 mL n-dodecanol containing alkylphosphonic acid was dissolved in 300 mL methanol. This solution was pumped through the column for 20 h at room temperature, followed by washing the column with methanol and water in sequence, to remove unabsorbed alkylphosphonic acid.

Chromatography

The HPLC equipment consisted of a Shimadzu 10A Liquid Chromatograpic pump, SPD-10A US-Vis photometric detector, a Rheodyne 7125 injection system, and a Shimadzu C-R6A integrator. The column temperature was controlled at 28 ± 1°C through a water bath. A methanol-water mixture containing 50 mM sodium chloride, 5 mM acetic acid-sodium acetate buffer at pH 2.0, 4.0, 6.0, and 5 mM Tris-HCl buffer at 8.0,10.0, were used as mobile phases. The flow rate of the mobile phase was set at 0.5 $mL.min^{-1}$. The detection wavelength was set at 254 nm. Prior to the study, the columns were equilibrated each time with 100 mL of the mobile phase.

RESULTS AND DISCUSSIONS

Stability and Regeneration Study

The chemical stability of the alkylphosphonic acid modified ceria-zirconia, was investigated by flushing with methanol-5mM buffer solution (50:50) at pH2.0 and pH10.0, respectively. The retention of biphenyl and N,N’-dimethylaniline was tested at intervals after the column was equilibrated with methanol-water mixture (60:40). The results are shown in Figure 1. It is obvious from

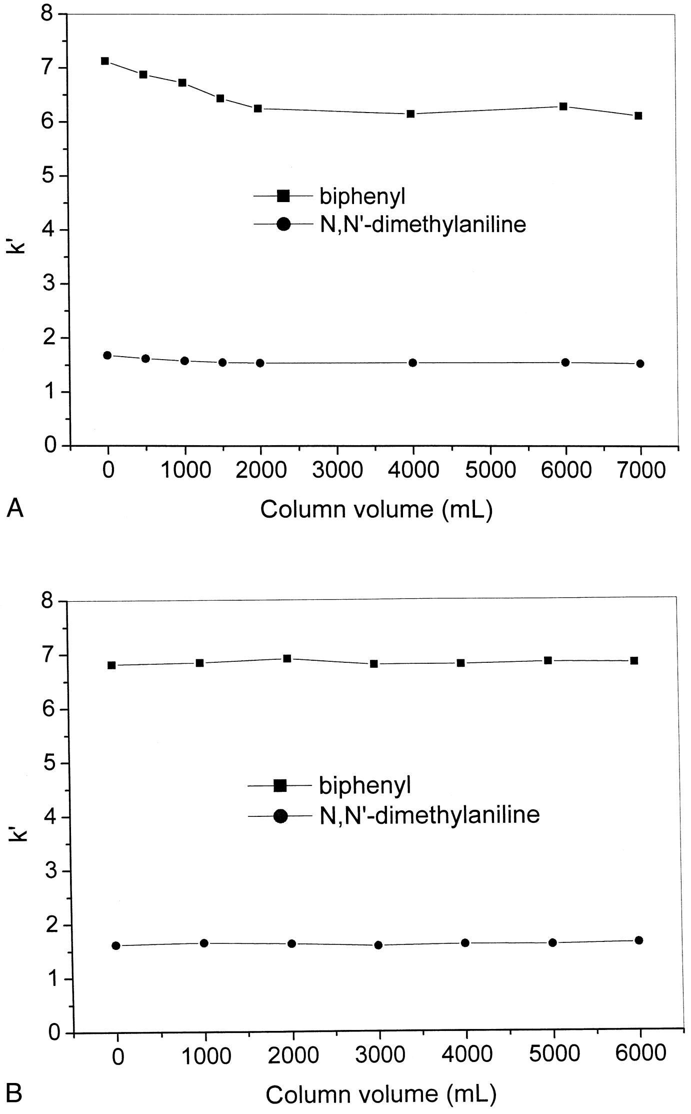

Figure 1. Plots of capacity factor of the test solutes versus the number of column volume of solution perfused through the column. Solutions used in the stability test, methanol-buffer (50/50, v/v) containing 50 mM sodium chloride, 5 mM acetic acid at pH 2.0 (a) and 50 mM sodium chloride, 5 mM Tris at pH 10.0 (b).

Figure 1, that PA-CeO_2-ZrO_2 stationary phase was very stable at pH2.0, as indicated by nearly constant retention of biphenyl and N,N'-dimethylaniline.

On the other hand, there appears to be some decrease of retention of the test solutes when PA-CeO_2-ZrO_2 was exposed to methanol-5mM Tris solution at pH10.0. A reasonable explanation is that alkylphosphonic acid was partly stripped from the surface by the attacking of the hydroxyl ion that was announced to be a competing Lewis base. However, the retention of test solutes reached a constant value after 2000 column volume. As the surface of ceria-zirconia is so complex, there may exist adsorption sites of different energy. Only the alkylphosphonic acid adsorbed on the weaker sites can be displaced from the surface of ceria-zirconia.

In addition, it has to be noted that no compaction of the column bed was observed throughout the course of the stability test. Based on the result mentioned above, it can be concluded that the PA-CeO_2-ZrO_2 stationary phase is relatively stable for chromatographic use.

Attempts to regenerate the stationary phase were conducted by equilibrating the column with the original alkylphosphonic acid solution. At first, the column was directly flushed with 500 mL of alkylphosphonic acid solution after being exposed to pH10.0 solution. There was no sign of resumption of column, as the retention of biphenyl never increased. Then, the column was first fully washed with water, and afterwards equilibrated with 500 mL alkylphosphonic acid solution. The retention of biphenyl has been increased, partially, by the treatment. But, further treatment under this condition fell short of returning the column to its original state.

Retention of PAHs

The reversed-phase chromatographic properties of the alkylphosphonic acid modified ceria-zirconia stationary phase, were evaluated with polycyclic aromatic hydrocarbons. Figure 2 illustrates the relationship of the logarithmic capacity factor of PAHs and the volume percent of methanol in the mobile phase. Straight lines with the correlation coefficient above 0.999 were obtained. With the decrease of methanol in the mobile phase, selectivity among the PAHs was improved. Excellent separation of the seven PAHs was readily observed with methanol-water (60:40) as the mobile phase, shown in Figure 3.

As can be seen in Figure 3, the elution order is in accordance with the order of increasing hydrophobic area of the molecules. For PAH isomers, such as phenanthrene and anthracene, their breadth-to-length ratio (B/L) which are 1.46 and 1.57, respectively,[34] determined their retention, i.e., the isomer of higher B/L was more retarded.

The chromatographic properties mentioned above are all analogous to those reported on silica-based reversed-phase columns. Thus, PA-CeO_2-ZrO_2 can

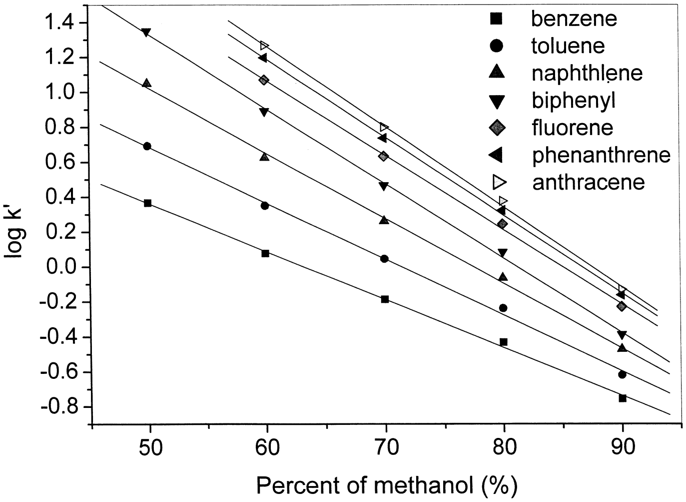

Figure 2. Plots of logarithmic capacity factor of PAHs versus the volume percent of methanol in the mobile phase. Mobile phase: water at various volume percent of methanol; flow rate: 0.5 mL/min.

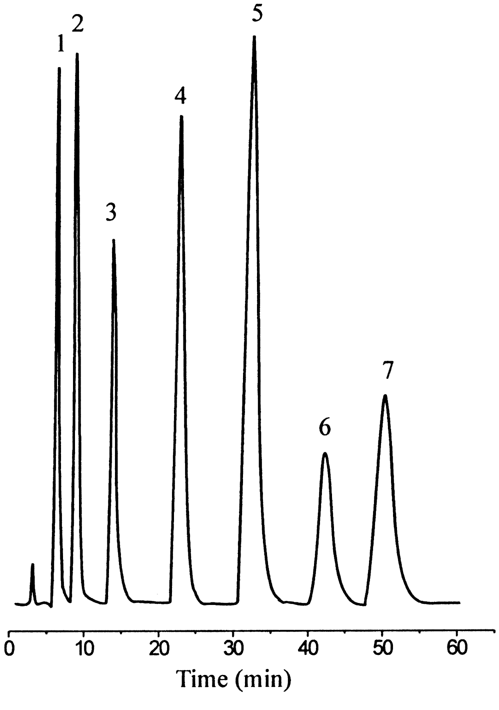

Figure 3. Separation of polycyclic aromatic hydrocarbons. Mobile phase: methanol/water (60/40, v/v); flow rate: 0.5 mL/min. Peaks: 1:benzene, 2:toluene, 3:naphthalene, 4:biphenyl, 5:fluroene, 6:phenanthrene, 7:anthracene.

be used as a hydrophobic stationary phase for fast separation of non-polar solutes.

Retention of Basic Solutes

It was well known that the retention of charged species depended largely on the pH value of the mobile phase. To thoroughly investigate the chromatographic properties of the PA-CeO_2-ZrO_2, the retention of basic solutes was investigated over a wide range of pH. As shown in Figure 4, the retention pattern of basic solutes falls into two categories according to different chromatographic behavior. For those amines without substituted groups on nitrogen atom, the capacity factors were small at pH 2.0, then reached the maximum at pH 4.0. As the pH arrived at 6.0, their retention decreased dramatically and then kept a slow increase at higher pH (Figure 4(a)). The retention behavior of basic solutes within this group, under extreme low and high pH condition, is understandable.

As most of the solutes protonated at pH2.0, the hydrophobic interaction between solutes and stationary phase was very weak. Besides, electrostatic exclusion from the positively charged support further contributed to the decrease of solute retention. At pH above 6.0, deprotonation of the solutes led to the gradual increase of retention. The interesting retention behavior occurred at pH 4.0, whereby, capacity factors of solutes in this group were much higher than those expected according to hydrophobic mechanism. In addition, peak tailing and broadening was apparent at pH 4.0.

The behavior can be explained by dual mode retention mechanism, i.e., reversed phase and ion-exchange. As pK_a of the basic solutes in Figure 4(a) are between 3.88 and 5.08, these compounds are partially protonated at pH 4.0, resulting in hydrophobic interaction, as well as cation-exchange interaction with the exposed hydroxyl group of alkylphosphonic acid. However, the ion-exchange interaction was not notable for those amines with non-polar substitutes on a nitrogen atom. Sigmodial curves typical for hydrophobic mechanism were obtained for these solutes, as shown in Figure 4(b). This may be a result of shielding effect as the cation-exchange sites have been protected by non-polar groups.

To further elucidate the retention mechanism of basic solutes on PA-CeO_2-ZrO_2 stationary phase, the logarithmic capacity factor of basic solutes was plotted against their hydrophobic constant[33] at pH 4.0 and pH 10.0, respectively. The linear correlation results are shown in Figure 5. If hydrophobic interaction was the dominant mechanism for retention of solutes, a linear relationship of plot logk'-logP would exist. Otherwise, secondary interaction, such as ion-exchange interaction would contribute to the retention mechanism.

It can be seen, in Figure 5, that an acceptable linear relationship of logk'-logP with the correlation coefficient of 0.97 was obtained at pH 10.0, illustrating

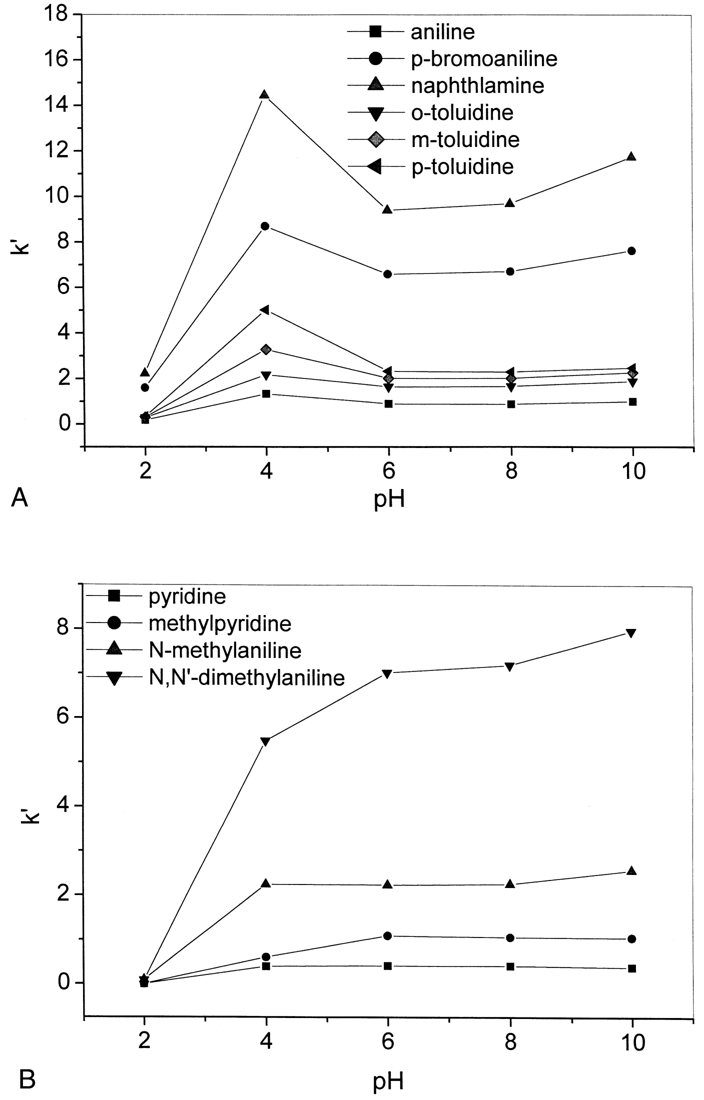

Figure 4. Plots of capacity factor of basic solutes versus the pH of the mobile phase. Mobile phase: methanol-buffer (30/70, v/v) containing 50 mM sodium chloride, 5 mM acetate for pH 2.0, 4.0, 6.0, and 5 mM Tris for pH 8.0, 10.0; Flow rate: 0.5 mL/min.

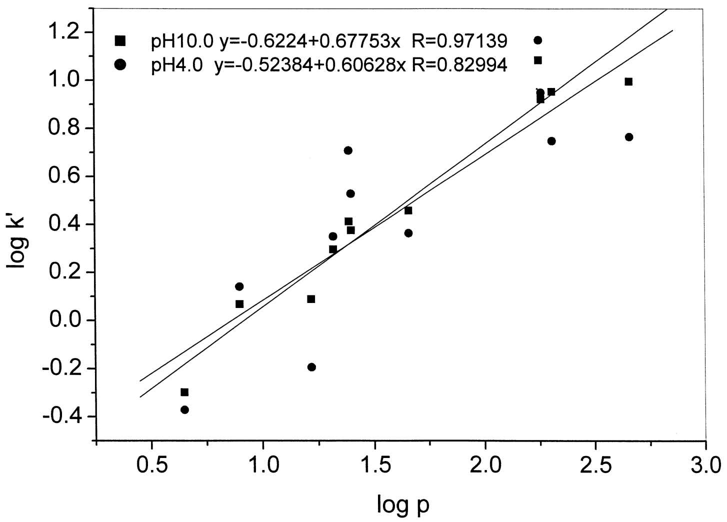

Figure 5. Plots of capacity factor of basic solutes versus their hydrophobic constant at pH 10.0 and pH 4.0. Mobile Phase: methanol-buffer (30/70, v/v) containing 50 mM sodium chloride, 5 mM acetate for pH 4.0, and 5 mM Tris for pH10.0; Flow rate: 0.5 mL/min. Basic compounds are pyridine, aniline, 3-methylpyridine, o-toluidine, m-toluidine, p-toluidine, N-methylaniline, p-bromoaniline, N,N'-dimethylaniline, 2-naphthylamine, N-ethyl-m-toluidine.

that basic solutes were retarded mostly by hydrophobic interaction mechanism at pH10.0. In contrast, bad linear correlation (R=0.83) occurred at pH 4.0, which confirmed the existence of ion-exchange interaction.

It should be noted that this kind of secondary interaction was beneficial for the increase of selectivity, although peak broadening limited the separation of basic solutes under this condition. Nevertheless, ideal separation of seven basic solutes was still available at pH 10.0, as shown in Figure 6.

Retention of Acidic Solutes

It was well documented that the bare zirconia had a strong affinity towards acidic solutes. The interaction was still apparent even after the zirconia was covered by a thick polymer layer.[21] In the current study, acidic solutes were tested on the PA-CeO_2-ZrO_2 stationary phase under various conditions. The results are shown in Table 1. Elution of the acidic solutes was not observed at all in methanol-water (80:20) without the competing agent in the mobile phase. This

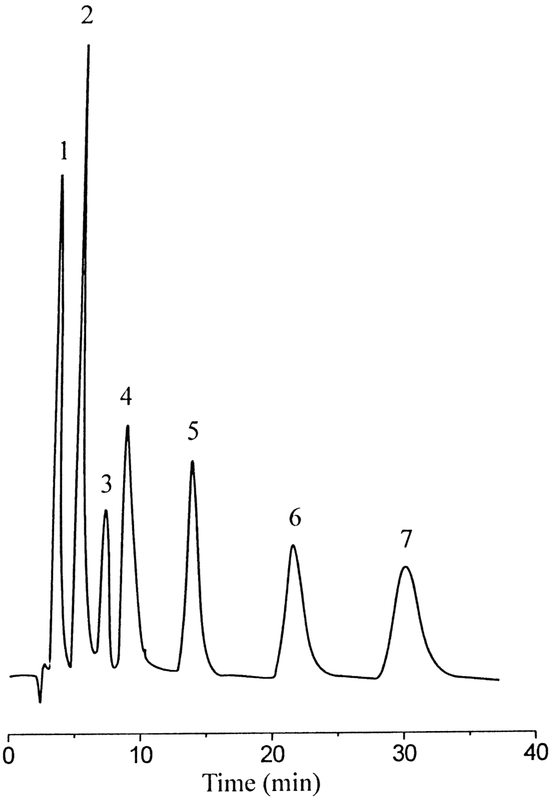

Figure 6. Separation of basic solutes. Mobile phase: methanol-buffer (30/70, v/v) containing 50 mM sodium chloride, 5 mM Tris at pH10.0; Flow rate: 0.5 mL/min. Peaks: 1: pyridine; 2: 3-methylpyridine; 3: o-toluidine; 4: 2,2'-bipyridine; 5: o-nitroaniline; 6: p-bromoaniline; 7: β-naphthylamine.

indicates that the ligand exchange sites on CeO_2-ZrO_2 surface are still accessible even after modification of alkylphosphonic acid. When methanol-acetate buffer (30:70) was used as mobile phase, most of the acidic solutes eluted with appropriate capacity factors.

Elution of m-phthalic acid and o-hydroxybenzoic acid was not observed even under this condition, probably due to the formation of a chelate ring. Addition of stronger competing agent such as sulfate and oxalate into the mobile phase, had no apparent effect on the capacity factors of acidic solutes without chelate groups, but contributed to a decrease of retention of carboxylic acids with complex ability, except for o-hydroxybenzoic acid, which forms a strong chelate

Table 1. Capacity Factor of Acidic Solutes in Various Mobile Phase Compositions

	k’		
Solutes	Mobile Phase A	Mobile Phase B	Mobile Phase C
Benzoic acid	5.31	5.10	4.88
p-Nitrobenzoic acid	5.13	5.20	4.95
3,5-Dinitrobenzoic acid	3.89	3.95	3.75
Cinnamic acid	6.88	6.99	6.23
o-Phthalic acid	7.06	3.38	1.52
m-Phthalic acid	eno	eno	5.58
p-Hydroxybenzoic acid	3.49	3.5	2.91
o-Hydroxybenzoic acid	eno	eno	eno

Mobile phase A: methanol/water (30/70,v/v) containing 5mM acetic acid and 50mM sodium chloride at pH2.0.
Mobile phase B: addition of 10mM sodium sulfate in mobile phase A.
Mobile phase C: addition of 10mM oxalic acid in mobile phase A.
eno: elution not observed in 2h.

ring with the metal oxide. Unfortunately, the stationary phase was not very stable on addition of sulfate and oxalate into the mobile phase. This may ascribe to displacement of alkylphosphonic acid by these strong competing agents.

Column Efficiency

Since resolution in chromatography depends both on selectivity and on efficiency, we are concerned with the efficiency of the column. In most cases, the column efficiency of zirconia-based stationary phases was lower than that of silica-based stationary phases. This is determined by the intrinsic physical properties and surface heterogeneity of zirconia. Results of our studies show introducing ceria into zirconia greatly increased the column efficiency, as better pore structure was obtained for the ceria-zirconia composite. We expect to further increase the column efficiency by chemically masking the adsorptive sites on the surface of supports. In fact, the column efficiency increased approximately 1.6 times after modification of ceria-zirconia by alkylphosphonic acid. However, the presence of a secondary interaction was disadvantageous to column efficiency.

Table 2 illustrates the column efficiency of PA-CeO_2-ZrO_2 measured with various solutes, at different pH of the mobile phases. It can be seen from Table 2, that plate counts determined by neutral compounds is relatively high and exhibits little variance at different pH. But, pH of the mobile phase has a significant

Table 2. Capacity Factor and Column Efficiency Measured at Various pH

	pH 10.0		pH 4.0		pH 2.0	
Solutes	k'	N	k'	N	k'	N
Benzene	4.86	2568	4.90	2178	4.86	2527
Phenol	3.54	129	2.19	678	1.78	977
p-Toluidine	2.59	2019	5.06	226	0.35	924

Mobile phase: methanol/water (30/70, v/v) containing 50mM sodium chloride and 5mM acetate buffer for pH 2.0, pH 4.0 and 5mM Tris buffer for pH 10.0.
k'= capacity factor; $N = 5.54 (t_r/W)^2$.

effect on peak width of charged species. Phenol exhibits a broader and tailing peak at higher pH, due to stronger ligand interaction with the support, although the efficiency at low pH is also not satisfied. P-toluidine has a comparable peak shape to benzene at pH 10.0, whereby hydrophobic interaction is almost the sole retention mechanism; however, it shows very broad peaks in the presence of secondary interactions at pH 4.0. Based on the above result, it is necessary to minimize the secondary interaction if high column efficiency is expected.

CONCLUSIONS

The study on the chromatographic characterization of alkylphosphonic acid modified ceria-zirconia, has shown that this stationary phase has potential as reversed-phase material. The retention mechanism of PA-CeO_2-ZrO_2 was analogous to that of alkyl-silica bonded phases, with respect to retention of neutral compounds in methanol-water mixtures and retention of basic solutes in methanol-Tris buffer solution at pH 10.0. Cation-exchange interaction between primary amines and the stationary phase was present in methanol-acetate buffer at pH 4.0. Ligand exchange interaction of acidic solutes with the bulk ceria-zirconia support persist, although, the surface of support was blocked with alkylphosphonic acid, especially for those carboxylic acids with complex ability.

ACKNOWLEDGMENTS

Financial support of this research by a grant from the National Nature Science Foundation of China and Hubei Provincial Nature Science Foundation of China is gratefully acknowledged.

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Received September 15, 2000
Accepted October 11, 2000

Manuscript 5397