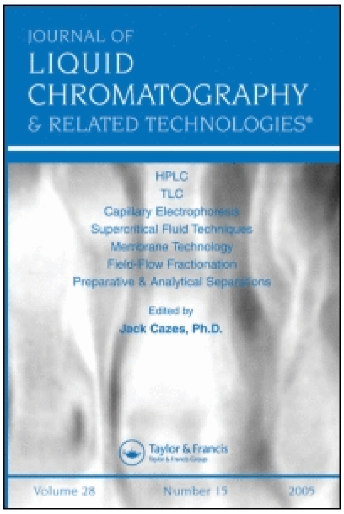

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Preparation and Chromatographic Characterization of Zirconia Modified with Dodecylamine-*N*,*N*-dimethylenephosphonic Acid

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Preparation and Chromatographic Characterization of Zirconia Modified with Dodecylamine-*N*,*N*-dimethylenephosphonic Acid

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ABSTRACT

A new zirconia-based stationary phase was prepared for the first time by modified zirconia with dodecylamine-*N*,*N*-dimethylenephosphonic acid (DDPA). The adsorption mode that DDPA adsorbed on zirconia with only one phosphonic group was studied by the coloration reaction. The resultant stationary phase (DPZ) has not only long alkyl groups, but also amino group and free phosphonic groups. The chromatographic characterization of the stationary phase was strongly influenced by these polar groups. Despite the lower surface coverage of the DPZ, the capacity factors of polar solutes on DPZ are greater than that on ODS due to the various retention mechanism provided by the polar groups.

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The free phosphonic group imparts the negative charges to the stationary phase at high pH and blocks most of the Lewis acid sites on the surface of zirconia, so excellent separations were obtained not only for PAHs and amines, but also for phenols. Separations of benzoic acids and positional isomers of dihydroxylbenzene and nitrophenol on DPZ were improved greatly.

Key Words: Zirconia; Stationary phase; Dodecylamine-*N*,*N*-dimethylenephosphonic acid; HPLC.

INTRODUCTION

Silica is the most widely used packing material for high performance liquid chromatography (HPLC), however, the use of silica-based stationary phase is usually limited to the pH range of about 2 to 8. In addition, the separation of basic solutes on silica-based stationary phase was unsatisfactory; so, the use of alternative packing materials, such as zirconia, titania, alumina, has been of great interest for decades. Among these materials, zirconia arouses much fascination due to its excellent chemical (from pH 1 to 14), thermal (up to 200°C), and mechanical stability.[1]

Zirconia stationary phases, modified by coating with a polymer or cladding with a carbon layer, can be used in HPLC under very aggressive conditions.[2–7] For example, polybutadiene-coated zirconia (PBD-ZrO_2)[5–7] had been tested with 5000 column volumes of eluent at $>150^\circ$C with no changes in the retention characteristics of the column. Zirconia can also be modified with Lewis-bases due to the strong Lewis acid–base interaction, such as inorganic phosphate,[8,9] organic phosphonic acids,[10–14] stearic acid,[15,16] 2,4,6,-trinitrophenol.[17] The alkylphosphonic acid (APA), which was synthesized from dodecanol and fosfomycin, was used for modification of zirconia–magnesia and zirconia–ceria composites.[10–13] The APA-modified composites, which have high stability in reversed-phase chromatographic conditions, were used for separating aromatic hydrocarbons, disubstituted benzene isomers, basic compounds, bases, and nucleosides.

However, zirconia also has some disadvantages. The surface chemistry of zirconia significantly influences the chromatography of certain solutes. The Lewis acid sites on the surface of zirconia is always troublesome, carboxylic acids and phenols strongly adsorb on these sites of zirconia, leading to severe peak broadening and tailing. Up to now, neither polymer-coated zirconia or Lewis base-modified zirconia can be used for separating Lewis base compounds successfully. It is unrealistic to hope the polymers block all of the Lewis acid sites on the surface of zirconia without sacrificing resolution and efficiency (optimized polymer coatings of PBD-ZrO_2 only block 60% of the Lewis acid sites[18]). Although the interaction between zirconia and Lewis bases (such as organic phosphonate) is very strong, it is almost impossible to block all of the Lewis acid sites with these modifiers; benzoic acids still refused to be eluted on these stationary phases.[10–13]

A few references discussed the separation of phenols or acids on zirconia, attempts had been undertaken on both polymer-coated zirconia and Lewis base-modified zirconia. Great improvement was made by covalently linked vinlyphosphonic acid (VP) to the PBD network to get VP–PBD-ZrO_2.[18] On this zirconia, the recovery for three hard Lewis bases (catechol, benzoic acid, and salicylic acid) is 100%, but the peak shape is not acceptable, and the column efficiency is very low; VP must be added to mobile phase as additive to get satisfactory separations. Other attempts, such as modified zirconia with PEI-SUCC and carboxymethyl dextran[19–21] were also performed. In these works, Dunlap et al. hoped the carboxyl of these polymers would block the Lewis acid sites. But the Lewis acid sites on the stationary phases are still available to the solutes, so fluoride or phosphate must be added to the mobile phase as additives when separating Lewis basic solutes. Improvement was also made on Lewis base-modified zirconia. Ethylenediamine-*N*,*N*′-tetramethylphosphonic acid (EDTPA)-modified zirconia (PEZ)[22–25] is quite different from APA-modified zirconia (APA-ZrO_2), it seems that EDTPA blocked the Lewis acid sites of zirconia thoroughly; the separation of Lewis bases is also dissatisfactory—acid proteins (Lewis base compounds) do not retain at all ($k < 0$) on this stationary phase, just like benzoic acids have done on zirconia modified with inorganic phosphate.[8,9]

Both APA-ZrO_2 and PEZ are useful zirconia-based stationary phases modified with Lewis base. APA-ZrO_2 can be used for separating various solutes due to the hydrophobicity provided by long alkyl group, but benzoic acids can not be eluted on this stationary phase. On the contrary, PEZ almost has no hydrophobicity, but benzoic acids can be eluted easily on PEZ. In order to separate acidic compounds (Lewis bases) on zirconia with moderate retention, a new zirconia stationary phase is prepared in this work. Dodecylamine-*N*,*N*-dimethylenephosphonic acid (DDPA), a compound with a long alkyl as APA and multi-phosphonic groups as EDTPA, was selected as modifier. The adsorption mode of DDPA on zirconia was investigated by coloration reaction of the stationary phase (DPZ) with ammonium molybdate. The chromatographic property of various solutes, such as polycyclic aromatic hydrocarbons (PAHs), basic and acidic derivatives on DPZ was studied in detail.

EXPERIMENTAL

Chemicals

Zirconia (5–7 μm) was prepared in our laboratory according to the method reported elsewhere,[12] surface area was 29 m^2/g and average pore diameter was 9.1 nm, measured by nitrogen adsorption/desorption isotherms on a model ST-03A specific surface area analyzer (The Analytical Instrument Plant, Beijing, China). Ammonium molybdate solution was prepared by dissolving 1.3 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 100 mL diluted sulfuric acid (137 mL/863 mL); Tin(II) chloride solution was prepared by dissolving 2.5 g $SnCl_2$ in 100 mL glycerol; phosphorous acid (50%), dodecylamine, formaldehyde (37%) are analytical reagents, obtained from Shanghai General Chemical Reagent Factory (Shanghai, China). Other chemicals were obtained from commercial sources and were of reagent grade or better. Distilled water was boiled for 15 min before use, to remove dissolved carbon dioxide.

DDPA was synthesized by a Mannich-type reaction according to Ref.[26]. Briefly, 0.01 mol dodecylamine was added to 30 mL HCl (1 : 1), and then heated to 80°C with stirring; after dodecylamine was dissolved, 0.25 mol phosphorous acid and 0.25 mol formaldehyde were added, the mixture was allowed to react at 80°C for 4 hr. The reaction was cooled and adjusted to pH2 with pyridine, the white product was filtered, washed by 0.01 mol/LHCl, and recrystallized with ethanol.

Columns

DPZ

The modification procedure of zirconia with DDPA was improved on the basis of the method described in Ref.[22]: 7.0 g zirconia was placed in 250 mL round-bottomed flask and suspended in 150 mL 2-propanol containing 1.0 g DDPA. After 8 hr at reflux with stirring, the particles were allowed to settle and the DDPA solution was decanted, the particles were filtered and washed several times with hot 2-propanol to remove any physical adsorbed DDPA.

DDPA-modified zirconia (DDPA-ZrO_2, DPZ) was dried at 100°C in a clean vacuum oven overnight, and slurry-packed into 15 cm × 4.6 mm stainless-steel HPLC column at 5500–6000 psi.

Surface coverage analysis of DPZ was characterized by elemental reanalysis, with Model-1106 elemental analyzer (Carlo Erba, Italy).

ODS

Conventional octadecyl silica chromatographic column (ODS) [Chromatorex, 5 μm, 100 Å, 15 cm × 4.6 mm was commercially purchased from Elite Chromatographic company (Dalian, China).

Coloration Reaction of Phosphate/Phosphonate-Modified Zirconia

In order to investigate the adsorption mode of DDPA on zirconia, various zirconia packings were tested, including: (A) native ZrO_2; (B) phosphate-modified ZrO_2[8]; (C) APA-modified ZrO_2;[10] (D) zirconia dipped 48 hr in a DDPA solution (at 25°C); (E) DDPA-modified ZrO_2 to be fresh prepared; (F) DDPA-modified ZrO_2 to be used 2 months in RP-HPLC (DDPA was not added to mobile phase). All of the zirconia stationary phases were washed thoroughly with hot 2-propanol, methanol, or water until phosphate/phosphonate was not detected in filtrate any more.

The testing process was performed as follows: 0.1 g zirconia packings were added into 10 mL test tubes, respectively, then in each tube was added 5 mL methanol/water, 2 mL ammonium molybdate solution, after 5 min, 0.5 mL $SnCl_2$ solution was added.

Chromatography

All studies were carried out with an Elite (II) HPLC system (Dalian, China), the data was processed by using Echrom 98 ChemStation software (ver 2.0) (Dalian, China).

Methanol/water or methanol/buffer was used as mobile phase. Acetate or Tris buffers were used to control the pH of the mobile phase when investigating the influence of eluent pH on the capacity factor of solutes. The pH values were measured in the aqueous buffer. Before use, the mobile phases were generally filtered through a G-4 fritted glass funnel and degassed in an ultrasonic bath for 5 min.

Solutes were dissolved in methanol/water mixtures. The wavelength used for detection was 254 nm. Column dead time was measured with methanol as probe.

RESULTS AND DISCUSSION

Adsorption Mode of DDPA on Zirconia

The surface coverage of DPZ was 3.3 μmol/m^2 according to the results of elemental analysis (N = 0.134%, C = 1.664%). It is equivalent to that of EDTPA-modified zirconia (3.0 μmol/m^2),[22] but only half of that of phosphate-modified zirconia (6.1 μmol/m^2).

The results of coloration reaction were given in Table 1. It can be seen, that only zirconia modified with DDPA reacted with a solution of ammonium molybdate to give a blue product (phosphomolybdic blue), which indicates that there are free phosphonic groups on the surface of DPZ. Furthermore, DPZ was washed many a time with 2-propanol, and even used for 2 months in RP-HPLC, the free phosphonic groups on surface were still detected, so the free phosphonic groups do not come from the DDPA physically adsorbed on zirconia, but from the chemicals adsorbed on zirconia.

From the results of the coloration reaction, the possible adsorption mode of DDPA on zirconia proved that DDPA adsorbs on zirconia only with one phosphonic group, as shown in Fig. 1(A). This kind of adsorption made the surface of DPZ change from cation to zwitterion, and to anion from low to high pH. This structure is similar to the embedded polar bonded stationary phases[27–30] that have proven to be very useful for separations of polar compounds containing basic functionalities, the typical structure is shown in Fig. 1(B). Therefore, it can be inferred that the similar structure of DPZ can be used for the separations of acidic compounds.

Overview of the Column Performance

The chromatographic property of DPZ was compared with ODS by using the benzene, aniline and phenol as probes. Owing to the lower surface coverage

Table 1. The coloration reaction of different zirconia packings.

Packings	Color
Native zirconia	White
Phosphate-modified zirconia	White
Alkylphosphonic acid-modified zirconia	White
Zirconia dipped in DDPA solution	Blue
DDPA-modified zirconia	Blue
DDPA-modified zirconia (used 2 months)	Blue

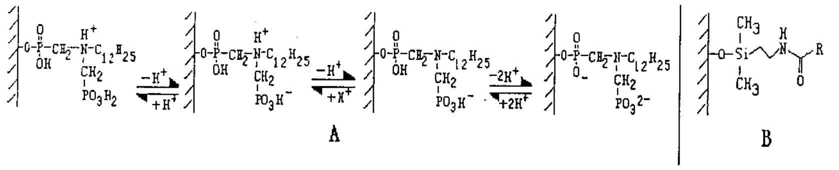

Figure 1. The possible adsorption mode of DDPA on zirconia (A) and the typical structure of embedded polar bonded stationary phase (B).[28]

and shorter alkyl group (C_{18} for ODS, C_{12} for DPZ), the hydrophobicity of DPZ is weaker than that of ODS (benzene: $k_{ODS}/k_{DPZ} = 6.76/3.09$). However, polar solutes have greater capacity factors on DPZ (aniline: $k_{ODS}/k_{DPZ} = 0.76/1.40$; phenol: $k_{ODS}/k_{DPZ} = 1.56/3.78$) due to the various retention mechanisms provided by polar groups of DPZ. Separation of the mixture was shown in Fig. 2.

Separation of PAHs

The separation of PAHs was carried out on DPZ with methanol/water as the mobile phase. The long alkyl group of DDPA provided hydrophobicity for DPZ, and excellent separation of the PAHs was achieved, as shown in Fig. 3. The retention data of capacity factor, relative retention, and column efficiency were given in Table 2. Compared to APA-ZrO$_2$,[10–13] the selectivity and column efficiency of DPZ are greatly improved.

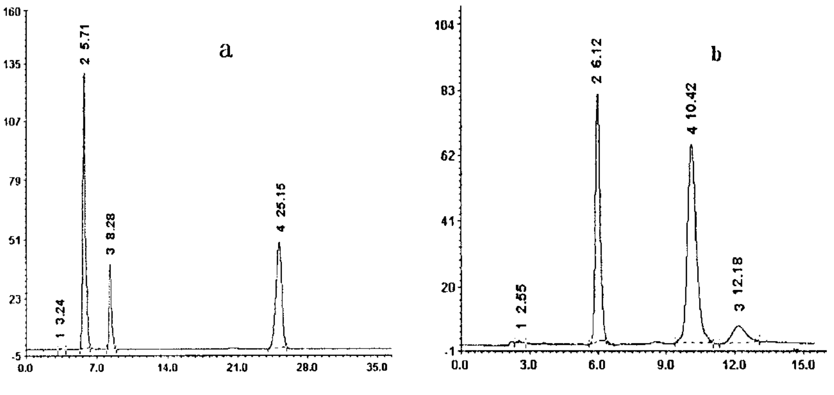

Figure 2. Separation of a mixture of benzene, phenol, and aniline on (a) ODS and (b) DPZ. Mobile phase: methanol/water (50/50, v/v); flow rate: 0.5 mL/min. Solutes: 1, system peak; 2, aniline; 3, phenol; 4, benzene.

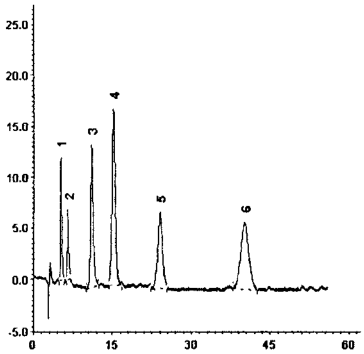

Figure 3. Separation of a mixture of PAHs on DPZ. Mobile phase: methanol/water (70/30, v/v), flow rate: 0.5 mL/min. Solutes: 1, benzene; 2, toluene; 3, naphthalene; 4, biphenyl; 5, fluorene; 6, phenanthrene.

Separation of Basic Compounds

The influence of mobile phase pH on the retention values of basic compounds is shown in Fig. 4. Retention behaviors of basic compounds on DPZ are similar to that on APA-ZrO_2: The low retention was observed at pH 2.0, then the retention increase with increasing pH between pH 2 and 4, little changes in retention above pH 6.7 were observed. Because of the cation exchange interaction on DPZ between protonated cation of basic compound and dissociated phosphonic group, the maximum retention values are at pH 3.8 or 4.7 on DPZ. But the main retention mechanism for basic compounds on APA-ZrO_2 is hydrophobic interaction, so the maximum is at $\mathrm{pH} > 7$.[11]

The separations of basic compounds, as shown in Fig. 5, was achieved by using methanol/Tris buffer (50/50, v/v, pH 9.5) as mobile phase. Excellent selectivity and high resolution were obtained.

Table 2. Separation of PAHs.

	Benzene	Toluene	Naphthalene	Biphenyl	Fluorene	Phenanthrene
k	0.97	1.57	3.48	5.38	9.24	16.29
α	1.00	1.62	3.58	5.54	9.51	16.78
N	14,700	15,200	23,800	23,800	27,500	24,500

Note: k, capacity factor; α, relative retention; N, theoretical plate number (N/m); chromatographic conditions as shown in Fig. 3.

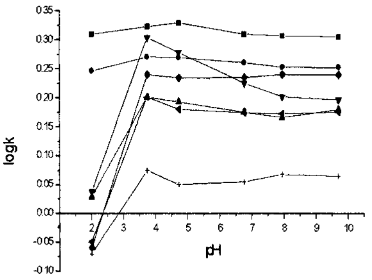

Figure 4. Influence of the mobile phase pH on the capacity factors of basic compounds. Mobile phase: methanol/buffer (70 : 30, v/v) containing 10 mmol/L HAc for pH 2.02, 3.74, 4.72, 6.76, and containing 10 mmol/L Tris for 7.90, 9.70. Solutes: (■) *o*-nitroaniline; (●) *m*-nitroaniline; (▲) *o*-toluidine; (▼) *m*-toluidine; (◆) *N*,*N*-dimethylaniline; (◀) *N*-methylaniline; (+) pyridine.

Separation of Acidic Compounds

As mentioned above, acidic solutes (such as phenols and acids) strongly adsorb on the surface of zirconia due to the Lewis acid–base interaction. This similar problem also occurs with the silica-based stationary phases: basic solutes (such as amines) strongly adsorb on silica due to the interaction between amines and residual silanols. One of the effective methods to

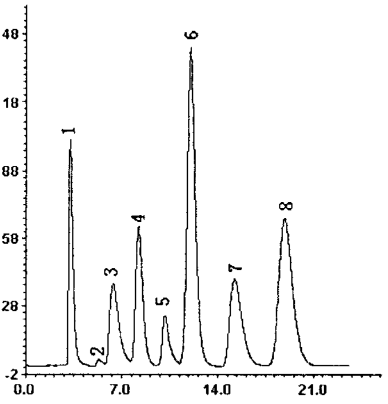

Figure 5. Separation of a mixture of basic compounds on DPZ. Mobile phase: methanol/buffer (50/50, v/v), pH = 9.50, 10 mmol/L Tris, flow rate: 0.5 mL/min. Solutes: 1, pyridine; 2, 1,5-diaminonaphthalene; 3, aniline; 4, *N*-methylaniline; 5, *m*-toluidine; 6, *N*,*N*-dimethylaniline; 7, *p*-nitroaniline; 8, *o*-nitroaniline.

eliminate this undesired interaction is the preparation of embedded polar bonded stationary phases, as shown in Fig. 1(B). Since amide bonded phases[27–30] can be used to eliminate the undesired interaction between basic solutes with residual silanols, the free phosphonic group of DPZ could also be used to suppress the undesired Lewis acid–base interaction between acidic compounds and zirconia.

Separation of Phenols

Capacity factors of phenol and *o*, *m*, *p*-nitrophenol isomers decrease almost linearly with the increase of the percent of methanol, as shown in Fig. 6(a), indicating the major interaction between solutes and DPZ is hydrophobic interaction, for the reason that DDPA blocked most of the Lewis acid sites on zirconia. The influence of mobile phase pH on the capacity factors of phenol and nitrophenols is shown in Fig. 6(b). The capacity factors change little at pH 2.0–6.7, however the capacity factors of nitrophenol isomers decrease, obviously, at pH 8.0–9.7. There are two reasons for this: at the pH $>$ 8.0, the phenols ionize to anions, the hydrophobic interaction decreases, so the capacity factors deceased; the coulombic repellent interaction between ionized nitrophenols and the dissociated free phosphonic groups also decreased the capacity factors.

2,4-Dinitrophenol and 2,4,6-trinitrophenol are hard Lewis bases, the influence of methanol content on their capacity factors is quite different from phenol and nitrophenol isomers, which do not decrease linearly with the increase in percent of methanol in mobile phases. It indicates that a few Lewis acid sites still exist on DPZ. According to the solvophobic theory,

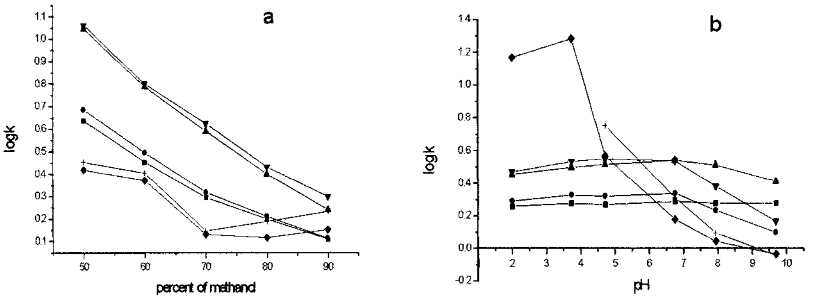

Figure 6. Influence of the methanol content (a) and pH of mobile phase (b) on the capacity factors of phenols. Mobile phase: as shown in Fig. 4. Solutes: (■) phenol; (●) *o*-nitrophenol; (▲) *m*-nitrophenol; (▼) *p*-nitrophenol; (◆) 2,4-dinitrophenol; (+) 2,4,6-trinitrophenol.

when the solvophobic interaction is stronger than Lewis acid–base interaction, the retention of acidic compounds (Lewis base) decreases with increasing the percent of methanol in the mobile phases. When the solvophobic interaction is weaker than Lewis acid–base interaction, the retention increases with increasing the percent of methanol, as shown in Fig. 6(a).

The satisfactory separation of phenol derivatives is shown in Fig. 7. DPZ also shows the potential of separating the isomers of disubstituted benzenes (see Fig. 8). One should note that the *o*-dihydroxybenzene (due to the chelating effect) and nitrophenols (due to the strong Lewis acid–base interaction) usually could not be eluted from the zirconia packings,[31] but all of them were eluted in 15 min and completely separated on DPZ.

Separation of Benzoic Acids

The influence of the methanol content and mobile phase pH on the capacity factors of benzoic acids is shown in Fig. 9. The Lewis acid–base interaction is so strong that most of the benzoic acid derivatives can't be eluted at pH 2.0–3.8 in 2 hr. The retention decreases dramatically with the increasing of mobile phase pH at pH > 4. The free phosphonic group fully dissociated and imparted negative charges on the surface of stationary phases at pH 9.7, and the coulombic repellent interactions make the retentions of benzoic acids decrease to $k < 0$.

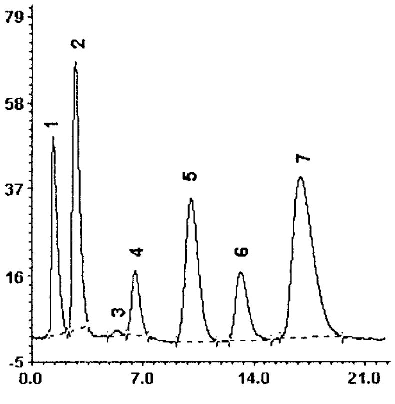

Figure 7. Separation of a mixture of phenols on DPZ. Mobile phase: methanol/buffer (50/50, v/v), pH 8.00, 10 mmol/L HAc; flow rate: 1.0 mL/min. Solutes: 1, 2,4-dinitrophenol; 2, *p*-dihydroxybenzene; 3, *o*-chlorophenol; 4, phenol; 5, *o*-cresol; 6, 4-chloro-2-methylphenol; 7, 2,4-dimethylphenol.

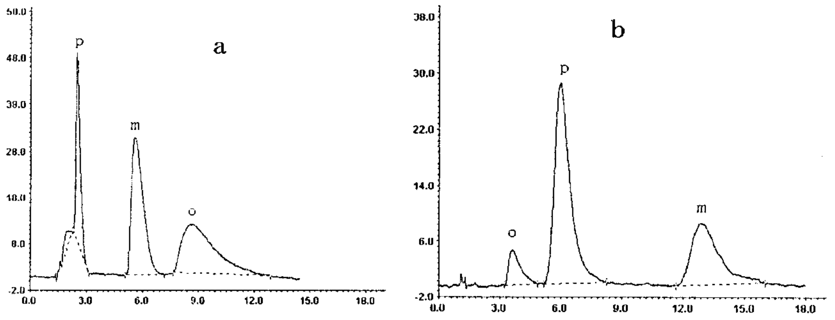

Figure 8. Separation of positional isomers of dihydroxybenzene (a) and nitrophenols (b) on DPZ. Flow rate: 1.0 mL/min. (a) Mobile phase: methanol/buffer (50/50, v/v), pH 3.50, 10 mmol/L HAc; (b) Mobile phase: methanol/buffer (50/50, v/v), pH 8.00, 10 mmol/L Tris.

This chromatographic property is quite different from that of zirconia modified with EDTPA, of which the Lewis acid sites have been completely blocked, so benzoic acids were not retained at all. The difference in chromatographic properties between the two stationary phases may arise because there are more negative charges on EDTPA than on DDPA.

The chromatogram of benzoic acids without any additives in mobile phase is shown in Fig. 10. Although the efficiency is low mainly due to the Lewis acid–base interaction, it shows great improvement compared with

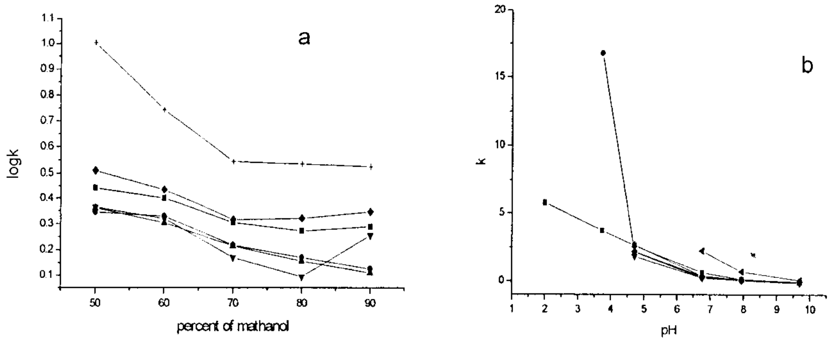

Figure 9. Influence of the methanol content (a) and the pH (b) of the mobile phase on the capacity factors of benzoic acids. Mobile phase: as shown in Fig. 4. Solutes: (■) benzoic acid; (●) *m*-nitrobenzoic acid; (▲) *p*-nitrobenzoic acid; (▼) 3,5-dinitrobenzoic acid; (◆) *p*-hydroxybenzoic acid; (+) *o*-aminobenzoic acid.

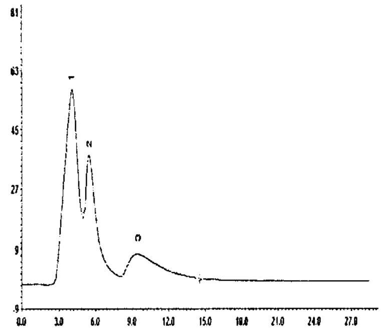

Figure 10. Separation of benzoic acids on DPZ without any additive in mobile phase. Mobile phase: methanol/water (70/30, v/v). Flow rate: 0.5 mL/min. 1, 3,5-dinitrobenzoic acid; 2, *p*-hydroxybenzoic acid; 3, *o*-aminobenzoic acid.

the unmodified zirconia and zirconia modified with other Lewis base modifiers (such as APA-ZrO$_2$).

Stability of the Stationary Phase

Taking toluene as probe, the stability of DPZ was investigated. A plot of the capacity factor of toluene against the volume of purge eluent is shown in Fig. 11. The mobile phase is methanol/Tris buffer (50/50, v/v, 10 mmol/L, pH 9.50). The retention of toluene is basically constant after 5500 mL at pH 9.5, which indicated that DPZ is stable for routine chromatographic analysis.

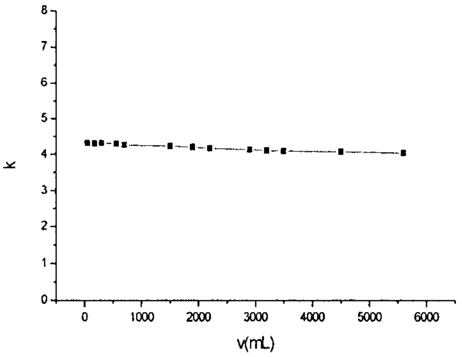

Figure 11. Investigation of stability of DPZ. Purge solution: methanol/Tris buffer (50/50, v/v), pH 9.50, 10 mmol/L. Flow rate: 0.5 mL/min. Test solute: toluene.

CONCLUSION

A new zirconia stationary phase was prepared by modifying zirconia with DDPA. DDPA adsorbed on zirconia with only one phosphonic acid group, the another phosphonic acid group is free. This kind of adsorption influenced the chromatographic properties of the stationary phase (DPZ), because the free phosphonic groups imparted the negative charges to the surface of the zirconia, and blocked most of the Lewis acid sites. Excellent separations were obtained not only for PAHs and amines but also for phenols, some isomers of phenols which usually can not be eluted on zirconia-based stationary phase, were also separated on baseline. Benzoic acids can also be eluted without any additives in mobile phase.

The polar groups of DPZ, which consist of amino groups and phosphonic groups, make this new stationary phase useful for the separation of polar compounds. Also, the amino group can be protonated at low pH, the phosphonic acid group dissociated at high pH, so the surface of DPZ can change from cationic to zwitterion, and to anionic from low to high pH. Therefore, it can be used to adjust the capacity factors, eluted order, and selectivity of polar solutes by controlling the pH of mobile phase.

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