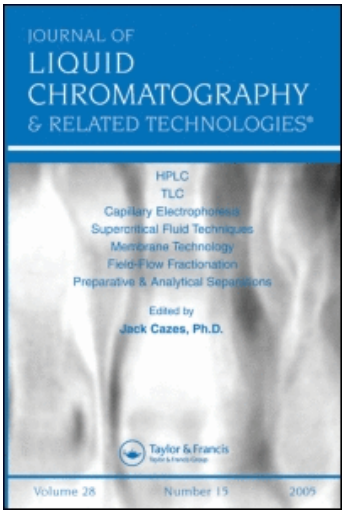

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 31 August 2001

J. LIQ. CHROM. & REL. TECHNOL., 24(13), 1881–1894 (2001)

CAPILLARY ELECTROCHROMATOGRAPHY WITH ALKYLPHOSPHONATE-MODIFIED MAGNESIA-ZIRCONIA AS THE CHROMATOGRAPHIC SUPPORT MATERIAL

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ABSTRACT

A capillary electrochromatography with a new packing material, alkylphosphonate-modified magnesia-zirconia stationary phase (APMZ), was developed. The influence of some parameters such as pH, concentration of methanol and TRIS in the buffer on EOF, and chromatographic behavior of APMZ in CEC is investigated.

It was found that, with a slight change in EOF over pH range from 4.5 to 9.3, the APMZ behaves as reserved-phase packing for polycyclic aromatic hydrocarbons (PAHs) and basic compounds. Furthermore, the ion-exchange interaction between APMZ and basic compounds at low pH was observed.

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INTRODUCTION

There has been increasing interest in capillary electrochromatography (CEC) with a packed column, because it brings together the advantages of the capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC), providing higher efficiency and more flexible selectivity than HPLC and CE.

To date, the most commonly used packing materials for CEC are the silica-based C_{18} stationary phases commonly used in HPLC.[1] This is probably due to chromatographers wishing to enter the area of CEC choosing these packing materials for their studies, and to the lack of packing materials specifically designed for CEC.[1] The electroosmotic flow (EOF) properties of the reversed-phase packing materials are dependent on the number and acidity of free silanol groups present on the packing and the extent of their ionization, the surface area of the packing, and the eluent pH.[2,3] Therefore, it is expected that the packing materials from different manufactures should exhibit a difference in the EOF with a given eluent, because they use different silica and bonding chemistries for producing packing materials.[1,4] Furthermore, some of the silica-based reversed-phase packing materials have low chemical stability in the high pH media, and others show low or no EOF.[4]

These shortcomings of the traditional reversed-phase packing materials limit, to some extent, the applications of CEC. However, little work has focused on the development of new packing materials specially designed for CEC.[1,5-8]

In the past decade, zirconia and modified-zirconia have been reported to be the greatest potential for use as chromatographic packings, due to their high chemical and mechanical stability.[9-13]

Recently, we have prepared a magnesia-zirconia composite for liquid chromatographic support and evaluated it in normal-phase HPLC mode. The mix-oxide has a larger surface area, better pore size distribution than those of a bare zirconia, and gave a satisfactory separation of basic compounds.[14] Since it has the great affinity for phosphonates, the magnesia-zirconia composite has been modified with an alkylphosphonate as a packing material for reverse-phase liquid chromatography.

The alkylphosphonate-modified magnesia-zirconia composite (APMZ) has been characterized by using elemental analysis, FTIR, ^{13}C CP-MAS NMR spectrometry, and liquid chromatography.[15-18] The results show that the new material has a hydrophobic surface, and can be used as a reverse-phase stationary phase for separation of polycyclic aromatic hydrocarbons,[19] nucleobases and nucleosides,[18] as well as basic compounds.[20] Furthermore, the new packing material presents higher stability over pH range from 2 to 10.[17]

To our knowledge, the properties of zirconia-based stationary phases have not been studied in CEC. In this paper, we demonstrate the usefulness of the new

packing material APMZ in CEC. The dependence of EOF properties in APMZ packed capillary column on various conditions such as pH, ionic strength, electrolytes, and the proportion of organic modifier in the eluents are investigated. The applications of the APMZ packed capillary column in the separation of basic compounds and polycyclic aromatic hydrocarbons (PAHs) are described.

EXPERIMENTAL

Reagents and Materials

All fused-silica capillaries (75 μm I.D.) used were purchased from Hebei Yongnian Optical Fibre Factory (Hebei, China). Tris(hydroxymethyl) aminomethane (TRIS) and methanol were purchased from Shanghai General Chemical Reagent Factory. The other solvents were of analytical grade and used without further purification.

Spherical particles of magnesia-zirconia composite (MZ)(5 μm in average; Mg/Zr: 11.4: 88.6) were prepared in our laboratory by a sol-gel method reported previously.[14] The alkylphosphonate-modified magnesia-zirconia stationary phase (APMZ, 5 μm) was prepared by the method proposed previously.[16-18] Briefly, n-dodecanol (100 mL) and sodium hydride (2.0 g) was stirred for 30 min at room temperature in an inert atmosphere of nitrogen gas. After 2.0 g of fosfomycin was added, the mixture was allowed to react at 120°C for 48 h, also in an inert atmosphere of nitrogen gas.

The reaction was then cooled and adjusted to neutral with hydrochloric acid. Thereafter, the product (alkylphosphonate in n-dodecanol) was washed five times in a separatory funnel with distilled water to remove unreacted fosfomycin. 0.50 g of MZ was added into 10 mL of the alkylphosphonate solution, and stirred for 2 h at room temperature. Afterward, the mixture was filtered and washed with methanol. The resulting APMZ was dried in an oven at 100°C for 1 h, and then kept in a desiccator before use.

Apparatus

Experiments were carried out on an NT 1229 HPCE instrument (Beijing Institute of New Technology Applications, Beijing, China). The system comprised a 0-30-kV high voltage built-in power supply and a selectable fixed-wavelength UV detector. The detection was performed at 254 nm, and the chromatograms were recorded with a Type 3066 recorder (YEW, Japan). All experiments were performed at room temperature and no temperature was controlled.

Samples were electrokinetically introduced into the capillary by applying a voltage of 5 kV. The injection time was 3 s. 16 kV was applied to a capillary of 33 cm in length with a packed length of 18 cm to carry out the electrochromatography.

Procedures

The APMZ and MZ capillary columns were prepared in the same way. A suspension of the APMZ or MZ particles in isopropanol was slurry-packed into a capillary with an outlet frit by using a LC pump at a pressure of 100 bar. The outlet frit was created by sintering 40% sodium silicate solution with a thermal wire stripper. After packing, the inlet frit was created by wetting the stationary phase in the inlet end of the capillary with 20% sodium silicate solution, and then heating it gently with the thermal wire stripper. A narrow window (2-3 mm) for detection was created within 1-2 mm after the packed portion of the column. The column was flushed with the given mobile phase about 1h before performing the separation.

Mixtures of water and methanol containing TRIS were used as the mobile phase, whose pH was measured after mixing aqueous phase with methanol. The mobile phase was degassed for 20 min before use. All solutes were dissolved in the mobile phase. The column was flushed with the mobile phase, using pressure and then was conditioned on the instrument with the mobile phase for about 1 h before measurement. No pair of buffer vials (inlet and outlet) was used for more than 2 h run-time. Thiourea is chosen as a marker of EOF. To avoid bubble formation, 6 bar pressure was also applied to both ends of the capillary.

RESULTS AND DISCUSSION

Frit and Mobile Phase

The frits for CEC are usually made by ways[21] such as: 1) sintering with stationary phase; 2) sintering with stationary phase wetted with sodium silicate solution; 3) sintering with sodium silicate solution. Methods 2 and 3 were adapted in this study, since it was difficult to make a rigid frit with only APMZ or MZ to afford the pressure of packing or flushing the column on the HPLC pump. The inlet frit was made of stationary phase wetted with 20% sodium silicate solution; the outlet frit was made by sintering 40% sodium silicate solution. It is critical to control the strength and time of heating in order to get a good permeable and rigid frit.

The TRIS is chosen as the support electrolyte for excess heat. Moreover, it has no strong interaction with the APMZ, while the phosphate and tetraborate, which are usually used as support electrolyte in the mobile phase of CEC, have strong interaction with MZ, probably causing APMZ to be unstable.

Electroosmotic Flow

Electroosmotic Flow (EOF) is a driving force for the mobile phase in CEC. Therefore, it is essential to investigate the dependence of EOF on various factors for the new packings. Figure 1 shows the plot of the EOF as a function of pH for

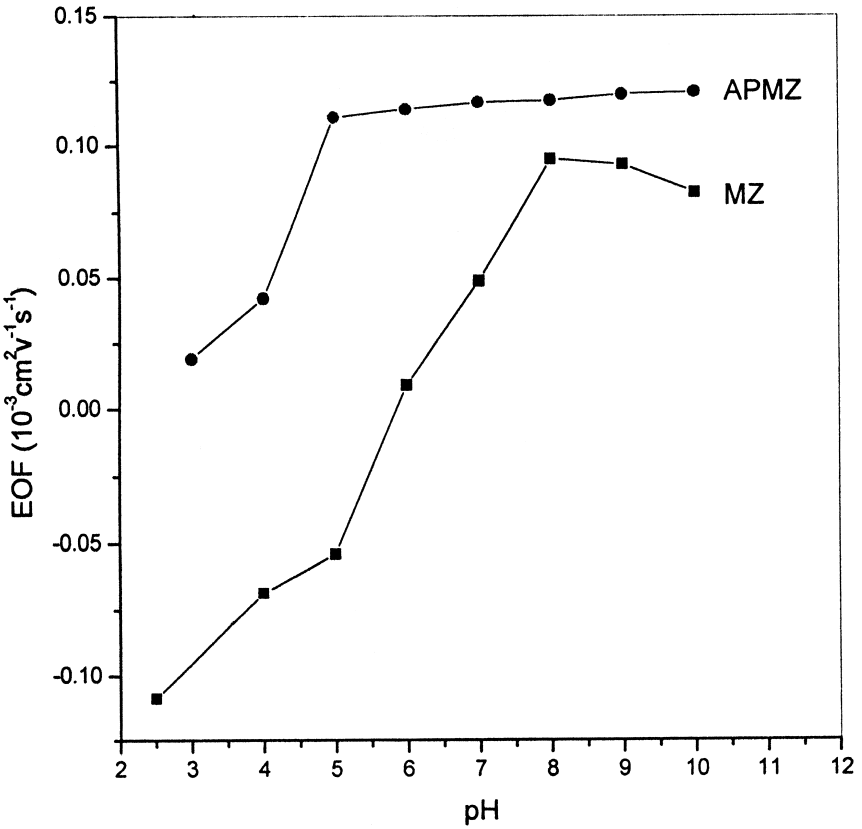

Figure 1. Influence of pH on the EOF in APMZ-packed CEC and MZ-packed CEC. 16KV, Mobile phase: 1.0 mol.L^{-1}TRIS buffer.

both the APMZ-packed column and the MZ packed column over the pH range of 3-10. A remarkable difference in the EOF for both columns could be observed.

In the APMZ-packed column; the direction of EOF was from anode to cathode in the pH range from 3-10 and the EOF increased with increasing pH of the mobile phase, but a slight increase in the EOF was observed in the pH range of 4.5-10. In MZ-packed column, a reversed EOF was observed when the pH of the mobile phase was below 6.0, though the direction of EOF is from anode to cathode and the EOF increases with increasing the pH of the mobile phase when the pH is greater than 6.0. These results can be explained on the basis of the surface characteristics of the MZ and APMZ.

The surface of MZ is highly heterogeneous, containing Lewis acid, Brönsted acid and Brönsted base sites. The Brönsted acid is deprotoned at higher pH and exhibits net negative charge on the surface of MZ, like silanol deprotonation on the silica-based packing, leading to the EOF from anode to cathode. However, the Brönsted bases are protoned at lower pH and results in a net positive charge on the surface of MZ, which leads to a reversal of EOF. No reversed EOF was observed on APMZ, which may be due to the surface modification of MZ with the alkylphosphonate that could provide negative charges even at lower pH. In addition, it should be pointed out that no remarkable change in EOF in APMZ-packed CEC is potentially capable of providing a stable EOF over a wide pH range, which would benefit reaching ionization equilibrium at a faster rate, thus improving migration time reproducibility.[24] At the same time, APMZ has greater stability than that of silica-base packing, which provides more broad pH range for optimization of pH condition.

The effect of the methanol content in the mobile phase and the electrical field on the EOF was also investigated. The EOF decreases with increasing the methanol content in the mobile phase, which is similar to the behavior observed on the commonly used silica-based packing. A linear relationship between the EOF and the electrical field (from 10kV to 20kV) was obtained on the APMZ with methanol-TRIS buffer at pH 8.0. The linear interpolation yielded a correlation coefficient of 0.997. This result indicates that the Joule heating in our experiment seemed to be negligible,[22] although no cooling system was used.

Repeatability

The mobile phase containing lower concentration of buffer is usually used in CEC for preventing the formation of bubble. However, low concentration of buffer is easy to produce gradient in the run, which is troublesome for the repeatability. Therefore, it is required to change the mobile phase frequently for keeping the mobile phase pH stable. In the study, 5 mL of mobile phase were used in each vial, and no pair of vials was used for more than 2 h of run-time. The

Table 1. Reproducibility of Migration Time of Test Solutes

Compound	Run to run (n=5)(%)	Day to day (n=4) (%)
Benzene	0.79	3.07
Toluene	0.93	2.18
Aniline	0.21	2.62
N-methylaniline	0.87	2.96
Pyridine	0.76	3.69

repeatability of the retention time of five compounds was tested with run to run and day to day. The R.S.D of them is satisfactory, as shown in Table 1.

Application

The chromatographic properties of the APMZ are evaluated by use of PAHs and some basic compounds. The dependence of the retention behavior of the solutes on some parameters such as pH value, methanol concentration, and TRIS concentration in the mobile phase was also investigated, respectively.

Separation of PAHs

Figure 2 depicts the change of the capacity factors of PAHs with the varying of concentration of methanol with methanol-buffer (1.0 mmol L^{-1} TRIS) at pH 9.0. At the investigated range, the capacity factors of PAHs linearly decrease with increasing the concentration of methanol. The result indicates that the packing behaves as a reverse-phased stationary phase and the hydrophobic interaction is dominant between PAHs and the packing.

Separation of the PAHs was carried out with methanol-TRIS buffer (88:18, v/v) at pH 8.75 as the mobile phases. Baseline separations of the PAHs, as shown in Figure 3, were achieved. It is expected that efficiency obtained in CEC should be higher than those obtained in HPLC because of the plug like profile of EOF. The efficiency of the CEC column is 37,700 plates.m^{-1} for benzene, which is higher than 10,000 plates/m for benzene obtained in HPLC.[17] As a new packing, the surface area, the structure and size of pore, and particle size of APMZ are not optimized; the efficiency of the APMZ are lower than those of commonly used silica-based packing.[23]

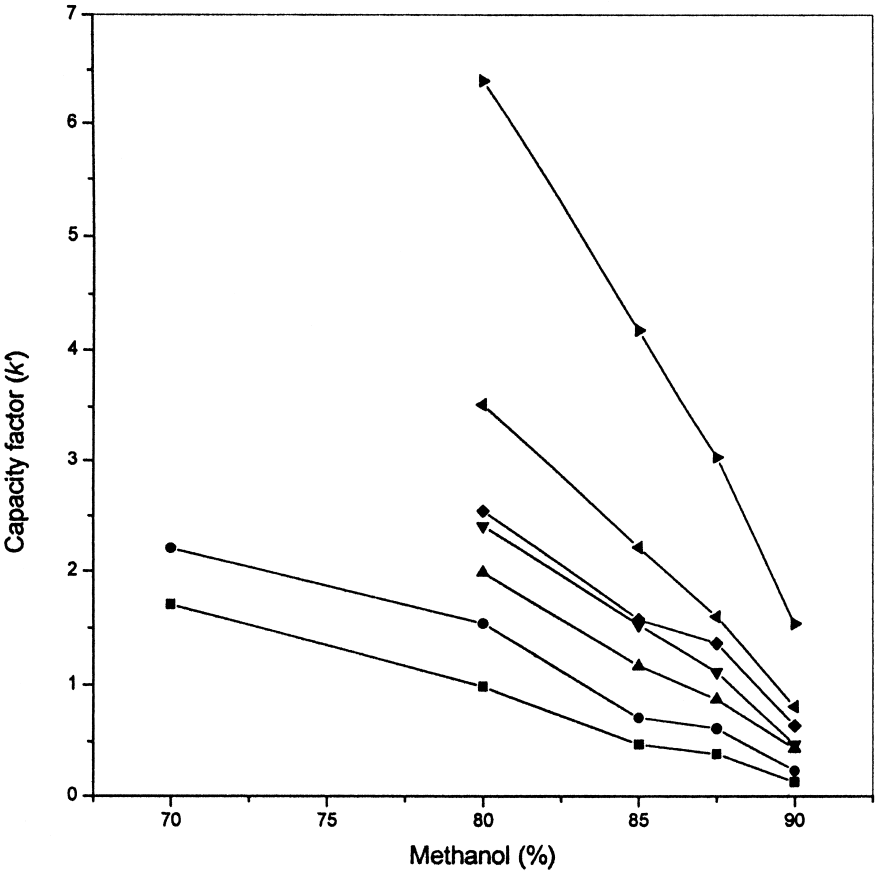

Figure 2. Influence of methanol concentration in the mobile phase on the capacity factors of PAHs. 16KV, Mobile phase: methanol-1.0 mmol.L^{-1}TRIS buffer, pH 9.0. ■ benzene; ● toluene; ▲ *p*-chlorotoluene; ▼ naphthalene; ◆ biphenyl; ◀ fluorene; ▶ fluoranthene.

Separation of Basic Compounds

The investigation of effect of the concentration of TRIS on the capacity factors is carried on with a buffer at pH 4.5 or pH 9.3. The TRIS concentration of the buffer was from 0.5 mmol.L^{-1} to 2.0 mmol.L^{-1}. Almost no change of capacity

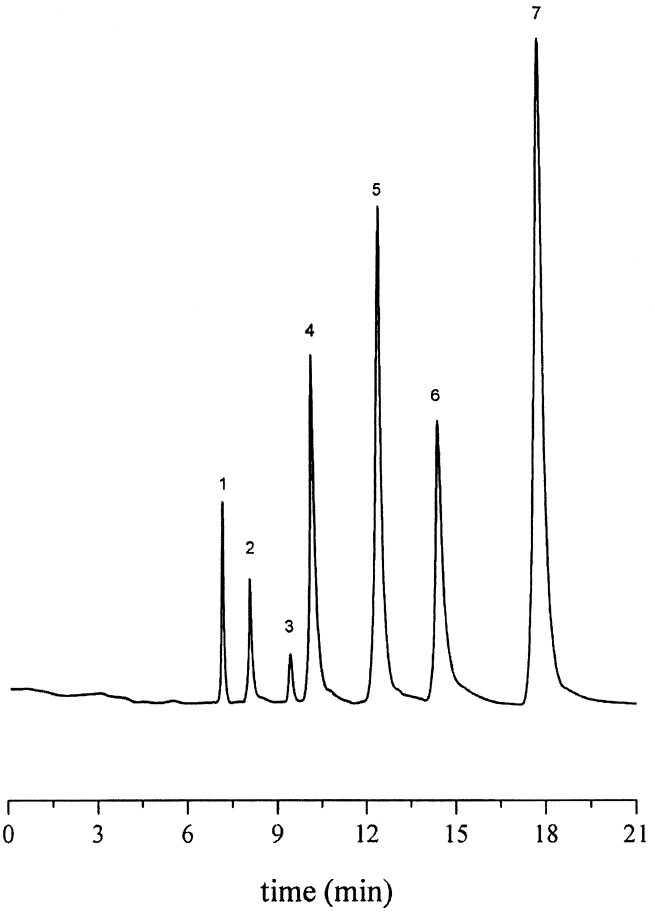

Figure 3. The separation of PAHs on APM. 16KV, Mobile phase: methanol-1.0 mmol.L^{-1} TRIS buffer (70:30, V/V) pH 9.0. 1. benzene; 2. toluene; 3. *p*-chlorotoluene; 4. naphthalene; 5. biphenyl; 6. fluorene; 7. fluoranthene.

factors of basic compounds is observed with the variation of TRIS concentration; this illustrates that TRIS doses do not interact with sample or stationary phase in APMZ CEC, and assists in interpretation of the interaction between basic compounds and the APMZ.

Figure 4 shows the capacity factor of basic compounds as a function of the mobile phase pH from 4.5 to 9.36. The measurement was performed with the mobile phase containing 80% (v/v) methanol. It is apparent from Figure 4 that the capacity factors of pyridine and *p*-phenylenediamine increase slowly, whereas

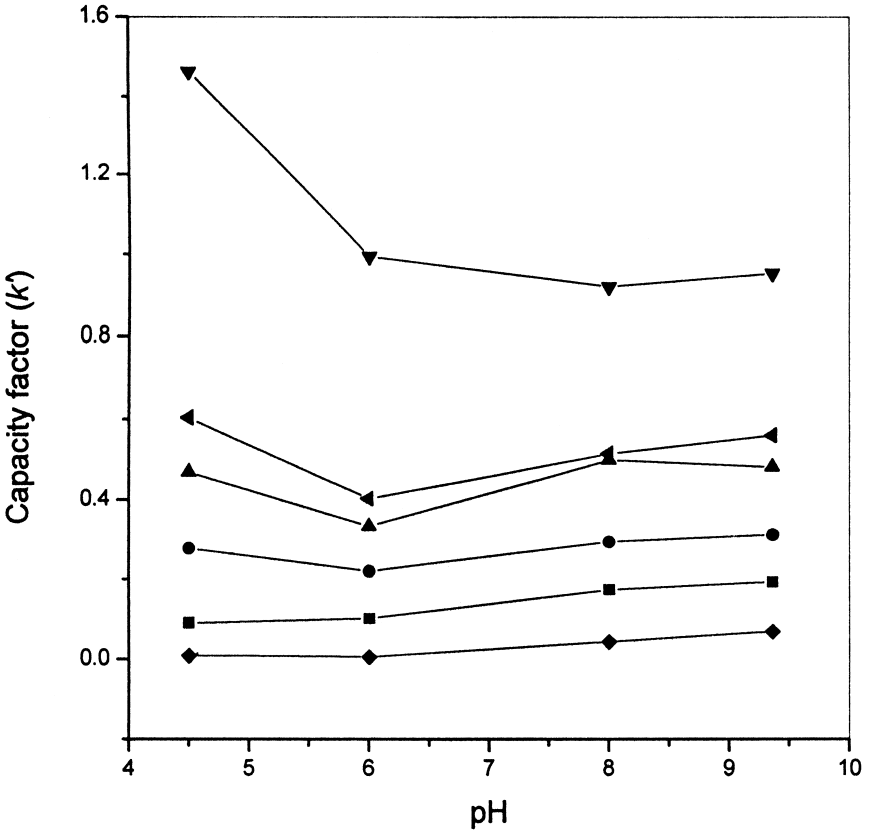

Figure 4. Influence of pH on the capacity factors of basic compounds. 16KV, Mobile phase: methanol-1.0 mmol.L^{-1}TRIS buffer (80:20, V/V). ◆ *p*-phenylenediamine; ■ pyridine; ● aniline; ▲ *m*-chloroaniline; ◀ N-methylaniline; ▼ N,N-bimethylaniline.

those of the rest of the four basic compounds decrease at the pH range of 4.5 - 6.0, and reach their minimum at pH6.0. Above pH 6.0, the capacity factors of all basic compounds change slightly.

Because the basic solutes shown in Figure 4 have pKa values in the range of 3.58-5.5, and are in ionic forms or partial ionic forms at pH 4.5, their capacity factors is affected by electrophoresis and ion-exchange interaction, as well as hydrophobic interaction. With the increase of pH value, the solutes are deprotoned gradually, so the electrophoresis and ion exchange interaction decrease dramatically, which results in the decrease of the capacity factors. Above pH 6.0, the solutes are mostly in neutral forms. In this case, their retention is dependent mainly on the hydrophobic interaction between solutes and the stationary phase;

so, the capacity factors change little with the pH increase, which is similar to that observed in HPLC.[20]

Figure 5 shows the influence of methanol concentration on the capacity factors of some basic compounds with methanol-buffer (1.0 mmol L^{-1}, pH 9.0) as the mobile phase. It can be seen from Figure 5, that the elution order of the compounds is consistent with their hydrophobicity. The increase of the methanol concentration reduces the retention of the basic compounds, which is similar to the behavior of PAHs. This result can be ascribed to the neutral form of the basic compounds at pH 9.0.

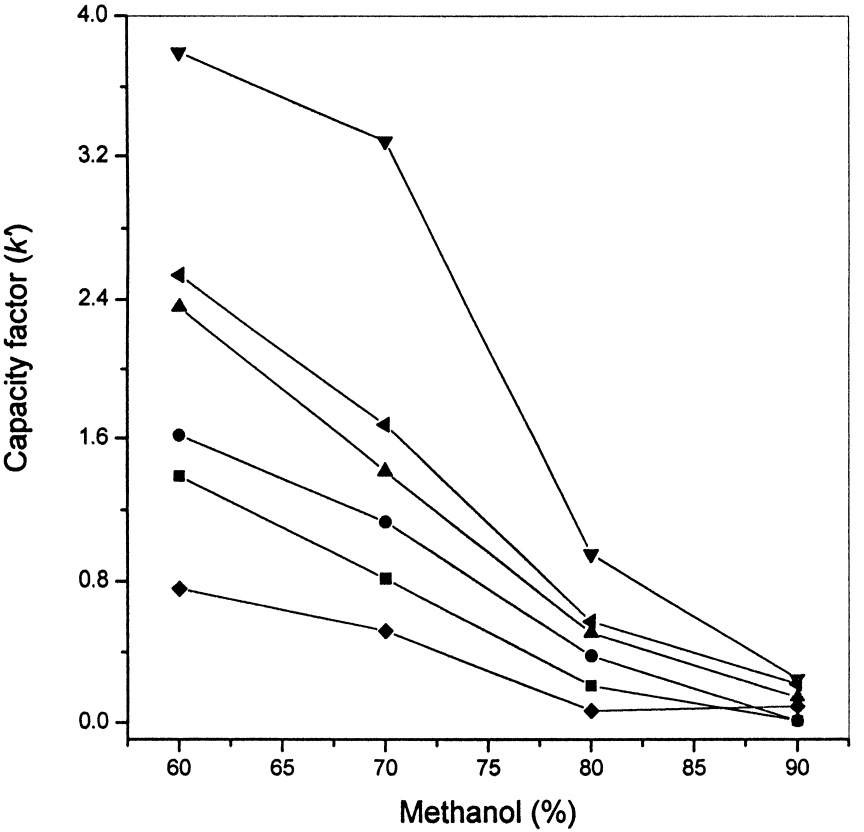

Figure 5. Influence of methanol concentration in the mobile phase on the capacity factors of basic compounds. 16KV, Mobile phase: methanol-1.0 mmol.L^{-1}TRIS buffer, pH 9.0. ◆ *p*-phenylenediamine; ■ pyridine; ● aniline; ▲ *m*-chloroaniline; ◀ N-methylaniline; ▼ N,N-bimethylaniline.

The separation of basic compounds was investigated on the APMZ. As shown in Figure 6, the baseline separation of the six basic compounds was achieved using methanol-TRIS buffer at pH 4.5 or 8.75 as mobile phases; and peak shapes are independent on the mobile phase pH value, indicating that APMZ has no strong irreversible interactions with basic compounds. In CEC with a silica-based stationary phase, the separation of basic analytes is problematic, since severe peak tailing is encountered due to strong interactions of the basic compounds with the ionized silanol groups.[23] Therefore, APMZ is superior to those silica-based reversed packing in the separation of basic compounds by CEC.

CONCLUSIONS

In the paper, the effect of pH, methanol concentration and ion strength of the mobile phase on the EOF and the chromatographic performance of the APMZ

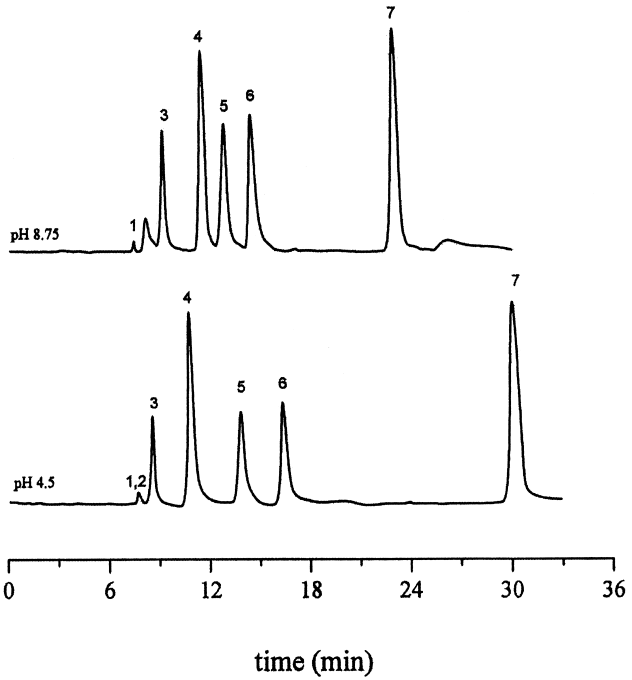

Figure 6. The separation of basic compounds. 16KV, Mobile phase: methanol-1.0 mmol.L^{-1}TRIS buffer (70:30, V/V). 1. thiourea; 2. *p*-phenylenediamine; 3. pyridine; 4. aniline; 5. *m*-chloroaniline; 6. N-methylaniline; 7. N,N-dimethylaniline.

in CEC has been investigated. Although, the efficiency of APMZ in CEC is inferior to that of a column packed with commercial silica-matrix packing reported,[23] APMZ can provide stable EOF and be used to separate basic compounds with symmetric peak in CEC, which implies the effectiveness and the potential of the new stationary phase in CEC.

In light of the fact that zirconia is easy to modify by phosphonates, it is expected that a series of stationary phase based on those zirconia-based particle can also be developed for CEC.

ACKNOWLEDGMENT

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

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Received November 7, 2000 Manuscript 5447
Accepted December 5, 2000