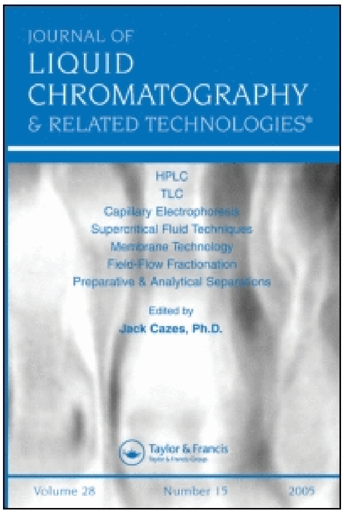

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

PREPARATION AND CHARACTERIZATION OF SILICA-ZIRCONIA SUPPORTS FOR NORMAL-PHASE LIQUID CHROMATOGRAPHY

Qing -He Zhang[a]; Yu -Qi Feng[a]; Shi -Lu Da[a]
[a] Department of Chemistry, Wuhan University, Wuhan, P. R. China

To cite this Article Zhang, Qing -He , Feng, Yu -Qi and Da, Shi -Lu(2000) 'PREPARATION AND CHARACTERIZATION OF SILICA-ZIRCONIA SUPPORTS FOR NORMAL-PHASE LIQUID CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 23: 10, 1461 — 1475

To link to this Article: DOI: 10.1081/JLC-100100427

URL: http://dx.doi.org/10.1081/JLC-100100427

J. LIQ. CHROM. & REL. TECHNOL., 23(10), 1461–1475 (2000)

PREPARATION AND CHARACTERIZATION OF SILICA-ZIRCONIA SUPPORTS FOR NORMAL-PHASE LIQUID CHROMATOGRAPHY

Qing-He Zhang, Yu-Qi Feng,* Shi-Lu Da

Department of Chemistry
Wuhan University
Wuhan 430072, P. R. China

ABSTRACT

Two silica-zirconia composites with different silica molar ratio (3.4% and 15.8%, respectively) were prepared as rigid microspheres by means of a sol-gel process. Scan electron micrograph, X-rays diffraction, nitrogen adsorption, and potentiometric titration were used to characterize the silica-zirconia composites and, for the comparison, bare zirconia. Addition of the silica into the zirconia prevented the formation of monoclinic zirconia. The silica-zirconia composites show larger specific surface area and more ideal pore structure than the bare zirconia. The normal phase chromatographic performance was studied by using isopropanol/cyclohexane, dichloromethane/cyclohexane and chloroform/cyclohexane as mobile phases. Basic solutes gave symmetrical peaks, and the acidic solutes exhibited better peak shape on the composite stationary phases than on the bare zirconia.

INTRODUCTION

Silica is the most common stationary phase support currently used in high performance liquid chromatography, because of its excellent mechanical strength and availability in a wide range of pore size and particle diameter. Unfortunately, the silica-based packings have two significant limitations.[1,2] One

www.dekker.com

is the undesirable adsorption towards basic compounds due to silanol interaction, leading to asymmetric peaks. The second is lack of pH stability because the silica backbone, Si-O-Si, hydrolyzes at pH>8, and the siloxane bond is unstable at pH<2.

Recently, inorgnic oxides such as alumina, titania, and zirconia have been used as the alternative of silica and have shown some advantages. Of the above mentioned oxides, zirconia was reported to be the greatest potential for use as chromatographic packings for separation of biological materials, owing to its high chemical stability. Nawrocki et al. have reviewed the physical-chemical properties of zirconia.[3] Unger and co-workers[2,4] have compared the chromatographic performance of zirconia, silica, alumina, and titania in normal phase HPLC mode. Their results indicate that the zirconia is superiority for the separation of basic compounds. Kurganov et al.[5] and Yu et al.[6] have prepared chemically bonded zirconia with silanes for hydrophobic and hydrophilic stationary phases. However, owing to the poor pore structure and the chemical heterogeneous surface, the zirconia supports usually exhibited low column efficiency in comparison to silica.[3]

The surface of zirconia is highly heterogeneous, consisting of Lewis acid, Bronsted acid, and Bronsted base sites, leading to the elution process being complicated.[3] Carr and co-workers have extensively studied carbon[7-9] and polymer[10-14] coatings on zirconia as surface modifiers of zirconia to create anion-exchange, hydrophobic and hydrophilic-type phases with a certain degree of success. Since bare zirconia has a great affinity for Lewis bases, Carr and co-workers have studied modification of zirconia with Lewis bases such as fluoride,[15] phosphate,[16] and ethylenediamine-N,N'-tetramethylphosphonic acid (EDTPA),[17,18] and their application in separation of proteins and purification of monoclonal antibodies. Recently, silica coated zirconia microspheres (1-2 μm) have been prepared by Shalliker et al.[19] Their properties were evaluated by means of Raman spectroscopy, differential thermal analysis, thermal gravimetric analysis, and electron microscopy. However, as yet, the chromatographic application of the composites has not been reported.

The pore structural characteristics of packings are a crucial factor for its chromatographic performance.[20,21] Nawrocki and co-workers have described the variance in the specific surface area, the specific pore volume and the mean pore size of zirconia with thermal treatment.[3] Carr and co-workers have compared the pore structures of zirconia synthesized with oil emulsion (OEM) and polymerization induced colloidal aggregation (PICA) methods by means of nitrogen sorption, mercury porosimetry, size exclusion chromatography, and NMR techniques.[22-24] They pointed out the presence of a considerable number of pore constructions for the zirconia synthesized with the different methods.[23] Shalliker and Douglas have reported that the specific surface area, the specific pore volume, and the mean pore size of zirconia could be controlled by sodium chloride impregnation technique, and indicated that pore structures of poor quality were obtained without sodium chloride.[25]

Recently, we have focused the attention on modifying zirconia with inorganic oxides by using sol-gel technique for improvement of its physical-chemical properties. A porous magnesia-zirconia composite in sphere (5 μm) with ideal pore structure and the surface properties have been prepared. The chromatographic evaluation of the composite showed that it had better chromatographic performance compared to the bare zirconia.[26]

The purpose of this study is to improve the pore structure and surface properties of zirconia through silica doping. Two silica-zirconia composites with different molar ratio were prepared by a sol-gel process. For comparison, zirconia was also prepared under the same condition. The bulk structure, the pore size distribution, the specific pore volume, the specific surface area, and the acid-base character were examined for the silica-zirconia and zirconia supports. The chromatographic performances in normal phase mode were investigated with a variety of compounds as probes by using dichloromethane/cyclohexane, chloroform/ cyclohexane, and isopropanol/cyclohexane as mobile phases.

EXPERIMENTAL

Chemicals

All reagents were obtained from commercial sources and were of analytical-reagent grade. Zirconyl chloride octahydrate ($ZrOCl_2 \cdot 8H_2O$), polyoxyethylenesorbitan trioleate (Tween 85), sorbitan monooleate (Span 80), acetone, ethanol, hydrochloride acid, and all the samples were obtained from Shanghai General Chemical Reagent Factory. Sodium metasilicate pentahydrate ($NaSiO_3 \cdot 9H_2O$) was obtained from Guangzhou Chemical Plant. Hexamethylenetetramine (HMTA) and urea were obtained from Chengdu Chemical Reagent Plant. Light petroleum (60-90°C), cyclohexane, isopropanol, chloroform, and dichloromethane were obtained from Tianjing Chemical Reagent Plant. Double distilled water was boiled for 15 min before the use to remove dissolved carbon dioxide.

Preparation of Silica-Zirconia Composite

Silica-zirconia microspheres were synthesized according to the procedures reported by our laboratory[26] and Kaneko et al.[27] with some modification. Briefly, sodium metasilicate pentahydrate (2.5 g or 10 g) was dissolved in 40 mL of distilled water, the pH value of the solution was adjusted to 1 by hydrochloric acid. Then zirconyl chloride octahydrate (64 g) was added by stirring the solution at room temperature. The resulting mixed solution was poured into light petroleum that containing span80 and Tween85. The mixture was homogenized for 10 min at a speed calibrated to yield particles with a diameter of approximately 5 µm. HMTA and urea were added to the emulsion to initiate the gelation reaction. The reaction mixture was stirred for at least 48 h. To remove adherent light petroleum, surfactants, and residual substances the hydrogel particles of the mixed oxides obtained were subjected to a multi-stage washing procedure, with light petroleum, acetone, ethanol, and water. Size classification of the silica-zirconia was done by a sedimentation/decanting/resuspension sequence. The resultant particles were collected by vacuum filtration and heated at 120 °C for 2 h to turn the hydrogels to xerogels. Thereafter the xerogels was slowly taken to temperature of 600°C at a heating rate of about 3°C/min and held at this temperature for 1 h to remove the organic residues. Zirconia was prepared under the same conditions but without the addition of sodium metasilicate pentahydrate.

Physicochemical Properties

The contents of silicon and zirconium on the surface of silica-zirconia composites were determined by means of a model XSAM800 Electron Energy Spectrometer (Kartos, AEI) according to Si2p (102.13ev) and Zr3d (182.61ev). Particle diameter distribution of silica-zirconia was measured with a model X-650 scanning electron microscope (Hitachi, Japan). X-ray diffraction (XRD) measurements were performed on a D/max-r diffractometer (Rigaku, Japan). A potentiometric titration method was used to investigate the surface acid-base properties of silica-zirconia and zirconia in aqueous solution according to the procedure proposed by Kita et al.[28] Nitrogen adsorption/desorption isotherms were measured on a model ST-03A surface analyser (The Analytical Instrument Plant, Beijing, China) at 77K. Pore diameter distributions for the packings were calculated from desorption data using the Barrett-Joyner-Halenda (BJH) method. The specific surface area was calculated using the BET equation in the range of linearity P/P_0 (0.05-0.30).

Chromatography

Chromatographic tests were carried out with standard HPLC equipment, a Shimadzu 10A liquid chromatographic pump; SPD-10A UV-Vis photometric detector; Rheodyne 7125 injection system (sample loop, 20 µL); Shimadzu C-R6A integrator (Shimadzu, Kyoto, Japan).

The packing materials, silica-zirconia and zirconia, were slurry-packed into 150×4.6 (i.d.) mm stainless-steel columns, respectively. The column temperature was controlled at 25°C. Isopropanol/cyclohexane, 50%-water-saturated dichloromethane/cyclohexane and 50%-water-saturated chloroform/cyclohexane were used as mobile phases, respectively. The flow rate of the mobile phase was set at 1.0 or 0.5 mL min^{-1}. Phenols, anilines, and substituted aromatic compounds were used as solutes. The detection wavelength was set at 254 nm. Columns were equilibrated with 150 mL of eluting solvents at a flow rate of 1.0 mL·min^{-1} before the injection of samples.

RESULTS AND DISCUSSION

Physical-Chemical Properties

The molar ratios of Si to Zr on the surface of silica-zirconia composites used in this study were 3.4% and 15.8%, respectively, which was determined by electron energy spectrometer.

The X-ray diffraction spectra of the zirconia and silica-zirconia composites are shown in Figure 1. No peak was observed for the silica in the composites, suggesting that the silica in the composites is amorphous. However, the peaks

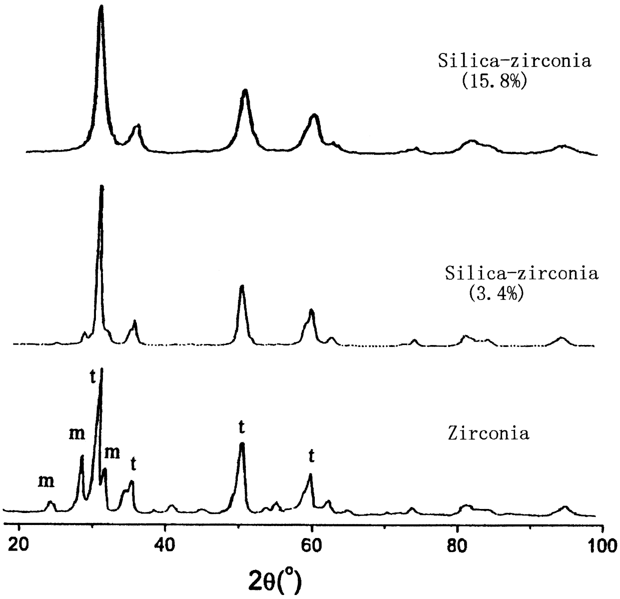

Figure 1. X-ray diffraction spectra of the zirconia and the silica-zirconia composites. A) zirconia; b) silica-zirconia (3.4%); c) silica-zirconia (5.8%) m = monoclinic, t = teragonal.

of zirconia are dependent on the molar ratio of Si to Zr. According to Figure 1, the bare zirconia and silica-zirconia with lower molar ratio (3.4%) were found to be of monoclinic and metastable tetragonal structure, whereas, the silica-zirconia (15.8%) is of tetragonal structure. These results show that the silica is a stabilizer of the tetragonal phase of zirconia in the silica-zirconia composite, which is similar to that of silica-coating zirconia obtained by Shalliker et al.[19]

The pore structure of the silica-zirconia composites and the zirconia were studied by nitrogen sorption method. All the three samples show type IV adsorption isotherms according to the IUPAC recommendation.[29] This type of isotherm is characteristic for mesoporous samples which do not contain an appreciable amount of macropores. The hysteresis loops for both of the silica-zirconia composites were of type H1, had nearly parallel adsorption and desorption branches that exhibit the silica-zirconia composites, and have good pore connectivity. As previously reported,[3] the inorganic oxides that exhibit the type IV adsorption isotherms with H1 hysteresis loops are the most suitable for chromatographic purposes. Therefore, the silica-zirconia composites may be acceptable as liquid chromatographic packings. In contrast to the silica-zirconia composites, the hysteresis loop for zirconia is of type H2 to H3, which is in agreement with that reported by Mercere,[30] suggesting that the zirconia have narrow necked pores with wide bodies. As mentioned above, the silica doping can improve the pore structure of zirconia, which might be result from the silica that partially occupying the zirconia framework, leading to the formation of an irregular crystal lattice structure.

The pore size distribution for the silica-zirconia composites and the bare zirconia was calculated from the desorption branches of isotherms using BJH method. The pore size centered in the mesopore range and the two silica-zirconia composites show more uniform pore diameter distribution than the zirconia.

The specific pore volume, the specific surface area, and the average pore diameter of the bare zirconia and the two silica-zirconia composites were calculated from the data obtained by nitrogen sorption method, and the results are shown in Table 1. It can be seen that the specific surface areas increases, however, the mean pore diameter decreases as the increase of the content of silica in the silica-zirconia composites.

The acid-base properties of the zirconia and the silica-zirconia composites were also measured by means of potentiometric titration method. The results were also shown in Table 1. Both of the acidic and basic sites were observed on the surface of the zirconia. The weak basic surface properties were observed for the silica-zirconia composites. However, the acidity increased with the increase of the content of silica in the composites. These results may be ascribed to the silanols that exhibit a bit of acidity.

Table 1

Physicochemical Properties of Silica-Zirconia and Zirconia Packings

Oxide	ZrO_2	SiO_2/ZrO_2 (3.4%)	SiO_2/ZrO_2 (15.8%)
Average particle size, µm	5	5	5
Average pore size, nm	24.4	8.6	5.9
Specific Surface, $m^2.g^{-1}$	21	29	76
Total pore volume, $cm^3.g^{-1}$	0.128	0.062	0.112
Bulk structure	Monoclinic, tetragonal	Monoclinic, tetragonal	Tetragonal
Acidity at pH 10, $mmol.g^{-1}$	0.08	0.14	0.70
Basicity at pH 3.5, $mmol.g^{-1}$	0.15	Not detected	0.09

Chromatographic Evaluation

The silica-zirconia and zirconia packings were, respectively, slurry-packed into three stainless-steel columns with the same size under the same condition. In order to compare their chromatographic characteristics, the two silica-zirconia composites and the bare zirconia were evaluated chromatographically in normal-phase mode with basic, acidic, and neutral compounds as probes, by using various mobile phases.

Chromatographic Behavior of Basic Compounds

The retention of a variety of basic compounds on the zirconia and both of the silica-zirconia composites were investigated by using 50%-water-staturared dichloromethane/cyclohexane (*v/v*, 30/70) as mobile phase. The pK_b and the capacity factors of the compounds were illustrated in Table 2. It can be seen that much greater k values for the basic compounds were observed on the silica-zirconia composites than that on zirconia. In order to investigate the contribution of silica in the silica-zirconia composite to the retention of the solutes, the ratio of the capacity factor on silica-zirconia ($k_{si\text{-}zr}$) to that on zirconia (k_{zr}) was plotted against the pK_b of the solutes, which was shown in Figure 2. It can be seen that the ratios of $k_{si\text{-}zr} / k_{zr}$ for the compounds decreased with the increase of pK_b of them. This result can be ascribed to the presence of acidic sites on the surface of silica-zirconia composite resulted from the silanol.

The effect of the mobile phase on the capacity factors of the basic compounds was investigated. Figure 3 are the capacity factors of pyridine with

Table 2

The Capacity Factors of Substances on the Silica-Zirconia and the Zirconia Supports*

Columns	pKb	ZrO_2	SiO_2/ZrO_2 (3.4%)	SiO_2/ZrO_2 15.8%)
o-toluidine	9.46	0.27	1.37	10.0
m-toluidine	9.26	0.39	3.01	33.5
p-toluidine	8.83	0.42	5.01	62.3
o-nitroaniline	14.25	0.71	1.99	11.75
m-nitroaniline	11.40	1.30	4.09	29.78
p-nitroaniline	11.96	2.78	8.07	51.94
aniline	9.34	0.42	2.95	38.52
β-aminonaphthalane	9.70	0.58	3.18	>60

---

* Mobile phase: 50% water-saturated dichloromethane/cyclohexane (30/70, v/v), flow rate: 1.0 $mL.min^{-1}$.

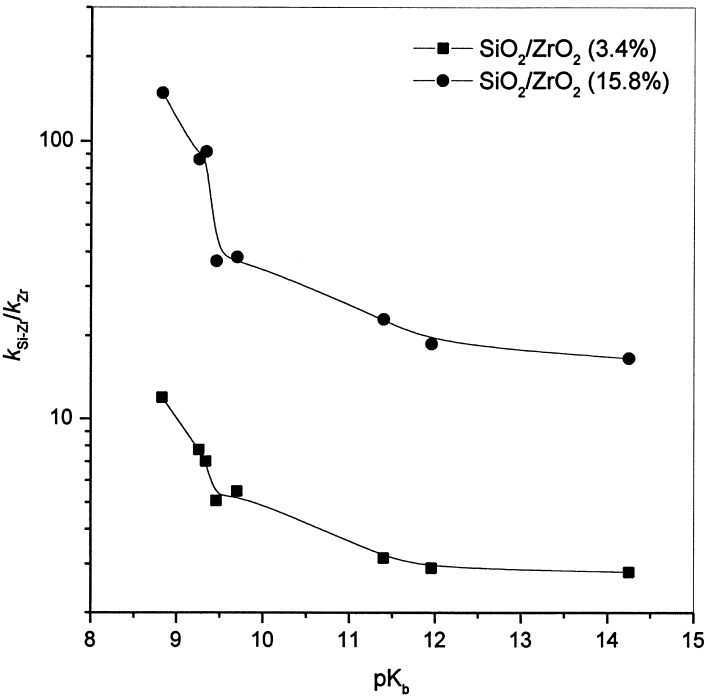

Figure 2. Relationship between the k_{Si-Zr}/k_{Zr} and the pK_b of the basic compounds. Data is from Table 2.

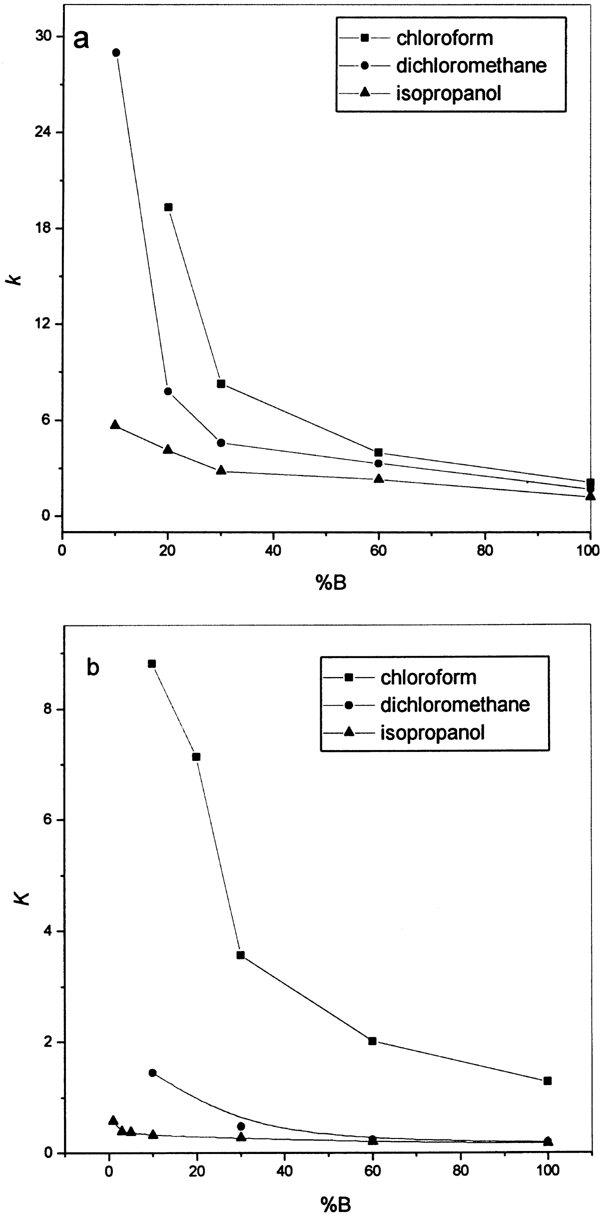

Figure 3. Influence of the polar solvents m(B) content of the mobile phase on capacity factors of pyridine on silica-zirconia (3.4%) (a) and zirconia (b) stationary phase.

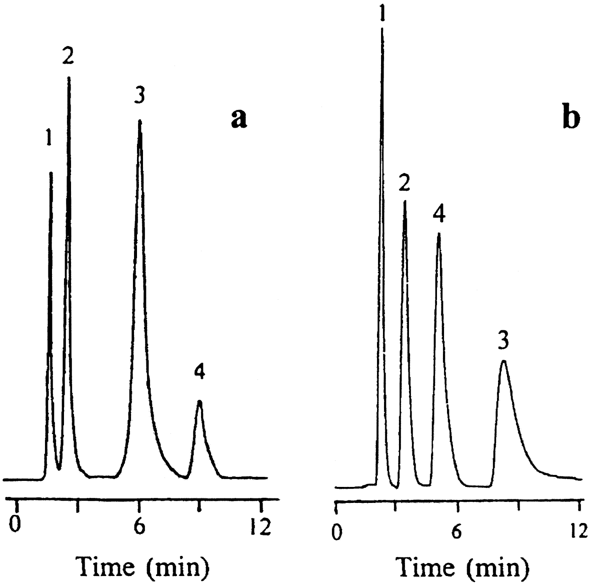

Figure 4. HPLC chromatogram of amino compounds on silica-zirconia composites: Column: 150 x 4.6 mm i.d.; flow rate: 1.0 mL/min; Col. Temp., 25°C. a) silica-zirconia (3.4%), mobile phase: 50% water-saturated dichloromethane/cyclohexane (60:40, v/v), b) silica-zirconia (15.8%, mobile phase: 50% water saturated chloroform/cyclohecane (60:40, v/v). Peaks: 1, N,N-dimethylaniline; 2, N-methylaniline; 3, aniline; 4, p-nitroaniline.

the concentration of the polar solvent in the mobile phases (isopropanol/cyclohexane, dichloromethane/cyclohexane, and chloroform/ cyclohexane). It can be seen that the capacity factors of pyridine decreased with the increase of the polar solvent in the mobile phase and the solvent strength increased with the order of chloroform<dichloromethane<isopropanol. Similar results have been obtained for the other basic compounds. This result agreed with the results on alumina and silica packings obtained by Snyder et al.[31]

Figure 4 are the chromatograms of N, N'-dimethylaniline, N-methylaniline, aniline, and *p*-nitroaniline on the silica-zirconia (3.4%) and silica-zirconia (15.8%) packings, respectively. It can be seen that the substances can be separated and give symmetrical peaks. However, the elution sequence of aniline and *p*-nitroaniline on the two packings is reversed. This result can be ascribed to the stronger acid properties of the silica-zirconia (15.8%).

Chromatographic Behavior of Acidic Compounds

The retention of phenol and nitrophenol isomers on silica-zirconia (3.4%), silica-zirconia (15.8%), and zirconia supports were studied under normal-phase conditions. The capacity factors of phenol on the three columns against the concentration of dichloromethane in the mobile phase were shown in Figure 5. It can be seen that the *k* values decreased with increasing the concentration of dichloromethane in the mobile phase and the *k* values of phenol on the packings increased with the order of silica-zirconia (3.4%) < zirconia < silica-zirconia (15.8%). The same result has been obtained for nitrophenols. These results seem strange, because the surface basicity of the zirconia is greater than that of the silica-zirconia composite (15.8%) as shown in Table 1. It is possible that the retention of the solutes is dependant not only on the acid-base property of the supports, but also on the crystal structure of the supports. The zirconia in silica-zirconia (15.8%) was a tetragonal structure, while bare zirconia and zirconia in silica-zirconia (3.4%) was a monoclinic structure with a tetragonal phase.

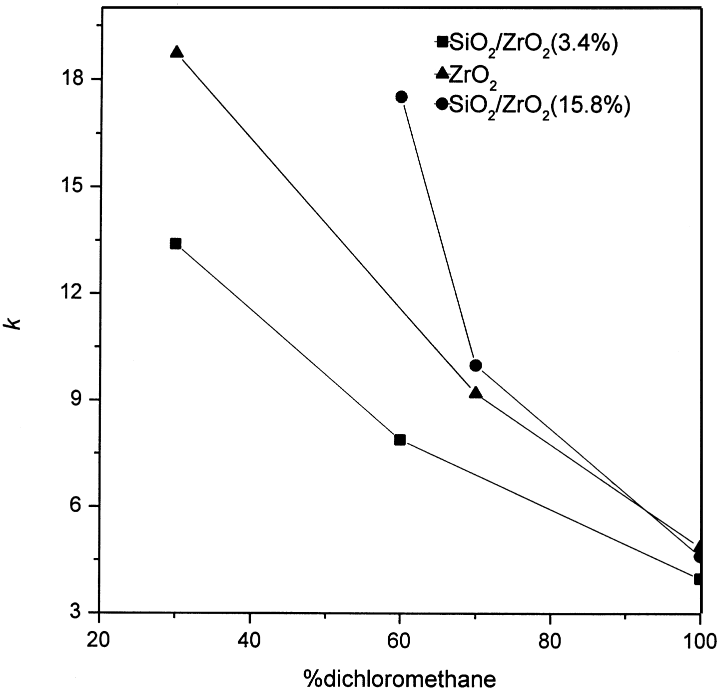

Figure 5. Influence of the dichloromethane content of the mobile phase on capacity factors of phenol on silica-zirconia (3.4%) (a) and silica-zirconia (b) stationary phase.

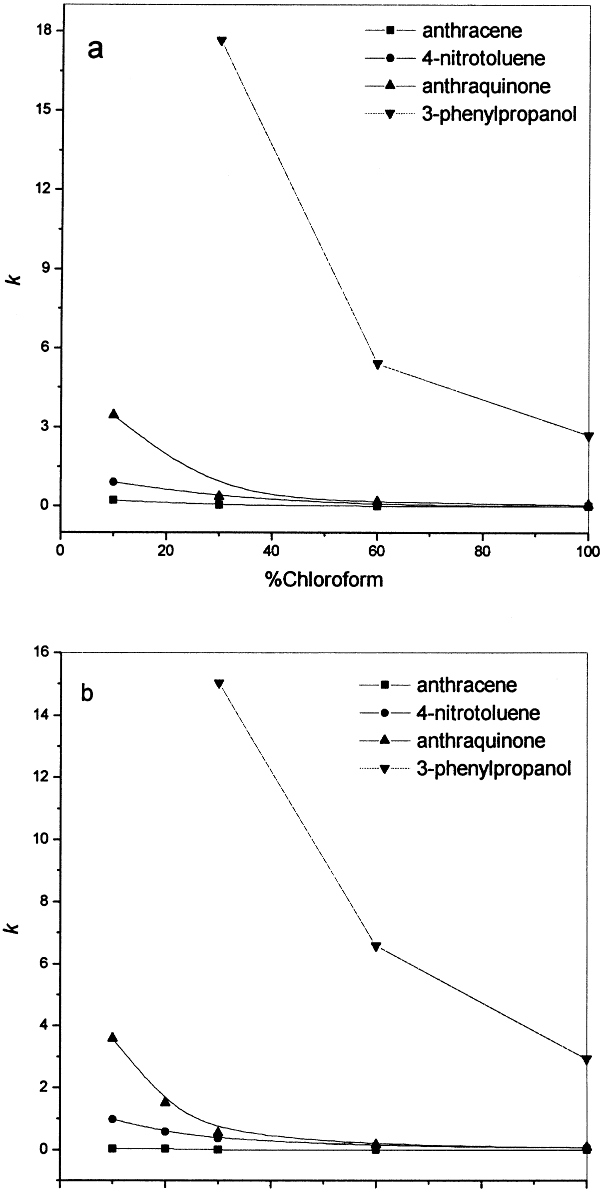

Figure 6. Influence of the chloroform content of the mobile phase on capacity of neutral solutes on silica-zirconia (3.4%) (a) and zirconia (b) stationary phase.

The peak asymmetry factors of phenol at 10% the peak high were found to be 1.8 on silica-zirconia (3.4%) (retention time 21.6 min), 2.0 on silica-zirconia (15.8%) (retention time 17.9 min), and 3.1 on zirconia (retention time 22.3 min), respectively. The result shows that introduction of silica into zirconia can improve the peak tailing of phenol.

Chromatographic Behavior of Neutral Compounds

Figure 6a and Figure 6b are the plots of capacity factors of some neutral compounds against the concentration of chloroform in chloroform/cyclohexane mobile phase on the silica-zirconia (3.4%) and the zirconia, respectively. It can be seen that the capacity factors decreased with increase of the concentration of chloroform in the mobile phase. The polycyclic aromatic hydrocarbons (PAHs) could be retarded, to some extent, on the stationary phases, which is probably due to the interaction between their π-electron and the Lewis acid site of the stationary phases. 4-Nitrotoluene, anthraquinone, and 3-phenylpropanol exhibited stronger retention than PAHs as shown in Figure 6, which may be due to the stronger polarity of them. The similar retention behavior of these neutral compounds was observed on both of the stationary phases by using isopropanol/cyclohexane and dichloromethane/cyclohexane as mobile phases. The elution strength of the solvents used for neutral compounds on silica-zirconia and zirconia was found to be in the order of chloroform < dichloromethane < isopropanol. This similar result was observed on silica-zirconia (15.8%) packings. The slightly smaller capacity factors was obtained on zirconia due to the smaller specific surface area of zirconia.

CONCLUSION

Two silica-zirconia packings with different silica molar ratios (3.4% and 15.8%, respectively) were synthesized by a sol-gel process. Addition of the silica into the zirconia microspheres can prevent the formation of monoclinic zirconia at calcination temperature 600°C. In comparison with zirconia, the silica-zirconia composites show more ideal pore connectivity for liquid chromatography. The specific surface increased but the average pore diameter decreased with the increase of the content of silica in the silica-zirconia composite. The acidic surface characteristics was observed for the silica-zirconia composites. Symmetrical peaks and stronger retention are obtained for the basic compounds on the silica-zirconia composites than on the zirconia. The peak tailing of acidic compounds on zirconia have been improved by introduction of silica into zirconia. Therefore, the improvement in chromatographic performance of the zirconia packings can be accomplished by the modification of the zirconia with silica.

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.

REFERENCES

1. H. Billiet, C. Laurent, L. De Galan, Trends Anal. Chem., **4**, 100 (1985).
2. U. Trudinger, G. Muller, K. Unger, J. Chromatogr., **535**, 111 (1990).
3. J. Nawrocki, M. P. Rigney, A. McCormick, P. W. Carr, J. Chromatogr., **657**, 229 (1993).
4. M. Grun, A. Kurganov, S. Schacht, F. Schuth, K. Unger, J.Chromatogr. A., **740**, 1 (1996).
5. A. Kurganov, U. Trudinger, T. Isaeva, K. Unger, Chromatographia, **42**, 217 (1996).
6. J. Yu, Z. E. Rassi, J. Liq. Chromatogr., **17**, 773 (1994).
7. T. P. Weber, P. W. Carr, Anal. Chem., **62**, 2620 (1990).
8. T. P. Weber, P. T. Jackson, P. W. Carr, Anal. Chem., **67**, 3042 (1995).
9. P. T. Jackson, T.-Y. Kim, P. W. Carr, Anal. Chem., **69**, 5011 (1997).
10. C. McNeff, Q. H. Zhao, P. W. Carr, J. Chromatogr. A, **684**, 201 (1994).
11. L. Sun, P. W. Carr, Anal. Chem., **67**, 3717 (1995).
12. C. J. Dunlap, P. W. Carr, J. Chromatogr. A, **746**, 199 (1996).
13. J. W. Li, P. W. Carr, Anal. Chem., **69**, 2193 (1997).
14. Y. Hu, P. W. Carr, Anal. Chem., **70**, 1934 (1998).
15. J. A. Blackwell, P. W. Carr, J. Chromatogr., **549**, 59 (1991).
16. W. A. Schafer, P. W. Carr, J. Chromatogr., **587**, 149 (1991).

17. A. M. Clausen, P. W. Carr, Anal. Chem., **70**, 378 (1998).

18. A. M. Clausen, A. Subramainian, P. W. Carr, J. Chromatogr., **831**, 63 (1999).

19. R. A. Shalliker, L. Rintoul, G. K. Douglas, S. C. Russell, J. Mater. Sci., **32**, 2949 (1997).

20. W. W. Yau, J. J. Kirkland, D. D. Bly, **Modern Size Exclusion Liquid Chromatography**, John Wiley & Sons, New York, 1979.

21. R. A. Shalliker, G. K. Douglas, J. Liq. Chrom. & Rel. Technol., **21**, 2413 (1998).

22. C. F. Lorenzano-Porras, P. W. Carr, A. V. McCormick, J. Colloid Interface Sci., **164**, 1 (1994).

23. C. F. Lorenzano-Porras, M. J. Annen, M. C. Flicking, P. W. Carr, A. V. McCormick, J. Colloid Interface Sci., **170**, 299 (1995).

24. C. J. Dunlap, P. W. Carr, A. V. McCormick, Chromatographia, **42**, 273 (1996).

25. R. A. Shalliker, G. K. Douglas, J. Liq. Chrom. & Rel. Technol., **20**, 1651 (1997).

26. Q.-H. Zhang, Y.-Q. Feng, S.-L. Da, Chromatographia, **50**, 654 (1999).

27. S. Kaneko, T. Mitsuzaka, S. Ohmori, M. Nakamura, K. Nobuhara, M. Masatani, J. Chromatogr. A, **669**, 1 (1994).

28. H. Kita, N. Henmi, K. Shimazu, H. Hattori, K. J. Taneba, J. Chem. Soc., Fraday Trans.1, **77**, 2451 (1981).

29. K. S. W. Sing, D. H. Everett, R. A. W. Haul, R. A. Pierotti, S. T. Rouquerol, Pure Appl. Chem., **57**, 603 (1985).

30. P. D. L. Mercera, J. G. Van Ommen, E. B. M. Doesburg, A. J. Burggraaf, J. R. H. Ross, Appl. Catal., **57**, 127 (1990).

31. L. R. Snyder, J. J. Kirkland, **Introduction to Modern Liquid Chromatography**, John Wiley & Sons, Inc., 1979

Received July 13, 1999
Accepted November 9, 1999
Author's Revisions December 22, 1999
Manuscript 5125