http://dx.doi.org/10.5012/bkcs.2012.33.8.2651

Chiral Separation on Sulfonated Cellulose Tris(3,5-dimethylphenylcarbamate)-coated Zirconia Monolith by Capillary Electrochromatography

Jeong-Mi Lee, Myung Duk Jang,† and Jung Hag Park*

*Department of Chemistry, Yeungnam University, Gyeongsan 712-749, Korea. *E-mail: jhpark@ynu.ac.kr*
†Department of Gas Energy Engineering, Kundong University, Andong 760-833, Korea
Received April 26, 2012, Accepted May 14, 2012

Sulfonated cellulose tris(3,5-dimethylphenylcarbamate) (SCDMPC)-coated zirconia monolith (ZM) was used as the chiral stationary phase in capillary electrochromatography for separation of enantiomers of ten chiral compounds in acetonitrile (ACN)-phosphate buffer mixtures as the eluent. Influences of the ACN content, buffer concentration and pH on chiral separation have been investigated. Separation data on SCDMPC-ZM have been compared with those on CDMPC-ZM. Resolution factors were better on SCDMPC-ZM than CDMPC-ZM while retention factors were in general shorter on the former than the latter. Best chiral resolutions on SCDMPC-ZM were obtained with the eluent of 50% ACN containing 50 mM phosphate at pH around 4.

Key Words : Chiral separation, Zirconia monolith, Sulfonated cellulose 3,5-dimethylphenylcarbamate, Capillary electrochromatography

Introduction

Capillary electrochromatography (CEC) is a hybrid technique of HPLC and CE, and has been increasingly utilized in studies on the development and evaluation of separation methods including chiral separations.[1-3] It provides high efficiency because of the flat profile of electroosmotic flow (EOF) to pump the mobile phase and ability to separate charged as well as uncharged compounds through electrophoresis and chromatographic separation.[3] Several reviews have been reported on enantioseparations using CEC as a separation technique.[1-4] Three types of columns including, open-tubular, particulate-packed and monolithic capillaries are used for CEC.[5] Monolithic columns are becoming attractive alternative to particle-packed columns in HPLC and electrochromatography.[6-8] Monolithic columns are devoid of problems and difficulties associated with packed capillary columns, including burdensome packing of stationary phase particles in a capillary and frits that cause formation of air bubbles during the analysis which results in reduction of separation efficiency, and tend to break easily.[9-12] The monolithic columns allow fast mass transfer at lower pressure drops, enabling much faster separations. The continuous monolithic bed in the capillary column also allows high linear velocities that enable high throughput screening and fast separations of enantiomers.[6-8,13]

Among various classes of chiral selectors[14,15] polysaccharides have been widely used as the chiral stationary phase (CSP). A great number of polysaccharide derivatives including cellulose, amylose, chitin, chitosan, galactosamine, curdlan, dextran, xylan, and inulin have been in use for chiral separations.[16] The derivatives of cellulose and amylose usually exhibit higher chiral recognition ability than the other type polysaccharides. A number of HPLC separations for a large number of chiral compounds in different classes on polysaccharide-immobilized silica[17-20] and polymer columns.[21] The polysaccharide-based columns can be used in normal phase, polar organic and reversed-phase mode.[22]

While silica-based stationary phases have received wide acceptance due to their well-studied surface chemistry, drawbacks of silica-based stationary phases are also well experienced, which includes restricted use in eluents of a limited pH range of 2-8 due to unstable nature of Si-O-Si bond in acidic as well as basic eluents[23,24] and applicability only in limited temperature range.[25] Zirconia is a viable alternative to silica as the support, due to its unique and extraordinary chemical, mechanical and thermal stability.[26-28] Zirconia particles are stable over the entire pH range and have been used for prolonged periods at temperatures up to 200 °C. The specific surface area and pore volume of zirconia are smaller in comparison with silica, but due to its higher density the surface area of zirconia is comparable to that of silica in terms of surface area per unit volume. The unique surface chemistry of zirconia extends different applications for its use in chromatography.[26,29] A number of zirconia-based CSPs have been evaluated in HPLC[30-34] and CEC.[35-37] Recently zirconia-based CSPs coated with cellulose derivatives have been used in HPLC[30,31,38,39] and CEC in particle-packed[35] and monolithic columns.[37] We recently reported chiral separation of basic compounds by CEC on cellulose 3,5-dimethylphenylcarbamate (CDMPC)-coated zirconia monolith (ZM) column in mobile phases of acidic[37,40] and basic pH.[41]

In this work, we report enantiomer separation by CEC on zirconia monolithic column modified with sulfonated CDMPC (SCDMPC).[42] The augmented EOF generated by the negative charges of the sulfonate groups and dissociated zirconol groups of the ZM surface as well will provide much

faster separation than native CDMPC. It is expected that the better chiral resolution in the shorter analysis time is obtained on SCDMPC. We have investigated chiral separation of a set of ten neutral and basic chiral compounds on the SCDMPC-ZM column in aqueous organic eluents of varying pH, organic and electrolyte compositions to evaluate the performance of the column and compared them with those on CDMPC-ZM.

Experimental

Materials. Fused silica capillaries (75 μm I.D., 365 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Zirconium butoxide, acetic acid, potassium dihydrogen phosphate and sodium hydroxide were purchased from Aldrich (Milwaukee, WI, USA). Cellulose (Avicel) was obtained from Merck (Darmstadt, Germany). Triphenylmethyl chloride and 4,4'-diphenylmethane diisocyanate of reagent grade were received from TCI (Tokyo, Japan). Polyethylene glycol (PEG) (MW = 10,000 g mol^{-1}), 3,5-dimethylphenyl isocyanate, tetrahydrofuran (THF) and pyridine were supplied by Aldrich (Milwaukee, WI, USA). All reagents used were reagent grade or better having higher than 99% purity. HPLC-grade acetonitrile (ACN) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Water was purified with an Elgastat UHQ water purification system (Bucks, UK). Chiral compounds including Tröger's base (**TB**), 2,2,2-trifluoro-1-(9-anthryl)ethanol (**TFAE**), *trans*-stilbene oxide (**TSO**), 1-(1-naphthyl)ethanol (**NE**), 4-phenyl-1,3-dioxane (**PD**), α-methylbenzyl cyanide (**MBC**), α-(trifluoromethyl)-benzyl alcohol (**TFMB**), epoxiconazole (**EPO**), benalaxyl (**BEN**) and tetramethrin (**TM**) were of the highest-purity available from Aldrich (Milwaukee, WI, USA) or TCI (Tokyo, Japan) and structures are shown in Figure 1.

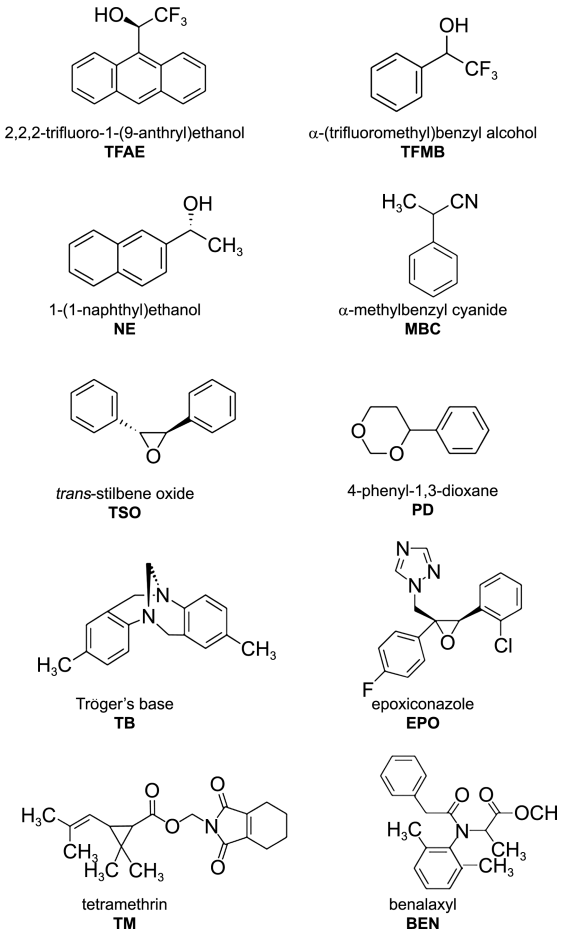

Figure 1. Structures of chiral compounds.

Instrumentation. An Agilent HP ^{3D}CE System (Palo Alto, CA, USA) equipped with a diode-array UV detector, a ± 30 kV high voltage power supply and an external nitrogen pressure was used for the CEC separations. Instrument control and data collection were performed with the ChemStation software. The morphology of the zirconia monoliths was examined by a field emission scanning electron microscope (FE-SEM S-4100, Hitachi, Japan). A syringe pump from Cole-Parmer (Vernon Hills IL, USA) was used to inject the SCDMPC solution into the zirconia monolithic capillary.

Column Preparation. Zirconia monolithic capillary column was prepared according to the method reported earlier.[37] Briefly, to a homogeneous hydrolysis solution consisted of PEG, water, acetic acid and 1-butanol at suitable concentrations, a required amount of zirconium butoxide was added. The resulting mixture was injected into the activated fused silica capillary up to the required length and allowed to react overnight at 35 °C. Then, the column was heated at 150 °C for 6 h. After completion of heating, the capillaries were cooled to room temperature and then characterized by SEM. CDMPC was prepared as per the reported method[43] and SCDMPC was prepared as per the reported method[42] and characterized by elemental analyses, IR and NMR spectroscopy. To perform the CDMPC and SCDMPC coatings on the surface of ZM bed, the ZM capillaries were initially washed with ethanol and then with THF. Then, a polymer solution of 8% by weight in THF was passed through the capillaries at a flow rate of 5 μL min^{-1} using a syringe pump to coat the entire zirconia monolithic bed of the capillary column. The capillary was finally rinsed with methanol and mobile phase, respectively.

Chromatography. CEC separations were carried out at 25 °C with an applied voltage of 5 kV and monitored at 214, 254 and 280 nm. An external pressure of 10 bars was applied to both buffer reservoirs. The mobile phases were mixtures of ACN and phosphate buffer of varying pH in different compositions. These mobile phases were filtered through a nylon membrane filter of 0.2-μm pore size and degassed prior to use. The monolithic capillary columns were equilibrated for 8-10 h in order to reduce baseline noise before CEC runs. Samples dissolved in the mobile phase were injected electro-kinetically at 10 kV for 1 s. Separations were done at the applied voltage of 10 kV. Migration times of two consecutive injections were in agreement within 3%. Fresh mobile phase was replenished after each run of sample. The capillaries with total length of 35 cm and monolithic bed length of 25 cm were used for separation. The dead time was measured by injecting acetone.

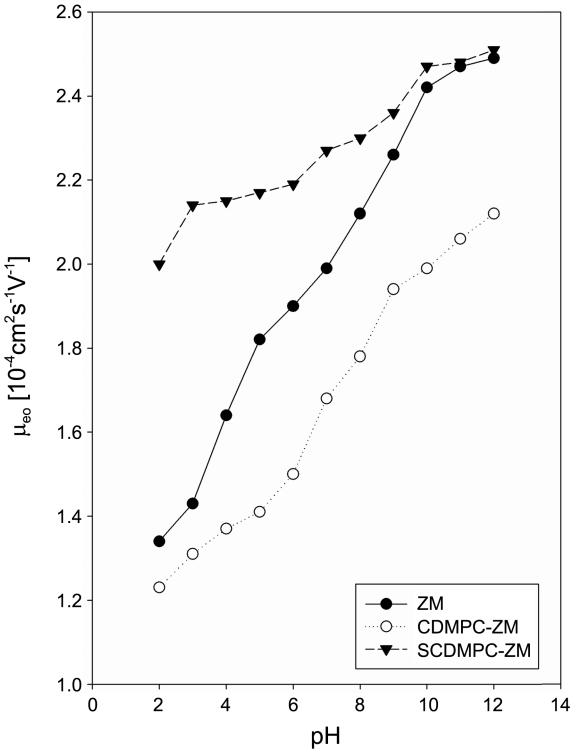

Figure 2. Variation of electroosmotic mobility, μ_{eo}, on bare and coated ZM columns. Conditions: column, length 35 cm (monolith bed 25 cm) × i.d. 50 μm; eluent, 50/50 (v/v) ACN/phosphate buffer (50 mM); marker, acetone; voltage, 5 kV; injection, 10 kV for 1 s; detection, 280 nm; temperature, 25 °C.

Results and Discussion

EOF Behavior of SCDMPC-ZM. Zirconol groups on the zirconia monolith (ZM) surface can undergo Brönsted acid-base reactions ($ZrOH \rightleftarrows ZrO^- + H^+$ (1); $ZrOH_2^+ \rightleftarrows ZrOH + H^+$ (2)) and either positive or negative surface charge can develop depending on the pH of the mobile phase. Point of zero charge on zirconia occurs at pH between 5 and 6,[28] and thus the direction of EOF can be either cathodic above this pH or anodic below this pH.[44] In organic-phosphate buffer mixtures cathodic EOF was invariably observed for both native and modified zirconia regardless of pH.[37,44] Phosphate ions as Lewis bases are able to bind strongly to the Lewis acid sites of zirconia surface[26] to provide extra negative charges and hence cathodic EOF, regardless of the mobile phase pH. Figure 2 shows variation of electroosmotic mobility (μ_{eo}) measured by acetone on bare, CDMPC- and SCDMPC-ZM with pH. The magnitude of EOF increases with pH as more zirconol groups dissociate according to Eq. (1) to increase negative surface charges, resulting in increasing EOF. The magnitude of EOF on CDMPC-ZM is smaller than that on bare ZM since the number of exposed dissociable zirconol groups on CDMPC-ZM is decreased as the surface is covered by adsorbed polymer.[37] The magnitude of EOF on SCDMPC-ZM is even greater than on bare ZM due to additional negative charges of the sulfonate groups of SCDMPC.

Influence of the ACN Content. The effect of organic content in the eluent on chiral separation was examined by varying the ACN content from 40 to 70 v% for two typical analytes, EPO and TFAE, and resulting chromatograms are shown in Figure 3 along with selectivity factor (α), resolution factor (R_s) and the number of the theoretical plate for the early eluting enantiomer (N_1). ACN was chosen as the organic modifier as it provides the better separation efficiency.[35,45] Migration times for both analytes were decreased as the ACN content was increased. Enantioselectivity factors were increased when the ACN content was increased from 40 to 50% and then decreased upon further increase of the ACN content, showing a maximum at 50%. Resolution factors and theoretical plate counts showed similar trends to that for enantioselectivity. While variation of the selectivity factor is marginal that for resolution factor is much greater. The much greater variation of resolution factors with ACN composition is likely due to the much bigger changes in the plate counts, according to the equation, $R_s = 1/4 \cdot N^{1/2} \cdot (\alpha-1)$.[46] It is thought that ACN composition of 50 v% is optimal when migration time and resolution are taken into consi-

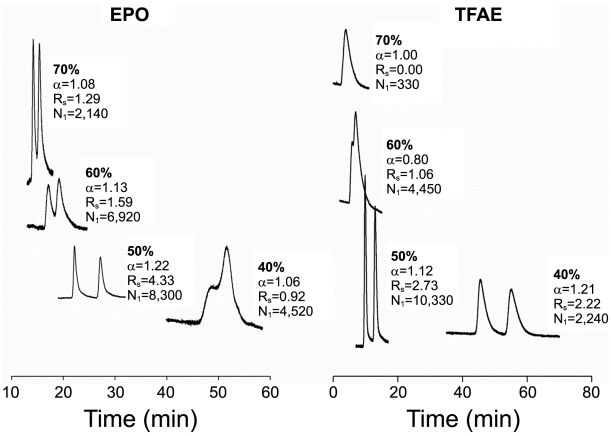

Figure 3. Effect of ACN content. Conditions: column, SCDMPC-ZM; eluent, ACN/phosphate buffer (50 mM, pH 4.4); detection, 254 nm. Other conditions are the same as in Fig. 2.

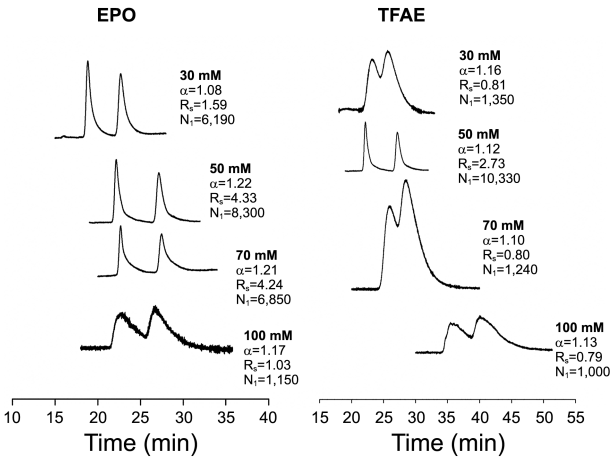

Figure 4. Effect of phosphate concentration. Conditions: eluent, 50/50 (v/v) ACN/phosphate buffer (pH 4.4). Other conditions are the same as in Fig. 2. Note that chromatograms were shifted vertically for viewing feasibility.

deration together.

Influence of the Phosphate Concentration. SCDMPC-ZM is likely to show cation-exchange behavior. The enantio-separations of EPO and TFAE as the representative analyte were studied by varying the concentration of phosphate from 30 mM to 100 mM, and resulting chromatograms are shown in Figure 4 along with selectivity, resolution and the plate count for the early eluting enantiomer. As the phosphate concentration was increased, the magnitude of EOF was decreased due to the reduced double-layer thickness (2.06, 1.87, 1.79 and 1.72 [$\times 10^{-4}$ $cm^2s^{-1}V^{-1}$] at 30, 50, 70 and 100 mM, respectively). The shorter migration time of the positively charged EPO than neutral TFAE is an indication of cation-exchange effect. The migration of the cationic EPO is further augmented by the cathodic electrophoretic flow, which is co-directional with the EOF. The plate counts and resolutions were the highest at 50 mM and decreased with a further increase in the electrolyte concentration, which is most likely due to the increased Joule heating that causes molecular diffusion to increase. Enantioselectivity did not change significantly with the phosphate concentration. Phosphate concentration of 50 mM was thus chosen for further separations.

Influence of pH. The pH of the mobile phase will affect the electromigration behavior of a basic analyte as it determines the degree of ionization of the analyte and hence the electrophoretic migration behavior. Migration of nonioniz-

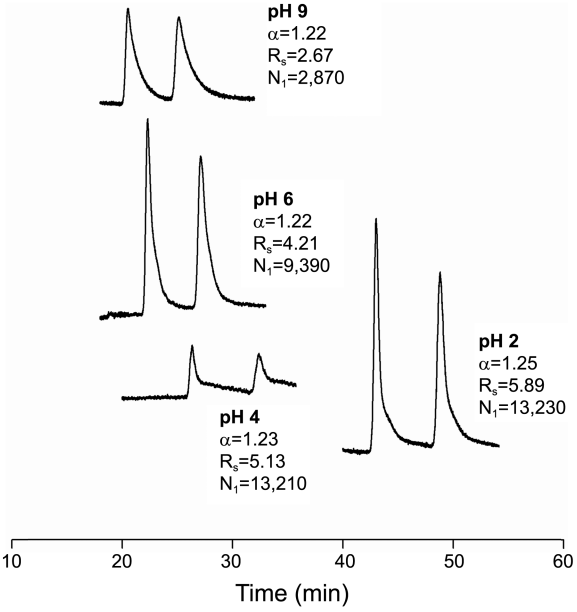

Figure 5. Effect of pH. Conditions: analyte, EPO; eluent, 50/50 (v/v) ACN/phosphate buffer (50 mM). Other conditions are the same as in Fig. 2.

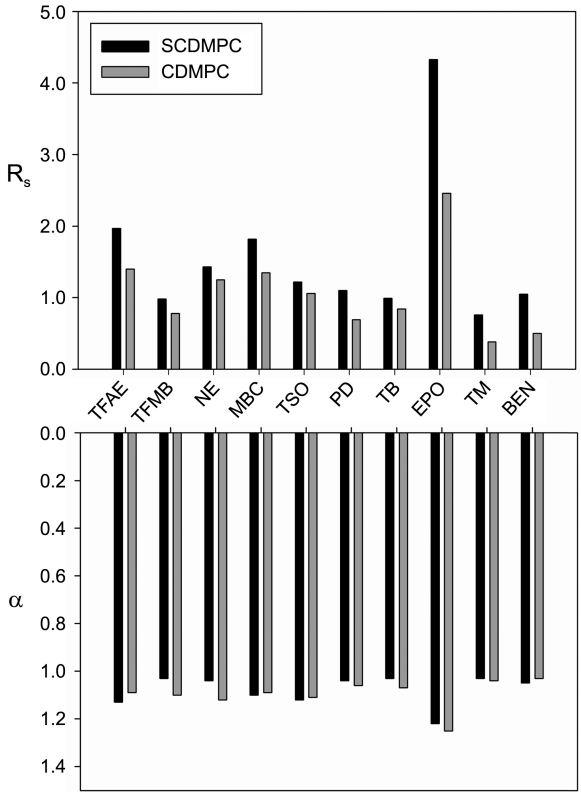

Figure 6. Comparison of resolution and selectivity of SCDMPC-ZM *vs*. CDMPC-ZM.

Table 1. Separation Data on CDMPC-ZM and SCDMPC-ZM*

	CDMPC-ZM					SCDMPC-ZM				
	t_{R1}/t_{R2}[a]	α[b]	R_s[c]	k_1/k_2[d]	N_1/N_2[e]	t_{R1}/t_{R2}	α	R_s	k_1/k_2	N_1/N_2
TFAE	27.2/30.1	1.09	1.40	0.35/0.49	3770/2970	25.8/28.9	1.12	2.73(1.95)[f]	0.52/0.80	10330/8400
TFMB	27.8/32.3	1.10	0.78	0.24/0.39	2010/2320	17.6/18.1	1.03	0.98(1.26)	0.09/0.13	16610/14820
NE	27.2/30.9	1.12	1.25	0.35/0.53	2000/1290	15.6/16.2	1.04	1.43(1.14)	-0.02/0.01	21450/18360
MBC	22.6/24.1	1.07	0.84	0.12/0.20	2350/2190	17.8/18.3	1.03	0.99(1.18)	0.11/0.14	15640/15190
TSO	23.4/26.0	1.11	1.06	0.20/0.31	1780/1440	13.1/14.6	1.12	1.22(1.15)	-0.18/-0.09	18530/17740
PD	25.2/28.5	1.06	0.69	0.07/0.18	1620/1430	17.1/17.7	1.04	1.10(1.67)	0.06/0.10	17790/14770
TB	23.0/26.4	1.09	1.35	0.09/0.18	3970/3360	21.3/23.1	1.08	2.53(1.87)	0.27/0.44	7010/5844
EPO	25.9/32.5	1.25	2.46	0.28/0.61	4160/3780	22.1/27.1	1.22	4.33(1.76)	0.42/0.69	8300/6720
TM	28.4/29.6	1.04	0.38	0.41/0.47	2400/1900	17.4/18.0	1.03	0.76(2.00)	0.08/0.12	11740/8720
BEN	26.0/26.9	1.03	0.50	0.29/0.33	4490/3500	20.4/21.3	1.05	1.05(2.10)	0.27/0.33	7960/6760

*Conditions: columns, ID, 50 μm, length, 35 cm, monolith bed, 25 cm; mobile phase, ACN/phosphate buffer (50 mM, pH 4.4); reservoir pressure, 10 bar; voltage, 5 kV; injection, 10 kV, 1 sec; detection; 214 nm, 254 nm; temperature, 25 °C; dead time marker, acetone. [a]Migration time. [b]Apparent selectivity factor, $\alpha = t_{R2}/t_{R1}$. [c]Resolution factor. [d]Retention factor. [e]Plate counts for the first- and second-eluting enantiomer. [f]In parentheses are the ratios of R_s on SCDMPC-ZM *vs*. CDMPC-ZM.

ing a neutral analyte is determined predominantly by EOF. The separation of a typical basic analyte, EPO, has been examined in 50:50 ACN/phosphate mixture of different pH, and the chromatograms are shown in Figure 5 along with selectivity, resolution and the plate count for the early eluting enantiomer. As pH was increased from 2 to 4 the migration time of EPO was decreased sharply due to increasing EOF as seen in Figure 2. Further increase of pH up to 9 produced only marginal decrease in the migration times. While EOF was increased to a small extent as pH was changed from 4 to 6 and 9, the increased migration by this increase in EOF was likely to be cancelled by decreased electrophoretic migration. With increasing pH the fraction of protonated form of EPO molecules decreases, which in turn reduces electrophoretic migration that is co-directional with EOF, resulting in decreased migration of the analyte. While selectivity factors did not change appreciably resolution factors were decreased in general with pH. The best enantioseparations were obtained at pH ~4.

Chiral Separation on SCDMPC-ZM. Enantioseparation data for ten analytes on SCDMPC-ZM in 50:50 ACN/phosphate buffer (50 mM, pH 4.4) are listed in Table 1, along with those on CDMPC-ZM. Bar graphs for resolution and selectivity factors on the two CSPs are shown in Figure 6 for straightforward comparison. Better resolutions were obtained for all the analytes in shorter migration times on SCDMPC-ZM than CDMPC-ZM. Enantiomers of three compounds were baseline separated on SCDMPC-ZM while only one compound was baseline separated on CDMPC-ZM.

Conclusions

Zirconia monolith modified with sulfonated cellulose 3,5-dimethylphenylcarbamate was used for the separation of enantiomers of a set of ten chiral compounds by CEC. The EOF behavior of bare, CDMPC- and SCDMPC-coated ZM column was studied in ACN/phosphate buffer eluents of pH ranging from 2 to 12. Increasing cathodic EOF with pH was observed for all ZM columns, with the highest EOF on SCDMPC-ZM. The SCDMPC-ZM column was evaluated by studying the influences of the ACN and phosphate composition, and pH of the eluent on enantiomer separation. Better separations in shorter analysis time for the analytes were obtained on SCDMPC-ZM than CDMPC-ZM. CEC separations in the mobile phase of 50% ACN containing 50 mM phosphate at pH of around 4 provided the best chiral resolutions for the analytes studied.

Acknowledgments. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2009-0070894).

References

1. Gubitz, G; Schmid, M. G. *Electrophoresis* **2004**, *23*, 3981.
2. Fanali, S.; Catarcini, P.; Blaschke, G.; Chankvetadze, B. *Electrophoresis* **2001**, *22*, 3131.
3. Lämmerhofer, M. *J. Chromatogr. A* **2005**, *1068*, 31.
4. Mangelings, D.; Vander Heyden, Y. *Electrophoresis* **2011**, *32*, 2583.
5. Ahuja, S. *Chiral Separation by Liquid Chromatography*; American Chemical Society: Chapter 1, Washington, D.C., U.S.A, 1991.
6. Kłodziǹska, E.; Moravcova, D.; Jandera, P.; Buszewski, B. *J. Chromatogr. A* **2006**, *1109*, 51.
7. Wu, R.; Hu, L.; Wang, F.; Ye, M.; Zou, H. *J. Chromatogr. A* **2008**, *1184*, 369.
8. Svec, F.; Huber, C. G. *Anal. Chem.* **2006**, *78*, 2100.
9. Ericson, C.; Liao, J.-L.; Nakazato, K.; Hjerten, S. *J. Chromatogr. A* **1997**, *767*, 33.
10. Hayes, J. D.; Malik, A. *Anal. Chem*. **2000**, *72*, 4090.
11. Behnke, B.; Grom, E.; Bayer, E. *J. Chromatogr. A* **1995**, *716*, 207.
12. Asiaie, R.; Huang, H.; Farnan, D.; Harvathe, C. *J. Chromatogr. A* **1998**, *806*, 251.
13. Zhu, G.; Zhang, L. H.; Yuan, H.; Liang, Z.; Zhang, W.; Zhang, Y. *J. Sep. Sci*. **2007**, *30*, 792.
14. Lämmerhofer, M. *J. Chromatogr. A* **2010**, *1217*, 814.
15. Wang, Z.; Ouyang, J.; Baeyens, W. R. G. *J. Chromatogr. A* **2008**, *862*, 1.
16. Chen, X. M.; Yamamoto, C.; Okamoto, Y. *Pure Appl. Chem.* **2007**, *79*, 1561.
17. Okamoto, Y.; Kawashima, M.; Hatada, K. *J. Am. Chem. Soc.* **1984**, *106*, 5357.
18. Okamoto, Y.; Yashima, E. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1020.
19. Girod, M.; Chankvetadze, B.; Blaschke, G. *J. Chromatogr. A* **2000**, *887*, 439.
20. Chankvetadze, B.; Kartozia, I.; Breitkreutz, J.; Okamoto, Y.; Blaschke, G. *Electrophoresis* **2001**, *22*, 3327.
21. Dong, X.; Wu, R.; Dong, J.; Wu, M.; Zhu, Y.; Zou, H. *Electrophoresis* **2008**, *29*, 919.
22. Tachibana, K.; Ohnishi, A. *J. Chromatogr. A* **2001**, *906*, 127.
23. Wehrli, A.; Hildenbrand, J. C.; Keller, H. P.; Stampeli, R.; Frei, R. W. *J. Chromatogr.* **1978**, *149*, 199.
24. Glajch, J. L.; Kirkland, J. J.; Kohler, J. *J. Chromatogr.* **1987**, *384*, 81.
25. McNeff, C. V.; Zigan, L.; Johnson, K.; Carr, P. W.; Wang, A.; Weber-Main, A. M. *LC-GC* **2000**, 5, 514.
26. Nawrocki, J.; Rigney, M.; McCormick, A.; Carr, P. W. *J. Chromatogr. A* **1993**, *657*, 229.
27. Dunlap, C. J.; McNeff, C. V.; Stoll, D.; Carr, P. W. *Anal. Chem*. **2001**, *11*, 599A.
28. Nawrocki, J.; Dunlap, C. J.; McCormick, A.; Carr, P. W. *J. Chromatogr. A* **2004**, *1028*, 1.
29. Nawrocki, J.; Dunlap, C. J.; Carr, P. W.; Blackwell, J. A. *Biotechnol. Prog.* **1994**, *10*, 561.
30. Castells, C. B.; Carr, P. W. *Anal. Chem*. **1999**, *71*, 3013.
31. Castells, C. B.; Carr, P. W. *J. Chromatogr. A* **2000**, *904*, 17.
32. Park, S. Y.; Park, J. K.; Park, J. H.; McNeff, C. V.; Carr, P. W. *Microchem. J.* **2001**, *70*, 179.
33. Park, J. H.; Lee, J. W.; Kwon, S. H.; Cha, J. S.; Carr, P. W.; McNeff, C. V. *J. Chromatogr. A* **2004**, *1050*, 151.
34. Kim, I. W.; Choi, H. M.; Yoon, H. J.; Park, J. H. *Anal. Chim. Acta* **2006**, *569*, 151.
35. Gwon, J.; Jin, J. H.; McNeff, C. V.; Park, J. H. *Electrophoresis* **2009**, *30*, 3846.
36. Lee, M. R.; Gwon, J.; Park, J. H. *Bull. Korean Chem. Soc.* **2010**, *31*, 82.
37. Kumar, A. P.; Park, J. H. *J. Chromatogr. A* **2010**, *1217*, 4494.
38. Park, J. H.; Whang, Y. C.; Jung, Y. J.; Okamoto, Y.; Yamamoto, C.; Carr, P. W.; McNeff, C. V. *J. Sep. Sci.* **2003**, *26*, 1331.
39. Kwon, S. H.; Okamoto, Y.; Yamamoto, C.; Cheong, W.; Moon, M. H.; Park, J. H. *Anal. Sci.* **2006**, *22*, 1525.
40. Kumar, A. P.; Park, J. H. *J. Chromatogr. A* **2011**, *1218*, 5369.
41. Kumar, A. P.; Park, J. H. *J. Chromatogr. A* **2011**, *1218*, 6548.

42. Zheng, J.; Bragg, W.; Hou, J.; Lin, N.; Chandrasekaran, S.; Shamsi, S.A. *J. Chromatogr. A* **2009**, *1216*, 857.
43. Okamoto, Y.; Kawashima, M.; Hatada, K. *J. Chromatogr.* **1986**, *363*, 173.
44. Randon, J.; Huguet, S.; Piram, A.; Puy, G.; Demesmay, C.; Rocca, J.-L. *J. Chromatogr. A* **2006**, *1109*, 19.
45. Krause, K.; Girod, M.; Chankvetadze, B.; Blaschke, G. *J. Chromatogr. A* **1999**, *837*, 51.
46. Bowser, M.; Bebault, G. M.; Peng, X.; Chen, D. D. Y. *Electrophoresis* **1997**, *18*, 2928.