Anal. Chem. **2003,** *75,* 1348−1354

Application of Cyclam-Capped *β*-Cyclodextrin-Bonded Silica Particles as a Chiral Stationary Phase in Capillary Electrochromatography for Enantiomeric Separations

Yinhan Gong and Hian Kee Lee*

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Republic of Singapore 117543

Two novel types of substituted cyclam-capped *β*-cyclodextrin (*β*-CD)-bonded silica particles have been prepared and used as chiral stationary phases in capillary electrochromatography (CEC). The two stationary phases have a chiral selector with three recognition sites: *β*-CD, cyclam, and the latter's sidearm. They exhibit excellent enantioselectivities in CEC for a wide range of compounds as a result of the cooperative functioning of the anchored *β*-CD and cyclam. After inclusion of the metal ion (Ni^{2+}) from the running buffer into the substituted cyclams and their sidearm ligands, the bonded stationary phases become positively charged and can provide extra electrostatic interactions with ionizable solutes and enhance the dipolar interactions with some polar neutral solutes. This enhances the host−guest interaction with some solutes and improves chiral recognition and enantioselectivity. These new types of stationary phases exhibit great potential for fast chiral separations in CEC.

Development and application of new chiral stationary phases (CSPs) with high selectivities to separate chiral molecules is one of the most active areas of liquid chromatography (LC).[1−4] Chiral separations are important in various fields,[5−7] such as natural product research, stereospecific synthesis, chiral drugs in the pharmaceutical industry, and chiral compounds in environmental studies. Many chiral separations have been accomplished using *β*-cyclodextrin-type stationary phases as CSPs in LC.[5,8,9] Recently, it was shown that the combination of a crown ether and *β*-CD as a buffer additive in capillary electrophoresis (CE) sometimes produced better enantioseparations than did either selector alone as a result of cooperative functioning of the *β*-CD and the crown ether.[10−12] However, many crown ethers, cyclams, and derivatized CDs with high UV/vis absorption characteristics or poor solubility in water are not suitable to be used as CE additives for direct detection. Alternatively, they can be bonded onto silica support to be used as CSPs in LC to separate chiral compounds. Although crown ether-capped *β*-CD has been used to model the receptor sites of enzymes for a long time,[13−15] its use as a stationary phase selector for chromatography has seldom been studied.[16] We have previously reported that the crown ether-capped *β*-CD-bonded silica particles showed excellent enantioselectivities when used as CSPs in LC.[3] Since cyclams have similar structures and properties to crown ethers, it was of interest to us to prepare cyclam-capped *β*-CD-bonded silica particles for study of their enantioselectivities when used as new CSPs in LC. To the best of our knowledge, we were among the first to report a convenient method involving successive multiple-step liquid−solid-phase reactions on the silica surface to synthesize cyclam-capped *β*-CD-bonded silica particles.[4]

Capillary electrochromatography (CEC) is a modern LC technique combining the high efficiency of CE with the high selectivity usually obtained in high-performance liquid chromatography (HPLC).[17] As in HPLC, the stationary phase packed-capillary columns are used for the separation of solutes of interest. The mobile phase in CEC is transported through a capillary column by means of electroosmotic flow (EOF) instead of pressure, as in HPLC. Neutral solutes are separated by partitioning between the mobile and the stationary phases. Charged solutes have an additional electrophoretic mobility in the applied electric field in CEC, and the separation is achieved by the combined

* Corresponding author. Tel: (65)-6874-2995. Fax: (65)-6779-1691, E-mail: chmleehk@nus.edu.sg.

(1) Dermaux, A.; Lynen, F.; Sandra, P. *J. High Resolut. Chromatogr.* **1998**, *21*, 575−576.
(2) Wistuba, D.; Schurig, V. *J. Chromatogr., A* **2000**, *875*, 255−276.
(3) Gong, Y.; Xue, G.; Bradshaw, J. S.; Lee, M. L.; Lee, H. K. *J. Heterocycl. Chem.* **2001**, *38*, 1317−1321.
(4) Gong, Y.; Xue, G.; Xiang, Y.; Bradshaw, J. S.; Lee, M. L.; Lee, H. K. *Tetrahedron Lett.* **2002**, *43*, 2463−2466.
(5) Armstrong, D. W. In *Chiral Separations by Liquid Chromatography*; Ahuja, S. Ed.; American Chemical Society: Washington, DC, 1991.
(6) Blaschke, G.; Chankvetadze, B. *J. Chromatogr., A* **2000**, *875*, 3−25.
(7) Koide, T.; Ueno, K. *J. Chromatogr., A* **2001**, *909*, 305−315.
(8) Schurig, V.; Wistuba, D. *Electophoresis* **1999**, *20*, 2313−2328.
(9) Li, S.; Lloyd, D. K. *Anal. Chem.* **1993**, *65*, 3684−3690.
(10) Armstrong, D. W.; Chang, L. W.; Chang, S. S. C. *J. Chromatogr., A* **1998**, *793*, 115−134.
(11) Huang, W. X.; Xu, H.; Fazio, S. D.; Vivilecchia, R. V. *J. Chromatogr., B* **1997**, *695*, 157−162.
(12) Kuhn, R.; Wagner, J.; Walbroehl, Y.; Bereuter, T. *Electrophoresis* **1994**, *15*, 828−834.
(13) Park, J. W.; Lee, S. Y.; Park, K. K. *Chem. Lett.* **2000**, *6*, 594−595.
(14) Willner, I.; Goren, Z. *J. Chem. Soc., Chem. Commun.* **1983**, 1469−1470.
(15) Suzuki, I.; Obata, K.; Anzai, J.; Ikeda, H.; Ueno, A. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1705−1710.
(16) Zeng, Z. R.; Liu, M. *Chromatographia* **1998**, *48*, 817−822.
(17) Carter-Finch, A. S.; Smith, N. W. *J. Chromatogr., A* **1999**, *848*, 375−385.

10.1021/ac0204909 CCC: $25.00
Published on Web 02/08/2003

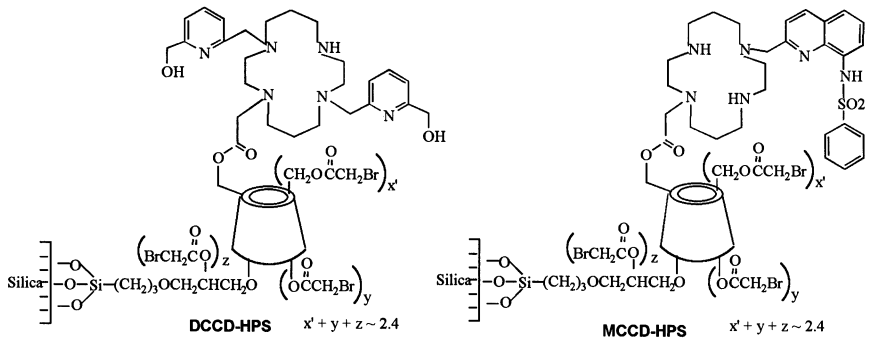

Figure 1. Structures of the bonded silica particles.

effects of partitioning and electrophoresis. Like other CE techniques, CEC provides a flat flow profile of the mobile phase and provides the possibility of using small size particles as stationary phase. This greatly increases efficiencies of separations.[8,17] Therefore, application of new CSPs with high enantioselectivity in CEC has good potential for high-resolution enantiomeric separations.

In this paper, we report the application of two novel types of cyclam-capped β-CD-bonded silica particles, mono-(8-benzenesulfonamidoquinoline-2-ylmethyl)-substituted cyclam and 1,8-di-(2-hydroxymethylpridine-6-ylmethyl)-substituted cyclam-capped (3-(2-*O*-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica (MCCD-HPS and DCCD-HPS), as CSPs in CEC for separation of a wide range of chiral compounds. Under the running buffers, methanol/Tris−HCl and acetonitrile/Tris−HCl with or without 2 mM $Ni(ClO_4)_2$, baseline enantioseparations for many solutes were achieved. A comparison of enantioseparations between the columns packed with crown ether-capped β-CD-bonded silica particles and cyclam-capped β-CD-bonded silica particles is studied.

EXPERIMENTAL SECTION

Materials. β-CD was purchased from Sigma (St. Louis, MO) and was dried in a 0.1-mmHg vacuum at 120°C for 12 h. Bare silica gel particles (1.5 μm, 100 Å) were obtained from Alltech Exsil (Deerfield, IL). 3-Glycidoxypropyltrimethoxysilane was obtained from Merck (Schuchardt, Hobenbrum, Germany) and redistilled under reduced pressure before use. Water was processed with a Barnstead (Dubuque, IA) NANOpure ultrapure water system. HPLC-grade acetonitrile, dichloromethane, triethylamine, methanol, and 2-propanol were all purchased from Fisher Scientific (Fair Lawn, NJ). Analytical-grade sodium hydride (60%) and sodium hydroxide were all purchased from Fluka (Buchs, Switzerland). Analytical-grade calcium hydride was purchased from Spectrum (New Brunswick, NJ). Phosphoric acid (85%) was obtained from Carlo Erba (Milan, Italy). Hydrochloric acid, Tris (base) and $Ni(ClO_4)_2$ were purchased from J. T. Baker (Phillipsburg, NJ). Bromoacetyl bromide was obtained from Aldrich (Milwaukee, WI). Racemic drugs were obtained from Sigma and Merck. Mono-(8-benzenesulfonamidoquinoline-2-ylmethyl)-substituted cyclam and 1,8-di-(2-hydroxymethylpridine-6-ylmethyl)-substituted cyclam ligands were kind gifts from Professor J. S. Bradshaw; their synthetic strategies have been reported.[4]

Apparatus. CEC was performed on an HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode-array UV detector, an autosampler, and ChemStation software. The CE system allows application of a pressure up to 10 bar at the inlet or outlet vial. A home-built ultrahigh-pressure packing system[18] was used for packing the capillary columns. A model DSF-150-C1 air-driven supercritical fluid (SCF) pump (Haskel, USA) was used for packing capillary columns.[18] A Shimadzu (Tokyo, Japan) LC-9A HPLC pump was used for flushing the capillary column. Buffer pH was determined using a Metrohm (Herisau, Switzerland) 692 pH meter.

Preparation of Bonded Stationary Phases. The preparation of the cyclam-capped β-CD-bonded silica particles was previously described in detail.[4] Briefly, β-CD was anchored onto silica particles at its C(2) position, derivatized primarily at the more reactive and less sterically hindered[19,20] C(6) position by treatment with 7 equiv of bromoacetyl bromide to form bromoacetate-substituted-(3-(2-*O*-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica particles (BACD-HPS). Finally, BACD-HPS was reacted with excess mono-(8-benzenesulfonamidoquinoline-2-ylmethyl)-substituted cyclam and 1,8-di-(2-hydroxymethylpridine-6-ylmethyl)-substituted cyclam to form cyclam-capped β-CD-bonded silica particles MCCD-HPS and DCCD-HPS. The amount of anchored β-CD and substituted bromoacetate moieties in BACD-HPS were 161 and 544 μmol g^{-1}, respectively, as determined by elemental analysis. The degree of substitution of bromoacetate was calculated to be 3.4. The amount of cyclam moieties in the bonded silica was 168 μmol g^{-1} in MCCD-HPS and 157 μmol g^{-1} in DCCD-HPS. The structure of the bonded silica particles is shown in Figure 1.

Chromatographic Procedure. The bonded silica particles were packed into fused-silica tubing to fabricate 30-cm (effective length, 38.5 cm total length) × 75-μm-i.d. columns by using a supercritical fluid packing method that was developed by Lee and co-workers.[18] The internal column frits and on-column UV detection window were fabricated using a resistive heating device (InnovaTech, UK). The freshly packed column was flushed with the running buffer for 3 h before being installed in the CE instrument. It was stabilized with pressure on both vials by gradually increasing the applied voltage to 25 kV. The column temperature was set at 20 °C. All runs were performed with a

(18) Lippert, J. A.; Xin, B.; Wu, N.; Lee, M. L. *J. Microcolumn Sep.* **1999**, 11, 631−643.
(19) Pikramenou, Z.; Nocera, D. G. *Inorg. Chem.* **1992**, *31*, 532−536.
(20) Easton, C. J.; Lincoln, S. F. *Modified Cyclodextrins: Scaffolds and Templates for Supramolecular Chemistry*; Imperial College Press: London, 1999; Chapter 2.

Table 1. Comparison of Enantioseparations under Two Different Organic Modifiers in Running Buffer

solutes[a]	running buffer[b]	voltage, (kV)	V_{EOF}, (mm s^{-1})	separation data[c]			
				T_{R1}(min)	k_1'	α	R_S
propranolol	ACN/Tris−HCl (70:30 v/v)	10	0.42	15.23	0.28	1.38	1.82
	MeOH/Tris−HCl (70:30 v/v)	10	0.30	21.76	0.32	2.59	4.01
tolperisone	ACN/Tris−HCl (70:30 v/v)	15	0.67	10.15	0.36	1.13	1.17
	MeOH/Tris−HCl (70:30 v/v)	15	0.37	19.17	0.42	1.36	1.75

[a] Conditions: 30-cm (effective length, 38.5 cm total length) × 75-μm i.d. fused-silica capillary column packed with 1.5-μm MCCD-HPS particles; 10 mM Tris−HCl (pH = 8.6). [b] ACN = acetonitrile; MeOH = methanol. [c] k_1' is the retention factor for the enantiomer eluted out first. α is the selectivity factor; $\alpha = k_2'/k_1'$. R_S is the resolution. T_{R1} is the retention time for the enaniomer eluted out first.

pressure of 10 bar on both inlet and outlet vials to prevent bubble formation in the column.

The mobile phases used were mixtures of methanol/10 mM Tris−HCl (pH = 8.6) buffer, acetonitrile/10 mM Tris−HCl (pH = 8.6) buffer or acetonitrile/10 mM Tris−HCl buffer (pH = 8.6) containing 2 mM $Ni(ClO_4)_2$ by volume ratios. The Tris−HCl buffer was prepared by dissolving the desired amount of Tris (base) in water to achieve a 10 mM concentration and then adding concentrated hydrochloric acid to achieve the required pH. The Tris−HCl buffer containing 2 mM $Ni(ClO_4)_2$ was prepared by dissolving the desired amount of Tris (base) and $Ni(ClO_4)_2$ in water to achieve a 10 mM Tris and 2 mM $Ni(ClO_4)_2$ concentration and then adding concentrated hydrochloric acid to achieve the required pH. All samples were dissolved in acetonitrile/Tris−HCl buffer mixtures having an acetonitrile content ∼5−15% higher than the running buffer. The sample concentration was ∼1−5 mg/mL. The slight mismatch in the injection sample solution−running buffer composition causes a baseline perturbation that was used as the marker of the electroosmotic flow.[21] All running buffers and sample solutions were degassed with helium for 15 min and then sonicated for 10 min before use. Samples were injected electrokinetically by applying a voltage of 6 kV for ∼3−5 s. All solutions and solvents were filtered through 0.22-μm Millex-GV tips (Millipore, Bedford, MA). Supporting evidence for chiral separation was accomplished by comparing UV spectra for enantiomers from 200 to 254 nm.

RESULTS AND DISCUSSION

Enantioseparations with Acetonitrile/Tris−HCl as Running Buffer. Tris−HCl buffer is a low-ionic-strength buffer without metal ions and has a low dielectric constant. Consequently, Joule heating is low when this running buffer is used in CEC. Thiourea, a traditional EOF marker, was strongly retained on the DCCD-HPS and MCCD-HPS-packed columns and therefore cannot be used as marker. As was suggested by Lelièvre et al.,[21] we used baseline perturbations as the marker of EOF. A Van Deemter plot for a fused-silica column packed with 1.5-μm porous bonded-silica MCCD-HPS is shown in Figure 2. Using α-methylbenzylamine as solute and acetoniltrile/10 mM Tris−HCl (70:30 v/v) as running buffer, an optimized plate height (2.19 μm, N = 456,621 plates m^{-1}) was obtained under the applied voltage of 10 kV at a linear velocity of EOF of 0.42 mm s^{-1}. This velocity was used as the optimum linear velocity.

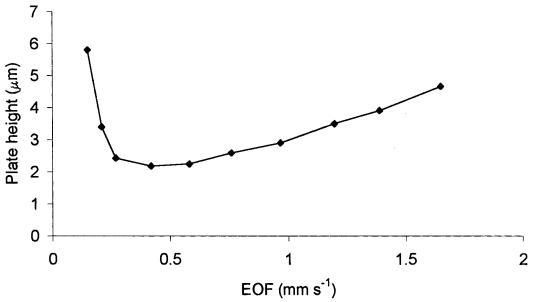

Figure 2. van Deemter plot for α-methylbenzylamine for the enantiomer eluted out first. Conditions: 30-cm (effective length, 38.5 cm total length) × 75-μm i.d. fused-silica capillary column packed with 1.5-μm MCCD-HPS particles; acetonitrile/Tris−HCl buffer (10 mM, pH = 8.6) (70:30 v/v); applied voltages vary from 2.5 kV to 25 kV in steps of 2.5 kV.

The influence of different organic modifiers in the running buffer on the enantioseparation has been studied. Table 1 lists the linear velocity of EOF (V_{EOF}), the retention and enantioseparation data of two chiral compounds using methanol/Tris−HCl and acetonitrile/Tris−HCl as running buffers. Better enantioseparation, however, with lower V_{EOF} and longer retention times was obtained under the methanol/Tris−HCl running buffer than under the acetonitrile/Tris−HCl running buffer at the same composition of Tris−HCl buffer when other chromatographic conditions were invariant. The better enantioseparation may be the result of the increased selectivity in the methanol/Tris−HCl running buffer, the longer retention under this running buffer condition that allows increased interaction of the analytes with the stationary phase, or both. Since the enantioselectivity in methanol/Tris−HCl is higher than that in the acetonitrile/Tris−HCl running buffer (Table 1), it appears that it is selectivity in the former running buffer that is the dominating mechanism in enhancing separation. We also found that acetonitrile afforded a more stable electroosmotic flow than methanol or 2-propanol on the columns packed with cyclam-capped β-CD-bonded CSPs.

Table 2 lists the retention and enantioseparation data of some chiral compounds under varying compositions of acetonitrile/Tris−HCl running buffer. Increasing the proportion of buffer produces higher retention and better enantiomeric selectivity. This observation implies that the solutes exhibit reversed-phase type behavior on MCCD-HPS. The main chiral recognition mechanism appears to be the result of the formation of an inclusion complex in which the hydrophobic portion of the solute is included in the capped β-CD cavity, and the cyclam moiety provides further host−guest interaction, H−H interaction, or dipolar interaction with the

(21) Lelièvre, F.; Yan, C.; Zare, R. N.; Gareil, P. *J. Chromatogr., A* **1996**, *723*, 145−156.

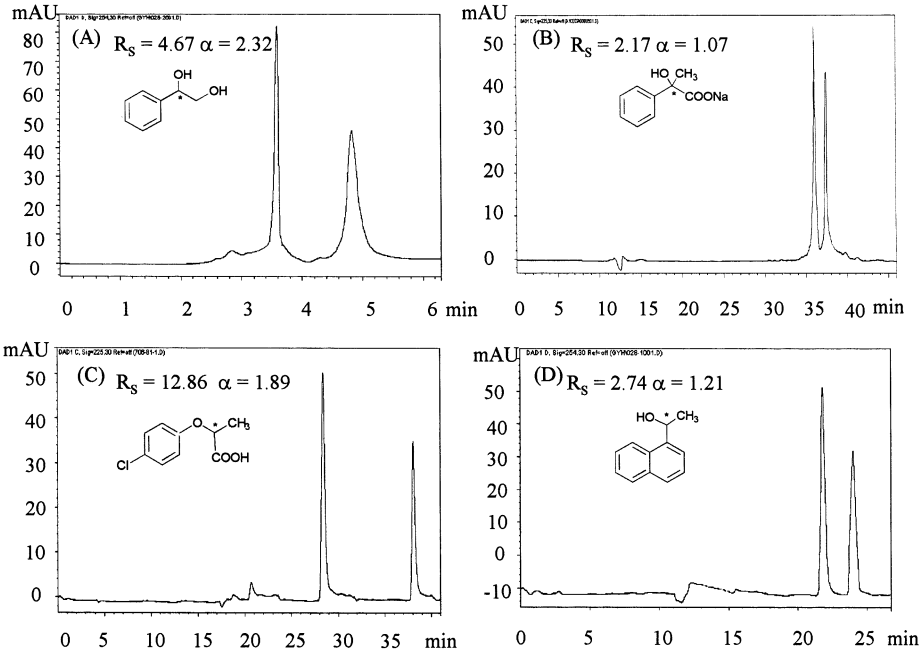

Figure 3. Typical chromatograms for enantiomeric separations using acetonitrile/Tris−HCl as running buffer. Conditions: 30-cm (effective length, 38.5 cm total length) × 75-μm i.d. fused-silica capillary column packed with bonded silica particles; (A) MCCD-HPS-packed column, 1-phenyl-1,2-ethanediol, Tris−HCl (10 mM, pH = 8.6)/acetonitrile (50:50 v/v), 20 kV, 254-nm UV detection; (B) MCCD-HPS-packed column, methyl mandelate, Tris−HCl (10 mM, pH = 8.6)/acetonitrile (30:70 v/v), 10 kV, 225-nm UV detection; (C) DCCD-HPS-packed column, 2-(4-chlorophenoxy)-propionic acid, Tris−HCl (10 mM, pH = 8.6)/acetonitrile (50:50 v/v), 10 kV, 225-nm UV detection; (D) DCCD-HPS-packed column, α-methyl-1-naphthalene-methanol, Tris−HCl (10 mM, pH = 8.6)/acetonitrile (30:70 v/v), 10 kV, 254-nm UV detection.

Table 2. Influence of Acetonitrile Content in Running Buffer on Enantioseparations

solutes	separation factors	acetonitrile/Tris−HCl buffer (v/v)[a] 90:10	70:30	50:50	30:70
ketoprofen	k_1'	0.36	0.43	0.67	1.26
	α	1.09	1.14	1.43	1.81
	R_S	1.22	1.61	3.52	5.83
phenylalanine	k_1'	0.45	0.57	1.25	1.32
	α	1.24	1.32	2.34	2.57
	R_S	1.17	2.83	3.85	4.68
mandelic acid benzyl ester	k_1'	0.23	0.28	0.53	0.91
	α	1.14	1.38	1.42	1.53
	R_S	1.11	1.82	2.14	3.03

[a] 10 kV applied voltage; other terms and separation conditions as in Table 1.

solute. The sidearm of cyclam also supplies an extra ligand site for the solute.

Typical chromatograms of enantiomeric separations on MCCD-HPS and DCCD-HPS-packed columns under Tris−HCl running buffer conditions are shown in Figure 3. The two CSPs are synthesized from small (1.5-μm) silica particles, and they have a chiral selector with three recognition sites, β-CD, cyclam, and its sidearm, and they exhibit excellent enantioselectivities and column efficiencies for separating chiral compounds. Accordingly, they show good potential for fast chiral separations with high resolutions under high voltages. As shown in Figure 3A, fast separation of enantiomers of 1-phenyl-1,2-ethanediol was achieved within 5 min with high resolution (R_S = 4.67) and high selectivity (α = 2.32). Table 3 lists typical enantioseparation data on the MCCD-HPS- and DCCD-HPS-packed columns under several running buffer conditions. For most of the enantioseparations, the resolution values (R_S) are >1.5 and the selectivity factors (α) are >1.3. Compared to other reported CE and LC techniques,[7,22−24] better enantioselectivity and resolution for most of the chiral solutes are obtained on the MCCD-HPS- and DCCD-HPS-packed columns in CEC. For example, the enantiomeric resolution for the enantiomers of indapamide was higher in CEC using MCCD-HPS as chiral stationary phase (R_S = 2.87) than in capillary zone electrophoresis (CZE) with β-CD as chiral additive (R_S = 1.50);[22] the enantioselectivities and resolution values for enantiomers of propranolol (α = 2.59, R_S = 4.01) and warfarin (α = 2.13, R_S = 5.27) were higher in CEC using DCCD-HPS as chiral stationary phase than in nano-HPLC with cellulose-coated silica as the stationary phase (propranolol, α = 1.38, R_S = 1.64; warfarin, α = 1.60, R_S = 1.91);[23] the enantioselectivities and resolution values for enantiomers of metoprolol (α = 2.32, R_S = 7.75) and 2-(4-chlorophenoxy)-propionic acid (α = 1.69, R_S = 7.85) were higher in CEC using MCCD-HPS as chiral stationary phase than in HPLC with β-CD-bonded silica particles as stationary phase (metoprolol, α = 1.21, R_S = 3.2; 2-(4-chlorophenoxy)-propionic acid, α = 1.27, R_S = 2.6);[24] only two stereoisomers of 2-amino-1,2-diphenylethanol, which has two chiral centers, was obtained in CEC with chiral crown ether-bonded polyacrylamide gels as CSP,[7] whereas baseline separation of the four stereoisomers was easily achieved on a DCCD-HPS-packed column in CEC. The bromoacetate-substituted-(3-(2-*O*-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica

(22) Wu, W. H.; Stalcup, A. M. *J. Liq. Chromatogr.* **1995**, *18*, 1289−1315.
(23) Krause, K.; Girod, M.; Chankvetadze, B.; Blaschke, G. *J. Chromatogr., A* **1999**, *837*, 51−63.
(24) Chang, S. C.; Reid, G. L.; Chen, S.; Chang, C. D.; Armstrong, D. W. *Trends Anal. Chem.* **1993**, *12*, 144−153.

Table 3. Typical Enantioseparation Data of Chiral Compounds Studied on Columns Packed with MCCD-HPS and DCCD-HPS Particles

solutes	running buffer[a]	column	voltage (kV)	separation data[b]		
				k_1'	α	R_S
ketoprofen	ACN/Tris−HCl (90:10)	MCCD-HPS	10	0.36	1.09	1.22
indapamide	ACN/Tris−HCl (70:30)	MCCD-HPS	10	0.30	1.88	2.87
4-methyl-4-phenylhydantoin	ACN/Tris−HCl (70:30)	MCCD-HPS	10	0.27	1.47	2.21
isoproterenol	MeOH/Tris−HCl (70:30)	MCCD-HPS	10	0.28	2.33	4.33
tolperisone	MeOH/Tris−HCl (70:30)	MCCD-HPS	15	0.42	1.36	1.75
methyl mandelate	ACN/Tris−HCl (70:30)	MCCD-HPS	10	1.88	1.07	2.17
α-methylbenzylamine	ACN/Tris−HCl (70:30)	MCCD-HPS	10	0.79	1.11	1.27
proglumide	ACN/Tris−HCl (70:30)	MCCD-HPS	10	1.60	1.08	1.92
oxprenolol	ACN/Tris−HCl (70:30)	MCCD-HPS	10	0.32	2.43	6.01
pindolol	ACN/Tris−HCl (50:50)	MCCD-HPS	10	0.34	1.37	3.78
1-phenyl-2-propanol	ACN/Tris−HCl (50:50)	MCCD-HPS	10	0.65	1.25	2.19
metoprolol	ACN/Tris−HCl (50:50)	MCCD-HPS	10	0.54	2.32	7.75
indoprofen	ACN/Tris−HCl (50:50)	MCCD-HPS	10	0.75	1.27	5.33
1-phenyl-1,2-ethanediol	ACN/Tris−HCl (50:50)	MCCD-HPS	20	0.26	2.32	4.67
bromopheniramine	ACN/Tris−HCl (30:70)	MCCD-HPS	10	1.21	2.12	7.86
mandelic acid benzyl ester	ACN/Tris−HCl−$Ni(ClO_4)_2$ (70:30)	MCCD-HPS	20	0.55	1.74	3.04
phenylalanine	ACN/Tris−HCl−$Ni(ClO_4)_2$ (50:50)	MCCD-HPS	10	1.43	2.83	9.67
2-(4-chlorophenoxy)-propionic acid	ACN/Tris−HCl−$Ni(ClO_4)_2$ (30:70)	MCCD-HPS	15	0.89	1.69	7.85
α-methyl-1-naphthalene-methanol	ACN/Tris−HCl (70:30)	DCCD-HPS	10	0.92	1.21	2.74
propranolol	MeOH/Tris−HCl (70:30)	DCCD-HPS	15	0.32	2.59	4.01
warfarin	ACN/Tris−HCl (70:30)	DCCD-HPS	15	0.43	2.13	5.27
2-(4-chlorophenoxy)-propionic acid	ACN/Tris−HCl (50:50)	DCCD-HPS	10	0.62	1.89	12.86
2-hydroxyl-α-phenethyl alcohol	ACN/Tris−HCl−$Ni(ClO_4)_2$ (30:70)	DCCD-HPS	15	1.11	1.70	5.11
4-chloro-α-phenethyl alcohol	ACN/Tris−HCl−$Ni(ClO_4)_2$ (30:70)	DCCD-HPS	15	0.86	1.36	1.81
4-methyl-α-phenethyl alcohol	ACN/Tris−HCl−$Ni(ClO_4)_2$ (30:70)	DCCD-HPS	15	0.75	1.07	0.93
2-phenylcyclohexanone	ACN/Tris−HCl−$Ni(ClO_4)_2$ (50:50)	DCCD-HPS	10	0.30	1.83	1.76
labetalol	ACN/Tris−HCl−$Ni(ClO_4)_2$ (70:30)	MCCD-HPS	15	0.39	$\alpha_{1,2} = 1.35$ $\alpha_{2,3} = 1.30$ $\alpha_{3,4} = 1.46$	$R_{S1,2} = 3.93$ $R_{S2,3} = 2.13$ $R_{S3,4} = 1.46$
2-amino-1,2-diphenylethanol	ACN/Tris−HCl−$Ni(ClO_4)_2$ (70:30)	DCCD-HPS	20	0.22	$\alpha_{1,2} = 1.27$ $\alpha_{2,3} = 1.29$ $\alpha_{3,4} = 1.22$	$R_{S1,2} = 1.84$ $R_{S2,3} = 1.92$ $R_{S3,4} = 1.21$

[a] ACN = acetonitrile; MeOH = methanol; buffer, 10 mM Tris−HCl with/without 2 mM $Ni(ClO_4)_2$ (pH = 8.6). [b] Terms and other separation conditions as in Table 1.

particles (BACD-HPS), the intermediate product for the synthesis of crown ether and cyclam-capped CD-bonded silica particles, have been previously packed into fused-silica tubing to fabricate 75-μm-i.d. × 30-cm effective length columns (38.5 cm total length, same as DCCD-HPS- and MCCD-HPS-packed columns) and used in CEC for comparative studies.[25] Under the same separation conditions (ACN/Tris−HCl (50:50), 10 kV), for the enantiomers of 1-phenyl-2-propanol, only partial enantioseparation ($\alpha = 1.08$, $R_S = 0.63$) on the BACD-HPS-packed column was achieved;[25] however, baseline separation ($\alpha = 1.25$, $R_S = 2.19$) was achieved on the MCCD-HPS-packed column. Greater retention was obtained on the MCCD-HPS-packed column ($k_1' = 0.65$, $V_{EOF} = 0.27$ mm s^{-1}) than on the BACD-HPS-packed column ($k_1' = 0.47$, $V_{EOF} = 0.28$ mm s^{-1}). The cooperative function of the capped β-CD, cyclam, and its sidearm is important for retention and enantioseparation on the cyclam-capped β-CD-bonded stationary phases.

Enantiomeric Separations on Cyclam-Capped β-CD-Bonded Silica-Packed Columns with Acetonitrile/Tris−HCl−$Ni(ClO_4)_2$ as Running Buffer. It is generally accepted that the origin of an electroosmotic flow in a packed CEC capillary column is the result of the negatively charged silica surface when the silanol groups on the inside wall surface of the capillary column and on the surface of the packed silica particles are deprotonated in the buffer.[26,27] When the running buffer contains $Ni(ClO_4)_2$, the cyclam selectors of MCCD-HPS and DCCD-HPS include Ni^{2+} from the buffer to form inclusion complexes that are positively charged. As a result, the net negative charge on MCCD-HPS and DCCD-HPS surface decreases, and the velocity of EOF also decreases. Table 4 shows that the V_{EOF} in the column packed with MCCD-HPS under the acetonitrile/Tris−HCl buffer containing 2 mM $Ni(ClO_4)_2$ is much lower than that under the acetonitrile/Tris−HCl buffer at the same acetonitrile composition.

After inclusion of Ni^{2+} from the running buffer into the substituted cyclam and the sidearm ligands, the cylcam-capped β-CD selector becomes positively charged. The positively charged cyclam-capped β-CD can undergo extra electrostatic interactions with ionizable solutes and enhance the dipolar interactions with some polar neutral solutes.[3,4] This can strengthen the host−guest interaction with some solutes and improve chiral recognition and selectivity. Therefore, cyclam-capped β-CD-bonded stationary phases exhibit higher chiral selectivities under the acetonitrile/Tris−HCl−$Ni(ClO_4)_2$ buffer than under the acetonitrile/Tris−HCl buffer. Increasing the concentration of Ni^{2+} from 1 mM to 5 mM results in only a slight increase in the retention and selectivity.

(25) Gong, Y.; Lee, H. K. *Helv. Chim. Acta* **2002**, *85*, 3283−3293.

(26) Stevens, T. S.; Cortes, H. J. *Anal. Chem.* **1983**, *55*, 1365−1370.

(27) Luis, A. C.; Kimberly, J. R.; Rafael, A. M.; Adam, M. F. *Electrophoresis* **1997**, *18*, 2162−2174.

Table 4. Comparison of Enantioseparations under Two Running Buffer Conditions

solutes	running buffer[a]	V_{EOF} (mm s^{-1})	separation data[b] k_1'	α	R_S
mandelic acid benzyl ester	acetonitrile/Tris−HCl−$Ni(ClO_4)_2$ (70:30)	0.31	0.54	1.74	3.05
	acetonitrile/Tris−HCl (70:30)	0.42	0.28	1.38	1.82
phenylalanine	acetonitrile/Tris−HCl−$Ni(ClO_4)_2$ (50:50)	0.27	1.43	2.83	9.67
	acetonitrile/Tris−HCl (50:50)	0.34	1.25	2.34	3.85

[a] 10 kV applied voltage; buffer, 10 mM Tris−HCl with/without 2 mM $Ni(ClO_4)_2$ (pH = 8.6). [b] Terms and other separation conditions as in Table 1.

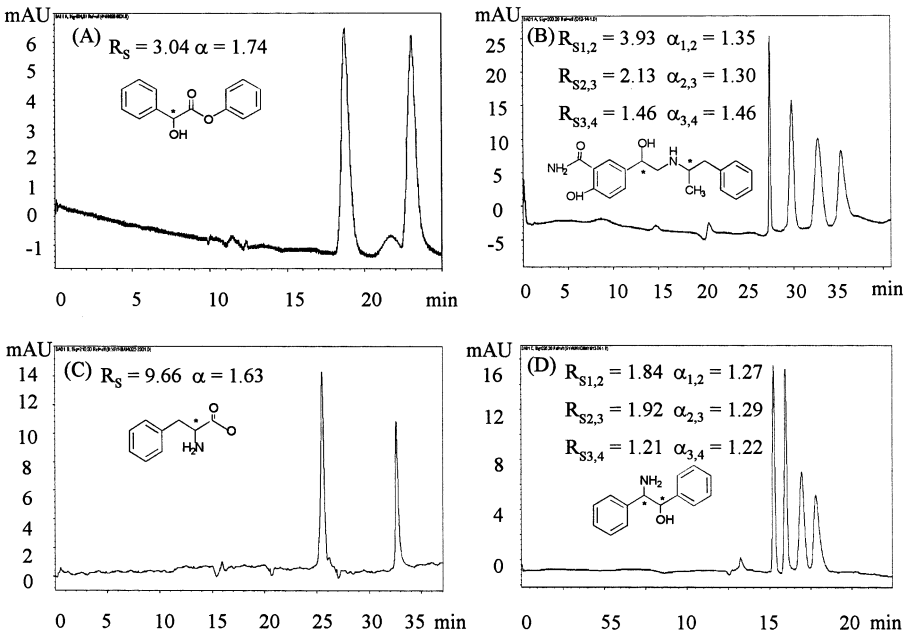

Figure 4. Typical chromatograms for enantiomeric separations using Tris−HCl (10 mM, pH = 8.6) containing 2 mM $Ni(ClO_4)_2$/acetonitrile as running buffer. Conditions: 30-cm (effective length, 38.5 cm total length) × 75-μm i.d. fused-silica capillary column packed with bonded silica particles; (A) MCCD-HPS-packed column, mandelic acid benzyl ester, 10 mM Tris buffer (pH = 8.6) containing 2 mM $Ni(ClO_4)_2$/acetonitrile (30:70 v/v), 20 kV, 200-nm UV detection; (B) MCCD-HPS-packed column, labetalol, 10 mM Tris buffer (pH = 8.6) containing 2 mM $Ni(ClO_4)_2$/acetonitrile (30:70 v/v), 15 kV, 254-nm UV detection; (C) DCCD-HPS-packed column, phenylalanine, 10 mM Tris buffer (pH = 8.6) containing 2 mM $Ni(ClO_4)_2$/acetonitrile (30:70 v/v), 15 kV, 210-nm UV detection; (D) DCCD-HPS-packed column, 2-amino-1,2-diphenylethanol, 10 mM Tris buffer (pH 8.6) containing 2 mM $Ni(ClO_4)_2$/acetonitrile (30:70 v/v), 20 kV, 210-nm UV detection.

To reduce the background absorption and obtain satisfactory enantioselectivity for most of the studied samples, 2 mM $Ni(ClO_4)_2$ was chosen as an additive in the running buffer. As shown in Table 4, the retention factor (k_1') and enantioselectivity (α) are higher under the acetonitrile/Tris−HCl−$Ni(ClO_4)_2$ buffer with all other conditions invariant for the enantiomers of mandelic acid benzyl ester and phenylalanine. Typical chromatograms of enantiomeric separations under the acetonitrile/Tris−HCl−$Ni(ClO_4)_2$ running buffer are shown in Figure 4.

Comparison of Enantioseparations between the Columns Packed with Cyclam-Capped *β*-CD-Bonded Phases and Crown Ether-Capped *β*-CD-Bonded Phases. All of the crown ether-capped *β*-CD-bonded silica particles[3] and cyclam-capped *β*-CD-bonded silica particles were separately packed into fused-silica tubing to fabricate 75-μm-i.d. × 30-cm effective length columns (38.5-cm total length) for the comparative studies. As discussed above, after inclusion of the metal ion (Ni^{2+}) from the running buffer into the cyclam unit, particles MCCD-HPS and DCCD-HPS are positively charged. Since the sidearms in the cyclams can also include Ni^{2+} ions that have higher positive charges than Na^+ or K^+ ions and the positively charged cyclam center is nearer to the *β*-CD, as compared to the 4′-aminobenzo-18-crown-6 and 4′-aminobenzo-15-crown-5-capped *β*-CD-bonded particles,[3,4] MCCD-HPS and DCCD-HPS have stronger electrostatic and dipolar interactions with solutes and, accordingly, show better selectivies than the crown ether-capped *β*-CD-bonded phases. It was found that for labetalol and 2-amino-1,2-diphenylethanol, which have two chiral centers, only partial separation of the four stereoisomers can be achieved on the 4′-aminobenzo-18-crown-6- and 4′-aminobenzo-15-crown-5-capped *β*-CD-bonded silica particles AB15C5−CD-HPS and AB18C6−CD-HPS. However, baseline separation of the four stereoisomers is easily achieved on bonded phases MCCD-HPS and DCCD-HPS (shown in Figure 4). The sidearms of the cyclams can provide additional ligand sites to interact with solutes. This also increases chiral recognition. For the enantiomers of 2-phenylcyclohexanone, no enantioseparation was obtained on the column packed with crown ether-capped *β*-CD-bonded silica particles. However, baseline enantioseparation (Table 3) was obtained on the columns packed with the cyclam-capped *β*-CD-bonded silica particles.

CONCLUSIONS

Cyclam-capped β-CD-bonded silica particles MCCD-HPS and DCCD-HPS are novel types of bonded chiral stationary phases suitable for CEC. They exhibit excellent enantiomeric selectivties for a wide range of chiral compounds. The cooperative functioning of the β-CD, cyclam, and its sidearm is important for chiral recognition. The cyclam-capped β-CD-bonded stationary phases show better enantioselectivity than the crown ether-capped β-CD-bonded stationary phases. With the metal ion included from the running buffer, the positively charged cyclam-capped β-CD-bonded silica particles provide extra electrostatic interactions with ionizable solutes and enhances the dipolar interactions with some polar neutral solutes. This additionally improves the chiral recognition and selectivity for CEC for some solutes. The composition of acetonitrile in the running buffer can influence the retention and separation selectivity. The results demonstrate that cyclam-capped β-CD-bonded silica particles have great potential for fast chiral separations when used as chiral stationary phases in CEC as a result of their excellent enantioselectivities.

ACKNOWLEDGMENT

The authors thank the National University of Singapore for financial support of this work. Y.G. gratefully acknowledges an Ang Kok Peng Memorial Fund Scholarship award from the National University of Singapore that allowed a study leave at Brigham Young University. The authors thank Professor Milton L. Lee for advice and technical support on packing capillary columns, and Professor Jerald S. Bradshaw and Dr. Xue Guoping for their kind gifts of cyclam materials and for their technical assistance with the synthesis of the bonded silica particles.

Received for review July 29, 2002. Accepted December 19, 2002.

AC0204909