Enantiomeric Separations in Capillary Electrochromatography with Crown Ether-Capped β-Cyclodextrin-Bonded Silica Particles as Chiral Stationary Phase

by **Yinhan Gong** and **Hian Kee Lee***

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Republic of Singapore 117543 (tel: (65)-6874-2995; fax: (65)-6779-1691; e-mail: chmleehk@nus.edu.sg)

Dedicated to Professor *Dieter Seebach* on the occasion of his 65th birthday

Two novel types of crown ether capped β-cyclodextrin (β-CD) bonded silica, namely, 4′-aminobenzo-*X*-crown-*Y* (*X* = 15, 18 and *Y* = 5, 6, resp.) capped [3-(2-O-β-cyclodextrin)-2-hydroxypropoxy] propylsilyl-appended silica, have been prepared and used as stationary phases in capillary electrochromatography (CEC) to separate chiral compounds. The two stationary phases have a chiral selector with two recognition sites: crown ether and β-CD. They exhibit excellent enantioselectivity in CEC for a wide range of compounds. After inclusion of metal ions (Na^+ or K^+) from the running buffer into the crown ether units, the stationary phases become positively charged and can provide extra electrostatic interaction with ionizable solutes and enhance the dipolar interaction with polar neutral solutes. This enhances the host-guest interaction with the solute and improves chiral recognition and enantioselectivity. Due to the cooperation of the anchored β-CD and the crown ether, this kind of crown ether capped β-CD bonded phase shows better enantioselectivity than either β-CD- or crown ether bonded phases only. These new types of stationary phases have good potential for fast chiral separation with CEC.

Introduction. – Separation of enantiomers is one of the most active areas of chromatography [1–3] and is important in various fields [4–6], *e.g.*, natural-product research, stereospecific synthesis, development of chiral drugs in the pharmaceutical industry, and in environmental studies. Chromatographic methods are typically employed for chiral separation, including high-performance liquid chromatography (HPLC) [7], gas chromatography (GC) [8], micellar electrokinetic capillary chromatography (MECC) [9] and, more recently, capillary electrochromatography (CEC) [10]. To improve chiral separation, two approaches can be used: *a*) optimization of chromatographic conditions, and *b*) use of new chiral stationary phases (CSPs) or chiral additives in the mobile phase.

GC and LC were the first tools to be employed in the separation of enantiomers [4]. However, conventional LC cannot utilize small particles and/or long columns to obtain high efficiencies because of the pressure limitations of normal pumping systems. Although GC usually offers higher efficiencies than LC due to the larger number of available theoretical plates, most chiral compounds of interest have low volatilities. This limits the use of GC for chiral separations [10]. During the last several years, capillary electrophoresis (CE) has become a powerful technique for chiral separations when chiral selectors are added to the running buffers [11]. CEC is a modern liquid chromatography technique combining the high efficiency of CE with the high selectivity usually obtained by HPLC [2]. The mobile phase is transported through a

capillary containing the stationary phase by means of electro-osmosis instead of pressure. Neutral solutes are separated by partitioning between the mobile and the stationary phase. Charged solutes have an additional electrophoretic mobility in the applied electric field, and the separation is achieved by the combined 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, which increases the efficiency of separation [2][12]. Therefore, using the new CSPs with high enantioselectivity in CEC has good potential for high-resolution enantiomeric separation.

Both cyclodextrins and crown ethers have been shown to be effective chiral selectors [9]. Many chiral separation techniques have been accomplished with cyclodextrin-type CSPs in HPLC [12] and CEC [13][14]. One drawback in using cyclodextrins is the low binding constant for most guest molecules [15]. It was reported that crown ether-capped β-CDs [15–17] exhibited high binding constants for several guest molecules due to the cooperation of the β-CD and the crown ether. Although crown ether capped β-CD has already been used to model the receptor sites of enzymes for a long time [16], its use as a stationary-phase selector for chromatography has seldom been studied [18]. It was reported that benzo-aza-15-crown-5 capped β-CD used as stationary phase in GC showed excellent enantioselectivity [18]. Recently, it was shown that combination of a crown ether and β-CD as CE additive sometimes produce better enantiomer separation than did either selector alone [9][19][20]. However, many crown ethers and CD derivatives with high UV/VIS absorption characteristics and/or poor solubility in H_2O are not suitable as CE additives for direct detection. Alternatively, they can be bonded onto silica support for use as CSPs in LC [21][22]. 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 crown ether capped β-CD bonded silica particles for use as new CSPs in LC [21].

In this paper, we report the application of two types of crown ether capped β-CD-bonded silica particles, 4′-aminobenzo-15-crown-5 and 4′-aminobenzo-18-crown-6-capped [3-(2-*O*-β-cyclodextrin)-2-hydroxypropoxy] propylsilyl-appended silica (AB15C5-CD-HPS and AB18C6-CD-HPS), as CSPs in CEC to separate a wide range of chiral compounds. Under the MeCN/phosphate buffer and MeCN/Tris · HCl running buffer conditions, baseline-enantiomeric separation for a wide range of solutes was achieved on the columns packed with these two CSPs. The enantiomeric separation of the columns packed with β-CD-bonded, crown ether bonded, and crown ether-capped β-CD-bonded silica particles has been compared.

Results and Discussion. – *Enantiomeric Separation with MeCN/Tris · HCl as Running Buffer*. The synthesis of crown ether capped β-CD-bonded 3-μm-porous silica particles AB15C5-CD-HPS and AB18C6-CD-HPS was described previously [21]. The structures are shown in *Fig. 1*. *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. MeCN was chosen as the organic modifier because it shows higher and more-stable electro-osmotic flow (EOF) than MeOH and i-PrOH on the columns packed with crown ether capped β-CD-bonded CSPs. Thiourea,

a traditional EOF marker, was strongly retained on the AB15C5-CD-HPS- and AB18C6-CD-HPS-packed columns and, therefore, cannot be used as marker. As was suggested by *Lelièvre et al.* [13], we used baseline perturbations as the EOF marker. A *Van Deemter* plot for a fused-silica column packed with AB15C5-CD-HPS is shown in *Fig. 2*. Using 1-(2-hydroxyphenyl)ethanol as solute and MeCN/*Tris* · HCl (10 mM) 70 : 30 (*v*/*v*) as running buffer, an optimized plate height (4.59 μm) for the enantiomer eluted first was obtained under the applied voltage of 10 kV at a linear EOF velocity of 0.45 mm s^{-1}, which was used as an optimum.

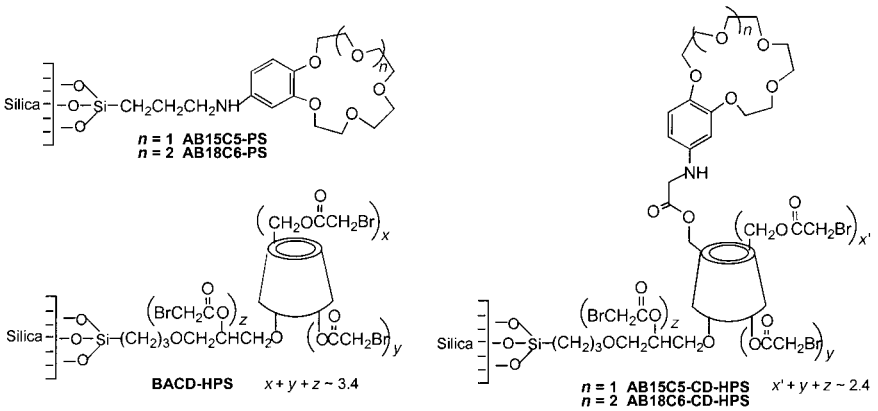

Fig. 1. *Structures of the silica-bonded particles*

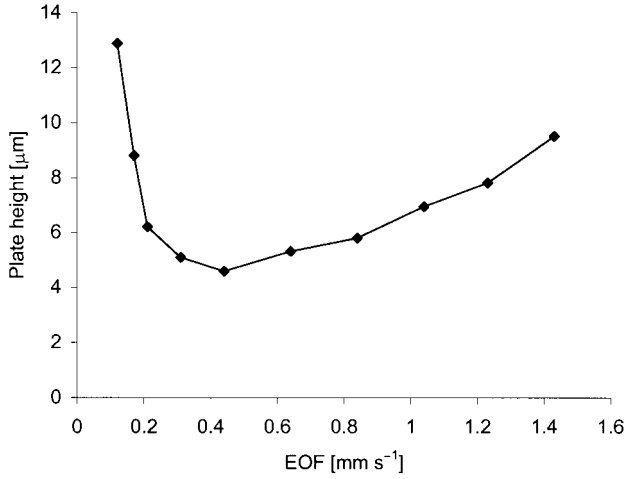

Fig. 2. Van Deemter *plot for 1-(2-hydroxyphenyl)ethanol for the enantiomer eluted first.* Conditions: 38.5 cm × 75 μm i.d. fused silica capillary column (30 cm to the detection window) packed with AB15C5-CD-HPS particles; MeCN/*Tris* · HCl buffer (10 mM, pH = 8.8) 70 : 30 (*v*/*v*); applied voltages vary from 2 to 20 kV in steps of 2 kV.

Table 1 lists the retention and separation data of 1-(2-hydroxyphenyl)ethanol under different compositions of the MeCN/Tris · HCl running buffer. With lower MeCN

content, higher selectivity was obtained, but at the expense of analysis time. The column efficiency generally decreases with decreasing MeCN content from 70 to 30%. The highest column efficiency (N_1) and the highest enantiomer-separation resolution ($R_S = 4.81$) are achieved at 70% MeCN within a short analysis time. The effect of the concentration of the *Tris* buffer on the separation has been studied. It was found that the most-rapid and most-efficient separations were obtained with a 10 mM *Tris* buffer. Therefore, MeCN/*Tris* · HCl (10 mM) 70 : 30 (*v*/*v*) was used as optimum running-buffer condition.

Table 1. *Influence of MeCN Content on the Retention and Separation of the Enantiomers of 1-(2-Hydroxylphenyl)ethanol*[a])

Running buffer	Retention and separation data[b])				
(MeCN/*Tris* · HCl)	t_{R1} [min]	k_1'	N_1 [plates m^{-1}]	α	R_S
30 : 70	33.37	0.47	94,417	1.76	1.93
50 : 50	13.69	0.39	135,237	1.71	3.67
70 : 30	10.56	0.35	187,969	1.64	4.81
90 : 10	9.77	0.28	162,994	1.61	3.89

[a]) Conditions: 38.5 cm × 75 μm i.d. fused-silica capillary (30 cm to the detection window) packed with AB15C5-CD-HPS particles; 12 kV applied voltage; 10 mM *Tris* · HCl (pH = 8.8). [b]) t_{R1} is the retention time for the enantiomer eluted first; k_1' is the retention factor for the enantiomer eluted out first; N_1 is the column efficiency for the enantiomer eluted out first; α is the selectivity factor ($= k_2'/k_1'$; R_S is the resolution).

Typical chromatograms of enantiomeric separations on AB15C5-CD-HPS and AB18C6-CD-HPS with MeCN/*Tris* · HCl as running buffer are shown in *Fig. 3*. Since both CSPs have a chiral selector with two recognition sites (crown ether and β-CD), they show excellent selectivity for separation of enantiomers. Accordingly, they show good potential for fast chiral separation under high voltages. As shown in *Fig. 3, b*, fast separation of enantiomers of 1-phenylpropan-1-ol was achieved within 6 min with high-resolution ($R_S = 2.67$) and high selectivity ($\alpha = 1.61$). Fast enantiomer separation of 1-(2-hydroxyphenyl)ethanol (*Table 2*) was also obtained within 6 min with high-resolution ($R_S = 2.03$) and high selectivity ($\alpha = 1.64$) under an applied voltage of 20 kV. *Table 2* lists typical enantiomeric-separation data on the AB15C5-CD-HPS- and AB18C6-CD-HPS-packed columns under several running-buffer conditions. For most of the enantiomer separations, the resolution values (R_S) are > 1.5 and the selectivity factors (α) are > 1.3. Compared to other reported CE and LC techniques [23–25], better enantioselectivities and resolution for most of the chiral solutes are obtained on the AB15C5-CD-HPS and AB18C6-CD-HPS packed columns in CEC. For example, the enantiomeric resolution for the enantiomers of indapamide was higher in CEC with AB15C5-CD-HPS as chiral stationary phase ($R_S = 2.76$) than in capillary zone electrophoresis (CZE) with β-CD as chiral additive ($R_S = 1.50$) [23]; the enantioselectivities and resolution values for the enantiomers of metoprolol ($\alpha = 1.59$, $R_S = 4.95$) and 2-(4-chlorophenoxy)propanoic acid ($\alpha = 1.67$, $R_S = 4.16$) were higher in CEC with AB18C6-CD-HPS as chiral stationary phase than in HPLC with β-CD-bonded silica particles as stationary phase (metoprolol: $\alpha = 1.21$, $R_S = 3.2$; 2-(4-chlorophenoxy)propanoic acid: $\alpha = 1.27$, $R_S = 2.6$) [24]; the enantioselectivities and resolution values for the enantiomers of pindolol ($\alpha = 1.98$, $R_S = 7.74$) and isoproterenol ($\alpha = 1.31$, $R_S = 2.53$)

were higher in CEC with AB15C5-CD-HPS as chiral stationary phase than in CEC with β-CD-bonded, negatively charged polyacrylamide gels as stationary phase (pindolol: $\alpha = 1.04$, $R_S = 0.93$; isoproterenol: $\alpha = 1.03$, $R_S = 0.68$) [25]. The cooperative functioning of the crown ether and the capped β-CD is important for the enantiomer separations on these crown ether capped β-CD-bonded stationary phases. The chiral discrimination depends on host-guest interaction, hydrophobic interaction, H-bonding, and dipolar interaction between the solute and the two selectors. Additionally, since there is a cap effect [17] exerted by the benzo-crown ether moiety in the two CSPs, and the spacers are connected at the wider torus rim of the β-CD, the two CSPs can provide a special stereochemical interation with the solute to enter the β-CD cavity through the wider torus rim to form a host-guest complex [21]. This enhances the chiral recognition.

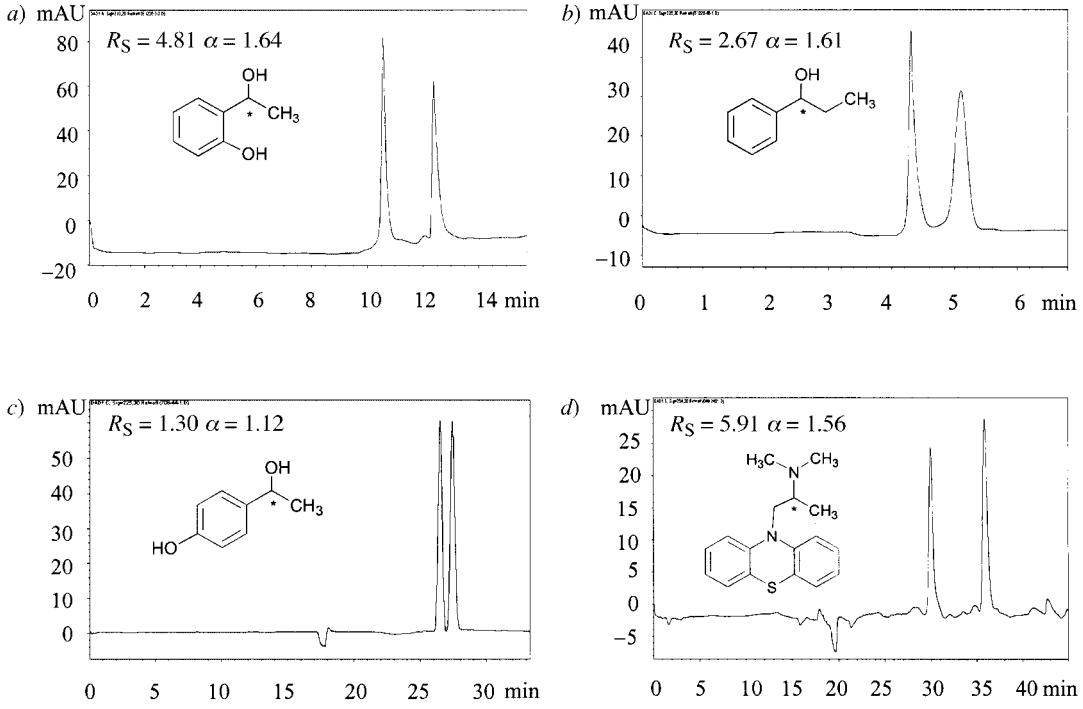

Fig. 3. *Typical chromatograms for enantiomeric separation with MeCN/Tris · HCl as running buffer.* Conditions: 75 μm i.d. × 30 cm effective length fused-silica capillary (38.5 cm total length) packed with bonded silica particles; *a*) AB15C5-CD-HPS-packed column, 1-(2-hydroxyphenyl)ethanol, *Tris* · HCl (10 mM, pH 8.8)/MeCN 30:70 (*v*/*v*), 12 kV, 215 nm UV detection; *b*) AB15C5-CD-HPS-packed column, 1-phenylpropan-1-ol, *Tris* · HCl (10 mM, pH 8.8)/MeCN 30:70 (*v*/*v*), 20 kV, 225 nm UV detection; *c*) AB18C6-CD-HPS-packed column, 1-(4-hydroxyphenyl)ethanol, *Tris* · HCl (10 mM, pH 8.8)/MeCN 50:50 (*v*/*v*), 12 kV, 225 nm UV detection; *d*) AB18C6-CD-HPS-packed column, promethazine, *Tris* · HCl (10 mM, pH 8.8)/MeCN 50:50 (*v*/*v*), 10 kV, UV 254 nm detection.

Enantiomer Separation with MeCN/Phosphate as Running Buffer. It is generally accepted that the origin of EOF in packed CEC columns is the negatively charged silica surface under the conditions where the silanol groups on the inside surface of the capillary column and on the surface of the packed silica particles are deprotonated in

Table 2. *Typical Enantiomer-Separation Data of Chiral Compounds Studied on Columns Packed with AB15C5-CD-HPS and AB18C6-CD-HPS Particles*

Solutes	Running buffer[a])	Column	Voltage [kV]	Separation data[b])		
				k_1'	α	R_S
1-(2-Hydroxyphenyl)ethanol	MeCN/Tris · HCl 70 : 30	AB15C5-CD-HPS	20	0.39	1.64	2.03
1-Phenylpropan-1-ol	MeCN/Tris · HCl 70 : 30	AB15C5-CD-HPS	20	0.30	1.61	2.67
1-(4-Methylphenyl)ethanol	MeCN/Tris · HCl 70 : 30	AB15C5-CD-HPS	10	0.41	1.28	1.17
2-(2,4,5-Trichlorophenoxy)propanoic acid	MeCN/Tris · HCl 70 : 30	AB15C5-CD-HPS	12	0.78	1.34	1.41
1-Phenylpropan-2-ol	MeCN/Tris · HCl 50 : 50	AB15C5-CD-HPS	12	0.75	1.34	2.39
1-(4-Hydroxyphenyl)ethanol	MeCN/Tris · HCl 50 : 50	AB15C5-CD-HPS	12	0.47	1.12	1.30
Oxprenolol	MeCN/phosphate 80 : 20	AB15C5-CD-HPS	10	0.66	1.11	0.89
Methyl 2-bromo-(2-phenyl)acetate	MeCN/phosphate 60 : 40	AB15C5-CD-HPS	12	1.65	1.14	1.22
Chloroquine	MeCN/phosphate 60 : 40	AB15C5-CD-HPS	10	0.70	1.25	1.85
2-(2,4-Dichlorophenoxy)propanoic acid	MeCN/phosphate 60 : 40	AB15C5-CD-HPS	10	1.16	1.61	2.48
Fenipentol	MeCN/phosphate 60 : 40	AB15C5-CD-HPS	12	1.08	1.67	2.11
Indapamide	MeCN/phosphate 40 : 60	AB15C5-CD-HPS	14	0.76	1.34	2.76
Isoproterenol	MeCN/phosphate 60 : 40	AB15C5-CD-HPS	12	0.63	1.31	2.53
Propranolol	MeCN/phosphate 60 : 40	AB15C5-CD-HPS	10	0.61	1.17	1.29
Nadolol	MeCN/phosphate 40 : 60	AB15C5-CD-HPS	16	1.25	1.23	1.14
Pindolol	MeCN/phosphate 40 : 60	AB15C5-CD-HPS	16	0.45	1.98	7.74
4-Phenylbut-3-en-1-ol	MeCN/phosphate 40 : 60	AB15C5-CD-HPS	14	0.65	1.57	5.26
Indoprofen	MeCN/Tris · HCl 50 : 50	AB18C6-CD-HPS	10	0.23	1.80	2.64
Ethyl-(1-naphthyl)amine	MeCN/Tris · HCl 50 : 50	AB18C6-CD-HPS	10	0.75	1.41	1.56
Nephril	MeCN/Tris · HCl 50 : 50	AB18C6-CD-HPS	10	0.86	1.06	0.93
Promethazine	MeCN/Tris · HCl 50 : 50	AB18C6-CD-HPS	10	0.54	1.56	5.91
Bromopheniramine	MeCN/phosphate 60 : 40	AB18C6-CD-HPS	12	0.92	1.54	2.42
2-(4-Chlorophenoxy)propanoic acid	MeCN/phosphate 60 : 40	AB18C6-CD-HPS	10	1.11	1.67	4.16
5-Methyl-5-phenylhydantoin	MeCN/phosphate 60 : 40	AB18C6-CD-HPS	12	1.24	2.44	9.58
Proglumide	MeCN/phosphate 40 : 60	AB18C6-CD-HPS	16	1.06	1.66	4.05
Metoprolol	MeCN/phosphate 40 : 60	AB18C6-CD-HPS	12	1.18	1.59	4.95
Labetalol	MeCN/phosphate 40 : 60	AB18C6-CD-HPS	16	1.16	$\alpha_{1,2} = 1.21$	$R_{S1,2} = 4.85$
					$\alpha_{2,3} = 1.09$	$R_{S2,3} = 1.43$
					$\alpha_{3,4} = 1.06$	$R_{S3,4} = 0.64$

[a]) Buffer, *Tris* · HCl (10 mM, pH = 8.8), H_3PO_4 · NaOH (5 mM, pH = 8.8) for AB15C5-CD-HPS-packed columns and H_3PO_4 · KOH (5 mM, pH 8.8) for AB18C6-CD-HPS-packed columns. [b]) For terms and other separation conditions, see *Table 1*.

the buffer [26][27]. Under the MeCN/phosphate buffer, the 4′-aminobenzo-15-crown-5 selector of AB15C5-CD-HPS includes Na^+, and 4′-aminobenzo-18-crown-6 selector of AB18C6-CD-HPS includes K^+ from the buffer to form an inclusion complex that is positively charged. As a result, the net negative charge on AB15C5-CD-HPS and AB18C6-CD-HPS surface decreases, and the velocity of EOF (v_{EOF}) also decreases. As shown in *Table 3*, v_{EOF} in the column packed with AB15C5-CD-HPS is higher with MeCN/*Tris*·HCl buffer (no metal ion) than with MeCN/phosphate buffer at the same MeCN content.

Table 3. *Comparison of* v_{EOF} *and Enantiomer Separation under Two Running-Buffer Conditions*

Solutes	Running buffer[a])	Voltage	v_{EOF}	Separation data[b])			
		[kV]	[mm s^{-1}]	t_{R1}[min]	k_1'	α	R_S
2-(2,4,5-Trichlorophenoxy)	MeCN/*Tris*·HCl 70 : 30	12	0.64	13.88	0.78	1.34	1.41
propanoic acid	MeCN/phosphate 70 : 30	12	0.38	25.39	0.93	1.67	2.12
1-(2-Hydroxyphenyl)	MeCN/*Tris*·HCl 70 : 30	16	0.83	11.02	0.51	1.63	4.07
ethanol	MeCN/phosphate 70 : 30	16	0.44	18.44	0.62	1.71	5.78

[a]) Buffer, 10 mM *Tris*·HCl (pH 8.8) and 5 mM H_3PO_4·NaOH (pH 8.8). [b]) For terms and other separation conditions, see *Table 1*.

After inclusion of metal ions (Na^+, K^+) from the running buffer, the crown ether-capped β-CD selector becomes positively charged and can supply extra electrostatic interaction with ionizable solutes and enhance the dipolar interaction with polar neutral solutes. This can enhance the host-guest interaction with the solute and improve chiral recognition and selectivity [21][22]. Therefore, crown ether capped β-CD-bonded stationary phases can exhibit higher chiral selectivity with MeCN/phosphate buffer than with MeCN/*Tris*·HCl buffer. As shown in *Table 3*, the retention factor (k_1'), enantioselectivity (α), and enantiomeric-separation-resolution (R_S) values are higher with MeCN/phosphate buffer (all other conditions invariant) for the enantiomers of 1-(2-hydroxyphenyl) ethanol and 2-(2,4,5-trichlorophenoxy)propanoic acid. It is found that the probability of bubble formation is high above 5 mM phosphate buffer because of the high conductivity of phosphate, even though a high volume percentage of MeCN is present. The optimized condition was then chosen as 5 mM phosphate buffer (pH 8.8). The typical enantiomer separation data for a wide range of chiral compounds are listed in *Table 2*. All the chiral solutes that can be separated with the MeCN/*Tris*·HCl buffer can also be separated with the MeCN/phosphate buffer. However, some solutes (*i.e.*, isoproterenol and metoprolol) can only be baseline-separated with the latter running buffer. Typical chromatograms of enantiomeric separation with MeCN/phosphate buffer are shown in *Fig. 4*. *Fig. 4*,*d* shows the separation of the four stereoisomers of labetabol, which has two chiral centers.

Comparison of Enantiomer Separations of the Columns Packed with AB15C5-CD-HPS and AB18C6-CD-HPS. The 4′-aminobenzo-18-crown-6 in AB18C6-CD-HPS better recognizes amines than the 4′-aminobenzo-15-crown-5 in AB15C5-CD-HPS [17]. The former can exhibit stronger dipolar interaction [17] and host-guest interaction [28] with the amine moiety of a solute than the latter. Accordingly, longer retention and

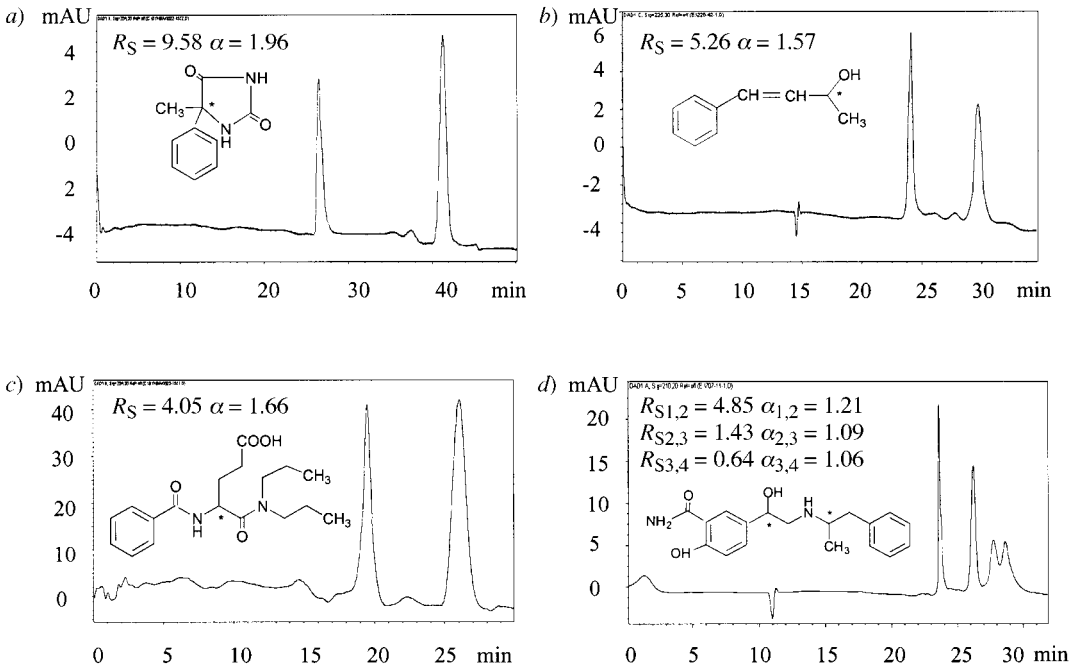

Fig. 4. *Typical chromatograms for enantiomeric separation with MeCN/phosphate as running buffer.* Conditions: 75 μm i.d. × 30 cm effective length fused-silica capillary (38.5 cm total length) packed with bonded silica particles; *a*) AB15C5-CD-HPS-packed column, 5-methyl-5-phenylhydantoin, $H_3PO_4 \cdot NaOH$ (5 mM, pH 8.8)/MeCN 40 : 60 (*v*/*v*), 12 kV, 254 nm UV detection; *b*) AB15C5-CD-HPS-packed column, 4-phenylbut-3-en-1-ol, $H_3PO_4 \cdot NaOH$ (5 mM, pH 8.8)/MeCN 60 : 40 (*v*/*v*), 14 kV, 225 UV detection; *c*) AB18C6-CD-HPS-packed column, proglumide, $H_3PO_4 \cdot KOH$ (5 mM, pH 8.8)/MeCN 60 : 40 (*v*/*v*), 20 kV, 254 nm UV detection; *d*) AB18C6-CD-HPS-packed column, labetalol, $H_3PO_4 \cdot KOH$ (5 mM, pH 8.8)/MeCN 60 : 40 (*v*/*v*), 18 kV, 210 nm UV detection.

better enantioselectivity can be obtained by the AB18C6-CD-HPS for some amine-containing solutes. *Table 4* compares typical data of enantiomeric separation on AB18C6-CD-HPS- and AB15C5-CD-HPS-packed columns. It is shown that AB18C6-CD-HPS exhibits better enantioselectivity (α) than AB15C5-CD-HPS for the same amine-containing solute under the same separation conditions.

Table 4. *Comparison of Enantiomer Separation of AB15C5-CD-HPS- and AB18C6-CD-HPS-Packed Columns*

Solutes	Running buffer[a])	Voltage	AB15C5-CD-HPS[b])		AB18C6-CD-HPS[b])	
		[kV]	k_1'	α	k_1'	α
Ethyl-(1-naphthyl)amine	MeCN/Tris · HCl 50 : 50	10	0.58	1.29	0.75	1.41
Isoproterenol	MeCN/phosphate 60 : 40	12	0.38	1.31	0.63	1.53
Pindolol	MeCN/phosphate 40 : 60	16	0.45	1.98	0.52	2.13
Metoprolol	MeCN/phosphate 40 : 60	12	0.87	1.23	1.18	1.59

[a]) Phosphate buffer: $H_3PO_4 \cdot NaOH$ (5 mM, pH 8.8) for the AB15C5-CD-HPS-packed column and $H_3PO_4 \cdot KOH$ (5 mM, pH 8.8) for the AB18C6-CD-HPS-packed column. [b]) For terms and other separation conditions, see *Table 1*.

Comparison of Enantiomer Separation of the Columns Packed with β-CD-Bonded, Crown Ether Bonded, and Crown Ether Capped β-CD-Bonded Silica Particles. The structures of all bonded silica particles are shown in *Fig. 1*. The β-CD-bonded silica BACD-HPS has only the β-CD selector. The crown ether bonded silica AB15C5-PS and AB18C6-PS contain only crown ethers as selectors. The crown ether capped β-CD-bonded silica AB15C5-CD-HPS and AB18C6-CD-HPS have a chiral selector with two recognition sites: crown ether and β-CD. Each particle type was separately packed into a fused-silica tubing to give a 75 μm i.d. × 30 cm effective-length column (38.5 cm total length). Under the same separation conditions, only partial enantiomer separation ($\alpha = 1.08$, $R_S = 0.63$) on the column packed with BACD-HPS was achieved for the enantiomers of 1-phenylpropan-2-ol, and no enantiomeric separation was obtained on the column packed with AB15C5-PS. However, baseline-separation ($\alpha = 1.34$, $R_S = 2.39$) was easily achieved on the column packed with AB15C5-CD-HPS. Similarly, for the enantiomers of isoproterenol, only partial separations on the BACD-HPS- ($\alpha = 1.09$, $R_S = 0.83$) and on the AB18C6-PS-packed columns ($\alpha = 1.05$, $R_S = 0.74$) were obtained; on the other hand, baseline separation was achieved on the AB18C6-CD-HPS packed column ($\alpha = 1.53$, $R_S = 2.72$). The columns packed with crown ether capped β-CD-bonded silica particles exhibit better enantioselectivity than the columns packed with either β-CD- or crown ether bonded silica particles only. These results show that the cooperation of crown ether and β-CD are an important contribution to the chiral separation with the crown ether capped β-CD-bonded stationary phases AB15C5-CD-HPS and AB18C6-CD-HPS.

Conclusions. – Crown ether-capped β-CD-bonded silica particles AB15C5-CD-HPS and AB18C6-CD-HPS are new types of bonded chiral stationary phases suitable for CEC. High enantiomer-separation resolution and reproducibility of chromatographic performance are obtained under both MeCN/phosphate buffer and MeCN/*Tris*·HCl running buffer. AB15C5-CD-HPS and AB18C6-CD-HPS exhibit high enantiomeric selectivity for a wide range of chiral compounds. The cooperation of the crown ether and the β-CD is important for the chiral recognition. The crown ether capped β-CD-bonded stationary phase shows better enantioselectivity than either crown ether bonded stationary phase or β-CD-bonded stationary phase only. With the metal ions included from the phosphate buffer, the positively charged crown ether-capped β-CD-bonded silica particles provide extra electrostatic interaction with ionizable solutes and enhance the dipolar interaction with polar neutral solutes. This additionally improves the chiral recognition and selectivity of CEC. The composition of MeCN in the running buffer influences the retention and resolution. The results demonstrate that crown ether capped β-CD-bonded silica particles have great potential for fast chiral separation when used as chiral stationary phases in CEC due to their excellent enantioselectivity.

Experimental Part

Materials. β-CD was purchased from *Merck* (Schuchardt, Hobenbrum, Germany) and was dried in 0.1 mm Hg vacuum at 120° for 12 h. Bare silica particles (3 μm, 100 Å) were obtained from *Merck* (D-Darmstadt). 3-Bromopropyl(triethoxy)silane and 3-glycidoxypropyl(trimethoxy)silane were obtained from *Merck* (*Schuchardt*) and redistilled under vacuum before use. H_2O was processed with a *Barnstead NANOpure* (Dubuque,

IA, USA) *Ultrapure* water system. HPLC-grade toluene, MeCN, CH_2Cl_2, Et_3N, MeOH, and i-PrOH were all purchased from *Fisher Scientific* (Fair Lawn, NJ, USA). Anal.-grade NaH (60%), NaOH, KOH and Na metal were all purchased from *Fluka* (Buchs, Switzerland). Anal.-grade CaH_2 was purchased from *Spectrum* (New Brunswick, NJ, USA). H_3PO_4 (85%) was obtained from *Carlo Erba* (Milan, Italy). HCl and *Tris* (=2-amino-2-(hydroxymethyl)propane-1,3-diol) were purchased from *J. T. Baker* (Phillipsburg, NJ, USA). $BrCH_2COBr$ was obtained from *Aldrich* (Milwaukee, WI, USA). Racemic drugs were obtained from *Sigma* (St. Louis, MO, USA) and *Merck*. 4′-Aminobenzo-15-crown-5 and 4′-aminobenzo-18-crown-6 were purchased from *Tokyo Chemical Industry* (Tokyo, Japan).

Apparatus. Cap. electrochromatography 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 and/or outlet vial. A *Shimadzu* (Tokyo, Japan) *LC-9A* HPLC pump was used for packing and flushing the cap. column. Buffer pH was determined with a *Metrohm* (CH-Herisau) *692* pH meter.

Preparation of Bonded Stationary Phases. The preparation of the crown ether-capped β-CD-bonded silica particles was previously described in detail [21]. Briefly, β-CD was anchored onto silica particles at its C(2)-atom, derivatized primarily at the more reactive and less sterically hindered [29][30] C(6)-atom by treatment with 7 equivs. of $BrCH_2COBr$ to form bromoacetate-substituted [3-(2-*O*-β-cyclodextrin)-2-hydroxypropoxy]-propylsilyl-appended silica particles (BACD-HPS). Finally, BACD-HPS was reacted with excess 4′-amino-benzo-15-crown-5 and 4′-aminobenzo-18-crown-6 to form crown ether-capped β-CD-bonded silica particles AB15C5-CD-HPS and AB18C6-CD-HPS. The amount of anchored β-CD and substituted bromoacetate moieties in BACD-HPS were 181 μmol g^{-1} and 618 μmol g^{-1}, resp., as determined by elemental analysis. The degree of substitution of bromoacetate was calculated to be 3.4. The amount of crown ether moieties in the bonded silica was 193 μmol g^{-1} for the benzo-15-crown-5 and 187 μmol g^{-1} for the benzo-18-crown-6. For comparison with crown ether-capped β-CD-bonded silica particles AB15C5-CD-HPS and AB18C6-CD-HPS, 4′-aminobenzo-15-crown-5 propylsilyl-appended silica (AB15C5-PS) and 4′-aminobenzo-18-crown-6 propylsilyl-appended silica (AB18C6-PS) were synthesized as reported [31] with 4′-aminobenzo-15-crown-5 and 4′-aminobenzo-18-crown-6 as the crown ether materials. The concentration of the anchored crown ether moieties in the bonded silica was 214 μmol g^{-1} for AB15C5-PS and 198 μmol g^{-1} for AB18C6-PS.

Chromatographic Procedure. The bonded silica particles were packed into fused silica tubing to give 75 μm i.d. × 30 cm effective length columns (38.5 cm total length) following the packing procedure described by *Boughtflower* [32]. The internal column frits and on-column UV detection window were made with a resistive heating device (*InnovaTech*, UK). The freshly packed column was flushed with the running buffer using a HPLC pump for 3 h before installing into the CE instrument. It was stabilized with pressure on both vials by gradually increasing the applied voltage to 20 kV. All runs were performed with a pressure of 10 bar on both inlet and outlet vials to prevent bubble formation in the column.

The mobile phase used was a mixture of MeCN/phosphate buffer or MeCN/*Tris*·HCl buffer soln. by volume ratios. The phosphate buffer was prepared by dissolving the desired amount of H_3PO_4 in H_2O to achieve a 5-mM conc. and then adding NaOH or KOH to achieve the required pH. Similarly, the *Tris*·HCl buffer was adjusted to the desired pH with conc. HCl. All samples were dissolved in MeCN/*Tris*·HCl buffer (or phosphate buffer) mixtures having an MeCN content of *ca.* 5–15% higher than the running buffer. The sample conc. was *ca.* 1–5 mg ml^{-1}. The slight mismatch in the composition of the injection-sample soln. and the running buffer causes a baseline perturbation that was used as the marker of the electo-osmotic flow [13]. All running buffers and sample solns. were degassed with He 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. The column temp. was set to 20°. All solns. 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 of the enantiomers from 200 to 254 nm.

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