Anal. Chem. **2000,** *72,* 310−317

A Family of Single-Isomer, Sulfated γ-Cyclodextrin Chiral Resolving Agents for Capillary Electrophoresis. 1. Octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin

Wenhong Zhu and Gyula Vigh*

Department of Chemistry, Texas A&M University, College Station, Texas, 77842-3012

The first member of the single-isomer, sulfated γ-cyclodextrin family, the sodium salt of octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin (ODAS-γCD) has been synthesized, analytically characterized, and used to separate, by capillary electrophoresis, a variety of neutral, acidic, basic, and amphoteric enantiomers in low pH background electrolytes. The anionic effective mobilities of the neutral and anionic analytes were found to increase with the concentration of ODAS-γCD. For weakly binding cationic analytes, the effective mobilities went from cationic high values, through zero, to increasingly larger anionic values as the concentration of ODAS-γCD was increased. For the strongly complexing cationic analytes, the effective mobilities became anionic even at very low ODAS-γCD concentrations and became smaller as the ionic strength of the background electrolyte increased with the increasing ODAS-γCD concentration. Separation selectivity followed the predictions of the charged resolving agent migration model: for neutral analytes it decreased as the concentration of ODAS-γCD was increased. For cationic analytes, selectivities were found to increase as the cationic effective mobilities approached zero, then decreased as the concentration of ODAS-γCD was increased further. The extent of peak resolution that could be realized with ODAS-γCD strongly depended on the magnitude of separation selectivity and the normalized electroosmotic flow mobility. ODAS-γCD proved to be a broadly applicable chiral resolving agent.

During the last five years, charged cyclodextrins (CDs) became reliable, widely used chiral resolving agents in capillary electrophoresis (CE).[1−4] Though both weak and strong electrolyte charged CDs yielded spectacular CE separations, the strong electrolyte CDs became favored because they could be used over a broad pH range without changes in their charge-states [2]. Most of these charged CDs were complex mixtures of isomers which differed in their degree and loci of substitution.[2] Recently, single-isomer anionic [7−9] and cationic[10,11] β-cyclodextrins were developed to eliminate both the batch-to-batch compositional variations and the associated, undesirable mobility and selectivity variations. The single-isomer anionic CDs were also meant to facilitate molecular-level studies of the chiral recognition process via NMR spectroscopy,[5] crystallography, and molecular modeling [6]. These single-isomer charged CDs were successfully used in aqueous,[7−13] hydroorganic,[14] and nonaqueous[15,16] background electrolytes (BEs) to separate the enantiomers of strong and weak acids, strong and weak bases, and amphiprotics. To provide single-isomer, sulfated cyclodextrin resolving agents with a larger cavity, the first member of the single-isomer, sulfated γ-cyclodextrin family, octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin, was synthesized, analytically characterized, and used for CE enantiomer separations, as described in this paper.

EXPERIMENTAL SECTION

Synthesis of the sodium salt of octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin. All the reagents used were obtained from Aldrich Chemical Co. (Milwaukee, WI), except γ-cyclodextrin, which was a generous gift from Cerestar USA (Hammond, IN). The synthetic procedure, a modification of what was used for the synthesis of the analogous β-cyclodextrin derivative,[7] is shown in Figure 1. The purity of every intermediate was monitored by gradient elution HPLC using a home-built system consisting of a Star 9010 ternary gradient pump (Varian, Walnut Creek, CA), a Rheodyne 7125 injection valve (Rheodyne, Cotati, CA), a UV 2050 variable wavelength UV detector (Varian), a DDL-31 evaporative

* Corresponding author. Phone: 409-845-2456. Fax: 409-845-4719. E-mail: vigh@mail.chem.tamu.edu.

(1) Beale, S. C. *Anal. Chem.* **1998**, *70*, 279R−300R.
(2) Chankvetadze, B. *Capillary Electrophoresis in Chiral Analysis*; J. Wiley: New York, 1997.
(3) Landers, J. P. *Handbook of Capillary Electrophoresis*; CRC Press: Boca Raton, FL, 1997.
(4) Khaledi, M. *Handbook of Capillary Electrophoresis*; J. Wiley: New York, 1998.
(5) Chankvetadze, B.; Endresz, G.; Blaschke, G. *Chem. Soc. Rev.* **1996**, *25*, 141−162.
(6) Lipkovitz, K. B. *Chem. Rev.* **1998**, *98*, 1829−1873.
(7) Vincent, J. B.; Sokolowski, A. D.; Nguyen, T. V.; Vigh, Gy. *Anal. Chem.* **1997**, *69*, 4226−4233.
(8) Vincent, J. B.; Kirby, D.; Nguyen, T. V.; Vigh, Gy. *Anal. Chem.*, **1997**, *69*, 4419−4428.
(9) Cai, H.; Nguyen, T. V.; Vigh, Gy. *Anal. Chem.* **1998**, *70*, 580−589.
(10) O'Keeffe, F.; Shamsi, S. A.; Darcy, R.; Schwinte, P.; Warner, I. M. *Anal. Chem.* **1997**, *69*, 4773−4782.
(11) Haynes, J. L.; Shamsi, S. A.; O'Keefe, F.; Darcey, R.; Warner, I. M. *J. Chromatogr., A* **1998**, *803*, 261−271.
(12) Vincent, J. B.; Vigh, Gy. *J. Chromatogr., A.* **1998**, *817*, 105−111.
(13) Cai, H.; Vigh, Gy. *J. Microcolumn Sep.* **1998**, *10*, 293−299.
(14) Cai, H.; Vigh, Gy. *J. Chromatogr., A* **1998**, *827*, 121−132.
(15) Vincent, J. B.; Vigh, Gy. *J. Chromatogr., A* **1998**, *816*, 233−241.
(16) Cai, H.; Vigh, Gy. *J. Pharm. Biomed. Anal.* **1998**, *16*, 615−621.

10.1021/ac991039t CCC: $19.00
Published on Web 12/17/1999

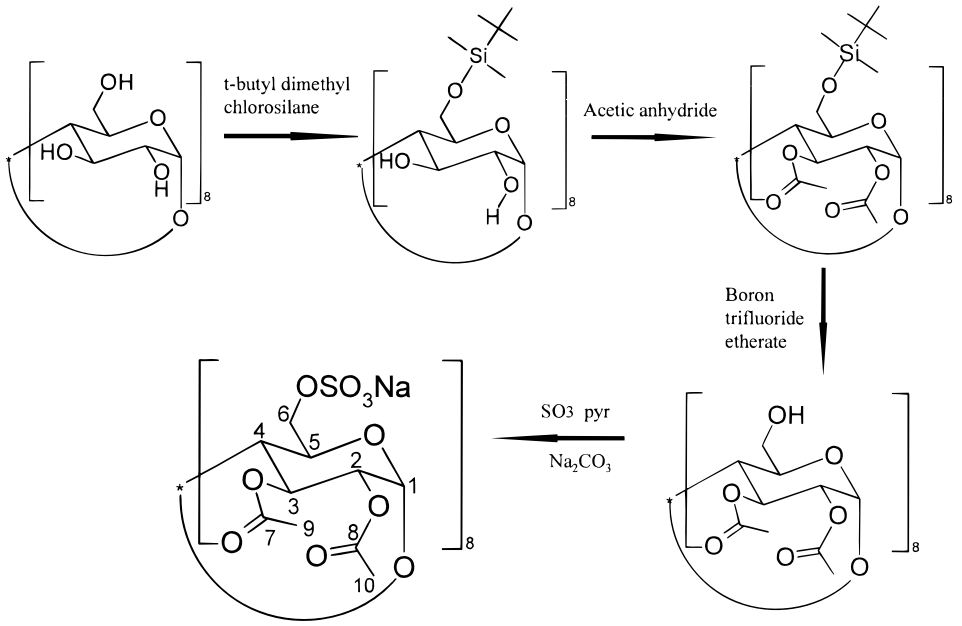

Figure 1. Synthesis scheme for octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin.

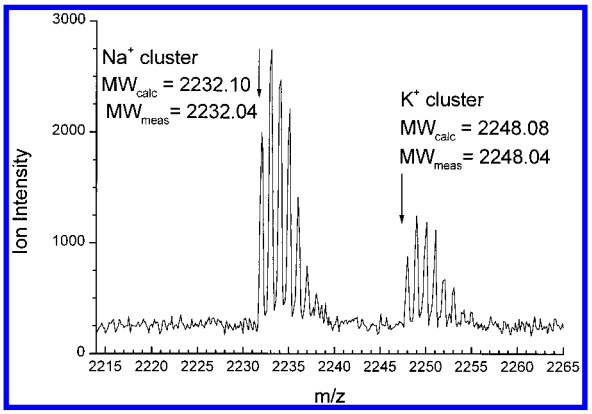

Figure 2. Partial mass spectrum of intermediate ***2*** obtained by MALDI-TOF-MS. For conditions see Experimental Section.

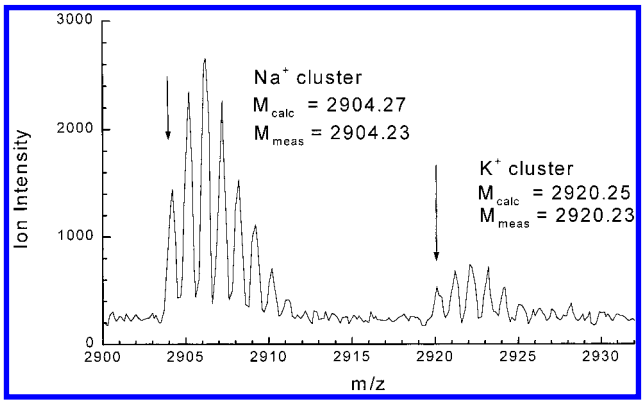

Figure 3. Partial mass spectrum of intermediate ***3*** obtained by MALDI-TOF-MS. For conditions see Experimental Section.

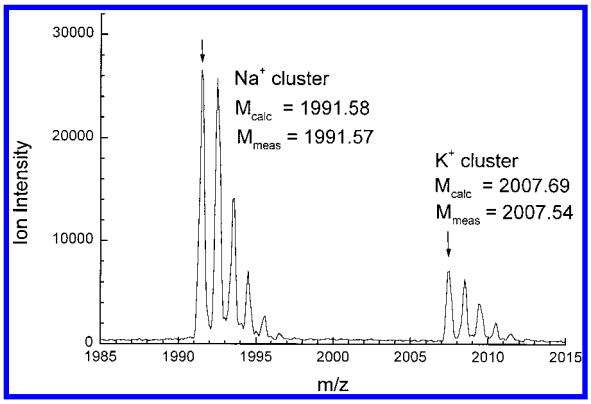

Figure 4. Partial mass spectrum of intermediate ***4*** obtained by MALDI-TOF-MS. For conditions see Experimental Section.

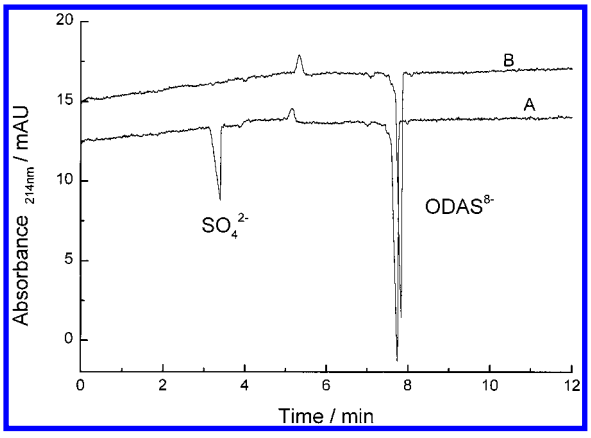

Figure 5. Indirect UV-detection electropherogram of ODAS-γCD before (A) and after (B) removal of sodium sulfate. BE: 20 mM *p*-toluenesulfonic acid, pH 4.1 adjusted with ϵ-aminocaproic acid. Wavelength: 214 nm. Applied potential: 10 kV. Thermostat temperature: 20 C. Capillary: 25-μm i.d. 39 cm/46 cm effective/total length, uncoated fused silica.

light scattering detector (Bodman Industries, Aston, PA), and an AD 406 data acquisition system operated under Gold 8.1 software control (Beckman Instruments, Fullerton, CA) running on a 486DX4 personal computer (Computer Associates, College Station, TX). The separations were obtained on either a 4.6-mm i.d. × 250-mm column packed with Zorbax silica or a 4.6-mm i.d. × 250-mm column packed with an experimental Zorbax 300 Bidentate C-18 stationary phase[17] (a generous gift by Dr. J. J. Kirkland, Hewlett-Packard, Newport Site, Newport, DE).

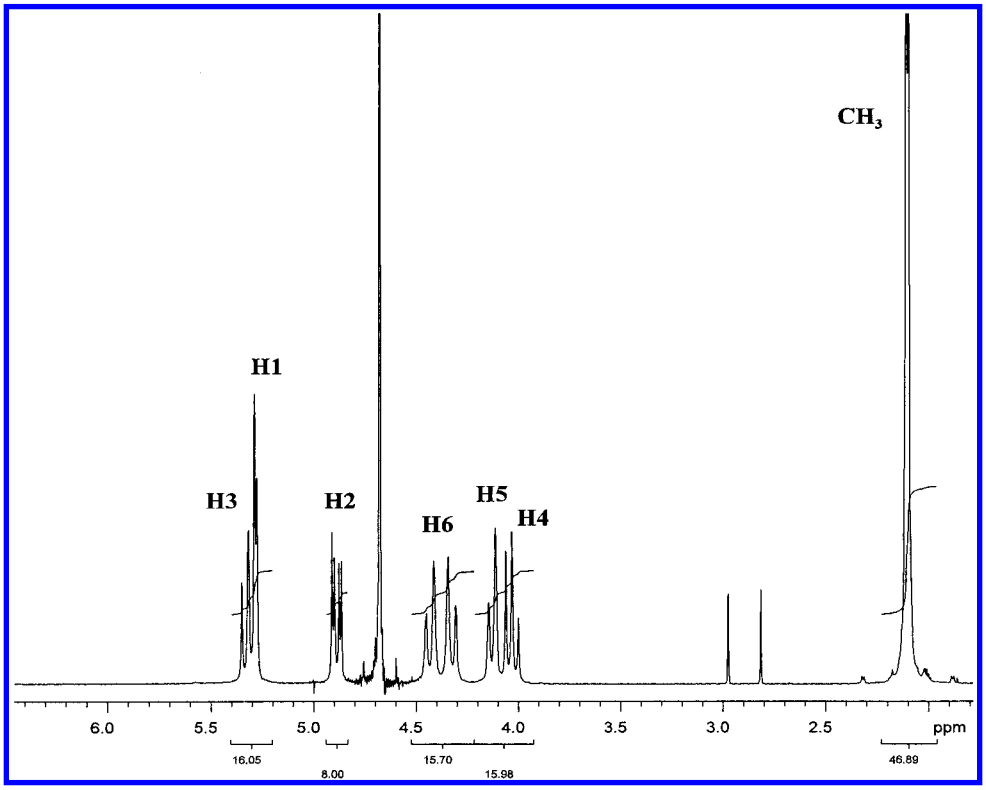

Figure 6. 300 MHz ^{1}H NMR spectrum of ODAS-γCD. Solvent: D_2O.

Table 1. Effective Mobilities of the Less Mobile Enantiomer (μ), Separation Selectivities (α), Measured Peak Resolution Values (Rs), Dimensionless EO Flow Mobility Values (β), and the Injector-to-Detector Potential Drop (U) in pH = 2.5 ODAS-γCD BEs

conc.	0 mM	5 mM					10 mM					25 mM				
name	μ^a	μ	α	Rs	β	U^b	μ	α	Rs	β	U	μ	α	Rs	β	U
NSA	−33.55	−30.74					−29.2					−26.89				
pindolol	22.34	−28.8	1.02	<0.5	−0.15	15	−26.3	1.02	2.4	−0.8	16	−24.57	1.01	1	−0.6	11
piperoxan	27.22	−24.9	1.05	1	−0.14	15	−23.8	1.04	1.4	−0.3	15	−22.87	1.03	2.2	−0.6	11
oxyphencyclamine	21.82	−21.3	1.04	0.8	−0.2	15	−18.6	1.03	0.6	−0.2	15	−17.87	1.02	1.9	−0.7	11
chlophedianol	24.04	−13.9	1.15	1.3	−0.02	12	−16	1.08	1.2	−1.8	20	−18.66	1.07	5.3	−0.7	11
terbutaline	22	−3.81	1.21	2.3	−2.3	12	−6.1	1.14	2.8	−1.7	16	−6.9	1.13	5.9	−1.4	11
aminoglutethimide	25.47	−17.9	1.27	2.4	−0.3	15	−16	1.2	7	−0.3	16	−17	1.15	9.7	−0.8	11
1-phenyl-1,2-ethanediol	0	−0.6	1	0	−16.8	16	−0.8	1	0	−8.2	15	−1.3	1	0	−9.5	11
1-phenyl-2-propanol	0	−1	1	0	−4.6	15	−2	1	0	−5.9	16	−3.54	1	0	−3.4	11
2-phenyl-1-propanol	0	−2.07	1.17	0.9	−2.7	15	−3	1.13	2.1	−3.1	16	−5.3	1.1	1.3	−2.2	11
methyl mandelate	0	−1.18	1	0	−5.3	15	−1.81	1	0	−6.3	16	−3.31	1	0	−3.6	11
4-phenyl-1,3-dioxane	0	−1.76	1.25	1	−6.9	15	−2.5	1.21	1.6	−4.5	16	−4.23	1.2	3.8	−2.2	11
a-methyl-a-phenylsuccinimide	0	−2.46	1.21	1.6	−4.4	12	−3.58	1.18	2.5	−3.1	16	−5.86	1.14	3.6	−1.8	11
2-phenyl-2-butanol	0	−3.05	1.24	1.9	−1.7	15	−4.1	1.22	5.1	−2.3	12	−6.9	1.18	4.1	−1.5	11
indapamide	0	−1.92	1.12	1.7	−2.3	12	−2.84	1.11	1.7	−2.6	16	−4.94	1.1	2.3	−2.2	11
2-phenyl-propionic acid	0	−7.27	1.13	2.7	−0.7	12	−8.14	1.11	1.8	−3.9	16	−8.34	1.1	1.7	−3.3	11
mandelic acid	0	−10.1	1	0	−0.5	15	−6.8	1	0	−1.4	16	−3.68	1	0	−3	11
2-phenylglycine	4.55	0.76	1.57	2.3	5.6	15	0.19	11.4	6.4	37	15	−0.23	−4.7	4.1	−51	11
tryptophan	10.22	−6.3	1.62	13.2	−0.5	12	−7.5	1.35	7.1	−4.1	16	−11.5	1.2	13.6	−0.6	9.7
dansyl-aspartic acid	11.41	−7.25	1.66	19.1	−0.5	12	−7.8	1.55	21	−1.2	15	−9.3	1.27	9.5	−0.7	11
dansyl-methionine	13	−8.58	1.2	8.9	−0.4	12	−9.03	1.07	3.2	−0.4	12	−11.11	1.06	5.8	−0.6	9.7
dansyl-phenylalanine	13.78	−8.6	1.22	9.6	−0.4	12	−9.5	1.16	8	−0.3	15	−12.28	1.07	9.7	−0.6	9.7

^{a} In 10^{-5} cm^2 V^{-1} s^{-1} units. ^{b} In kV units.

In the synthesis, γ-cyclodextrin (***1***) was first reacted according to the classical procedure of ref 18 with *tert*-butyldimethylchlorosilane and purified to obtain the first intermediate, octakis(6-*tert*-butyldimethylsilyl)-γ-CD (***2***). Progress of both the reaction and the purification processes was monitored by nonaqueous reversed-phase HPLC using a 10-min, 75:25 to 25:75 acetonitrile:chloroform gradient, at 2 mL/min, on the Zorbax Bidentate C-18 column. The ^{1}H and ^{13}C spectra of (***2***), obtained with a 300 MHz Unity spectrometer (Varian) agreed with those reported in ref 18. The

(17) Kirkland, J. J.; Adams, J. B.; van Straten, M. A.; Claessens, H. A. *Anal. Chem.* **1998**, *70*, 4344−4352.
(18) Takeo, K.; Mitoh, H.; Uemura, K. *Carbohydr. Res.* **1989**, *187*, 203−221.

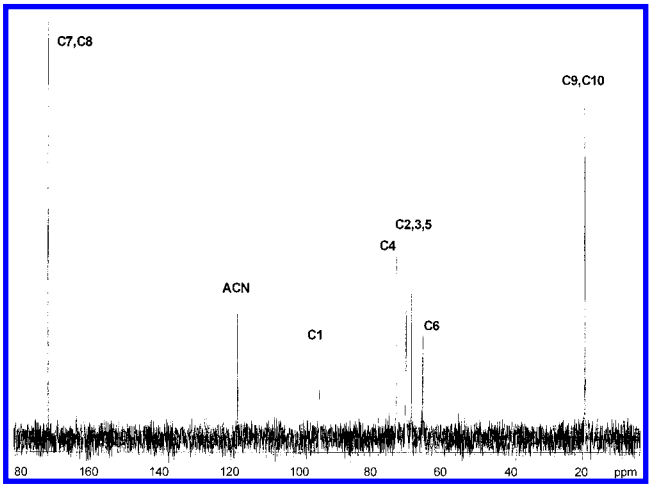

Figure 7. 300 MHz ^{13}C NMR spectrum of ODAS-γCD. Solvent: D_2O. C1−C6: respective carbon atoms in the glucose moieties. C7,8: CO carbon atoms in the acetyl functional groups connected to the C2, C3 carbons of the glucose moieties. C9,10: CH_3 carbon atoms in the acetyl functional groups connected to the C2, C3 carbons of the glucose moieties.

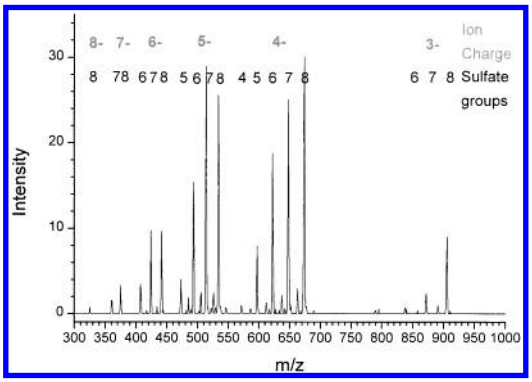

Figure 8. Mass spectrum of ODAS-γCD obtained by ESI-MS. For conditions see Experimental Section.

Na^+ and K^+ ion-adduct portion of the high-resolution MALDI/TOF mass spectrum of the parent molecule of (***2***), obtained on a Voyager Elite XL system (PerSeptive Biosystems, Framingham, MA) in delayed-extraction reflected-ion mode with α-cyano-4-hydroxy cinnamic acid as matrix, is shown in Figure 2. MW_{calc} is the calculated *m*/*e* value for the base isotope peak, while MW_{meas} is the actual, measured *m*/*e* value for the base isotope peak.

The next intermediate, octakis(2,3-diacetyl-6-*tert*-butyldimethylsilyl)-γ-CD (***3***), was obtained by exhaustive acetylation with acetic anhydride.[7,18] Once again, progress of both the reaction and the purification processes was followed by nonaqueous reversed-phase HPLC using a 30-min, 35:65:0 to 35:55:10 isopropyl alcohol:acetonitrile:*n*-hexane gradient, at 2 mL/min, on the Zorbax Bidentate C-18 column. The 300 MHz ^1H and ^{13}C spectra of purified (***3***) agreed with those reported in ref 18. The Na^+ and K^+ ion-adduct portion of the high-resolution MALDI/TOF mass spectrum of the parent molecule of (***3***) is shown in Figure 3.

The third intermediate, octakis(2,3-diacetyl)-γ-CD (***4***), was obtained by removing the *tert*-butyldimethylsilyl protecting groups with boron trifluoride etherate.[7,18] Completeness of the reaction and the purification process of (***4***) was monitored by isocratic normal-phase HPLC using the Zorbax silica column and 40:45:15 *n*-hexane:dichloromethane:methanol eluent at 1 mL/min. The 300 MHz ^1H and ^{13}C spectra of (***4***) agreed with those reported in ref 18. The Na^+ and K^+ ion-adduct portion of the high-resolution MALDI/TOF mass spectrum of the parent molecule of (***4***) is shown in Figure 4.

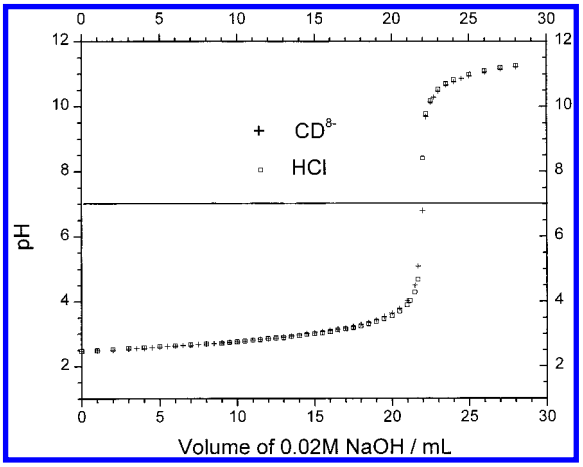

Figure 9. Potentiometric titration curves for 0.058 mmol ODAS-γCD and 0.46 mmol HCl.

Finally, pure intermediate (***4***) was reacted with SO_3·pyridine in DMF [19] to completely sulfate the primary hydroxyl groups of (***4***), then with Na_2CO_3 to exchange the pyridinium counterion with sodium [7] to obtain the sodium salt of octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin (ODAS-γCD). Indirect UV detection CE[20] with a 25 mM phthalic acid BE (pH adjusted to 8.5 with tris(hydroxymethyl)-aminomethane) was used to monitor the progress of the sulfation reaction. Indirect UV detection CE[20] with a 20 mM *p*-toluenesulfonic acid BE (pH adjusted to 4.1 with ϵ-aminocaproic acid) was used to monitor the subsequent removal of excess sodium sulfate.[7] The indirect-UV detection electropherograms of ODAS-γCD before and after sodium sulfate removal are shown in Figure 5. The 300 MHz ^1H and ^{13}C spectra of ODAS-γCD are shown in Figures 6 and 7 and are consistent with the postulated structure (for numbering of the H and C atoms see Figure 1). The negative-ion ESI-MS spectrum of ODAS-γCD was obtained as described in ref 21 with a Vestec model 201-A single-quadrupole mass spectrometer (PerSeptive Biosystems, Framingham, MA). To obtain the mass spectrum, ODAS-γCD was dissolved at a concentration of 5 mg/mL in a 75/25 (v/v) mixture of methanol/water and introduced into the ESI source with a model 341B SAGE syringe pump (Orion Research, Boston, MA) at a flowrate of 1.5 μL/min. The ESI-MS spectrum of ODAS-γCD is shown in Figure 8, confirming the presence of eight sulfate groups on the product. The top numbers in Figure 8 show the respective ion charges, while the lower numbers in each ion charge cluster indicate the number of sulfate groups left on the respective ion after the ESI process, as in ref 21. According to the spectrum, there are two isomeric contaminants in the sample, the more intense one is the monodeacetylated isomer (yielding ions 688 and 546 with 9 sulfate groups; ions 891, 662, and 525 with 8 sulfate groups; ions 637, 505, and 417 with 7 sulfate groups; ions 611 and 484 with 6 sulfate groups; and ions 789 and 586 with 5 sulfate groups). The less

(19) Bernstein, S.; Joseph, J.; Nair, U. US Patent 4,020,160, 1977.
(20) Nardi, A.; Fanalli, S.; Foret, F. *Electrophoresis* **1990**, *11*, 774−776.
(21) Bondarenko, P. V.; Wolf, B.; Cai, H.; Vincent, J. B.; Macfarlane, R. D.; Vigh, Gy. *Anal. Chem.* **1998**, *70*, 3042−3045.

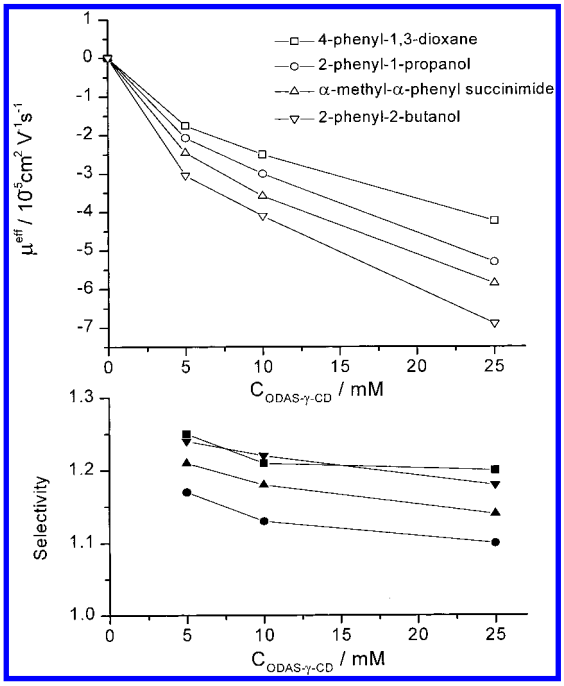

Figure 10. Effective mobilities (top panel, open symbols) and separation selectivities (bottom panel, filled symbols) for the non-charged analytes as a function of the ODAS-γCD concentration. Symbols: square, 4-phenyl-1,3-dioxane; circle, 2-phenyl-1-propanol; up-triangle, α-methyl-α-phenyl-succinimide; down-triangle, 2-phenyl-2-butanol.

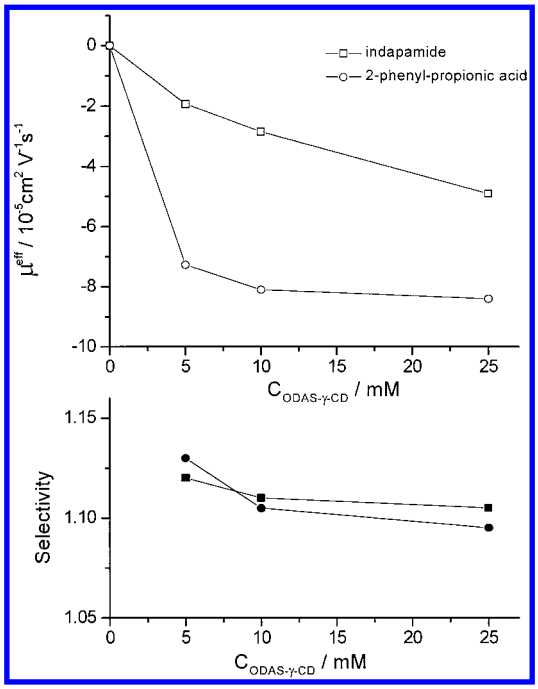

Figure 11. Effective mobilities (top panel, open symbols) and separation selectivities (bottom panel, filled symbols) for the weak-acid analytes as a function of the ODAS-γCD concentration. Symbols: square, indapamide; circle, 2-phenyl-propionic acid.

intense contaminants are due to the dideacetylated isomers (yielding ions 911, 677, 537, and 444 with 9 sulfate groups; ions 652 and 517 with 8 sulfate groups; and ion 627 with 7 sulfate groups).

Electrophoretic Separations Using ODAS-γCD. All CE separations were carried out on a P/ACE 2100 CE instrument

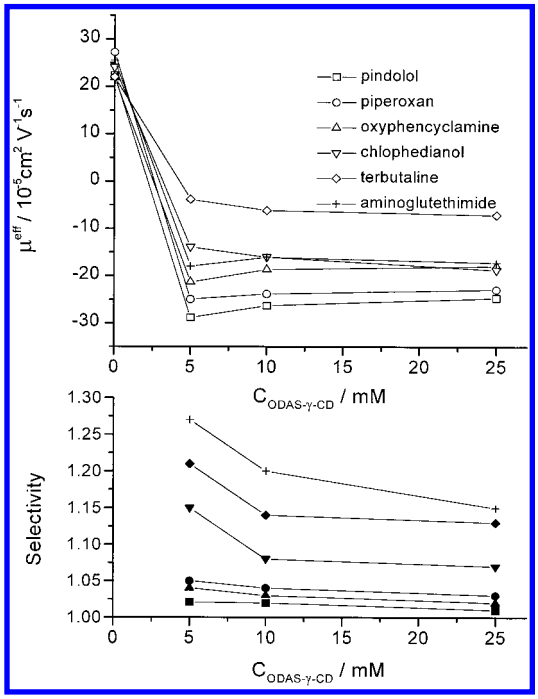

Figure 12. Effective mobilities (top panel, open symbols) and separation selectivities (bottom panel, filled symbols) for the weak-base analytes as a function of the ODAS-γCD concentration. Symbols: square, pindolol; circle, piperoxan; up-triangle, oxyphencyclamine; down-triangle, chlophendianol; cross, aminoglutethimide.

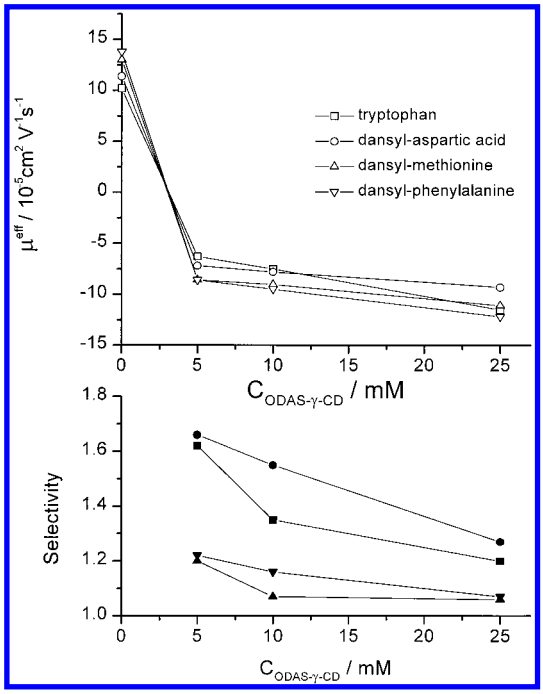

Figure 13. Effective mobilities (top panel, open symbols) and separation selectivities (bottom panel, filled symbols) for the amphoteric analytes as a function of the ODAS-γCD concentration. Symbols: square, tryptophan; circle, dansyl-asparatic acid; up-triangle, dansyl-methionine; down-triangle, dansyl-phenylalanine.

(Beckman Instruments, Fullerton, CA); its variable wavelength UV detector was operated at 214 nm. The cartridge coolant of the P/ACE 2100 was thermostated at 20 °C. The separations were carried out in 25-μm i.d. bare fused silica capillaries (Polymicro Technologies, Phoenix, AZ) with $L_d = 19.5$ cm and $L_t = 26.5$ cm.

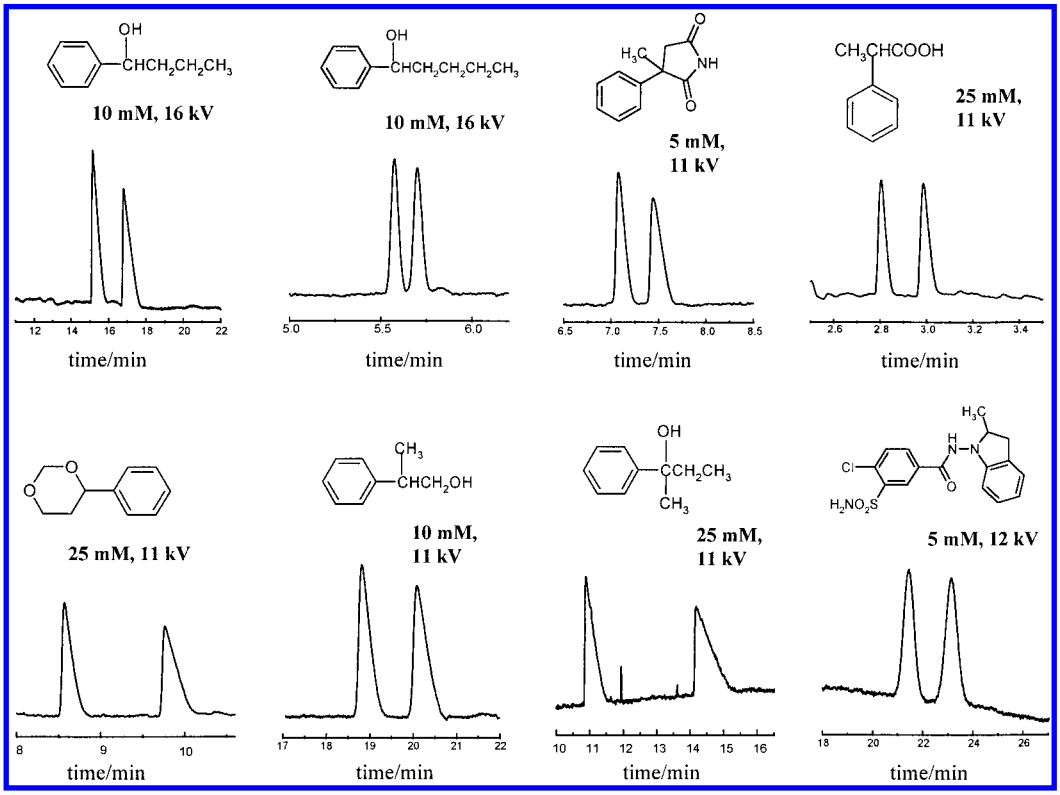

Figure 14. Typical electropherograms of chiral noncharged and weak-acid analytes. The numbers next to the structures indicate the ODAS-γCD concentrations (mM) and the applied effective potentials (kV). Capillary: 25 μm i.d. 19 cm/26 cm effective/total length, uncoated fused silica. Other conditions: see Experimental Section.

The 0.5 mM samples (including 2-naphthalene sulfonic acid, NSA^-), dissolved in the respective BEs, were injected by 4 psi nitrogen for 1 s. To remain on the linear portion of Ohm's plot, power dissipation was maintained between 500 and 700 mW/m by varying the applied potential between 15 and 20 kV.

According to the predictions of the charged resolving agent migration model (CHARM model) of CE enantiomer separations,[22] when a strong electrolyte resolving agent, such as ODAS-γCD is used, only two BEs, one with a low pH and another with a high pH are required to locate the best possible separation selectivity for any monoprotic weak electrolyte analyte. Since previous work with the single-isomer sulfated β-CDs[7−9,12−16] indicated that much more favorable dimensionless electroosmotic flow mobility values (β values,[23] vide infra) and, consequently, greater peak resolution values could be achieved in the low pH BEs, all measurements were carried out in the 25 mM phosphoric acid buffer the pH of which was adjusted to 2.5 with LiOH.[7] The 5, 10, and 25 mM ODAS-γCD BEs were prepared by weighing out the required amounts of the sodium salt of ODAS-γCD into 25-mL volumetric flasks and bringing the volumes to the mark with the pH 2.5 stock BE solution. It has been shown that even small neutral molecules, such as mesityl oxide complex with charged cyclodextrins and dynamically acquire ionic charge. Thus, they cannot be used as accurate electroosmotic flow (EOF) mobility (μ_{EO}) markers.[24] Therefore, NSA^- was used as a secondary EOF marker. The true effective mobility of NSA^- ($\mu^{eff}_{NSA^-}$) was first determined in each ODAS-γCD BE using the external EOF marker method.[24] Since NSA^- was added to every sample, the actual μ_{EO} could be obtained from the observed and effective mobilities of NSA^- as $\mu_{EO} = \mu^{obs}_{NSA^-} - \mu^{eff}_{NSA^-}$, from which the effective mobilities of the enantiomers (μ^{eff}_R and μ^{eff}_S) were obtained as $\mu^{eff}_R = \mu^{obs}_R - \mu_{EO}$, and the separation selectivities, α, were calculated as $\alpha = \mu^{eff}_R/\mu^{eff}_S$ where subscript S arbitrarily refers to the enantiomer which was less mobile in the 5 mM ODAS-γCD BE. (Please, note, that subscripts R and S used in the present context do not designate absolute configurations.) The normalized electroosmotic flow mobility values, β, were calculated as $\beta = \mu_{EO}/\mu^{eff}_S$.[23] The peak resolution values, Rs, were calculated, as usual, from the migration times and peak widths (at the baseline) of the respective enantiomer peaks.

To allay concerns that sulfate crowding may cause ODAS-γCD to behave as a weak electrolyte in the low pH BE,[25] the sodium salt of ODAS-γCD was percolated through a H-form strong cation exchanger resin column (Dowex 50X 8-100). A 9-mL aliquot of the effluent containing 0.058 mmol ODAS-γCD was titrated with 20 mM NaOH. For comparison purposes, 0.46 mmol of HCl was also titrated under identical conditions. The two potentiometric titration curves, shown in Figure 9, are indistinguishable, indicating that ODAS-γCD indeed behaves as a strong electrolyte.

RESULTS AND DISCUSSION

Effective Electrophoretic Mobilities of 2-Naphthalene Sulfonate in ODAS-γCD BEs. The effective mobilities of NSA^-, $\mu^{eff}_{NSA^-}$, in each ODAS-γCD BE, are listed in the first line of Table 1. The $\mu^{eff}_{NSA^-}$ values decrease as the ODAS-γCD concentration

(22) Williams, B. A.; Vigh, Gy. *J. Chromatogr.* **1997**, *777*, 295−309.
(23) Rawjee, Y. Y.; Vigh, Gy. *Anal. Chem.* **1994**, *66*, 619−627.
(24) Williams, B. A.; Vigh, Gy. *Anal. Chem.* **1997**, *69*, 4445−4451.
(25) Discussions following lecture L102 at the HPCE '99, Palm Springs, CA, February 1999.

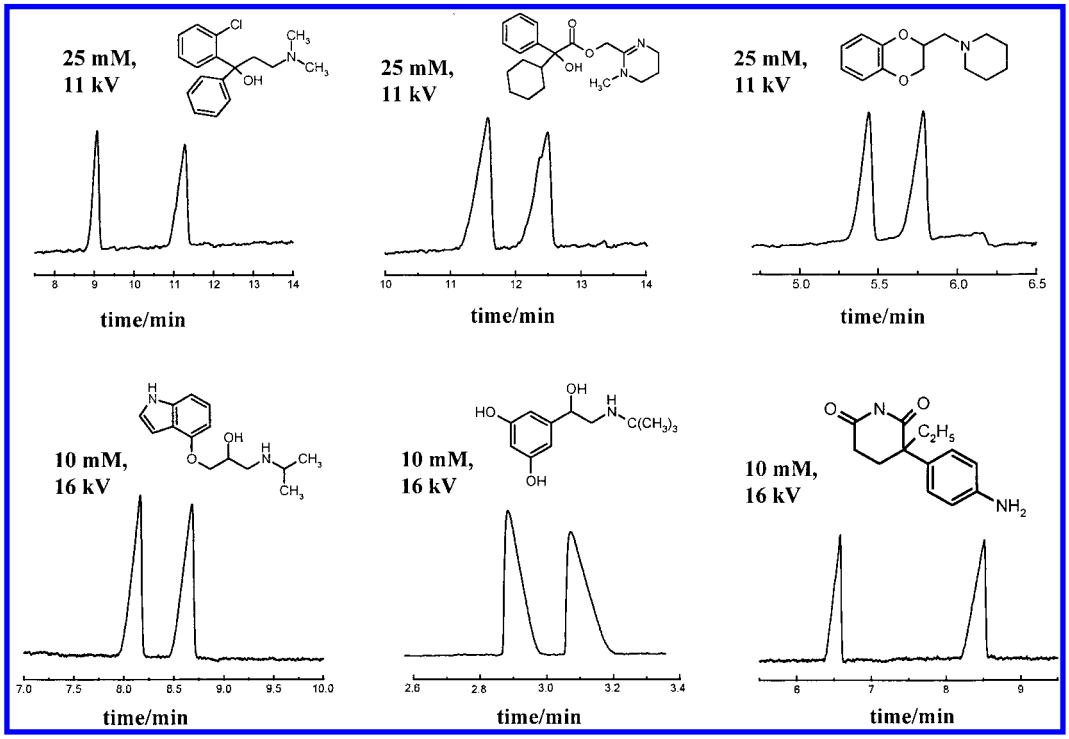

Figure 15. Typical electropherograms of chiral weak base analytes. The numbers next to the structures indicate the ODAS-γCD concentrations (mM) and the applied effective potentials (kV). Capillary: 25 μm i.d. 19 cm/26 cm effective/total length, uncoated fused silica. Other conditions: see Experimental Section.

is increased, just as it was found with BEs that contained heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-βCD).[7] This decrease is the result of the interplay between the increased BE viscosity,[7] increased ionic strength,[26] and increasing degree of complexation as the ODAS-γCD concentration is increased. However, the $\mu^{\text{eff}}_{\text{NSA}^-}$ values decrease much less upon addition of ODAS-γCD than HDAS-βCD,[7] indicating that ODAS-γCD binds NSA^- less strongly than HDAS-βCD and suggesting that one can expect significant binding-related mobility—and, perhaps, separation selectivity—differences between the two sulfated cyclodextrins, HDAS-βCD and ODAS-γCD.

Separation of Enantiomers in ODAS-γCD BEs. A series of nonionic, weak-acid, weak-base, and amphoteric enantiomers were separated with the pH 2.5 ODAS-γCD BEs. Table 1 lists the effective mobilities of the less mobile enantiomers (μ), the separation selectivities (α), the measured peak resolution values (Rs), the corresponding dimensionless EOF mobility values (β) and the injector-to-detector potential drop values (U).

As established by Friedl et al.,[26] the effective mobilities of the polyanionic analytes depend very strongly on the ionic strength of the BE. Therefore, the measured μ^{eff} values cannot be used to calculate meaningful complexation constants; they only permit qualitative comparison of the migration behavior. For the weakly complexing nonionic analytes and weak-acid analytes, the effective anionic mobility increases as the concentration of ODAS-γCD is increased (top panels in Figures 10 and 11), indicating that complexation overrides the mobility-reducing effects of both higher ionic strength and higher viscosity. As predicted by the CHARM model,[22] separation selectivity decreases as the concentration of ODAS-γCD is increased (bottom panels in Figures 10 and 11). The selectivity values for noncharged analytes are less distorted by ionic-strength effects, because separation selectivity for the noncharged analytes is a single-term expression[13,22] and, for the most part, both the numerator and the denominator of the selectivity expression are influenced by ionic-strength effects similarly.

For weak bases and amphiprotic substances, the effective mobilities become anionic upon the addition of as little as 5 mM of ODAS-γCD (top panels in Figures 12 and 13). For aminoglutethimide, oxyphencyclamine, piperoxan, and pindolol, the effective anionic mobilities are larger than -15×10^{-5} cm^2 V^{-1} s^{-1} at 5 mM ODAS-γCD concentration, indicating that their interactions with ODAS-γCD are very strong. As the concentration of ODAS-γCD is increased further, the rapidly increasing ionic strength of the BE reduces the effective (anionic) mobilities of these analytes.[26] For the weakly binding terbutaline and chlophedianol in Figure 12 and the amphiprotic substances in Figure 13 (with effective anionic mobilities smaller than -10×10^{-5} cm^2 V^{-1} s^{-1} at 5 mM ODAS-γCD concentration), the anionic effective mobilities increase as the concentration of ODAS-γCD is increased, indicating that the counteracting ionic-strength effects are weaker. The separation selectivities for both the weak bases and the amphiprotic substances studied here decrease as the concentration of ODAS-γCD is increased further, in agreement with the predictions of the CHARM model[22] and the observations found with the single-isomer sulfated β-CDs.[7–9]

The peak resolution values (Table 1) depend not only on the separation selectivities but also, very sensitively, on the β values and the magnitude of the effective potential drop. A few typical separations obtained with ODAS-γCD are shown in Figures 14–

(26) Friedl, W.; Reijenga, J. C.; Kenndler, E. *J. Chromatogr., A* **1995**, *709*, 163–170.

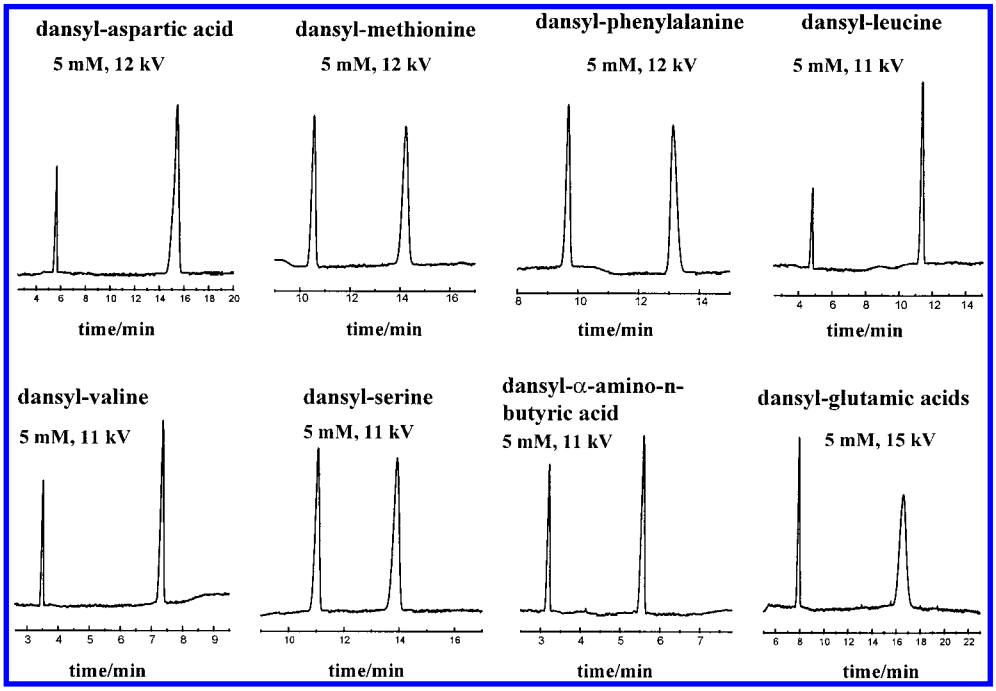

Figure 16. Typical electropherograms of chiral amphoteric analytes. The numbers next to the structures indicate the ODAS-γCD concentrations (mM) and the applied effective potentials (kV). Capillary: 25 μm i.d. 19 cm/26 cm effective/total length, uncoated fused silica. Other conditions: see Experimental Section.

16. The numbers next to the electropherograms indicate the actual ODAS-γCD concentrations and effective separation potentials. Figure 14 shows the separation of the enantiomers of noncharged and weak-acid analytes, Figure 15 those of the basic analytes, Figure 16 the amphoterics. In general, the peak-resolution values are quite adequate, even when the separations take only 10−15 min. It is noteworthy that ODAS-γCD affords much greater peak resolution values for the dansyl amino acids than the corresponding HDAS-βCD.

CONCLUSIONS

A new, single-isomer sulfated CD, the sodium salt of octakis-(2,3-diacetyl-6-sulfato)-γ-cyclodextrin, has been synthesized, analytically characterized, and used to separate the enantiomers of a variety of noncharged, weak-acid, weak-base, and amphiprotic analytes in low pH BEs. Both the effective mobilities and the separation selectivites were found to be in agreement with the predictions of the CHARM model of CE enantiomer separations.[22]

ACKNOWLEDGMENT

Partial financial support of this project by the Texas Coordination Board of Higher Education ARP program (Project Number 010366-016), the NSF-REU program (Grant Number CHE9322109), the R.W. Johnson Pharmaceutical Research Institute (Springhouse, PA), J&W Scientific, Inc. (Folsom, CA), and Regis Technologies (Morton Grove, IL) is gratefully acknowledged. We are indebted to Beckman Instruments (Fullerton, CA) and Arqule (Boston, MA) for the loan of the P/ACE unit and the DDL-31 evaporative light scattering detector, respectively, to Dr. J. J. Kirkland (Hewlett-Packard, Newport, MD) for the Bidentate C18 Zorbax column used for the nonaqueous reversed-phase HPLC separations, and to Cerestar (Hammond, IN) for the γ-cyclodextrin sample used in the synthesis of ODAS-γCD.

Received for review September 8, 1999. Accepted November 5, 1999.

AC991039T