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A Family of Single-Isomer Chiral Resolving Agents for Capillary Electrophoresis. 2. Hepta-6-sulfato-β-cyclodextrin

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A new, hydrophilic, single-isomer charged cyclodextrin, the sodium salt of hepta-6-sulfato-β-cyclodextrin has been synthesized, characterized, and used for the capillary electrophoretic separation of the enantiomers of numerous noncharged, acidic, basic, and zwitterionic analytes. Hepta-6-sulfato-β-cyclodextrin proved to be a much stronger complexing agent for all the analytes tested, in both low-pH and high-pH background electrolytes, than the previously synthesized, moderately hydrophobic heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin. The separation selectivities of the two single-isomer, differently functionalized charged cyclodextrins often proved to be complementary. In agreement with the predictions of the charged resolving agent migration model, separation selectivity for the noncharged analytes decreased as the concentration of hepta-6-sulfato-β-cyclodextrin was increased. For acidic, basic, and zwitterionic analytes, selectivity could increase, decrease, or pass a maximum, depending on the binding strength of the enantiomers and ionic mobilities of both the complexed and noncomplexed forms of the enantiomers.

In part 1 of this series,[1] we described the synthesis and capillary electrophoretic (CE) use of a new, moderately hydrophobic, single isomer, fully charged anionic cyclodextrin, heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin. The material was found to obey the separation characteristics predicted by the charged resolving agent migration model (CHARM model) of CE enantiomer separations[2] and proved successful for the separation of enantiomers belonging to very different compound classes.[1] However, it is well-known from the CE use of noncharged cyclodextrins that different functional groups connected to the 2- and 3-positions of the glucose moieties of the cyclodextrins result in different enantioselectivities.[3] It is reasonable to expect that the same holds true for the charged cyclodextrins as well. Unfortunately, the commercially available charged cyclodextrins are complicated mixtures of isomers which differ in their degree of substitution and their substitution patterns. It was pointed out in ref 1 that resolving agent mixtures are not as desirable as pure, single-isomer resolving agents, because (i) the different isomers may possess different chiral selectivities, (ii) they may possess different, finite complexation rates, which lead to kinetic broadening, and (iii) the composition of a particular mixture of resolving agent isomers might change from batch to batch and might result in irreproducible separations. Therefore, we proceeded to synthesize another, well-characterized, single-isomer charged cyclodextrin, hepta-6-sulfato-β-cyclodextrin that (i) offers intermolecular interactions different from the previously described first member of the family,[1] (ii) has the same functional groups on the 2- and 3-positions of the glucose moieties as native β-cyclodextrin and, (iii) is a maximally charged strong electrolyte that can be used at any pH without compromising either separation selectivity or efficiency.[2,4,5] This paper describes the synthesis, characterization, and use of hepta-6-sulfato-β-cyclodextrin (HS-βCD), the most hydrophilic member of our new, single-isomer, charged cyclodextrin family.

EXPERIMENTAL SECTION

Synthesis of Hepta-6-sulfato-β-cyclodextrin. The chemicals used in the synthesis and the CE application of hepta-6-sulfato-β-cyclodextrin were purchased from Aldrich Chemical Co. (Milwaukee, WI), except for β-cyclodextrin, which was a gift from Cerastar (Hammond, IN). The sodium salt of hepta-6-sulfato-β-cyclodextrin was synthesized according to Figure 1 by combining known derivatization and purification steps. The first intermediate, heptakis-[6−(*tert*-butyldimethyl)silyl]-β-cyclodextrin was synthesized according to ref 6 and purified by gradient elution preparative column chromatography[7] on silica gel using a simple eluent system, *n*-hexane/ethyl acetate/ethanol.[6] (The 50 mm i.d., 300 mm long preparative HPLC column packed with 30 nm pore size, 10 μm irregular silica (Merck, Darmstadt, Germany) was generously loaned by Dr. Y. Y. Rawjee of Smith-Kline Beecham, King of Prussia, PA) The second intermediate was prepared by acetylation with acetic anhydride[6] and purification by gradient elution preparative column chromatography on silica gel using *n*-hexane/ethyl acetate/ethanol as eluent.[6] In the third step, the protecting silyl group was removed by boron trifluoride etherate[6] and the product was repurified as in the first step. The pure third intermediate was sulfated with SO_3·pyridine,[8] freed of the sodium sulfate byproduct,[1] and, finally, deacetylated by dissolving the last intermediate in water, raising the pH of the solution to 12 with NaOH, adding 25% methanol, and stirring the mixture for 12 h. Once indirect UV detection CE[1,9] indicated that the reaction was complete, the reaction mixture was poured into ethanol, and the solid was collected by filtration, washed with ethanol, and dried

(1) Vincent, B. J.; Sokolowski, A. D.; Nguyen, T. V.; Vigh, Gy. *Anal. Chem*. **1997**, *69*, 4226.
(2) Williams, B. À.; Vigh, Gy. *J. Chromatogr.* **1997**, *777*, 43.
(3) Fanali, S. *J. Chromatogr., A* **1996**, *735*, 77.
(4) Friedl, W.; Kenndler, E. *Anal. Chem.* **1993**, *65*, 2003.
(5) Rawjee, Y. Y.; Vigh, Gy. *Anal. Chem.* **1994**, *66*, 428.
(6) Takeo, K.; Hisayoshi, M.; Uemura, K. *Carbohydr. Res.* **1989**, *187*, 203.
(7) Vigh, Gy.; Quintero, G.; Farkas, Gy. *J. Chromatogr.* **1989**, *484*, 237.
(8) Bernstein, S.; Joseph, J.; Nair, U. U.S. Patent 4,020,160, 1997.
(9) Nardi, A.; Fanalli, S., Foret, F. *Electrophoresis* **1990**, *11*, 774.

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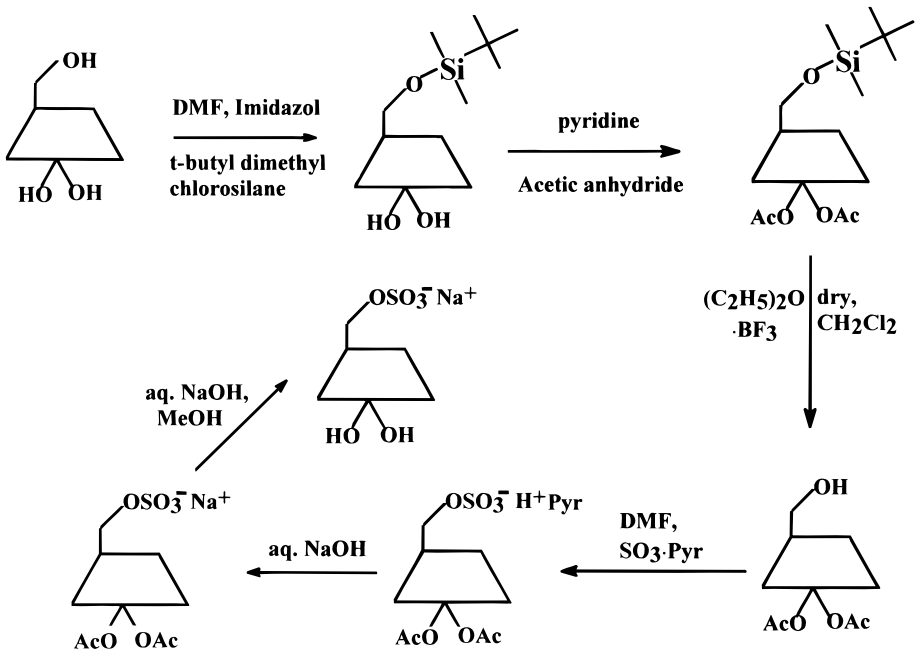


Figure 1. Synthesis scheme for hepta-6-sulfato-β-cyclodextrin.

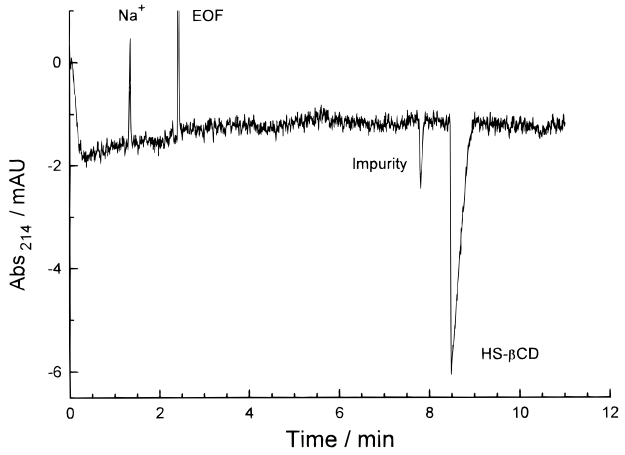


Figure 2. Indirect UV detection electropherogram of a typical hepta-6-sulfato-β-CD sample: BE, 20 mM *p*-toluenesulfonic acid, pH 8, adjusted with Tris; wavelength, 214 nm; applied potential, 25 kV, thermostat temperature, 25 °C; capillary, 25 μm i.d.; 39/45 cm effective/total length; uncoated fused silica.

in a vacuum oven at 80 °C yielding the end product, pure hepta-6-sulfato-β-CD. The purity of the final product was determined by indirect UV detection CE using 20 mM *p*-toluenesulfonic acid (PTSA) as background electrolyte (BE), whose pH was adjusted to 8 with tris(hydroxymethyl)aminomethane (Tris).[1] The electropherogram of a typical hepta-6-sulfato-β-CD sample is shown in Figure 2. The 200 MHZ ^{1}H NMR spectrum of the final product is shown in Figure 3; the narrow, well-defined lines also attest to the purity of the final product.

Electrophoretic Separations Using Hepta-6-sulfato-β-cyclodextrin. All CE experiments were carried out with a P/ACE 2200 CE system (Beckman Instruments, Fullerton, CA). The wavelength of the UV detector was set at 214 nm, the separation temperature was 20 °C. Untreated, 25 μm i.d. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ) were used with a nominal total length of 45 cm and nominal injector-to-detector length of a 39 cm. The samples were pressure injected for 1 s at 5 psi.[10] The power dissipation was maintained between 500 and 700 mW/m, brought about by 12−20 kV applied potentials.

As in part 1, both a low-pH and a high-pH buffer stock solution was prepared by adding 0.0250 mol of concentrated phosphoric acid (for the low-pH buffer) and 0.0250 mol ethanolamine (for the high-pH buffer) to enough deionized water (Milli-Q, Millipore, Milford, MA) to obtain solutions of ~0.95 L. Using a combination glass electrode and a precision pH meter (Corning Science Products, Corning, NY), these solutions were titrated to pH 2.5 and pH 9.5, respectively, with aqueous LiOH and aqueous methansulfonic acid to achieve solutions with similar ionic strengths. After the solutions were quantitatively transferred to 1 L volumetric flasks, they were diluted to the mark with deionized water and their pH was measured again.

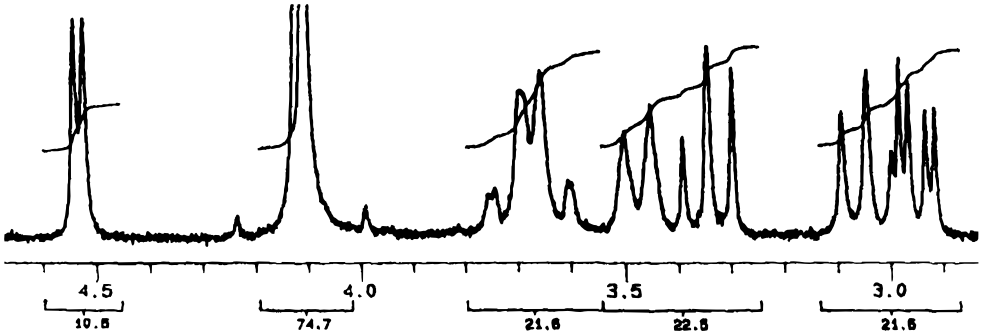


Figure 3. ^{1}H NMR spectrum of a typical hepta-6-sulfato-β-CD sample. Solvent, D_2O.

Table 1. Effective Mobilities of the Less Mobile Enantiomer (μ, in 10^{-5} cm^2/(V s)), Separation Selectivities (α), Measured Peak Resolution Values (Rs), Dimensionless EO Flow Mobilities (β), and Injector-to-Detector Potential Drop (U in kV) in Low-pH (pH 2.5) HS-β-CD BEs

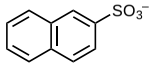
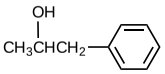
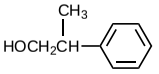
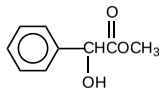
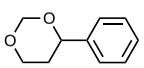
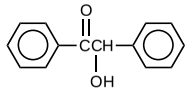
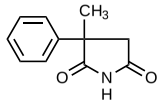
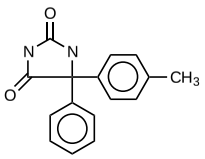
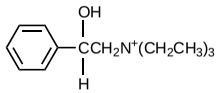
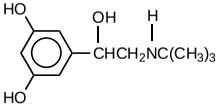
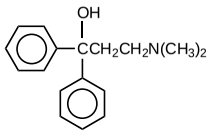
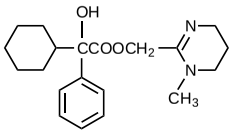
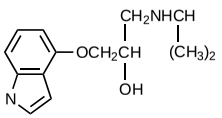
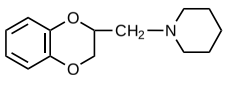
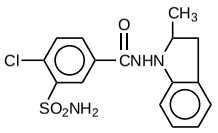
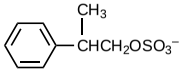
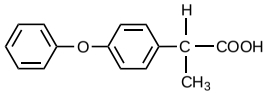
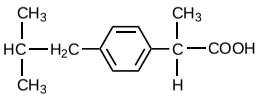
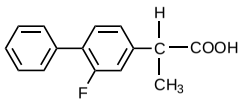
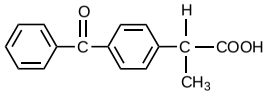
structure	10 mM					15 mM					30 mM					50 mM				
	μ	α	Rs	β	U	μ	α	Rs	β	U	μ	α	Rs	β	U	μ	α	Rs	β	U
Marker																				
	−32.33 (±0.03)					−31.73 (±0.09)					−30.42 (±0.08)					−29.19 (±0.09)				
Neutrals																				
	−19.4	1.05	1.0	−0.1	16.8	−19.7	1.03	0.8	−0.1	17.2	−21.9	1.02	<0.5	−0.2	12.9	−22.4	<1.01	<0.5	−0.3	10.3
	−22.1	1.03	1.3	<−0.1	17.2	−22.2	1./02	0.8	−0.1	17.2	−24.2	1.01	<0.5	−0.2	12.9	−24.0	<1.01	<0.5	−0.3	10.3
	−7.1	1.10	3.2	−0.3	2.8[a]	−8.0	1.10	5.4	−0.4	17.2	−9.6	1.10	4.7	−0.1	12.9	−11.8	1.07	2.1	−0.1	1.7[a]
	−20.7	1.05	1.9	−0.1	16.8	−20.9	1.03	1.3	−0.1	17.2	−23.6	1.02	1.1	−0.2	12.9	−23.6	1.01	1.0	−0.3	10.3
	−25.4	1.10	3.3	<−0.1	16.8	−25.7	1.05	2.3	−0.2	17.2	−25.1	1.03	2.2	−0.2	12.9	−25.1	1.02	1.7	−0.3	10.3
	−21.6	1.05	1.3	<−0.1	17.2	−21.7	1.04	0.9	−0.1	17.2	−24.0	1.02	0.9	−0.2	12.9	−23.2	1.02	0.8	<−0.1	10.3
	−27.9	1.03	1.3	<−0.1	17.2	−27.8	1.01	0.8	−0.2	17.2	−26.1	1.00	0.0	−0.2	12.9	−25.3	1.00	0.0	−0.3	10.3
Bases																				
	1.1	1.12	1.0	1.5	2.8[a]	0.7	2.49	4.3	1.8	2.8[a]	0.2	4.76	4.3	2.1	2.1[a]	−0.9	1.96	4.7	−4.9	1.7[a]
	−18.3	1.12	2.9	−0.1	17.2	−18.6	1.10	2.7	<−0.1	17.2	−17.2	1.07	3.9	−0.1	12.9	−15.5	1.07	4.0	−0.2	12.9

Table 1 (Continued)

structure	10 mM					15 mM					30 mM					50 mM				
	μ	α	Rs	β	U	μ	α	Rs	β	U	μ	α	Rs	β	U	μ	α	Rs	β	U
	−20.0	1.05	1.9	−0.2	17.2	−18.7	1.05	2.0	−0.2	17.2	−17.5	1.03	2.2	−0.2	12.9	−17.4	1.03	1.9	−0.1	10.3
	−38.3	1.00	0.0	−0.3	17.2	−34.9	1.01	<0.5	−0.2	17.2	−28.6	1.02	1.3	−0.2	12.9	−25.4	1.03	2.4	−0.3	10.3
	−23.4	1.01	0.7	−0.2	17.2	−20.9	1.01	0.7	−0.1	17.2	−18.8	1.01	1.0	−0.2	12.9	−17.3	<1.01	<0.5	<−0.1	10.3
	−29.5	1.03	1.0	<−0.1	17.2	−27.8	1.02	1.0	−0.2	17.2	−24.0	1.02	1.2	−0.2	12.9	−21.9	1.02	1.5	−0.3	10.3
Acids																				
	−7.6	1.19	9.5	−0.3	17.2	−8.3	1.18	9.4	−0.2	17.2	−10.6	1.15	8.3	−0.2	12.9	−12.8	1.11	7.6	−0.1	12.9
	−34.1	1.01	0.7	−0.3	17.2	−31.8	1.01	1.0	−0.2	17.2	−30.4	1.01	<0.5	−0.2	12.9	−28.7	1.01	<0.5	−0.3	10.3
	−35.7	<1.01	<0.5	−0.2	17.2	−34.3	<1.01	<0.5	−0.2	17.2	−29.7	<1.01	<0.5	−0.3	12.9	−27.7	<1.01	<0.5	<−0.1	10.3
	−38.5	1.00	0.0	−0.3	17.2	−35.1	1.00	0.0	−0.2	17.2	−30.4	1.00	0.0	−0.3	12.9	−27.7	1.00	0.0	<−0.1	10.3
	−36.7	1.00	0.0	−0.2	17.2	−34.6	1.00	0.0	−0.2	17.2	−30.0	1.00	0.0	−0.2	12.9	−26.7	1.00	0.0	−0.3	10.3
	−25.6	1.02	1.1	−0.2	17.2	−25.6	1.01	0.9	−0.1	17.2	−25.5	1.01	0.9	−0.2	12.9	−25.4	1.01	0.9	−0.3	10.3

	−32.8	1.02	0.8	−0.2	17.2	−31.0	1.01	0.7	−0.2	17.2	−28.5	1.01	<0.5	−0.3	12.9	−26.6	<1.01	<0.5	−0.3	10.3
	−6.4	1.04	0.9	−0.1	2.8[a]	−7.4	1.05	1.0	<−0.1	17.2	−9.3	1.03	0.9	−0.2	12.9	−11.9	1.02	0.8	−0.1	1.7[a]
Zwitterions																				
	−9.9	1.03	1.1	−0.2	2.8	−10.3	1.03	1.7	<−0.1	17.2	−11.9	1.02	1.3	−0.3	12.9	−16.5	1.02	0.7	−0.2	1.7[a]

[a] Analyte injected from the short (detector) side of the capillary.

The low-pH and the high-pH BEs, which contained 10, 15, 30, and 50 mM HS-βCD, were prepared from the buffer stock solutions. As in part 1,[1] the external EO flow marker method described in ref 10 was used to determine the true effective mobility ($\mu^{\text{eff}}_{\text{NSA}-}$) of 2-naphthalenesulfonic acid (NSA^-) in each HS-βCD BE (Table 1). When NSA^- is added to each analyte sample, it can serve as an internal mobility marker for the calculation of the effective mobilities of the analytes, because the electroosmotic flow mobility, μ_{EO}, can be obtained as $\mu_{\text{EO}} = \mu^{\text{obs}}_{\text{NSA}-} - \mu^{\text{eff}}_{\text{NSA}-}$, and the effective mobilities of the enantiomers as $\mu^{\text{eff}}_{\text{R}} = \mu^{\text{obs}}_{\text{R}} - \mu_{\text{EO}}$. Separation selectivity, α, was calculated as $\alpha = \mu^{\text{eff}}_{\text{R}}/\mu^{\text{eff}}_{\text{S}}$, where subscript S refers to the less mobile enantiomer. The dimensionless electroosmotic mobility values, β, were calculated as $\beta = \mu_{\text{EO}}/\mu^{\text{eff}}_{\text{S}}$.[5] The observed peak resolution values, Rs, were calculated as usual, $\text{Rs} = 2\,(t_{\text{S}} - t_{\text{R}})/(w_{\text{S}} - w_{\text{R}})$, where t_{S} and t_{R} are the observed migration times and w_{S} and w_{R} are the observed peak widths of enantiomers.

RESULTS AND DISCUSSION

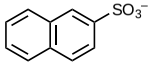
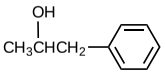
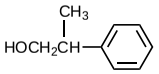
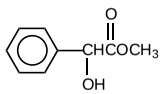
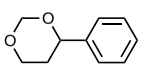
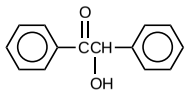
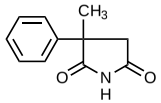
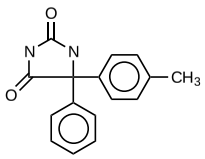
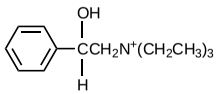
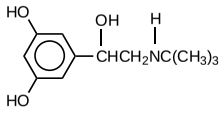
Effective Electrophoretic Mobilities of 2-Naphthalenesulfonate in HS-βCD BEs. Since the knowledge of accurate NSA^- effective mobilities, $\mu^{\text{eff}}_{\text{NSA}-}$, is crucial for the calculation of meaningful selectivities, the $\mu^{\text{eff}}_{\text{NSA}-}$ determinations were repeated five times in each BE. The results are shown in the first lines of Tables 1 (low-pH BEs) and 2 (high-pH BEs). Just as with the heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-βCD),[1] the $\mu^{\text{eff}}_{\text{NSA}-}$ values decrease as the HS-βCD concentration is increased because the viscosity and the ionic strength of the BE, as well as the extent of complexation with HS-βCD, increase. However, the $\mu^{\text{eff}}_{\text{NSA}-}$ values are about 10−15% larger with HS-βCD than with HDAS-βCD,[1] indicating that HS-βCD complexes more strongly than the acetylated analog. In the high-pH BEs, $\mu^{\text{eff}}_{\text{NSA}-}$ is again consistently lower than in the low-pH BEs, indicating that the high-pH BE constituents (ethanol amine/ethanol ammonium and methanesulfonate) compete more strongly with NSA^- for binding to HS-βCD than the low-pH BE constituents (phosphoric acid/dihydrogen phosphate and lithium ion), just as they did with HDAS-βCD.[1]

Separation of Enantiomers in HS-βCD-Containing BEs. A series of neutral, strong base, weak base, weak acid, and zwitterionic enantiomers were separated with both low-pH and high-pH BEs. Many of these compounds are the same that we used for the characterization of HDAS-βCD[1] in order to facilitate the comparison of the complexing properties and selectivities of the two differently funtionalized CDs. The effective mobilities of the less mobile enantiomers (μ), the separation selectivities (α), the measured peak resolution values (Rs), the corresponding dimensionless EO flow values (β), and the injector-to-detector potential drop (U) are listed in Tables 1 and 2.

For neutral analytes, both at low-pH and at high-pH, the anionic effective mobilities are about 5−10 times higher with HS-βCD than with HDAS-βCD (Tables 1 and 2 in ref 1), indicating much stronger complexation with HS-βCD. Some of the increased mobility could be due to the higher charge-to-mass ratio of HS-βCD over that of HDAS-βCD, but the decrease in molecular weight (∼35%) alone is not enough to explain the large increase of mobilities. At identical charged cyclodextrin concentrations, the separation selectivities with HS-βCD are significantly lower than with HDAS-βCD. For example, for methyl mandelate in the low-

(10) Williams, B. A.; Vigh, Gy. *Anal. Chem.* **1997**, *69*, 4410−4418.

Table 2. Effective Mobilities of the Less Mobile Enantiomer (μ, in 10^{-5} cm^2/(V s)), Separation Selectivities (α), Measured Peak Resolution Values (Rs), Dimensionless EO Flow Mobilities (β), and Injector-to-Detector Potential Drop (U in kV) in High-pH (pH 9.5) HS-β-CD BEs[a]

structure	10 mM					15 mM					30 mM					50 mM				
	μ	α	Rs	β	U	μ	α	Rs	β	U	μ	α	Rs	β	U	μ	α	Rs	β	U
Marker																				
	−24.65 (±0.05)					−24.01 (±0.07)					−22.53 (±0.04)					−20.04 (±0.09)				
Neutrals																				
	−7.2	1.12	<0.5	−7.3	17.2	−11.3	1.03	<0.5	−2.3	17.2	−13.3	1.03	<0.5	−1.7	12.8	−13.1	1.02	<0.5	−1.5	10.3
	−8.2	1.10	<0.5	−6.3	17.2	−11.2	1.04	<0.5	−2.6	17.2	−14.6	1.02	<0.5	−1.9	12.9	−13.1	1.02	<0.5	−1.5	10.3
	−1.2	1.75	1.6	−44.4	17.2	−1.5	1.59	1.6	−22.8	17.2	−2.2	1.44	1.9	−11.1	12.9	−2.8	1.40	1.3	−7.4	10.3
	−7.5	1.13	<0.5	−6.6	17.2	−10.5	1.07	<0.5	−4.2	17.2	−12.7	1.04	0.8	−1.7	12.8	−11.8	1.03	0.8	−1.6	10.3
	−15.7	1.15	4.9	−2.4	17.2	−17.5	1.08	5.8	−2.0	17.2	−17.3	1.04	4.5	−1.6	12.9	−14.9	1.04	2.5	−1.3	*1.7
	−18.0	1.07	1.7	−2.2	17.2	−18.7	1.07	1.9	−1.8	17.2	−18.4	1.06	3.0	−1.3	12.8	−16.6	1.06	4.0	−1.1	*1.7
	−19.8	1.12	49	−2.1	17.2	−21.5	1.05	3.1	−2.1	17.2	−20.6	1.03	3.9	−1.4	12.9	−18.0	1.01	1.6	−1.1	*2.1
Bases																				
	9.9	1.05	0.7	3.8	17.2	9.4	1.07	0.9	2.5	17.2	7.6	1.13	2.0	2.5	12.8	5.0	1.24	2.4	3.3	12.8
	−5.5	1.51	2.6	−9.0	17.2	−7.4	1.31	2.9	−3.6	17.2	−10.1	1.15	4.5	−2.3	12.8	−10.0	1.12	6.1	−2.1	10.3

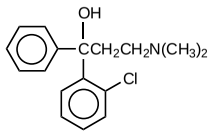
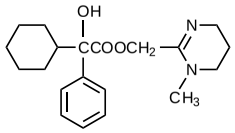
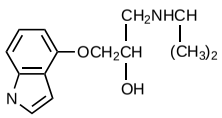
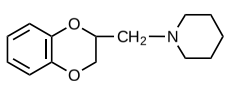
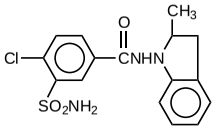
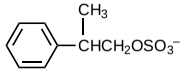
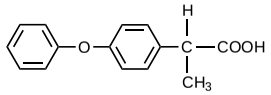
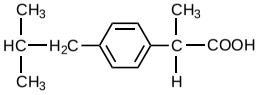
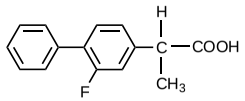
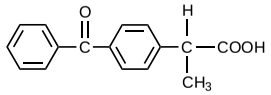
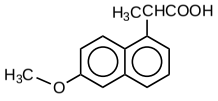
	−17.1	1.13	4.0	−2.4	17.2	−16.8	1.09	5.3	−1.9	17.2	−15.8	1.06	5.1	−1.8	12.8	−13.8	1.04	3.7	−1.4	12.8
	−30.0	1.01	1.0	−1.7	17.2	−26.9	1.02	1.0	−1.6	17.2	−19.7	1.03	2.2	−1.5	12.9	−15.5	1.04	3.0	−1.3	1.7[a]
	−12.0	1.00	0.0	−4.3	17.2	−12.1	1.00	0.0	−3.8	17.2	−11.2	1.01	0.7	−2.1	12.8	−9.7	1.01	<0.5	−2.1	10.3
	−20.7	1.03	0.8	−1.9	17.2	−20.9	1.02	0.8	−2.1	17.2	−18.6	1.02	0.9	−1.6	12.9	−15.9	1.00	0.0	−1.2	1.7[a]
Acids																				
	−4.8	1.15	1.2	−10.1	17.2	−6.1	1.12	1.9	−5.4	17.2	−7.2	1.12	4.0	−3.2	12.8	−8.2	1.11	5.6	−2.3	12.9
	−23.1	1.02	0.9	−2.3	17.2	−22.5	1.02	0.7	−2.0	17.2	−21.0	1.02	0.7	−1.4	12.9	−19.0	1.01	0.8	−1.1	2.1[a]
	−25.7	1.01	0.8	−2.0	17.2	−25.0	1.01	1.0	−1.4	17.2	−23.0	1.01	0.8	−1.2	12.9	−20.1	1.01	0.8	−1.1	*2.1
	−29.5	1.01	<0.5	−1.7	17.2	−28.5	1.01	<0.5	−1.3	17.2	−24.3	1.01	<0.5	−1.2	12.9	−20.2	1.00	0.0	−1.1	1.7[a]
	−29.3	1.01	<0.5	−1.8	17.2	−28.4	1.01	<0.5	−1.6	17.2	−24.2	1.00	0.0	−1.2	2.1	−20.3	1.00	0.0	−0.7	12.9
	−19.7	1.01	<0.5	−2.1	17.2	−20.2	1.01	<0.5	−1.7	17.2	−19.5	1.01	<0.5	−1.5	12.9	−18.0	<1.01	<0.5	−0.7	12.9
	−20.9	1.03	0.9	−1.9	17.2	−21.2	1.03	1.0	−2.1	17.2	−21.1	1.02	1.4	−1.4	12.9	−18.8	1.01	2.0	−0.7	12.9

Table 2 (Continued)

structure	10 mM					15 mM					30 mM					50 mM				
	μ	α	Rs	β	U	μ	α	Rs	β	U	μ	α	Rs	β	U	μ	α	Rs	β	U
	−26.3	1.02	1.5	−2.0	17.2	−24.8	1.03	2.0	−1.8	17.2	−21.6	1.05	4.8	−1.4	12.9	−19.2	1.04	4.1	−1.1	1.7[a]
	−12.7	1.05	1.4	−4.0	17.2	−13.0	1.06	2.5	−2.4	17.2	−14.0	1.05	4.4	−1.6	12.8	−14.3	1.05	3.8	−1.4	10.3
	−16.7	1.06	2.5	−3.1	17.2	−17.4	1.05	3.1	−1.9	17.2	−18.4	1.01	3.8	−1.1	2.1[a]	−16.9	1.00	0.0	−1.1	1.7[a]

Zwitterions

[a] Analyte injected from the short (detector) side of the capillary.

Figure 4. Typical electropherograms of neutral analytes in low-pH BEs. The numbers next to the structures indicate the HS-βCD concentrations (mM): applied effective potential, 17 kV; capillary, 25 μm i.d., 39/45 cm effective/total length, uncoated fused silica. For other conditions, see the Experimental Section.

pH BE at 10 mM charged CD concentration, $\alpha = 1.31$ with HDAS-βCD,[1] but only $\alpha = 1.10$ with HS-βCD, while the effective mobilities are −0.5 vs −7.1 (in 10^{-5} cm^2/Vs units), confirming the presence of strong, but parasitic (nonselective) complexation with HS-βCD. Greater selectivity alone does not automatically translate to greater resolution, because β and the effective charge of the analyte also have important effects on peak resolution.[2] For example, for (α-methyl-α-phenylsuccinimide) in the low-pH BE at 10 mM charged CD concentration, $\alpha = 1.05$ with HS-βCD and $\alpha = 1.50$ with HDAS-βCD. Yet, Rs = 1.3 with HS-βCD and Rs < 0.5 with HDAS-βCD, because $\beta = 0$ with HS-βCD and $\beta = -57$ with HDAS-βCD. Thus, the successful use of the two differently functionalized charged CDs, HDAS-βCD and HS-βCD, requires very different experimental conditions.

Due to solubility limitations, weak acids could not be analyzed at low-pH with HDAS-βCD.[1] However, since HS-βCD complexes with all the analytes more strongly than HDAS-βCD, weak acids became soluble in HS-βCD-containing BEs and could be separated by CE with α values hovering around $\alpha = 1.02$ and Rs values ranging between 0.5 and 1. Flurbiprofen and ibuprofen, which could not be separated at low-pH with HS-βCD, were at least partially separated in the high-pH BEs with HS-βCD, similarly to what was observed with HDAS-βCD.[1]

For strong bases, the cationic effective mobility decreases (and eventually becomes anionic) as the concentration of HS-βCD is increased. Though very similar viscosities were measured in the high-pH BEs and the low-pH BEs, the cationic mobilities of the strong bases are much higher in the high-pH HS-βCD BEs than in the low-pH BEs, once again confirming that the high-pH BE constituents compete very effectively with the analyte for the HS-βCD. In agreement with the predictions of the CHARM model (Figure 6 in ref 3), separation selectivity increases with the concentration of HS-βCD as long as the component migrates cationically; then selectivity decreases as the strong base begins to migrate anionically. In fact, selectivity becomes so high around the changeover point that an Rs = 4 can be obtained even when

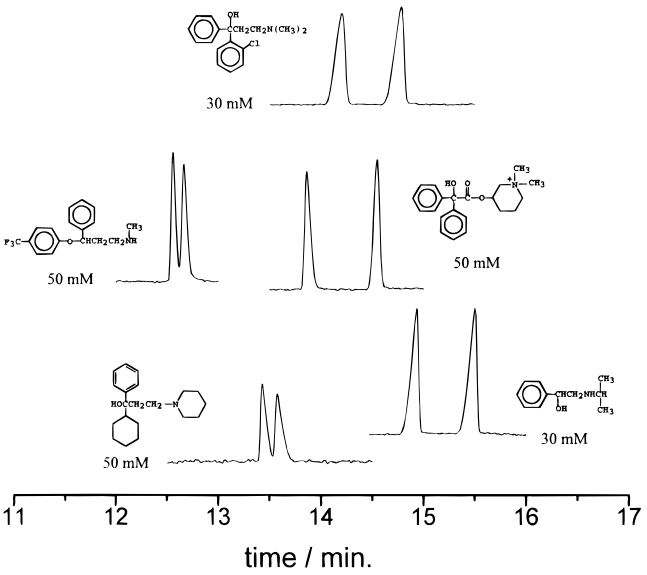


Figure 5. Typical electropherograms of weak base analytes in low-pH BEs. Conditions as in Figure 4, except applied effective potential, 12 kV.

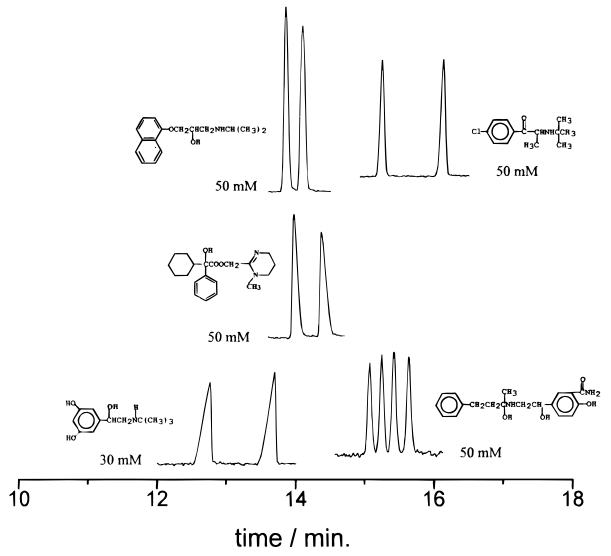


Figure 6. Typical electropherograms of weak base analytes in low-pH BEs. Conditions as in Figure 5.

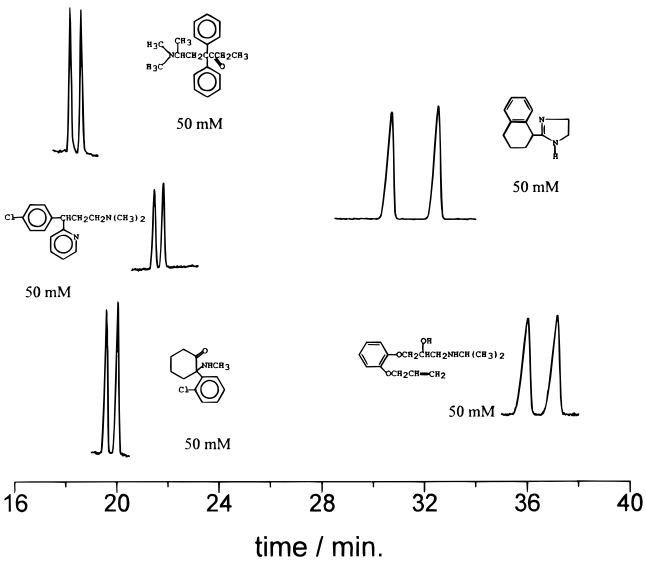


Figure 7. Typical electropherograms of weak base analytes in low-pH BEs. Conditions as in Figure 5.

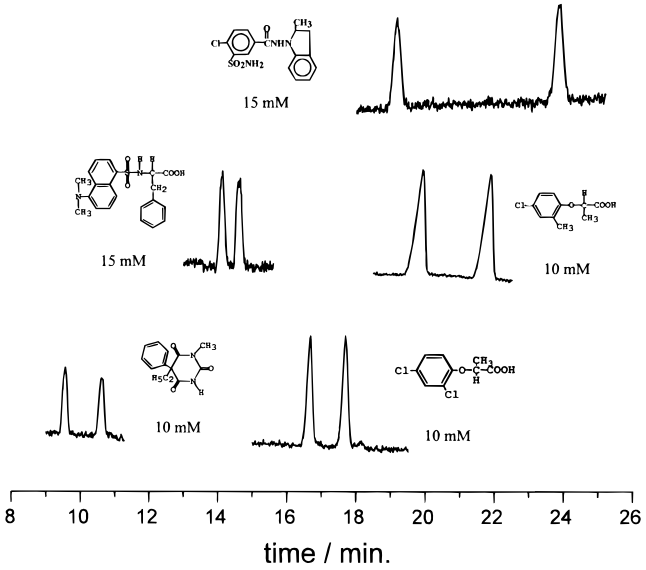


Figure 8. Typical electropherograms of weak acid and zwitterionic analytes in low-pH BEs. Conditions as in Figure 4.

the separation is carried out from the short side of the capillary (effective voltage 2.1 kV, Table 1).

In general, weak bases studied here migrate anionically both in the low-pH and the high-pH BEs: their effective mobilities decrease as the concentration of HS-βCD is increased. The only exception is terbutaline, which is only slightly dissociated in the high-pH BE and behaves more or less as a neutral species. In low-pH BEs (and for the most part, in high-pH BEs as well), separation selectivity is more or less constant, or decreases slightly, as the concentration of HS-βCD is increased. The only exception is oxyphencyclamine, which has a very high anionic mobility at very low HS-βCD concentrations, and whose selectivity increases with the concentration of HS-βCD. This again agrees with the predictions of the CHARM model.[3]

A few typical separations are shown in Figures 4−8: the numbers next to the electropherograms indicate the concentration of HS-βCD (in mM). All BEs were made with the low-pH stock buffer. Figure 4 shows the separation of neutral analytes, Figures 5−7 those of the basic analytes, and Figure 8 the weak acid and zwitterionic analytes. As with HDAS-βCD,[1] the peak resolution values are quite large, allowing for the optimization of these separations to meet diverse analytical objectives.

CONCLUSIONS

A new, hydrophilic, single-isomer charged cyclodextrin, the sodium salt of hepta-6-sulfato-β-cyclodextrin has been synthesized, characterized, and used to separate the enantiomers of neutral, weak acid, strong base, weak base, and zwitterionic analytes in pH 2.5 and pH 9.5 BEs. As predicted by the CHARM model of CE enantiomer separations[3] and first verified in part 1 of this series with heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin, separation selectivity for the neutral analytes decreases as the concentration of HS-β-CD increases. For charged analytes, α can increase, decrease, or pass a maximum, depending on the numeric values of the complexation constants and ionic mobilities. The dimensionless electroosmotic flow mobility, β,[6] greatly influences the magnitude of peak resolution obtained with HS-β-CD. HS-β-CD complexed more strongly with most analytes than HDAS-β-CD

and the two materials generally offered different separation selectivities, indicating that both materials could play an important role in the arsenal of separation scientists engaged in CE enantiomer separations.

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