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Characterization of highly sulfated cyclodextrins

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Abstract

A class of highly sulfated cyclodextrins (HS-CDs) was developed for enantiomeric separation of chiral compounds by capillary electrophoresis (CE). The HS-CDs were produced by a facile single-step direct sulfation of cyclodextrin using sulfur trioxide–trimethylamine complex in dimethylformamide. Characterization of the HS-CDs by electrospray ionization mass spectrometry and by CE using a well-established indirect detection method indicated the species have very narrow heterogeneity in terms of degree of sulfation. Elemental analysis of the HS-α-, β- and γ-CDs showed that the average sulfate contents were 11, 12, and 13 per CD molecule, respectively. The ^{13}C NMR of HS-CDs is consistent with the structural assignment of nearly complete sulfation at C-6 primary hydroxyl groups and partial sulfation at the C-2 secondary hydroxyls (>70%), while the C-3 hydroxyls remain unsubstituted. Enantiomeric separation by CE using the HS-CDs as chiral selectors showed that HS-α-, β- and γ-CDs complement each other by exhibiting different chiral selectivities, resulting in resolution of many chiral neutral, acidic and basic compounds of greatly varying structural features. The part of HS-CD that interacts with the guest molecule during complexation and, therefore, the receiving end of the cyclodextrin hydrophobic bucket was surrounded with largely regiospecifically substituted C-2 sulfates and intact C-3 hydroxyls, both at the equatorial positions. Such global regiospecific structural arrangement in HS-CDs provides differential diasteroisomeric complexation is proposed to be the principal contributing factor in the resolving racemates. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Enantiomer separation; Cyclodextrins

1. Introduction

The use of cyclodextrins for differential host–guest complexation of enantiomeric pairs of compounds is by far the most common strategy employed for chiral separation by capillary electrophoresis (CE) [1–3]. In contrast to liquid chromatography in which chiral selectors are immobilized to the solid support, chiral analyses by CE are greatly simplified by the use of separation buffer containing dissolved chiral selector. For enantiomeric separation of acidic and basic compounds by CE using neutral cyclodextrins, the complex migrates under the influence of the charge of the ionic analytes. For neutral racemates however, the uncharged cyclodextrins are not applicable because the analyte, the cyclodextrins, and the complex have no electrophoretic mobilities. The use of charged cyclodextrins [4–10] was therefore motivated by the need to separate enantiomers of neutral compounds.

This paper reports the characterization of a class of highly sulfated cyclodextrins (HS-CDs) developed for CE-based chiral separation. Enantiomeric separation by CE using the HS-CDs as chiral selectors showed that compounds with a wide variety of structural features were resolved under one generalized set of conditions using phosphate buffer containing 5% HS-CDs at pH 2.5. The chiral selec-

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tivities of the HS-α-, β-, and γ-CDs complement each other resulting in the resolution not only neutral but also basic and acidic racemates with widely varying structures [11–14].

2. Experimental

2.1. Materials

Hepta-6-sulfato β-CD with all sulfate esters at the C-6 primary hydroxyl of β-CD was synthesized according to the reported procedure [10]. HS-CDs (sodium as the counter ion) can be obtained as 20% (w/v) aqueous solutions from Beckman Coulter (Fullerton, CA, USA), and the synthesis is described later in the Experimental section. Sulfated β-CD with a low degree of sulfation was obtained from Cerestar (Hammond, IN, USA). Sulfated β-CD with an average degree of sulfation of 7 to 11 per β-CD was purchased from Aldrich (Milwaukee, WI, USA). Sulfobutyl ether β-cyclodextrin (SBE-β-CD) with an average of four sulfobutyl ether groups per β-CD moiety (100 m*M* in aqueous solution) was purchased from Perkin-Elmer ABI (Foster City, CA, USA).

2.2. Sample preparation

Racemates of drugs and pure enantiomers were obtained from Sigma (St. Louis, MO, USA) and from Research Biochemicals International (Natick, MA, USA). Basic compounds were dissolved in 0.5 *M* HCl at 10 to 20 m*M* as the stock solution and then diluted to a final working solution of 1 m*M* in water containing 0.25 m*M* pyrene-1,3,6,8-tetrasulfonate (PTS; Acros Chemicals, Pittsburgh, PA, USA). Neutral and acidic samples were dissolved in methanol or dimethylformamide (DMF) before dilution with water containing 0.25 m*M* PTS as the internal reference marker. Poly(ethylene oxide) (PEO, average M_r 300 000) was purchased from Aldrich. Methanol or DMF was used up to 25% in water as sample diluent for neutral species.

2.3. Synthesis of HS-β-CD

A solution of sulfur trioxide–trimethylamine complex (1.72 mol) in anhydrous DMF (300 ml) was added slowly over the period of 15 min to a 2-l round-bottom glass reactor containing β-cyclodextrin (88.1 mmol, dried overnight under reduced pressure in a 100°C vacuum and dessicated oven) dissolved in anhydrous DMF (500 ml). The reactor was stirred vigorously for an hour with an overhead mechanical stirrer and the reaction temperature was maintained at 50°C. A yellowish heavy gummy layer developed and the upper solvent layer was decanted while hot. The gummy product was washed with ethanol (2× 300 ml) and the resulting precipitate was solubilized and neutralized with 4.0 *M* sodium hydroxide (437.5 ml) to a homogeneous yellowish solution at pH of 10.0. The aqueous solution was concentrated to approximately 500 ml on a rotary evaporator under reduced pressure to remove trimethylamine and residual solvent. The concentrated liquid was filtered to eliminate particulate matter and the filtered aqueous solution was triturated in acetone and methanol to yield an off-white precipitate. The product was collected and dried in an oven maintained at 75°C under reduced pressure overnight to yield 200 g of the white powdery HS-β-CD (83.9 mmol).

2.4. Analysis

For all chiral separations, the analyses were performed in 5% HS-CDs in 25 m*M* triethylammonium phosphate, pH 2.5. All capillaries were rinsed with 0.4% PEO in 50 m*M* acetate (lithium salt) buffer at pH 4.75 solution at 50 p.s.i. for 4 min to reduce electroendoosmotic flow of the fused-silica capillary (1 p.s.i.=6894.76 Pa). The PEO rinsing procedure requires once daily to yield reproducible results. Samples were introduced by pressure injection at 0.3 p.s.i. for 4 to 8 s and detection was monitored at 200 nm. Fused-silica capillaries, 31.5 cm×25 μm I.D. were used for CE separations with an effective length of 10.5 cm in a P/ACE MDQ CE system (Beckman Coulter). The applied voltage was fixed at 30 kV with running current of 99 to 110 μA.

Indirect CE analysis [10,15–17] of various sulfated or sulfobutyl ether cyclodextrins was performed on a P/ACE 5500 CE system (Beckman Coulter) in a 27 cm×50 μm poly(vinyl alcohol) (PVA)-coated capillary (20 cm to the detector), and monitored at 254 nm. Sample was injected at 0.5 p.s.i. for 4 s at 5 mg/ml in water. Background buffer

electrolyte was 40 m*M* *p*-toluenesulfonic acid (TSA) in Tris base at pH 8.0 with an applied voltage of 10 kV at 11 μA.

For elemental and mass analysis, the HS-CDs were dialyzed in water to remove small-molecular-mass impurities using a dialysis membrane tube with an M_r 500 cut-off and the resulting dialysates were dried under reduced pressure to produce solid samples. Elemental analysis was performed by Galbraith Labs. (Knoxville, TN, USA). Mass spectra of HS-CDs were performed by Mass Consortium (San Diego, CA, USA) with a quadruple mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA) equipped with an electrospray ionization (ESI) device. Samples of HS-CDs were diluted in methanol–water (80:20) to 1.0 mg/ml for ESI mass spectrometry (MS). Nuclear magnetic resonance (NMR) was performed on a Varian XL-300 instrument at Beckman Coulter (Brea, CA, USA).

3. Results and discussion

In the indirect detection method for CE analysis of sulfated cyclodextrins, the non-UV absorbing sulfated cyclodextrins displace the ionic chromophore, TSA anion, which migrates electrophoretically to the anode to produce zones of lower UV absorbance than that of the background. The result is that the anionic analytes produce signals of negative absorbance as they pass the detector. The peaks are broader than those obtained with direct UV absorbance detection presumably due to imperfect match of mobilities between the sulfated cyclodextrins analytes and the TSA background chromophore. Characterization of the negatively charged cyclodextrins by CE with indirect detection using TSA as background electrolyte showed the distribution of varieties of sulfated-β-CD species in Fig. 1. The present HS-α-, β- and γ-CDs have a narrower heterogeneity (Fig. 1B, C and D, respectively) than other sulfated cyclodextrins (Fig. 1E–G). The presence of distinct species with degrees of sulfation from 1 to 6 and 1 to 5 is evident from the electropherograms in Fig. 1E (SBE-β-CD, 4SBE) and Fig. 1F (low sulfated-β-CD from Cerestar), respectively. The electropherogram of sulfated β-CD obtained from Aldrich (7–11 sulfates/β-CD) in Fig. 1G shows two major distinct populations of species with higher sulfate contents than those found in 4SBE and the low sulfated-β-CD. The electropherogram of the hepta-6-sulfato-β-CD[11] consisted of a single peak as expected (Fig. 1A). Each HS-CD in Fig. 1B′, C′ and D′ produced a peak with shorter migration time than that of the hepta-6-sulfato-β-CD, indicating a higher degree of sulfation. The fact that each HS-α, β- and γ-CD produced a distinct peak, which is resolved from that of the hepta-6-sulfato-β-CD is an indication that each HS-CD is not a gross mixture of broadly distributed sulfated species. The peak width of the HS-CD is not much wider than that of the hepta-6-sulfato-β-CD, indicating that the HS-CD have narrow molecular heterogeneity in terms of degree of sulfation. The results also suggest that the average degree of sulfation must be substantially greater than 7 and more likely greater than 10. If the mixture contains molecular species with significant amounts of 8, 9 or 10 sulfate esters, the expected electropherogram would be that one in which there is no distinct separation between the HS-CDs and hepta-6-sulfato-β-CD.

Elemental analysis of the HS-α, β- and γ-CDs showed that the average sulfate contents were 11, 12, and 13 per mol, respectively (data for HS-α- and γ-CDs were not shown). Table 1 summarizes the results of elemental analysis of three lots of HS-β-CDs after dialysis through a dialysis membrane with an M_r 500 cut-off. The amount of sulfation in HS-β-CD was determined from the sulfur/carbon ratios. The hepta-6-sulfato-β-CD produced 7.2 sulfates per mol of β-CD by elemental analysis, while the three different synthetic batches of HS-β-CD yielded sulfate contents ranging from 12.04 to 12.35 with an average of 12.2 sulfates per mol of β-CD, indicating the consistency of the synthetic process. More importantly, the slight differences in sulfation contents of each of the three synthetic lots of HS-β-CD provided comparable performance for CE-based enantiomeric separation of glutethimide racemates (data not shown).

The ESI-based negative-ion mass spectra of three lots of HS-β-CD are essentially identical with minor differences in the relative peak intensities. Fig. 2 shows the mass spectrum of the HS-β-CD lot 1 for representation. The most abundant species with mass units above 600 were the −3 charged ions (HS-β-

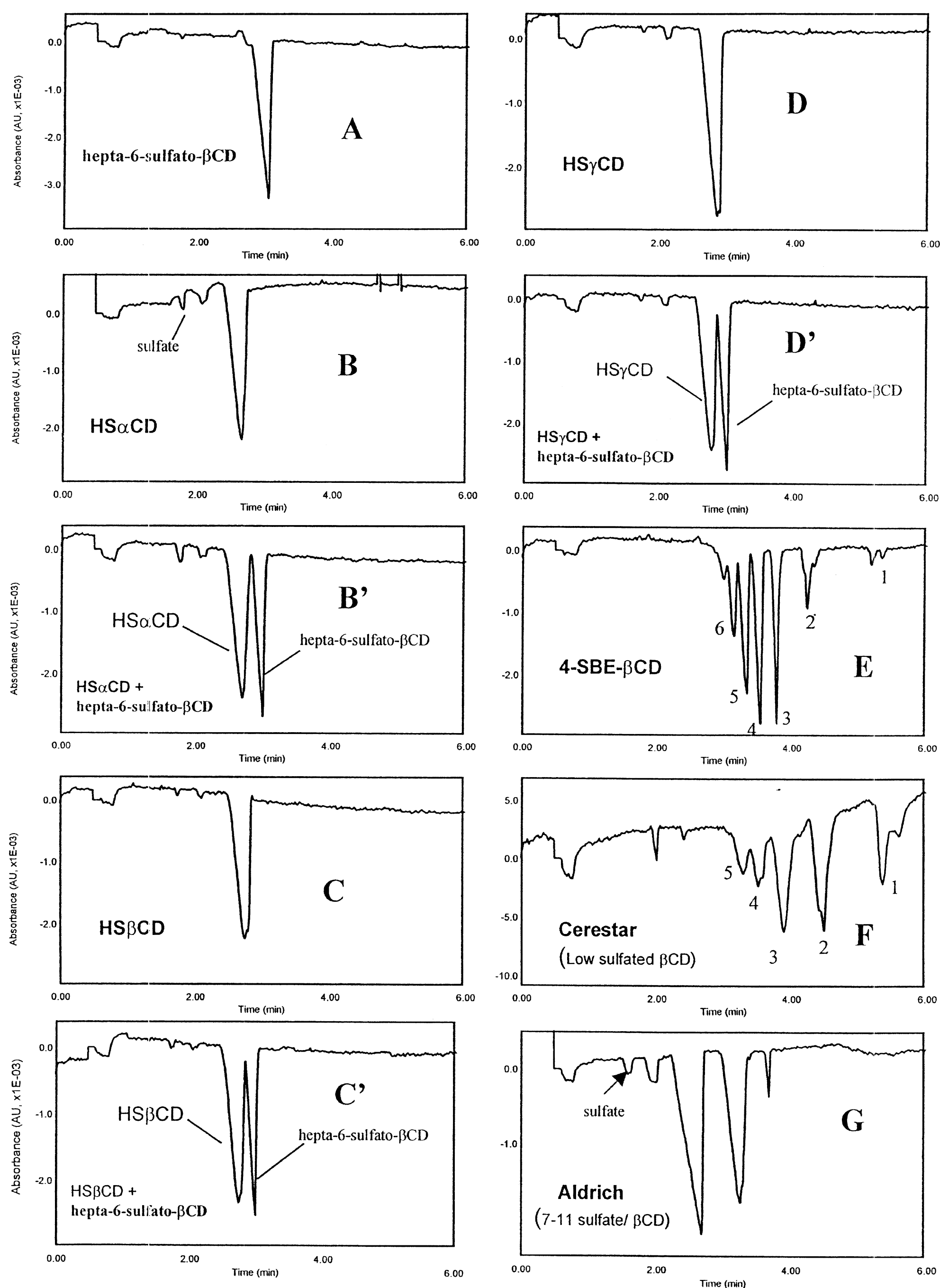

Fig. 1. Electropherograms of various sulfated cyclodextrins by indirect detection monitored at 254 nm. Conditions: 40 m*M* *p*-toluenesulfonic acid (TSA) with Tris base at pH 8.0. Applied voltage: 10 kV/13 μA; 27 cm (20 cm to the detector)×50 μm PVA-coated capillary; performed on a P/ACE 5500 CE system by Beckman Coulter. (A) Hepta-6-sulfato-β-CD; (B) HS-α-CD; (B′) HS-α-CD and hepta-6-sulfato-β-CD; (C) HS-β-CD; (C′) HS-β-CD and hepta-6-sulfato-β-CD; (D) HS-γ-CD; (D′) HS-γ-CD and hepta-6-sulfato-β-CD; (E) sulfobutyl ether-β-CD (SBE-4, Perkin-Elmer ABI); (F) low sulfated-β-CD (Cerestar); (G) sulfated β-CD (7–11 sulfates/β-CD, Aldrich).

Table 1
Elemental analysis of the highly sulfated β-cyclodextrins

	Hepta-6-sulfato-β-CD $C_{42}H_{63}Na_7O_{56}S_7$, Theoretical	Hepta-6-sulfato-β-CD Lot 1, Found	Tetradeca-2,6-sulfato-β-CD $C_{42}H_{54}Na_{17}O_{77}S_{14}$, Theoretical	HS-β-CD Lot 1, Found	HS-β-CD Lot 2, Found	HS-β-CD Lot 3, Found
% C	27.3	25.1	19.7	20.4	20.0	20.3
% H	3.4	4.2	2.2	3.7	3.0	3.4
% S	12.1	11.5	17.5	15.8	15.3	15.9
% Na	8.7	10.4	12.6	10.6	12.4	10.8
S/C	0.44	0.46	0.89	0.775	0.765	0.784
No. sulfation	7.0	7.2	14.0	12.21	12.04	12.35
No. S/glucose	1.0	1.03	2.0	1.74	1.72	1.76

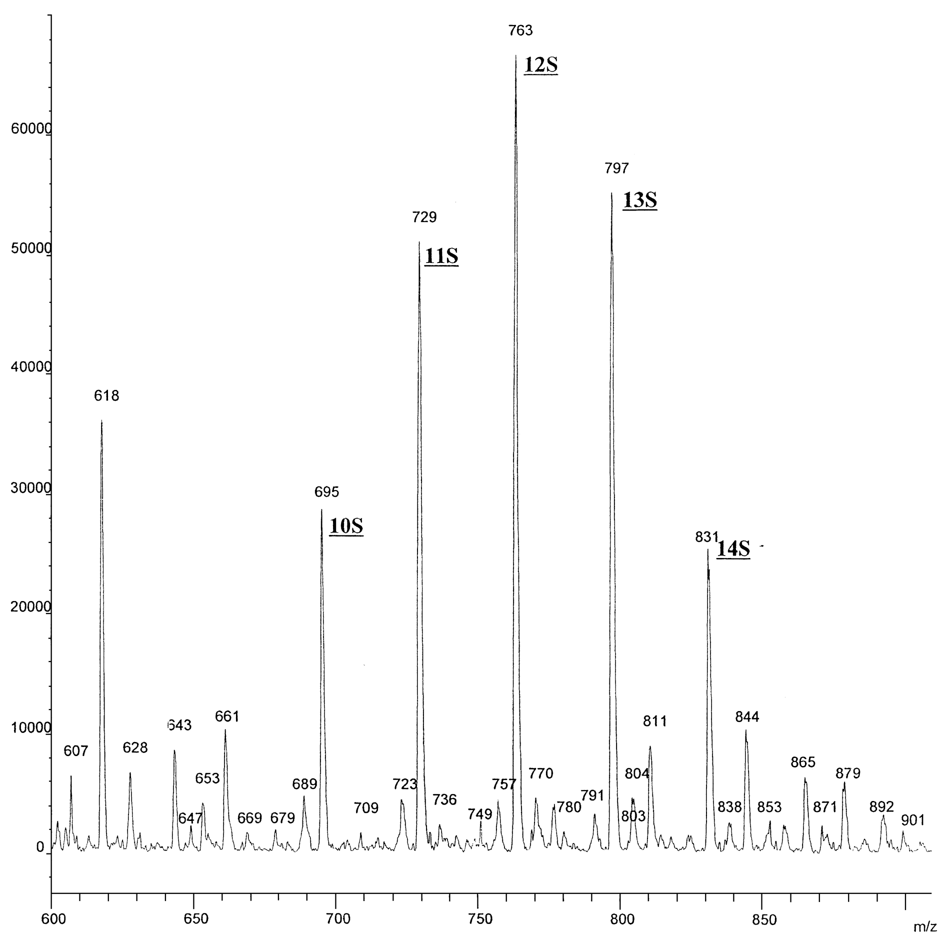

Fig. 2. ESI-MS of −3 charged species of HS-β-CD. A 1.0 mg/ml of HS-β-CD in methanol–water (80:20) solution was infused to the ESI device at 2 μl/min.

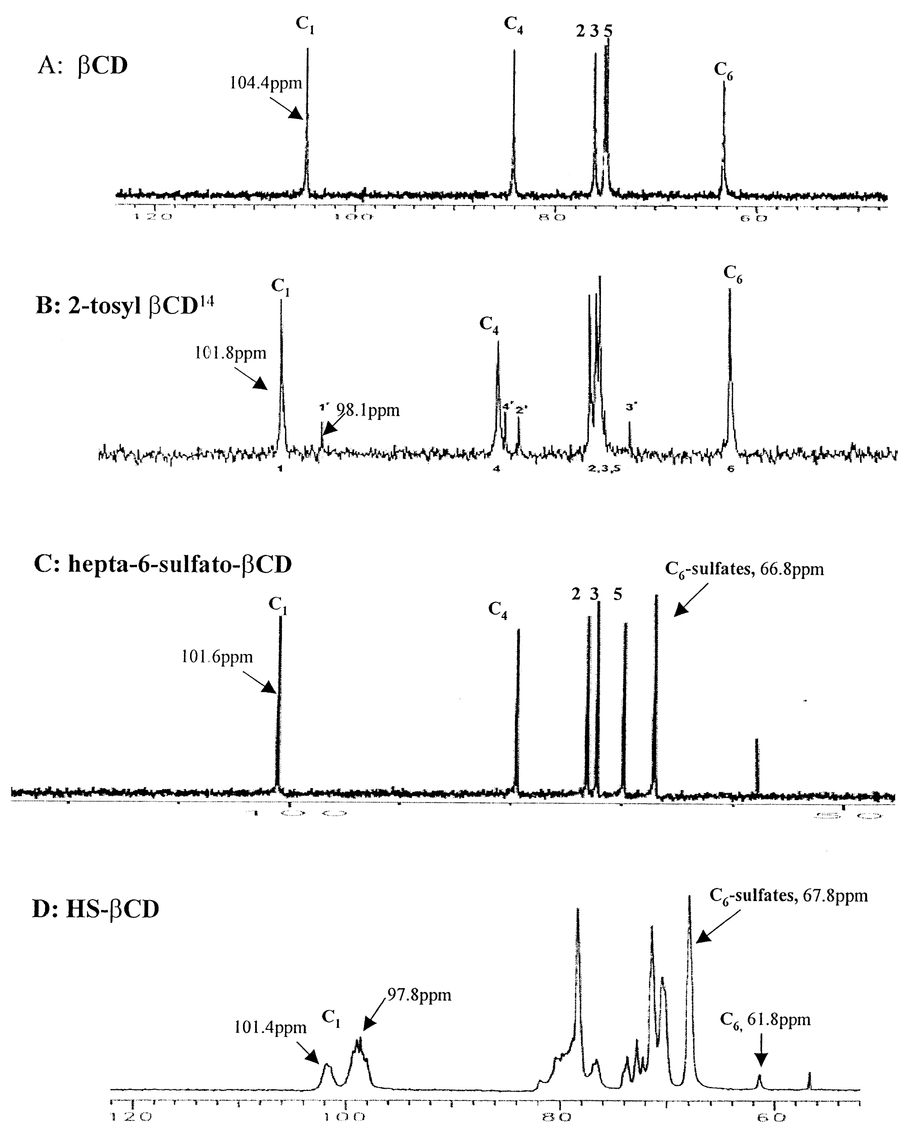

Fig. 3. ^{13}C NMR obtained from a Varian XL-300 instrument and Ref. [16]. (A) β-CD; (B) 2-toluenesulfonyl-β-CD[15] [from *Tetrahedron Lett.* 23 (1982) 3451–3454]; (C) hepta-6-sulfato-β-CD; (D) HS-β-CD.

CD −3 Na^+ ion). The mass units at 695, 729, 763, 797 and 831 correspond to 10, 11, 12, 13 and 14 sulfates, respectively, with the 11-, 12- and 13-sulfated species as the major components. The −2 charged species have relatively low abundance with three major peaks of 11-, 12- and 13-sulfated species also (data not shown). The average sulfation contents of HS-β-CDs measured by ESI-MS of the −3 charged species were slightly lower than the value obtained by elemental analysis. The loss of sulfate moieties in 6-sulfato β-CD in the gas-phase by ESI-MS was reported by Bondarenko et al. [17]. Thus, the distribution of sulfated species measured by ESI-MS may not be reliably reflected the sulfate

contents in HS-β-CD, nonetheless, the ESI-MS of the HS-β-CDs provided a direct evidence on the degree of sulfation. For HS-α- and γ-CDs, the ESI-MS results (data not shown) showed similarly that the −3 charged species as the most abundant ions with mass unit above 600 also.

The ^{13}C NMR provided most important structural information of HS-β-CD. Fig. 3A–D show the ^{13}C NMR of β-CD, hepta-6-sulfato-β-CD, 2-*p*-toluenesulfonyl-β-CD (2-tosyl-β-CD) [18] and HS-β-CD, respectively. The ^{13}C NMR of HS-β-CD in Fig. 3D indicated that nearly all the primary hydroxyl groups were converted to sulfate esters, while the secondary hydroxyl groups were also sulfated to a high degree. The chemical shift at 67.8 ppm in HS-β-CD is due to the C-6 sulfate esters, consistent with that of the C-6 sulfate esters at 66.8 ppm in hepta-6-sulfato-β-CD (Fig. 3C). A relatively smaller peak at 61.8 ppm is due to the residual non-sulfated C-6 species with an area of 4.8% of the total combined area of peaks at 67.8 and 61.8 ppm in Fig. 3D. Thus, the amount of C-6 sulfate esters in HS-β-CD is 6.69 per mole and the rest of the sulfate esters in HS-β-CD are either in the C-2 and/or C-3 positions.

Ueno and Breslow [18] reported the ^{13}C spectrum of the 2-monotosyl-β-CD in which the chemical shift of the C-1 next to the 2-tosyl group was 98.1 ppm, a significant upfield shift from the remainder of the C-1 at 101.8 ppm shown in Fig. 3B. Similar to the effect of 2-tosyl group on the ^{13}C chemical shift of the C-1, the sulfate ester in HS-β-CD at the C-2 position should produce a significant upfield chemical shift at C-1 in comparison with that of the non-sulfated species. Fig. 3D shows the ^{13}C NMR of HS-β-CD in which the chemical shift of the C-1 consists of two relatively broad, but baseline-separated peaks at 97.8 and 101.4 ppm. The broad peak centered at 97.8 ppm has a fine structure due to multiplicity, characteristic of isomers of discrete species with 4, 5 and 6 sulfates at C-2 hydroxyls. The area ratio of the 97.8 peak to 101.4 ppm peak is 3.2 to 1, indicating that 76.5% of the C-2 hydroxyl groups were sulfated, equivalent to 5.36 sulfate esters. Thus, the total sulfate content in HS-β-CD is 12.05 by ^{13}C NMR data (6.69 and 5.36 sulfate esters at C-6 and C-2, respectively), in good agreement with the 12.2 sulfates per HS-β-CD obtained by elemental analysis. The ^{13}C chemical shift of C-1 at 101.4 ppm could only be due to the non-sulfated C-2 and C-3 species. Meanwhile, the ^{13}C spectra of 2- and 3-monophosphate β-CDs reported by Siegel et al. [19] showed the upfield shift of C-1 in the 2-phosphate isomer from 100.8 to 99.7 ppm while the ^{13}C chemical shift at C-1 of the 3-phosphate isomer did not change. Both tosyl and phosphate ester at C-2 position provided similar upfield change on the ^{13}C chemical shift of C-1 as that of the sulfate ester at C-2 in HS-β-CD. The electronic effect of the C-2 sulfate ester of the HS-β-CD on the chemical shift of C-1 should be similar to that of the C-2 tosyl and phosphate ester groups. A ^{13}C NMR simulation using CambridgeSoft ChemDraw Ultra 4.5 software [20] showed that the monosulfated species of β-CD at the C-2 position produced an upfield shift of the C-1 from 101.2 to 97 ppm, fairly close to that of the value obtained in HS-β-CD and 2-tosyl-β-CD [17]. The C-3 monosulfated ester of β-CD shows a slightly downfield shift of the C-1 chemical shift from 100.5 to 100.8 ppm, however. Thus, the ^{13}C chemical shift data of the HS-β-CD are consistent with the assignment of the sulfate substitution exclusively at the C-2 secondary hydroxyls.

The data from the ^{13}C NMR spectra of HS-α- and γ-CDs (not shown) were similar to that of the HS-β-CD and consistent with the structural assignment of the sulfate ester formation at C-6 and C-2 positions only, while the C-3 hydroxyl position remained intact. In addition to the nearly complete sulfation at the C-6 of each glucose unit in cyclodextrins, only the C-2 secondary hydroxyls were sulfated, though not completely. In spite of the one-step synthetic procedure with three available sulfation sites in the glucopyranosyl moiety of the CD, the nearly complete sulfation at C-6 is expected due to the higher reactivity of its primary alcohol. However, the sulfation at the secondary hydroxyl occurred selectively at C-2 only, indicative of the higher acidity of the hydroxyl at the C-2 than C-3 as was proposed by Ueno and Brelsow [18]. Alternatively, the preferential sulfation at C-6 may stereochemically prevent or reduce the accessibility of sulfation at C-3. The sulfation occurred at C-2 equatorial hydroxyl position located at the rim of the wider opening of the cyclodextrin bucket, leaving a regiospecific C-3 hydroxyls available for potential hydrogen-bonding

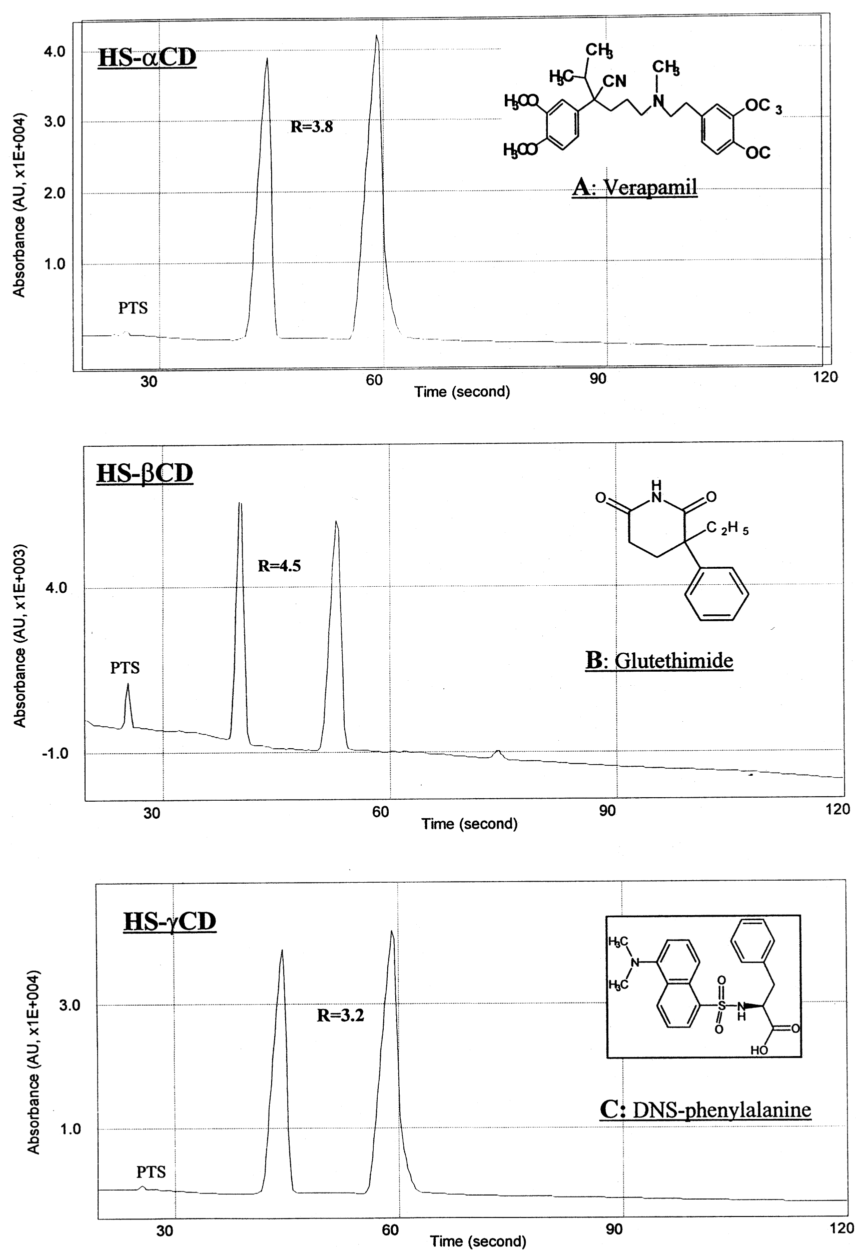

Fig. 4. Rapid enantiomeric separation by capillary electrophoresis with HS-CDs. Conditions: 5% HS-CD in 25 m*M* phosphate buffer, pH 2.5. Untreated fused-silica capillary 31.5 cm (10 cm to the detector)×25 μm; performed on a P/ACE MDQ CE system at 30 kV monitored at 200 nm; injection: 0.3 p.s.i./4 s; PTS is pyrenetetrasulfonate, a migration reference marker that does not complex with HS-CDs. (A) Verapamil (1.0 m*M*) in HS-α-CD at 30 kV/101 μA; (B) glutethimide (0.5 m*M*) in HS-β-CD at 30 kV/106 μA; (C) Dns-phenylalanine (1.0 m*M*) in HS-γ-CD at 30 kV/99 μA.

with guest molecules. The part of the HS-CD that interacts with the guest molecule during complexation and, therefore, the receiving end of the cyclodextrin hydrophobic bucket is surrounded with largely regiospecifically-substituted sulfates at the C-2 equatorial hydroxyl moiety. The fact that a large number of successful CE-based chiral separations of racemates with many variety of structural features are achieved using the combination of HS-α-, β- and γ-CDs [11–14] suggests that this stereospecific perturbation around the hydrophobic bucket is the principal contributing factor in the differential complexation of enantiomeric species. Furthermore, the surrounding sulfate moieties provide strong Coulombic interaction with basic drugs that would enhance their affinity. As such, rapid chiral resolution (less than 90 s) of basic and neutral racemic compounds like verapamil, glutethimide and Dns-phenylalanine can be resolved with resolution of more than 3.0 using the present HS-CDs as the chiral selector as

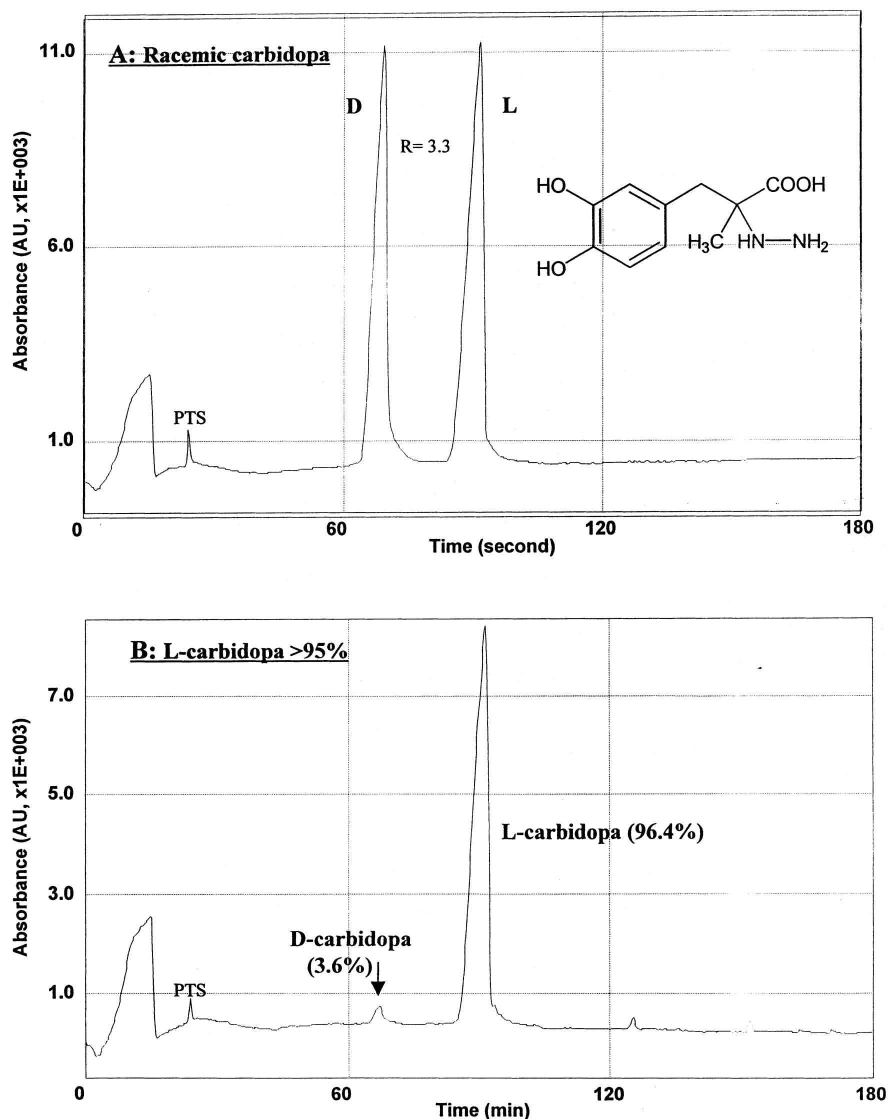

Fig. 5. Enantiomeric separation of carbidopa by capillary electrophoresis with HS-β-CD. Conditions as in Fig. 4. (A) Carbidopa racemate (2.0 m*M*) in HS-β-CD at 30 kV/109 μA; (B) L-carbidopa (1.4 m*M*) in HS-β-CD at 30 kV/110 μA.

shown in Fig. 4A–C, respectively. Similarly, the rapid analysis of the enantiomeric impurity in a commercial sample of L-carbidopa and racemic carbidopa by CE using HS-β-CD is shown in Fig. 5B and A, respectively.

4. Conclusion

Our data on the characterization of the highly sulfated cyclodextrins indicate that the HS-CDs are a mixture of species with narrow heterogeneity, i.e., with similar degrees of sulfation. The sulfate contents of HS-CDs were independently measured by elemental analysis and by ^{13}C NMR analysis. Furthermore, the ^{13}C NMR spectrum of HS-β-CD is consistent with the assignment that sulfation occurs at the C-6 and C-2 positions only and the C-3 hydroxyl is intact. Since the C-2 and C-3 positions are the sites of complexation with the racemates, the relative position of the C-2 sulfate, C-3 hydroxyl and the hydrophobic bucket of the cyclodextrins provide a regiospecific differential interaction with each enantiomer. A list of CE-based chiral analysis of racemic compounds with a wide variety of structural features is available on the web page at: www.Beckmancoulter.com/chiral38.html.

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References

[1] S. Fanali, J. Chromatogr. A 735 (1996) 77.
[2] R. Vespaletc, P. Bocek, Electrophoresis 18 (1997) 843.
[3] S. Terabe, Trends Anal. Chem. 6 (1989) 129.
[4] S. Mayer, M. Schleimer, V. Schurig, J. Microcol. Sep. 6 (1994) 43.
[5] R.J. Tait, D.O. Thompson, V.J. Stella, J.F. Stobaugh, Anal. Chem. 66 (1994) 4013.
[6] C. Dette, S. Ebel, S. Terabe, Electrophoresis 15 (1994) 799.
[7] B. Chankvetadze, G. Endresz, G. Blaschke, Electrophoresis 15 (1994) 804.
[8] T. de Boeer, R.A. de Zeeuw, G.J. de Jong, K. Ensing, Electrophoresis 21 (2000) 3220.
[9] A. Stalcup, K.H. Gahm, Anal. Chem. 68 (1996) 1360.
[10] J.B. Vincent, D.M. Kirby, T.V. Nguyen, G. Vigh, Anal. Chem. 69 (1997) 4419.
[11] K. Verleysen, P. Sandra, Electrophoresis 19 (1998) 2798.
[12] I.S. Lurie, N.G. Odeneal II, T.D. McKibben, J.F. Casale, Electrophoresis 19 (1998) 2918.
[13] F.-T.A. Chen, R.A. Evangelista, J. Chin. Chem. Soc. 46 (1998) 847.
[14] K. Verleysen, T. Van den Bosch, P. Sandra, Electrophoresis 20 (1999) 2650.
[15] A. Nardi, S. Fanali, F. Foret, Electrophoresis 11 (1990) 774.
[16] R.J. Tait, D.J. Skanchy, D.O. Thompson, N.C. Chetwyn, D.A. Sunshee, R.A. Rajewski, V.J. Stella, J.F. Stobaugh, J. Pharm. Biomed. Anal. 10 (1992) 615.
[17] P.V. Bondarenko, B. Wolf, H. Cai, J.B. Vincent, R.D. Macfarlane, G. Vigh, Anal. Chem. 70 (1998) 3042.
[18] A. Ueno, R. Breslow, Tetrahedron Lett. 23 (1982) 3451.
[19] B. Siegel, A. Pinter, R. Breslow, J. Am. Chem. Soc. 99 (1977) 2309.
[20] Chemdraw Pro version 4.5, CambridgeSoft Corporation, Cambridge, MA, 1997.