

Capillary electrophoretic separation of enantiomers in a high-pH background electrolyte by means of the single-isomer chiral resolving agent octa(6-*O*-sulfo)- γ -cyclodextrin

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Abstract

The new, alkali-stable, single-isomer, sulfated γ -cyclodextrin, the sodium salt of octa(6-*O*-sulfo)- γ -cyclodextrin (OS) was used for the first time to separate the enantiomers of non-ionic, acidic, basic and ampholytic analytes by capillary electrophoresis in high-pH aqueous background electrolytes. The effective mobilities and separation selectivities were found to follow trends similar to those observed earlier in acidic aqueous background electrolytes. OS proved to be a broadly applicable chiral resolving agent and afforded adequate peak resolution values with short separation times for a number of non-ionic, weak acid, weak base and ampholytic analytes.

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1. Introduction

During the last 5 years, single-isomer, sulfated cyclodextrins have become important; they are widely used chiral resolving agents in the capillary electrophoretic (CE) separation of enantiomers [1,2]. Using a selective protecting group chemistry approach, the primary hydroxy groups of cyclodextrins have been completely sulfated and products with isomeric purities in excess of 95 mol% have been successfully synthesized [3–7]. In order to broaden

the range of intermolecular interactions between the sulfated cyclodextrins and the analytes, single-isomer sulfated β -cyclodextrins have been prepared that carry hydroxy [4,7], acetyl [3,6] and methoxy [5,8–10] groups in the 2- and 3-positions of the glucopyranose units. Additional size-selectivity became available with the octakis(2,3-diacetyl-6-*O*-sulfo)- γ -cyclodextrin derivative [11–16]. To further broaden the choice of these single-isomer sulfated cyclodextrins, the sodium salt of octa(6-*O*-sulfo)- γ -cyclodextrin has also been synthesized recently [17]. Due to the absence of acetyl groups, this material was deemed to be especially compatible with alkaline background electrolytes (BGEs). This brief paper describes the first use of octa(6-*O*-sulfo)- γ -cyclodextrin in high-pH aqueous BGEs for the CE separation of the enantiomers of non-ionic, acidic, basic and ampholytic analytes.

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Table 1
Separation data for non-ionic and weak acid analytes in pH 9.4 OS BGE

Concentration Name	0 mM		5 mM				10 mM					15 mM					25 mM					50 mM						
	μ	μ	α	β	R_s	U	μ	α	β	R_s	U	μ	α	β	R_s	U	μ	α	β	R_s	U	μ	α	β	R_s	U		
1-Phenyl-2-propanol	0	-0.7	1	-69	0	11.2	-1.3	1	-35	0	13.4																	
2-Phenyl-2-butanol	0	-1.98	1	-30	0	14.9	-3.84	1	-11.3	0	11.2																	
Benzoin	0	-1.62	1	-36	0	14.9	-3.32	1	-16	0	11.2																	
4-Phenyl-1,3-dioxane	0	-0.8	1	-70	0	14.9	-1.8	1	-22	0	11.2																	
2-Phenyl-1-propanol	0	-1	1	-59	0	14.9	-2.3	1	-23	0	11.2																	
α -Methyl- α -phenylsuccinimide	-19.3	-17.8	1	-3.1	0	14.8	-17.3	1	-2.7	0	11.2																	
5-(4-Methylphenyl)-5-phenyl Hydantoin	-17.0	-13.8	1	-3.6	0	14.9	-14.3	1	-3.1	0	13.4																	
2-Phenyl propionic acid	-24.5	-21.9	1	-2.5	0	14.9	-19.9	1	-2.2	0	11.2																	
Indapamide	-10.1	-9.5	1	-5.3	0	14.9	-9.2	1	-5	0	11.2																	
Fenoprofen	-18.6	-17.3	1	-3	0	14.9	-16.5	1	-2.6	0	11.2																	
Ibuprofen	-19.1	-17.4	1	-3	0	14.9	-16.7	1	-2.5	0	11.2																	
Flurbiprofen	-18.5	-17.7	1	-2.9	0	14.9	-18.2	1	-2.4	0	11.2																	
Ketoprofen	-17.0	-15	1	-3.2	0	14.9	-15.4	1	-2.7	0	11.2																	

Effective mobilities of the less mobile enantiomer (μ , in $10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ units), separation selectivities (α), measured peak resolution values (R_s), dimensionless EO flow mobility values (β) and the injector-to-detector effective potential drop (U , in kV units) in pH 9.4 OS BGEs for non-ionic and weak acid analytes.

Table 2
Separation data for weak base and ampholytic analytes in pH 9.4 OS BGE

Concentration Name	0 mM						2.5 mM						5 mM					
	μ	μ	α	β	R_s	U	μ	μ	α	β	R_s	U	μ	μ	α	β	R_s	U
Oxyphencyclamine	10.7	-19.8	1.176	-2.1	2.6	16.4	-21.3	1.13	-2	2.3	14.9							
Piperoxan	3.8	-1.98	1	-25.5	0	16.4	-3.5	1	-13.5	0	14.9							
Pindolol	9.6	-7.5	1.08	-6.2	<0.5	16.4	-10.6	1.057	-4.3	0.5	14.9							
Terbutaline	3.26	-1	1	-33.7	0	16.4	-2.26	1	-21.6	0	14.9							
Tolperisone	2.8	-1.48	2.087	-15.9	0.9	16.4	-5.65	1.77	-4.9	3.1	14.9							
Chlophedianol	8.2	-3.99	1.394	-8.8	1	16.4	-6.84	1.298	-5.4	1.4	14.9							
DNS-aspartic acid	-26.5	-24.1	1	-2	0	16.4	-23.9	1	-2	0	14.9							
DNS-methionine	-15.8	-13	1.109	-3.4	1.3	16.4	-13.6	1.17	-3	2.6	14.9							
DNS-phenylalanine	-15.3	-17.5	1.153	-2.4	3.2	16.4	-20	1.148	-2.1	4.5	14.9							
DNS-serine	-15	-13.5	1	-3.6	0	16.4	-13.4	1	-3.6	0	14.9							
DNS-tryptophan	-13.2	-13.8	1.1	-3.2	1.3	16.4	-15.6	1.129	-2.7	2.5	14.9							
DNS-valine	-14.6	-12.9	1.103	-3.4	1.2	16.4	-13.5	1.169	-3.1	2.4	14.9							
Tryptophan	-6.8	-8.3	1	-5.9	0	16.4	-9.9	1	-4.9	0	14.9							
2-Phenylglycine	-12	-14.3	1	-3.4	0	16.4	-15.2	1	-3.2	0	14.9							
DNS-glutamic acid	-25.8	-23.5	1	-2.1	0	16.4	-23.2	1.01	-2.1	<0.5	14.9							
	10 mM						15 mM						25 mM					
	μ	α	β	R_s	U	μ	α	β	R_s	U	μ	α	β	R_s	U			
Oxyphencyclamine	-22.6	1.095	-1.7	4.4	13.4	-22.9	1.085	-1.6	6.7	11.2	-21.5	1.075	-1.4	7.8	8.2			
Piperoxan	-5.56	1.09	-7.1	<0.5	13.4	-6.73	1.08	-5.2	0.5	11.2	-8.75	1.07	-3.5	1.2	8.2			
Pindolol	-13.1	1.047	-3.2	1	13.4	-14.2	1.043	-2.6	1.4	11.2	-14.77	1.035	-2.1	2	8.2			
Terbutaline	-3.92	1	-11.2	0	13.4	-5.09	1	-7.5	0	11.2	-5.58	1	-5.8	0	8.2			
Tolperisone	-8.67	1.55	-3.2	6.2	13.4	-9.9	1.41	-2.8	7.3	11.2	-11.6	1.29	-2.2	10	8.2			
Chlophedianol	-10.2	1.244	-3.5	2.9	13.4	-12.4	1.212	-2.7	4.7	11.2	-14.2	1.169	-2	7.7	8.2			
DNS-aspartic acid	-24.1	1	-1.7	0	13.4	-24	1.01	-1.6	<0.5	11.2	-23	1.012	-1.4	1.6	8.2			
DNS-methionine	-15.3	1.262	-2.2	7.4	13.4	-16.6	1.289	-1.8	12.6	11.2	-18.8	1.281	-1.3	23.1	8.2			
DNS-phenylalanine	-25	1.103	-1.5	7.4	13.4	-25.8	1.066	-1.3	8.3	11.2	-26.4	1.052	-1.2	7.8	8.2			
DNS-serine	-14.7	1	-2.8	0	13.4	-15.2	1.01	-2.5	<0.5	11.2	-16.2	1.018	-1.8	1.3	8.2			
DNS-tryptophan	-19.4	1.13	-1.9	5.7	13.4	-21.4	1.115	-1.6	8.1	11.2	-23	1.086	-1.3	10.9	8.2			
DNS-valine	-15.7	1.258	-2.1	8	13.4	-17	1.276	-1.8	13.6	11.2	-19	1.26	-1.3	20.6	8.2			
Tryptophan	-10.7	1	-4.2	0	13.4	-11.6	1.019	-3.3	<0.5	11.2	-12.8	1.03	-2.4	1.2	8.2			
2-Phenylglycine	-14.8	1	-2.9	0	13.4	-15.9	1	-2.4	0	11.2	-15.4	1	-2.1	0	8.2			
DNS-glutamic acid	-23	1.027	-1.8	1.7	13.4	-22.8	1.049	-1.7	4.4	11.2	-22.2	1.085	-1.4	10.2	8.2			

Effective mobilities of the less mobile enantiomer (μ , in $10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ units), separation selectivities (α), measured peak resolution values (R_s), dimensionless EO flow mobility values (β) and the injector-to-detector effective potential drop (U , in kV units) in pH 9.4 OS BGEs for weak base and ampholytic analytes.

2. Experimental

All chemicals were purchased from Aldrich (Milwaukee, WI, USA), except for the sodium salt of octa(6-*O*-sulfo)- γ -cyclodextrin (OS) which was synthesized in our laboratory as described in Ref. [17] and is now commercially available [18]. The CE separations were carried out on a P/ACE 2100 CE instrument (Beckman-Coulter, Fullerton, CA, USA), equipped with a variable-wavelength UV detector (set to 214 nm). The capillary coolant was thermostated at 20 °C. The enantiomer separations were obtained with 25- μ m I.D., 150- μ m O.D., uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The injector-to-detector length of the capillary, L_d , was 19.5 cm, and the total length, L_t , was 26.5 cm.

All CE separations were carried out in 25 mM

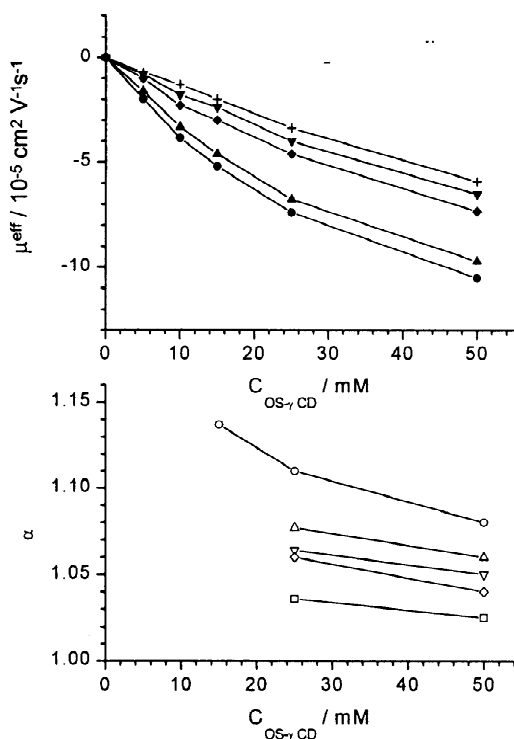


Fig. 1. Effective mobilities (top panel, full symbols) and separation selectivities (bottom panel, open symbols) for some of the non-ionic analytes as a function of the OS concentration of the BGE. Legends: \circ , \bullet : 2-phenyl-2-butanol; \blacktriangle , \triangle : benzoin; \blacktriangledown , \triangledown : 4-phenyl-1,3-dioxane; \blacklozenge , \lozenge : 2-phenyl-1-propanol; $+$: 1-phenyl-2-propanol.

ethanolamine BGEs adjusted to pH 9.4 with methanesulfonic acid (MSA). The 5, 10, 15, 25 and 50 mM OS BGEs were prepared by weighing out the required amounts of the sodium salt of OS into 25-ml volumetric flasks and bringing the volumes to mark with the pH 9.4 stock BGE solution. The external electroosmotic flow (EOF) marker method [19] proved that nitromethane (NM) did not complex with OS, i.e., its effective mobility was zero in OS-containing BGEs within the experimental error. Therefore, NM was used as the neutral marker. The analytes, dissolved in the OS BGEs, had a concentration of ~ 0.5 mM and were injected by 2 p.s.i. nitrogen for 2 s (1 p.s.i. = 6894.76 Pa). The applied potential (U_{appl}) was varied between 15 and 20 kV to maintain power dissipation between 500 and 700 mW/m and remain within the linear portion of Ohm's plot. The effective mobilities of the enantiomers (μ_R^{eff} and μ_S^{eff}) were obtained as $\mu_R^{\text{eff}} = \mu_R^{\text{obs}} - \mu_{\text{EO}}^{\text{eff}}$, the separation selectivities, α , as $\alpha = \mu_R^{\text{eff}} / \mu_S^{\text{eff}}$

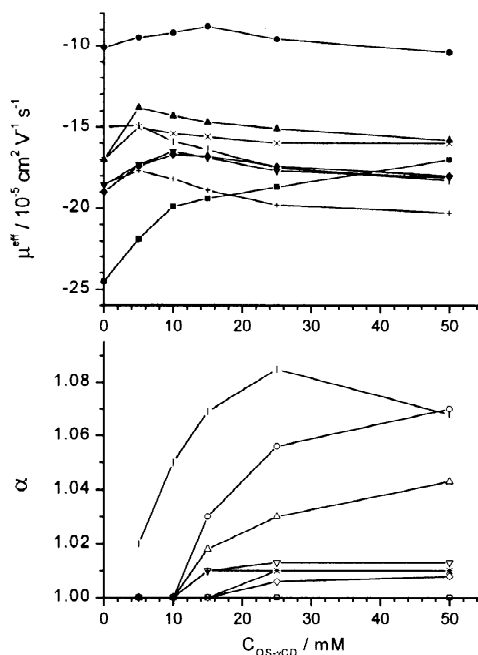


Fig. 2. Effective mobilities (top panel, full symbols) and separation selectivities (bottom panel, open symbols) for some of the weak acid analytes as a function of the OS concentration of the BGE. Legends: \blacksquare , \square : 2-phenylpropionic acid; \circ , \bullet : indapamide; \blacktriangle , \triangle : 5-(4-methylphenyl)-5-phenyl hydantoin; \blacktriangledown , \triangledown : fenpropfen; \blacklozenge , \lozenge : ibuprofen; $+$: flurbiprofen; \times : ketoprofen; vertical bar: ciprofibrate.

(where subscript S arbitrarily refers to the enantiomer whose effective mobility in the 1 mM OS BGE was smaller), and the normalized electroosmotic flow mobility values, β , as $\beta = \mu_{EO} / \mu_S^{eff}$ [20].

3. Results and discussion

A series of non-ionic, weak base and ampholytic enantiomers were separated with the pH 9.4 OS BGEs. Tables 1 and 2 list the observed effective mobilities of the less mobile enantiomers, the separation selectivities, the measured peak resolution values, the corresponding normalized EOF mobility values and the injector-to-detector effective potential drop values ($U = (U_{appl}L_d)/L_t$). Typical effective mobility and separation selectivity curves for some of the analytes tested are shown in Figs. 1–4.

For non-ionic analytes, the anionic effective mo-

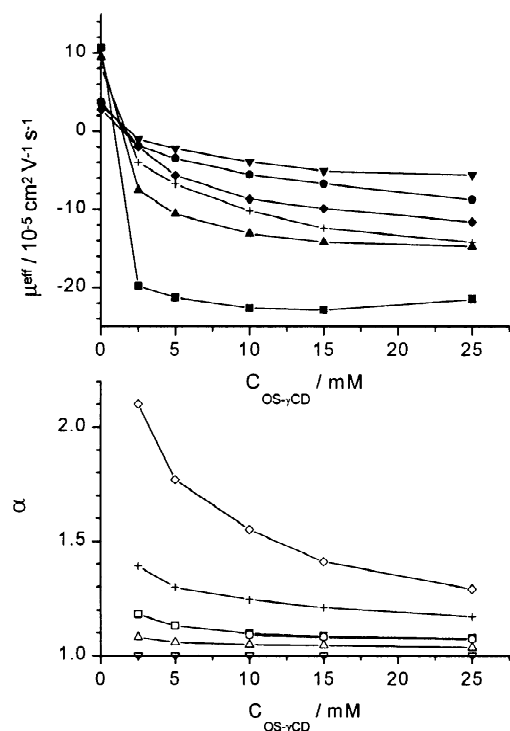


Fig. 3. Effective mobilities (top panel, full symbols) and separation selectivities (bottom panel, open symbols) for some of the weak base analytes as a function of the OS concentration of the BGE. Legends: ■, □: oxyphenacyclimine; ●, ○: piperoxan; ▲, △: pindolol; ▼, ▽: terbutaline; ◆, ◇: tolperisone; +: chlophedianol.

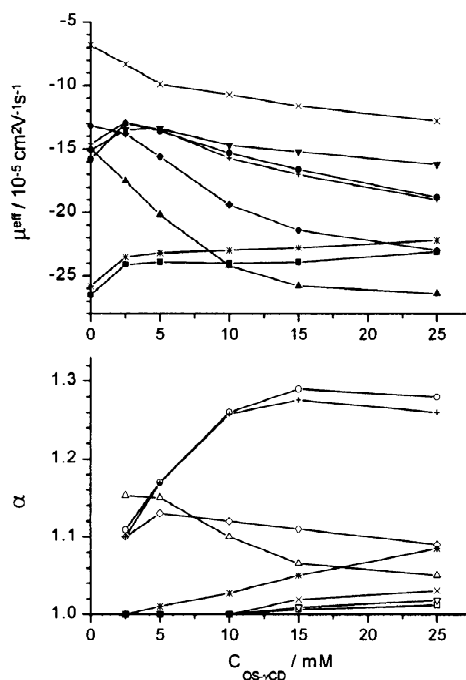


Fig. 4. Effective mobilities (top panel, full symbols) and separation selectivities (bottom panel, open symbols) for some of the ampholytic analytes as a function of the OS concentration of the BGE. Legends: ■, □: DNS-asp; ●, ○: DNS-met; ▲, △: DNS-phe; ▼, ▽: DNS-ser; ◆, ◇: DNS-trp; +: DNS-val; ×: tryptophan; *: DNS-glu.

bilities increase as the OS concentration is increased (top panel of Fig. 1), but the increase is smaller than in the low-pH OS BGEs [17], indicating that components of the ethanolamine–MSA buffer compete with the non-ionic analytes for OS more strongly than components of the H_3PO_4 –LiOH buffer [17]. Separation selectivity decreases as the OS concentration is increased (bottom panel of Fig. 1), in agreement with the predictions of the charged resolving agent migration model (CHARM model) [21].

For weak acids that are fully dissociated in the pH 9.4 BGE, complexation with OS can either decrease or increase the anionic effective mobilities (Fig. 2), depending on whether the dominant effect is an increase in the overall charge or the overall size [21]. Superimposed on these simple, complexation-induced effects are the ionic strength-related effects: the mobility of the non-complexed analyte that carries a single negative charge is depressed by the

increasing ionic strength much less than the mobility of the complexed analyte that carries nine negative charges. Thus, local effective mobility extrema and local separation selectivity extrema are commonly observed.

For weak bases, the initial effective mobilities in 0 mM OS are still cationic (top panel of Fig. 3) and range from 3 to $11 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, indicating that pH 9.4 is not high enough to completely deprotonate the amine groups in the weak bases. In the concentration range tested, the anionic effective mobilities increase with the OS concentration (above 2.5 mM) for all weak bases, except for oxyphencyclamine, indicating that complexation overcomes the mobility depressing effects of increasing ionic strength [22,23]. The binding of oxyphencyclamine with OS is the strongest and results in an anionic effective mobility maximum at an OS concentration of 15 mM. Since the effective mobilities of all weak bases are anionic above 2.5 mM, separation selectivities for the weak bases decrease

with increasing OS concentration, as predicted by the CHARM model [21].

For ampholytic analytes, the initial anionic effective mobilities (top panel of Fig. 4) can decrease (e.g., dansyl-glutamic acid and dansyl-aspartic acid), pass a local minimum (e.g., dansyl-methionine, dansyl-serine and dansyl-valine), or increase (e.g., dansyl-tryptophan and dansyl-phenylalanine) with increasing OS concentration, depending on the binding strength of the analytes and the interplay between increasing complexation and ionic strength-induced mobility depression, as explained in Ref. [23]. Correspondingly, separation selectivities (bottom panel of Fig. 4) decrease (e.g., for dansyl-phenylalanine), pass a local maximum (e.g., for dansyl-methionine, dansyl-valine, and dansyl-tryptophan), or keep increasing (the others), as the OS concentration is increased [23]. The separation selectivity values are generally larger than those observed with the corresponding β -CD analog, hepta(6-*O*-sulfo)- β -CD (HS) [4].

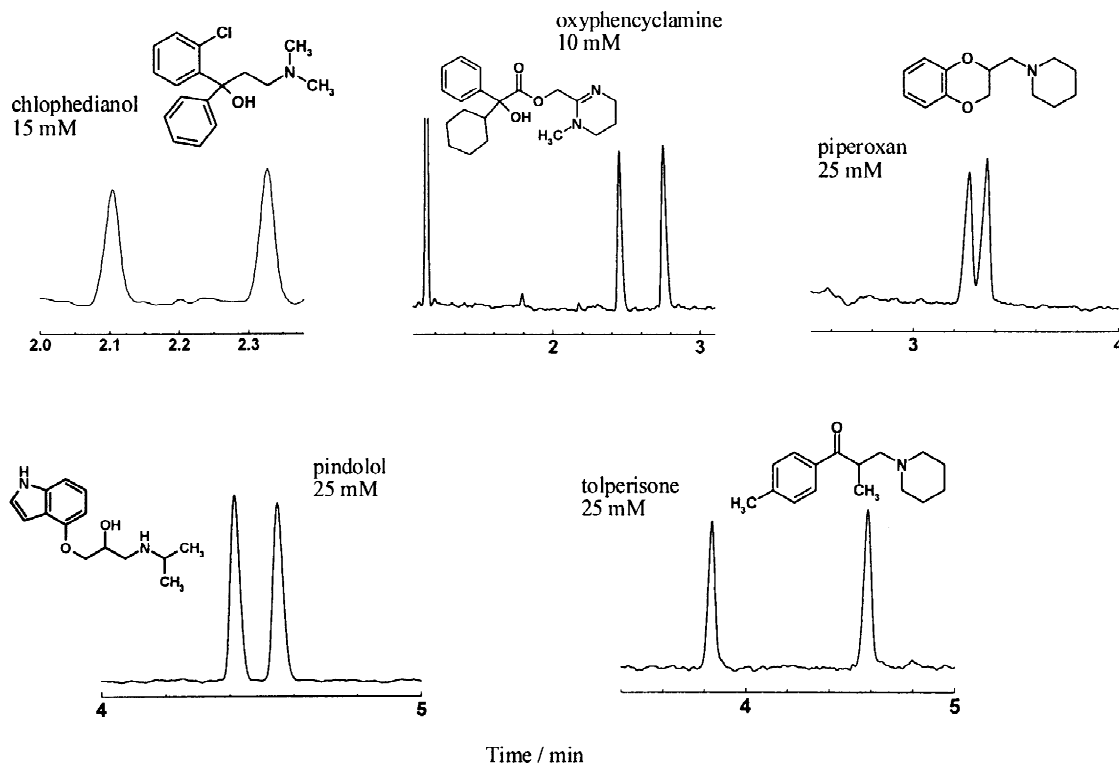


Fig. 5. Typical electropherograms of weak base analyte enantiomers. The numbers next to the structures indicate the OS concentrations (mM). Capillary: 25 μm I.D., L_d = 19.5 cm, L_t = 26.5 cm, uncoated fused silica. Other conditions: see Experimental section.

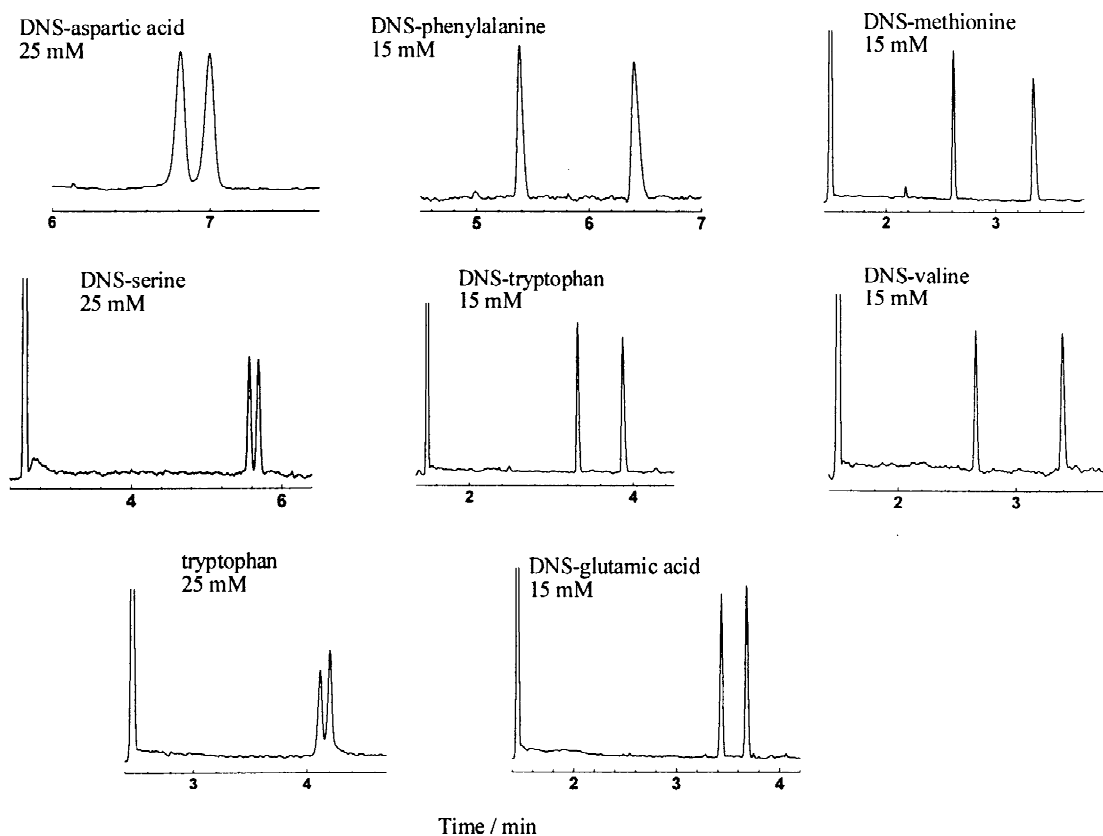


Fig. 6. Typical electropherograms of ampholytic analyte enantiomers. The numbers next to the structures indicate the OS concentrations (mM). Capillary: 25 μ m I.D., L_d =19.5 cm, L_t =26.5 cm, uncoated fused silica. Other conditions: see Experimental section.

Finally, Figs. 5 and 6 show typical electropherograms obtained with the high-pH OS BGEs. The numbers next to the electropherograms indicate the actual OS concentrations. Although the normalized electroosmotic flow mobilities are fairly large, and thus unfavorable, adequate peak resolution values were obtained in 5–10 min for all of the weak base and ampholytic analytes tested. In general, OS afforded greater peak resolution values for the enantiomers of the dansyl amino acids than the corresponding β -CD derivative, HS, studied earlier [4].

4. Conclusions

The new, single-isomer, alkali-stable, sulfated γ -CD, the sodium salt of octa(6-*O*-sulfo)- γ -cyclodex-

trin, has been used to separate the enantiomers of non-ionic, weak acid, weak base and ampholytic analytes in a pH 9.4 background electrolyte. The interactions between OS and many analytes were of a different strength than those observed with its β -cyclodextrin analog, hepta(6-*O*-sulfo)- β -cyclodextrin. Adequate, fast separations were obtained with OS in the high-pH background electrolyte for a large number of analytes.

Acknowledgements

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