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Nonaqueous capillary electrophoretic separation of enantiomers using the single-isomer heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin as chiral resolving agent

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Abstract

The sodium salt of the single isomer heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin has been used as chiral resolving agent for the nonaqueous capillary electrophoretic separation of the enantiomers of a variety of weak base pharmaceuticals in 100% methanol background electrolytes. As predicted by the charged resolving agent migration model of enantiomer separations, very large separation selectivities were observed for the cationic analytes around the heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin concentrations where the effective mobilities of the slower migrating enantiomers approached zero. Neutral analytes and acidic analytes complexed very weakly with heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin in the methanolic background electrolytes. Heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin proved to be a useful chiral resolving agent in 100% methanolic background electrolytes for a variety of weak base analytes that have limited solubilities in aqueous background electrolytes.

Keywords: Enantiomer separation; Background electrolyte composition; Cyclodextrins; Ciprofibrate; Fenoprofen; Ibuprofen

1. Introduction

Recently, we described the synthesis and capillary electrophoretic (CE) use of the first three members of a single isomer sulfated cyclodextrin family [1–3]. Compared to the randomly substituted charged cyclodextrins which (i) contain a large number of isomers with widely differing degrees of substitution and loci of substitution [4], and (ii) whose composition (and chiral resolving properties) can vary from batch-to-batch [5], the single isomer charged cyclodextrins (i) have well defined structures, (ii) can be produced reproducibly and (iii) are now commercially available in greater than 95% isomeric purity. These single isomer resolving agents were designed to be fully charged on the nonchiral face of the cyclodextrin (CD) molecule and offer, on the chiral face of the CD, the same hydrophilic (hydroxy), moderately hydrophobic (acetyl) and hydrophobic (methyl) functional groups as their well-studied and well-liked neutral CD counterparts: the native β-CD, the peracetyl β-CD and the permethyl β-CD [6–8]. The outstanding performance of single isomer fully charged CDs in difficult enantiomer separations using aqueous low-pH and high-pH background electrolytes (BGEs), according to the predictions of the charged resolving agent migration

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model (CHARM model) of enantiomer separations [9], has been demonstrated [10,11].

Often, neutral and weak electrolyte enantiomers are not sufficiently soluble in aqueous or hydro-organic BGEs. To mitigate the problem of limited aqueous solubility, enantiomer separations were attempted in nonaqueous solvents which, simultaneously, were good solvents for the cyclodextrin resolving agents as well [12–15]. The most commonly used solvents in these nonaqueous CE (NACE) enantiomer separations were formamide [12], *N*-methylformamide [12–14], and *N*,*N*-dimethylformamide [12], while the CDs were non-charged β- and γ-CDs and their neutral derivatives [12–14], except in Ref. [12], where a randomly substituted anionic β-CD was also tested in formamide solvent. Though the association constants between the CDs and the enantiomers in these formamide-derived solvents are very weak [12,13], good NACE enantiomer separations were obtained because sufficiently high CD concentrations could be reached in formamide, *N*-methylformamide and *N*,*N*-dimethylformamide. Unfortunately, the molar UV absorbances of formamide-derived solvents at low wavelengths are high and cause detection problems. To eliminate the formamide-related problems, Altria et al. [15] used acetonitrile–methanol (50:50) as NACE solvent and peracetylated β-CD as resolving agent.

During the synthesis of heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-β-CD) we found that it had a reasonably high solubility in methanol [1,4]. Therefore, we decided to investigate if HDAS-β-CD could be used for the NACE separation of enantiomers in methanol, one of the most commonly used reversed-phase HPLC solvents.

2. Experimental

NACE separations were carried out with a P/ACE 2100 CE unit (Beckman Instruments, Fullerton, CA, USA) using 23 cm (effective length 17 cm)×25 μm I.D.×150 μm O.D. untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The UV detector of the P/ACE unit was operated at 214 nm, the cartridge coolant was thermostated at 20°C. The samples were injected electrokinetically at 10 kV for 5 s. The separation potential was maintained at 25 kV. In the low-pH BGEs, the measured (and viscosity-corrected) currents were 10.8 (and 10.8) μA at 0 m*M* HDAS-β-CD, 11.6 (and 12.8) μA at 10 m*M* HDAS-β-CD, 12.2 (and 14.6) μA at 20 m*M* HDAS-β-CD, and 11.9 (and 19.0) μA at 40 m*M* HDAS-β-CD. In the high pH BEs, the measured (and viscosity-corrected) currents were 17.3 (and 17.3) μA at 0 m*M* HDAS-β-CD, 18.1 (and 19.9) μA at 10 m*M* HDAS-β-CD, 18.7 (and 22.4) μA at 20 m*M* HDAS-β-CD, and 17.0 (and 27.2) μA at 40 m*M* HDAS-β-CD.

All chemicals used in the BGE preparation were obtained from Aldrich (Milwaukee, WI, USA), except HDAS-β-CD (Cat. No. 733401, Regis Technologies, Morton Grove, IL, USA), which was synthesized as described in Ref. [1]. The acidic stock BE was prepared by adding 0.050 mole dichloroacetic acid and 0.025 mole of triethylamine to a 1 litre volumetric flask and filling it to the mark with HPLC grade methanol (EM Science, Gibbstown, NJ, USA). The basic stock BGE was prepared by adding 0.025 mol dichloroacetic acid and 0.050 mol of triethylamine (TEA) to a 1 litre volumetric flask and filling it to the mark with HPLC-grade methanol. The 10, 20 and 40 m*M* HDAS-β-CD BGEs were prepared by weighing out the required amounts of the sodium salt of HDAS-β-CD into 25 ml volumetric flasks and bringing the volumes to mark with either the acidic or the basic stock BE solutions.

The 0.5 m*M* benzyl alcohol (BA) samples were prepared with the respective HDAS-β-CD BGEs and used as external mobility markers. The effective mobility of BA (μ_{BA}^{eff}) was determined in each HDAS-β-CD BGE using the external electroosmotic flow (EOF) marker method described in [16]. The 0.5 m*M* racemic analyte sample solutions (prepared in the respective HDAS-β-CD BGEs) also contained 0.5 m*M* BA, so that the observed mobilities of both BA (μ_{BA}^{obs}) and the enantiomers (μ_1^{obs} and μ_2^{obs}) could be determined from the same NACE runs. Since μ_{BA}^{eff} was known in each HDAS-β-CD BGE, the electroosmotic flow mobility, μ_{EO}, could be obtained as $\mu_{EO}=\mu_{BA}^{obs}-\mu_{BA}^{eff}$, from which the effective mobilities of the analyte enantiomers were calculated as $\mu_1^{eff}=\mu_1^{obs}-\mu_{EO}$, and the separation selectivities, α, were calculated as $\alpha=\mu_1^{eff}/\mu_2^{eff}$ (subscript 2 arbitrarily refers to the enantiomer which

was less mobile in the 10 m*M* HDAS-β-CD BE). The dimensionless normalized EOF values, β, were calculated as $\beta=\mu_{EO}/\mu_2^{eff}$ [17]. The peak resolution values, R_s, were calculated as usual, by dividing the migration time difference of the two enantiomers with the sum of their half peak widths at the baseline.

3. Results and discussion

3.1. Selection of the composition of the BGEs

According to the CHARM model of CE enantiomer separations [9], one needs to use only two stock BGEs (a low-pH and a high-pH BGE) to locate the conditions under which separation selectivity for an enantiomer pair is at a maximum. Instead of trying to adjust, measure and report questionable pH* (apparent pH) values in the NACE solvents, we opted for reproducible buffer preparation and maximum buffering capacity by creating an acidic and a basic stock BGE in which the buffering species were half dissociated. We selected a moderately strong (in water) acid, dichloroacetic acid ($pK_a=1.3$ in water) and a moderately strong (in water) base, triethylamine ($pK_b=3.3$ in water) and used a mixture of 50 m*M* acid and 25 m*M* base to create the acidic stock BGE solution, and a mixture of 25 m*M* acid and 50 m*M* base to create the basic stock BGE solution. We expected that most weak bases studied here will be fully protonated in the acidic stock BGE, and that most weak acids studied here will be fully dissociated in the basic stock BGE.

The viscosities of the acidic and basic stock BGEs (measured with the P/ACE unit as a viscosimeter according to Ref. [18]) were identical within 1%. The nominal concentrations of both charge carrying species (dichloroacetate and triethyl ammonium) in the two stock BGEs were identical, 25 m*M* each. Yet, quite unexpectedly, the current in the basic stock BGE was about 70% higher than in the acidic stock BGE. The same conductivity difference remained when the stock BGEs were remade using different, fresh batches of reagents. We do not have yet a solid, experiments-backed explanation for the observed behavior.

3.2. Effective electrophoretic mobilities of neutral and weak acid analytes as a function of the composition of the BGEs

The effective electrophoretic mobilities of BA, μ_{BA}^{eff}, in the acidic and basic HDAS-β-CD BGEs are shown in the first lines in Tables 1 and 2. BA complexes very weakly with HDAS-β-CD and displays an ever so slightly negative effective mobility ($-0.08\cdot10^{-5}$ cm^2 V^{-1} s^{-1}), even at HDAS-β-CD concentrations as high as 40 m*M*. (The solubility limit of HDAS-β-CD is about 45 m*M* in the BGEs used). The same is true for the other, larger neutral analytes tested (such as, e.g., 4-phenyl-1,3-dioxane, α-methyl-α-phenyl succinimide, *trans*-2-phenyl-1-cyclohexanol).

The effective electrophoretic mobilities of the weak acids tested here (ciprofibrate, fenoprofen, ibuprofen) were zero (within experimental error) in the acidic stock BGE. This is not surprising, because their aqueous pK_a values are at least three units higher than the aqueous pK_a of dichloroacetic acid. When the concentration of HDAS-β-CD was increased to 40 m*M* in the acidic stock BGE, the effective mobility of ciprofibrate, fenoprofen and ibuprofen remained practically zero, i.e. they behaved as neutral analytes (see above).

The effective electrophoretic mobilities of ciprofibrate, fenoprofen and ibuprofen, measured in the basic BGEs, are listed in Table 3. In the absence of HDAS-β-CD, the effective mobilities are around $-15\cdot10^{-5}$ cm^2 V^{-1} s^{-1}, which are of the same order of magnitude as the previously observed effective mobilities in aqueous BGEs [17,19]. When the concentration of HDAS-β-CD is increased to 40 m*M*, the measured effective mobilities decrease to about $-13\cdot10^{-5}$ cm^2 V^{-1} s^{-1}. The mobility change can be attributed to three non-separable factors: increase in the solution viscosity, increase in the ionic strength and complexation of the analyte anions by HDAS-β-CD. When one corrects the effective mobilities for the HDAS-β-CD-related viscosity increase, as proposed in Ref. [20], the values become as large as about $-19\cdot10^{-5}$ cm^2 V^{-1} s^{-1}. Correction for the ionic strength effects would lead to even larger mobilities, a most unlikely scenario unless the analyte anions complex very very strongly with HDAS-β-CD. One could argue that if there is

Table 1
NACE data for weak base analytes in the acidic HDAS-β-CD BGEs

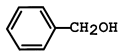
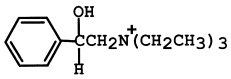
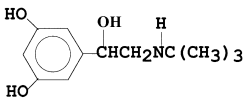
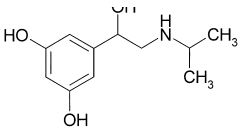
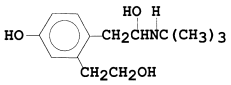
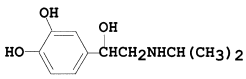
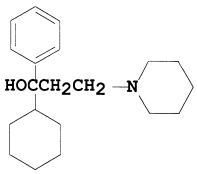
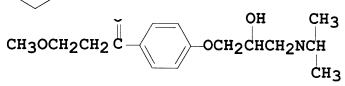
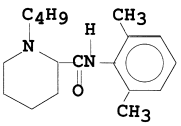
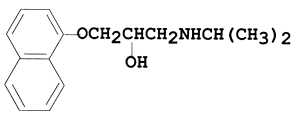
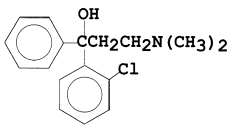
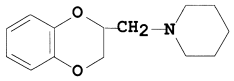
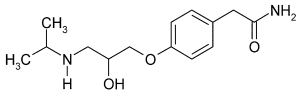
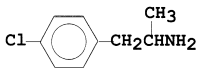
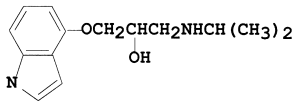
		0 m*M*	10 m*M*				20 m*M*				40 m*M*			
Structure	Ref. No.	μ	μ	α	β	R_s	μ	α	β	R_s	μ	α	β	R_s
Marker:														
	BZOH	0	−0.03 (±0.03)				−0.04 (±0.02)				−0.08 (±0.02)			
	Q^+	27.5	15.8	1.00	0.2	0.0	12.3	1.08	0.5	0.8	8.3	1.15	0.5	1.9
	B1	14.6	6.3	1.13	0.6	1.6	5.0	1.21	1.1	1.7	3.2	1.35	1.4	5.3
	B2	14.8	1.4	1.31	3.3	2.5	0.7	1.70	7.0	2.7	−0.02	−3.20	−184.0	0.9
	B3	15.3	7.8	1.08	0.5	1.2	6.0	1.19	1.0	1.2	4.3	1.22	1.0	1.9
	B4	15.8	5.5	1.16	0.8	2.0	4.1	1.19	1.4	1.7	2.8	1.28	1.6	2.7
	B5	15.9	7.5	1.00	0.6	0.0	5.4	1.03	1.1	0.4	3.1	1.14	1.4	1.8
	B6	16.5	5.2	1.05	0.9	0.6	3.8	1.17	1.5	1.5	2.2	1.53	1.9	4.0
	B7	17.1	7.4	1.08	0.7	0.6	6.3	1.13	0.9	0.9	5.2	1.18	0.9	1.8
	B8	17.2	3.9	1.05	2.5	3.0	0.3	2.57	20.7	2.7	−0.6	0.27	−7.7	4.3
	B9	17.9	1.8	1.29	2.5	3.0	0.3	2.57	20.7	2.7	−0.6	0.27	−7.7	4.3

Table 1. Continued

Structure	Ref. No.	0 m*M*	10 m*M*				20 m*M*				40 m*M*			
		μ	μ	α	β	R_s	μ	α	β	R_s	μ	α	β	R_s
	B10	20.3	2.7	1.03	1.5	0.4	1.2	1.26	4.8	1.3	0.5	1.40	8.7	1.8
	B11	20.8	2.8	1.06	1.7	1.4	1.4	1.35	4.2	2.0	0.2	1.68	19.1	1.3
	B12	21.2	5.3	1.17	0.9	2.0	0.7	1.69	8.0	2.0	0.4	2.29	10.6	3.3
	B13	27.7	16.2	1.00	0.3	0.0	4.3	1.10	1.4	1.1	2.8	1.23	1.6	2.6

Effective mobility of the less mobile enantiomer (μ, in 10^{-5} cm^2 V^{-1} s^{-1}), separation selectivity (α), dimensionless normalized EOF value (β) and measured peak resolution (R_s). Negative α values mean that the effective mobilities of the two enantiomers are opposite in sign.

complexation at all, its extent would be slightly different for fenoprofen, ibuprofen and ciprofibrate. Therefore, their effective mobility ratios (i.e., $\mu^{\text{eff}}_{\text{cipro}}/\mu^{\text{eff}}_{\text{feno}}$ and $\mu^{\text{eff}}_{\text{feno}}/\mu^{\text{eff}}_{\text{ibu}}$) were calculated in the 0 and 40 m*M* HDAS-β-CD BGEs and are listed in Table 3. The values are identical within experimental error, suggesting that these negatively charged analytes, just as the neutral analytes, do not complex strongly with HDAS-β-CD. The observed effective mobility decrease is simply an across-the-board, nonselective mobility decrease that is caused by a combination of ionic strength and viscosity effects, and cannot be simply corrected for by multiplying the measured effective mobilities with the measured viscosity ratios as suggested in Ref. [20].

Under the experimental conditions used, the mobility of the EOF is as high as $5\cdot10^{-5}$ cm^2 V^{-1} s^{-1}. For the neutral and anionic analytes, the dimensionless normalized EOF mobilities, the β values, are as large as -50 to -100. Therefore, one can conclude that there is no real chance to see separation of the enantiomers of neutral or acidic analytes in pure methanolic NACE with HDAS-β-CD as the resolving agent.

3.3. Effective electrophoretic mobilities of cationic analytes as a function of the composition of the BGEs

The effective electrophoretic mobilities of the less mobile enantiomer of a permanently charged, quaternary ammonium compound (Q^+) are shown in the second lines of Table 1 (acidic BGEs) and Table 2 (basic BGEs). In the absence of HDAS-β-CD, the effective mobilities of Q^+ are similar (27.5 vs. 27.6 in 10^{-5} cm^2 V^{-1} s^{-1} units) in the two BGEs. As the concentration of HDAS-β-CD is increased, the effective mobility of Q^+ decreases more rapidly in the acidic BGEs than in the basic BGEs. This indicates that the constituents of the acidic stock BGE interact less strongly with HDAS-β-CD than those of the basic stock BGE, i.e. the effective concentration of HDAS-β-CD available for complexation with Q^+ is higher in the acidic BGE.

The third and lower lines of Tables 1 and 2 show the effective electrophoretic mobilities of the weak bases studied. (The weak bases are listed in order of increasing effective mobility in the acidic stock BGE.) Considering the structure of the bases, it is

Table 2
NACE data for weak base analytes in the basic HDAS-β-CD BGEs

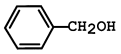
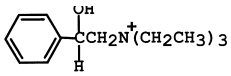
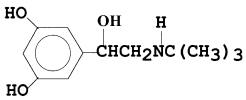
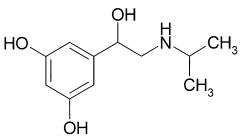
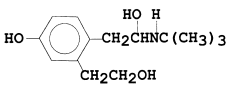
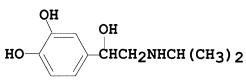
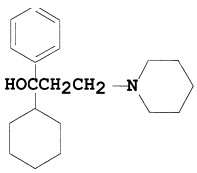
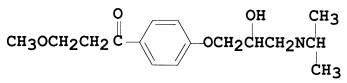
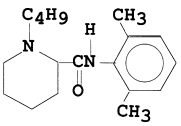
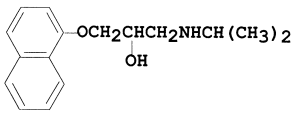
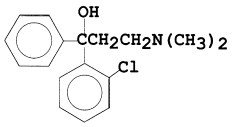
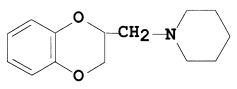
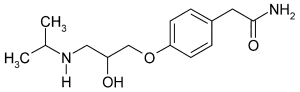
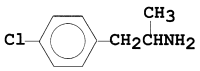
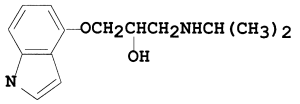
Structure	Ref. No.	0 m*M*	10 m*M*				20 m*M*				40 m*M*			
		μ	μ	α	β	R_s	μ	α	β	R_s	μ	α	β	R_s
Marker: 	BZOH	0	0				0				−0.00 (±0.03)			
	Q^+	27.6	18.2	1.00	0.2	0.0	16.1	1.00	0.2	0.0	10.1	1.09	0.4	1.5
	B1	3.2	1.8	1.03	1.8	<0.5	1.6	1.05	2.3	0.6	0.6	1.17	6.5	1.0
	B2	3.5	1.2	1.00	3.3	0.0	1.1	1.00	2.7	0.0	0.5	1.04	5.9	<0.5
	B3	5.6	2.6	1.00	1.4	0.0	1.4	1.07	2.9	0.9	1.4	1.07	2.9	0.9
	B4	reactive	reactive				reactive				reactive			
	B5	0.5	0.4	N/A	11.3	0.0	0.3	N/A	9.6	0.0	0.2	N/A	12.8	0.0
	B6	3.3	1.5	1.00	2.7	0.0	1.4	1.00	2.3	0.0	0.9	1.09	3.6	0.7
	B7	0.8	0.6	N/A	7.20	0.0	0.4	N/A	8.8	0.0	0.30	N/A	14.5	0.0
	B8	3.1	1.2	1.00	3.3	0.0	1.1	1.00	3.0	0.0	0.7	1.00	4.6	0.0
	B9	0.5	0.3	N/A	15.2	0.0	0.2	N/A	13.4	0.0	0.07	N/A	51.5	0.0
	B10	0.07	0.0	N/A	N/A	0.0	0.0	N/A	N/A	0.0	0.0	N/A	N/A	0.0

Table 2. Continued

Structure	Ref. No.	0 m*M*	10 m*M*				20 m*M*				40 m*M*			
		μ	μ	α	β	R_s	μ	α	β	R_s	μ	α	β	R_s
	B11	2.9	1.1	1.00	3.8	0.0	1.0	1.00	3.1	0.0	0.6	1.00	4.9	0.0
	B12	5.2	1.4	1.06	2.8	<0.5	1.3	1.07	2.5	0.7	0.9	1.12	3.5	0.9
	B13	3.8	1.8	1.00	2.3	0.0	1.7	1.00	1.8	0.0	0.9	1.04	3.1	<0.5

Effective mobility of the less mobile enantiomer (μ, in 10^{-5} cm^2 V^{-1} s^{-1}), separation selectivity (α), dimensionless normalized EOF value (β) and measured peak resolution (R_s).

reasonable to assume that all of them are fully protonated in the acidic stock BGE. This assumption is corroborated by the fact that the effective mobility values listed in the second column of Table 1 are in the (14 to 28)·10^{-5} cm^2 V^{-1} s^{-1} range, and are commensurable with the effective mobility values of the permanently charged Q^+. The effective mobility values in the second column of Table 2 are in the (0.1 to 6)·10^{-5} cm^2 V^{-1} s^{-1} range indicating that the weak bases are still about 0.1 to 30% protonated in the basic stock BGE. This means that, at half titration, TEA does not create a sufficiently basic medium to completely deprotonate all of the weak bases studied here (though TEA was sufficiently basic to completely deprotonate all of the weak acids studied, see above). As expected from the complexation behavior of the neutral analytes, the weak bases which are almost completely deprotonated do not complex sufficiently with HDAS-β-CD in the methanolic BGEs.

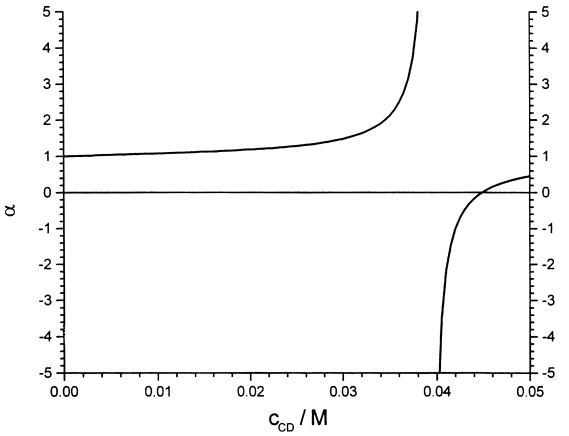


Fig. 1. Separation selectivity curve for a cationic enantiomer pair with HDAS-β-CD as the resolving agent, calculated according to Eq. 24 of the CHARM model [9]. Constants used: $\mu_R^0=\mu_S^0=15.6\cdot10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{RCD}^0=-5.5\cdot10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{SCD}^0=-5.7\cdot10^{-5}$ cm^2 V^{-1} s^{-1}, $K_{RCD}=60$, $K_{SCD}=66$.

Fig. 1 shows an example of the theoretical separation selectivity curve one can calculate with the CHARM model [9] for a fully protonated weak base enantiomer pair using HDAS-β-CD as resolving

Table 3
Effective mobilities and mobility ratios for the weak acid analytes in the basic HDAS-β-CD BGEs

Name	HDAS			
	0 m*M*		40 m*M*	
	μ_{eff}	μ_{eff} Ratio:	μ_{eff}	μ_{eff} Ratio:
Cipofibrate	−15.05	cipro./feno.=1.01	−13.12	cipro./feno.=1.02
Fenoprofen	−14.85	feno./ibu.=1.03	−12.89	feno./ibu.=1.03
Ibuprofen	−14.40	cipro./ibu.=1.05	−12.45	cipro./ibu.=1.05

agent. In general, separation selectivity will increase, initially slowly, then very rapidly, as the HDAS-β-CD concentration is increased (Segment 1). At a certain HDAS-β-CD concentration, the effective mobility of the stronger binding enantiomer (Enantiomer 2) approaches zero, while that of the weaker binding enantiomer, Enantiomer 1, remains a larger, positive value. This leads to a rapidly increasing α and, eventually, to a discontinuity in α ($\alpha \rightarrow \infty$, Segment 2). As the effective mobility of the stronger binding Enantiomer 2 crosses zero and becomes negative, α crosses over to the other side of the discontinuity. This results in large, negative α values ($\alpha \rightarrow -\infty$, Segment 3). As the HDAS-β-CD concentration is increased further, α first approaches zero from the negative side, then becomes zero when the effective mobility of Enantiomer 1 becomes zero (Segment 4). From then on, α is positive again (because the effective mobilities of both enantiomers are now negative), and approaches its limiting value when the HDAS-β-CD concentration becomes very large (Segment 5).

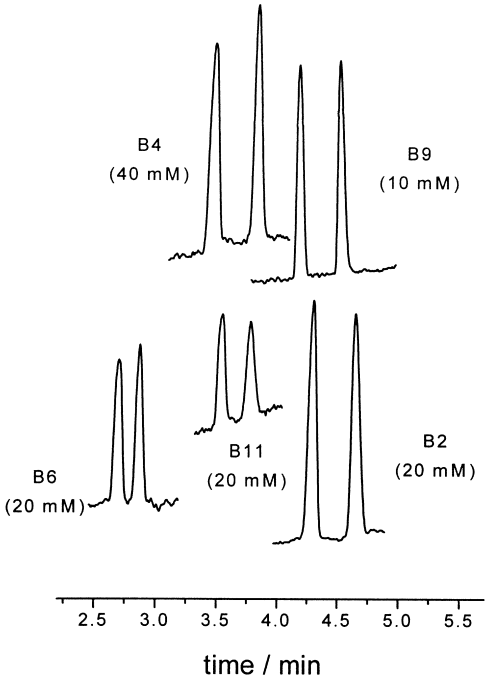


Fig. 2. Typical electropherograms of weak base analytes in the acidic methanolic HDAS-β-CD BGEs. The numbers in parentheses indicate the HDAS-β-CD concentrations (m*M*). Detector sensitivities: between 1 and 5 mAU full scale. Other conditions: see Section 2.

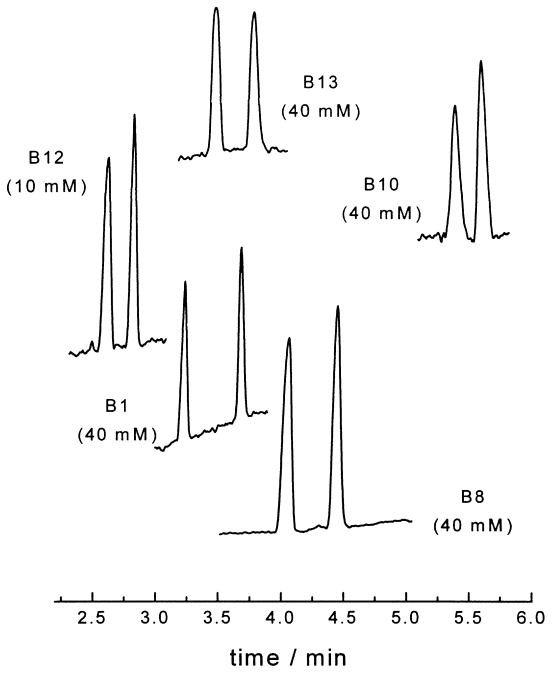


Fig. 3. Typical electropherograms of weak base analytes in the acidic methanolic HDAS-β-CD BGEs. The numbers in parentheses indicate the HDAS-β-CD concentrations (m*M*). Detector sensitivities: between 1 and 5 mAU full scale. Other conditions: see Section 2.

This general behavior is best demonstrated experimentally by the data in Table 1 for weak base B2 (Segments 1 and 3) and B9 (Segments 1, 2 and 5). Due to the 45 m*M* solubility limit of HDAS-β-CD, the rest of the weak bases only experience Segments 1 and 2 of the general selectivity curve shown in Fig. 1.

Since both the α and β values are generally favorable between 20 m*M* and 40 m*M* HDAS-β-CD concentrations, the observed peak resolution values are quite high and the separations are very fast in this methanolic acidic NACE system. A few typical weak base separations are shown in Figs. 2 and 3.

4. Conclusions

The NACE separation of enantiomers in pure methanol background electrolytes using the new,

moderately hydrophobic, single isomer charged cyclodextrin, the sodium salt of HDAS-β-CD was studied here. Excellent, rapid and rugged separations were obtained for the weak base enantiomers in acidic methanolic BGEs when the HDAS-β-CD concentrations were in the 20 to 50 m*M* range. The measured separation selectivities followed the theoretical predictions of the charged resolving agent migration model [9]. The binding constants of neutral analytes and weak acid analytes are very low in the methanolic HDAS-β-CD BGEs.

Acknowledgements

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