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Capillary electrophoretic separation of weak base enantiomers using the single-isomer heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin as resolving agent and methanol as background electrolyte solvent

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Abstract

The sodium salt of the single-isomer, heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin (HDMS-βCD) was used as resolving agent in the capillary electrophoretic (CE) separation of weak base enantiomers in pure methanol background electrolytes (BEs). According to the requirements of the charged resolving agent migration model of CE enantiomer separations (CHARM model), a high buffer-capacity, low pH methanolic BE was created from 25 mM phosphoric acid and 12.5 mM NaOH. In this BE, the solubility of HDMS-βCD was as high as 50 mM, permitting the realization of very high separation selectivities and short separation times for the fully protonated weak base enantiomers.

Keywords: Enantiomers; Capillary electrophoresis; Separation selectivities

1. Introduction

Recent reports [1–4] described the first successful separation of enantiomers by nonaqueous capillary electrophoresis (NACE). Most of these separations were achieved with formamide [1], *N*-methylformamide [1–3] and *N*,*N*-dimethylformamide [1] as solvents and native or derivatized β- and γ-cyclodextrins (CDs) as resolving agents. The formamide derivatives are good solvents for the polar CDs and permit the preparation of highly concentrated CD solutions. Unfortunately, they show strong absorbance at low UV wavelengths where most CE UV detectors operate. In order to eliminate the UV absorbance problem, a recent paper reported the use of a 1:1 mixture of acetonitrile and methanol as the BE solvent in combination with the hydrophobic peracetylated β-CD as resolving agent [4].

In addition to the neutral CDs, charged CDs (both weak electrolytes and strong electrolytes) are used ever more frequently for the facile CE separation of enantiomers [5]. The most popular

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strong-electrolyte charged CDs are the randomly sulfobutylated [1,6] and randomly sulfated [7] β-CDs. Because the isomer composition of the randomly substituted CDs can vary greatly from batch-to-batch (both in terms of the number of charged functional groups and their substitution positions on the CD), and because this compositional variability can lead to different separation selectivities [8], a family of pure, single-isomer, fully sulfated CDs was developed [9–11] which contain seven sulfate groups on the nonchiral face of the CD molecule. In these single-isomer sulfated CDs, there are either fourteen hydroxy groups (i.e. heptakis-6-sulfato-βCD, [10]), fourteen acetyl groups (i.e. heptakis(2,3-diacetyl-6-sulfato)-βCD, [9]), or fourteen methyl groups (i.e. heptakis(2,3-dimethyl-6-sulfato)-βCD, [11]) on the chiral face of the CD molecule. To aid in their rational use, a CE migration model, the charged resolving agent migration model (CHARM model) of CE enantiomer separations was developed [12]. The CHARM model predicts that for neutral and monoprotic analytes, the global separation selectivity maxima can be located simply by using a low pH and a high pH stock BE and varying the type and concentration of the charged resolving agent in these BEs.

Two of the single-isomer, heptasulfated β-CDs were designed to be soluble in simple, polar, organic solvents. This permitted the successful CE use of heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin in pure methanolic BEs [13], and the CE use of heptakis(2,3-dimethyl-6-sulfato)-β-cyclodextrin in methanol: water mixture BEs [14]. The objective of this paper is to explore the first-ever, possible CE use of heptakis(2,3-dimethyl-6-sulfato)-β-cyclodextrin for the separation of the enantiomers of weak base analytes in pure methanolic background electrolytes.

2. Experimental

The nonaqueous CE separations were carried out with a P/ACE 5010 CE unit (Beckman Instruments, Fullerton, CA) and 19/26 cm, 25 μm i.d. × 150 μm o.d. untreated fused silica capillaries (Polymicro Technologies, Phoenix, AZ). The cartridge coolant was thermostated at 15°C. The 214 nm filter of the UV detector was used to detect all analytes. The samples were injected electrokinetically at 10 kV for 1 s. The applied potential was maintained at 15 kV.

All chemicals used in the BE preparation were obtained from Aldrich Chemical (Milwaukee, WI), except HDMS-βCD (cat. no. 733402, Regis Technologies, Morton Grove, IL), which was synthesized as described in [11]. According to the dictates of the CHARM model of CE enantiomer separations [12], only a single acidic stock BE was used. The acidic stock BE was prepared by adding 0.025 mol phosphoric acid and 0.0125 mol of NaOH to a 1 l volumetric flask, then filling the flask to the mark with MeOH. The 12.5, 25 and 40 mM HDMS-βCD BEs were prepared by weighing out the required amounts of the sodium salt of heptakis(2,3-dimethyl-6-sulfato)-β-cyclodextrin into 25 ml volumetric flasks and bringing the volumes to mark with the acidic stock BE solution.

Nitromethane (N) samples, 0.5 mM, (external mobility marker) were prepared with each HDMS-βCD BE. The effective mobility of N (μ_N^{eff}) was determined in each HDMS-βCD BE using the external electroosmotic (EO) flow marker method [15]. μ_N^{eff} proved to be zero (within experimental error) at each of the HDMS-βCD concentrations tested, indicating that nitromethane could be added to each analyte sample and used as a direct EO flow mobility marker.

All test analytes (epinephrine, isoproterenol, metaproterenol, oxyphencyclimine, and propranolol) were obtained from Sigma (St. Louis, MO, USA). The 0.5 mM racemic analyte solutions, dissolved in the HDMS-βCD BEs, also contained 0.5 mM N, the EO flow mobility marker. Thus, the observed mobilities of both N (μ_N^{obs}) and the enantiomers (μ_1^{obs} and μ_2^{obs}) could be determined from the same runs. Since $\mu_N^{obs} = \mu_{EO}$, this allowed us to calculate the effective mobilities of the analyte enantiomers as $\mu_1^{eff} = \mu_1^{obs} - \mu_{EO}$. The separation selectivities, α, were calculated as $\alpha = \mu_1^{eff}/\mu_2^{eff}$ (subscript 2 arbitrarily refers to the enantiomer which proved less mobile in the 10 mM HDMS-βCD BE) [12]. The normalized electroosmotic flow mobility, β, was calculated as $\beta = \mu_{EO}/\mu_2^{eff}$

Table 1
Measured viscosities (using the P/ACE as a capillary viscosimeter [16]), measured currents and viscosity-corrected currents [17] for the HDMS-βCD-containing MeOH Bes

$C_{\text{HDMS-}\beta\text{CD}}$ (mM)	Viscosity (cP)	I_{meas} (μA)	$I_{\text{visc. corr.}}$ (μA cP^{-1})
12.5	0.697	3.0	2.1
25	0.797	3.4	2.71
40	0.906	3.6	3.26

[12], while peak resolution, *Rs*, was calculated by dividing the migration time difference of the two enantiomers with the total of their half widths.

The measured BE viscosities, the measured current values (at 569 V cm^{-1}) and the viscosity-corrected current values [16,17] are listed in Table 1.

3. Results and discussion

Table 2 shows that μ_{EO} remains approximately constant at around 5×10^{-5} cm^2 V^{-1} s^{-1} as the HDMS-βCD concentration is varied from 0 to 40 mM. Surprisingly, this μ_{EO} value is higher than what one would expect in an acidic, methanolic BE. This relatively strong EO flow helps in obtaining fast enantiomer separations for the weak bases.

Since all of the weak bases studied here (epinephrine, isoproterenol, metaproterenol, oxyphencyclimine and propranolol) are expected to be fully protonated in the phosphoric acid: dihydrogen phosphate acidic methanolic BE, their migration behavior should be very similar, and follow the predictions of the CHARM model [12]. Figs. 1 and 2 show the theoretical effective mobility and separation selectivity curves for a fully

Table 2
Electrophoretic data for the five weak base test analytes at a field strength of 569 V cm^{-1}

Analyte	[HDMS-βCD][a]	μ^{EO} [b]	μ_1^{eff} [c]	μ_2^{eff} [d]	α[e]	β[f]	*Rs*[g]
Epinephrine	0	4.62	15.50	15.50	1.00	0.3	N/A
	12.5	5.37	0.98	0.86	1.14	6.3	0.5
	25	5.25	−0.17	−0.59	0.29	−31.0	<0.5
	40	5.27	−0.57	−1.04	0.55	−9.0	3.3
Isoproterenol	0	5.50	16.13	16.13	1.00	0.3	N/A
	12.5	5.45	8.40	7.96	1.06	0.7	1.5
	25	5.32	6.56	6.17	1.06	0.9	1.7
	40	5.19	5.33	5.04	1.06	1.1	1.4
Metaproterenol	0	5.10	15.54	15.54	1.00	0.3	N/A
	12.5	5.00	8.06	7.29	1.10	0.7	2.8
	25	4.83	6.34	5.65	1.12	0.9	3.2
	40	5.49	5.13	4.60	1.12	1.2	2.3
Oxyphencyclimine	0	4.80	20.43	20.43	1.00	0.2	N/A
	12.5	5.42	8.14	7.87	1.03	0.7	0.9
	25	5.35	5.95	5.56	1.07	0.9	2.0
	40	5.18	4.74	4.35	1.09	1.2	2.4
Propranolol	0	5.74	16.63	16.63	1.00	0.3	N/A
	12.5	5.40	6.83	6.45	1.06	0.8	1.5
	25	5.14	5.14	4.48	1.15	1.1	3.7
	40	5.29	4.34	3.49	1.24	1.5	5.2

[a] HDMS-βCD concentration of BE (in mM).
[b] Electroosmotic flow mobility (μ^{EO}, in 10^{-5} cm^2 V^{-1} s^{-1} units).
[c] Effective mobility of the first enantiomer (μ_1^{eff}, in 10^{-5} cm^2 V^{-1} s^{-1} units).
[d] Effective mobility of the second enantiomer (μ_2^{eff}, in 10^{-5} cm^2 $\text{V}^{-1}\text{s}^{-1}$ units).
[e] Separation selectivity (α).
[f] Normalized EO flow mobility value (β).
[g] Measured peak resolution (*Rs*).

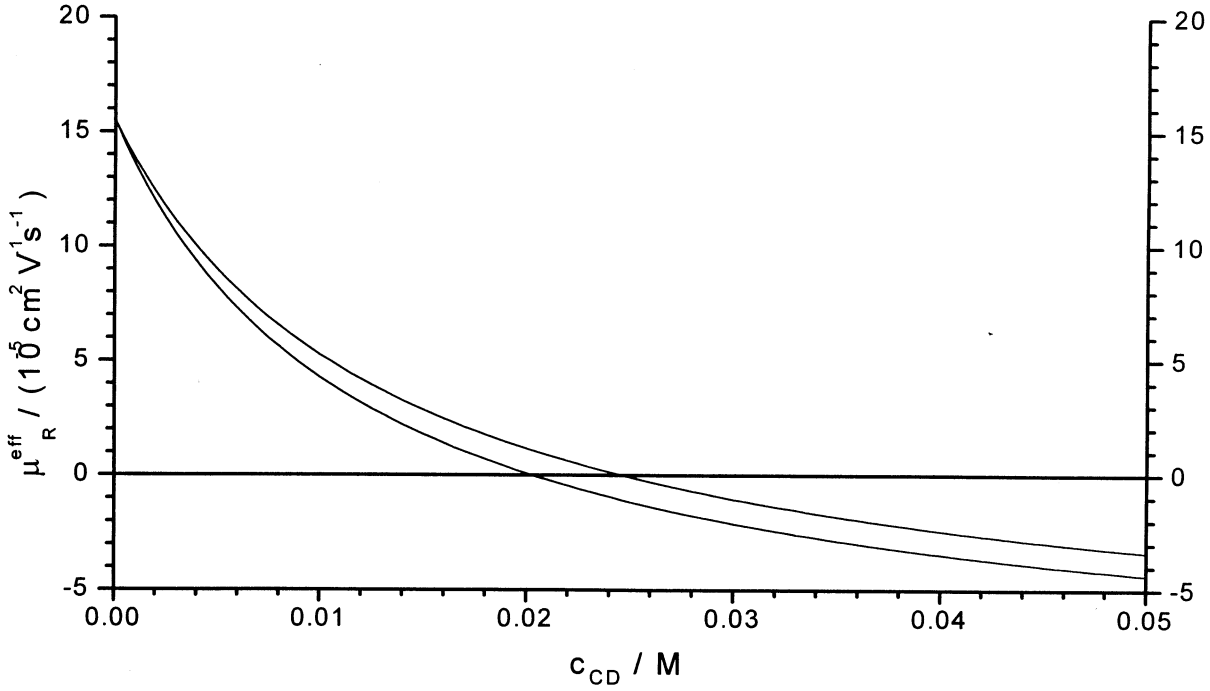

Fig. 1. Effective mobility curves for a fully protonated weak base enantiomer pair with HDMS-βCD as resolving agent, calculated according to the CHARM model (Eq. 29 in Ref. [12]). Constants used in the calculations: $\mu_R^0 = \mu_S^0 = 15.5 \times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{RCD}^0 = -8.4 \times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{SCD}^0 = -9.1 \times 10^{-5}$ cm^2 V^{-1} s^{-1}, $K_{RCD} = 75$, $K_{SCD} = 84$.

protonated weak base enantiomer pair that can be calculated with the CHARM model (with Eqs. 29 and 30 in Ref. [12]) and HDMS-βCD as resolving agent. The cationic effective mobility of the enantiomers decreases as the HDMS-βCD concentration is increased, then it becomes anionic: first for the stronger-complexing, slower enantiomer, then for both enantiomers. Simultaneously, separation selectivity will first increase, then approach a positive, infinitely large value, as the HDMS-βCD concentration approaches the cationic-to-anionic cross-over point on the mobility curve. As soon as the effective mobility of the stronger-binding enantiomer, enantiomer 2, becomes negative, separation selectivity crosses over to the other side of the discontinuity and becomes an infinitely large negative value. Upon further increase of the HDMS-βCD concentration, separation selectivity first crosses the $\alpha = 0$ line from the negative side, then moves toward its positive, limiting value, which always remains less than unity.

Table 2 lists the experimentally determined effective mobilities of the weak base enantiomers (μ^{eff}), the separation selectivities (α), the normalized electroosmotic flow mobilities (β), and the observed peak resolution (Rs) values at a field strength of 569 V cm^{-1}. Except for epinephrine, all the other bases maintain a cationic effective mobility across the experimentally accessible HDMS-βCD concentration range. Their cationic effective mobilities decrease — and the separation selectivities increase—as the HDMS-βCD concentration is increased, following the predictions of the CHARM model (see the first segment of the separation selectivity curve in Fig. 2). Since their migration direction does not become anionic in the HDMS-βCD concentration range studied, their binding strength must be relatively weak. Epinephrine, on the other hand, must bind much more strongly: it shows the entire expected mobility and separation selectivity behavior depicted by Figs. 1 and 2. At 12.5 mM HDMS-βCD concentration, the effective mobility of epinephrine is

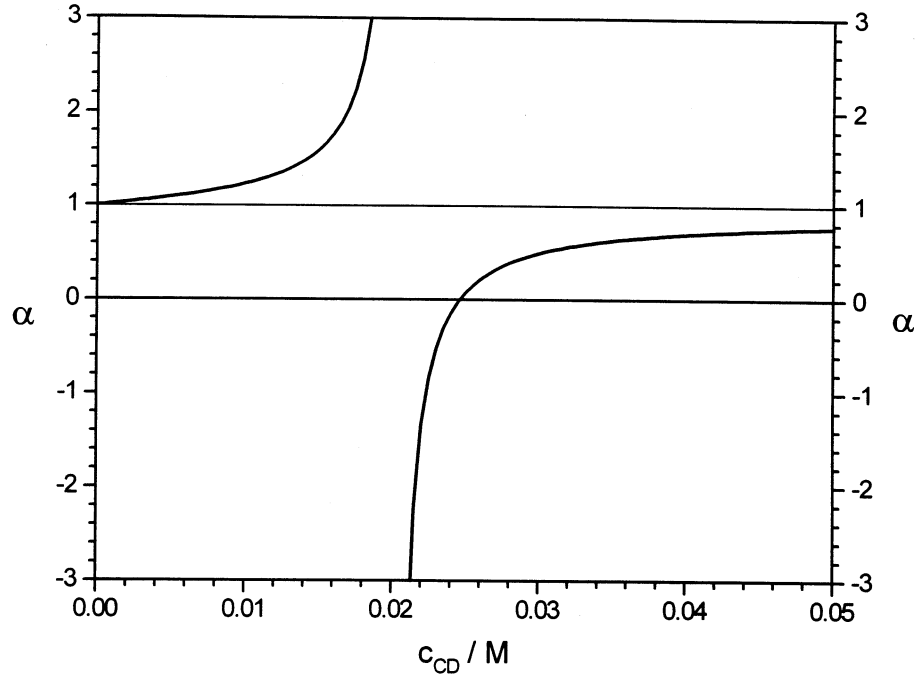

Fig. 2. Separation selectivity curve for a fully protonated weak enantiomer pair with HDMS-βCD as resolving agent, calculated according to the CHARM model (Eq. 30 in Ref. [12]). Constants used in the calculations: $\mu_R^0 = \mu_S^0 = 15.5 \times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{RCD}^0 = -8.4 \times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{SCD}^0 = -9.1 \times 10^{-5}$ cm^2 V^{-1} s^{-1}, $K_{RCD} = 75$, $K_{SCD} = 84$.

cationic, but then its migration direction turns anionic when the HDMS-βCD concentration is increased to 25 and 40 mM. Simultaneously, separation selectivity is best between 12.5 and 25 mM HDMS-βCD concentration, where the effective mobility changes from cationic to anionic (there is a selectivity discontinuity in this region).

Peak resolution (last column in Table 2) depends on both separation selectivity, the effective charge of the analytes and the normalized electroosmotic flow mobility. Since all three of these parameters vary simultaneously—but in different ways—as the concentration of HDMS-βCD is varied, no general trends can be assigned a priori, except to say that adequate resolution was obtained for all bases in less than 7 min. Representative electropherograms of the weak bases separated in this acidic, methanolic BE are shown in Figs. 3 and 4. Generally, the α and β values are quite favorable in the 10–40 mM HDMS-βCD concentration range. Therefore, good peak resolution can be accomplished very rapidly for the weak base analytes in this acidic, methanolic BE.

4. Conclusions

This paper demonstrates, for the first time, the successful use of the single-isomer, heptakis(2,3-dimethyl-6-sulfato)-β-cyclodextrin [11] for the CE separation of weak base enantiomers in pure methanol background electrolytes. The migration behavior of these analytes closely follows the predictions of the charged resolving agent migration model [12] and affords the development of good, rapid and rugged CE enantiomer separations for chiral weak bases.

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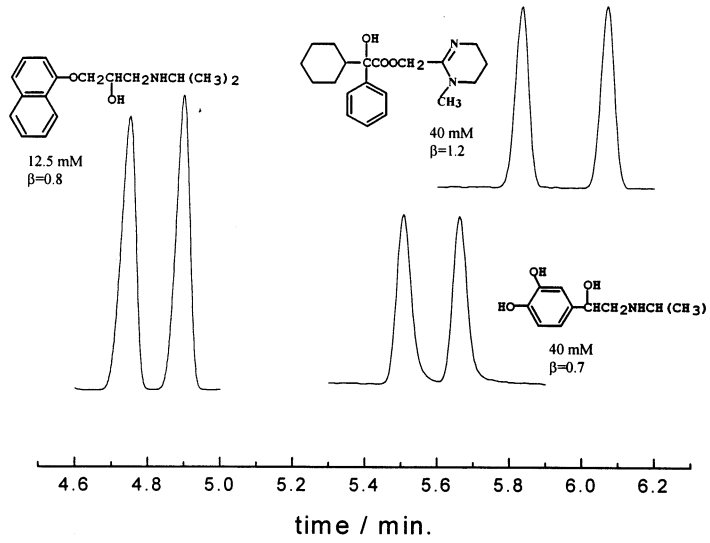

Fig. 3. Typical electropherograms of weak base analytes in the acidic, methanolic HDMS-βCD BEs. The numbers next to the structures indicate the HDAS-βCD concentrations (mM) and normalized electroosmotic flow mobilities (β). Detector sensitivities: between 1 and 5 mAU/full scale. Other conditions: see Section 2.

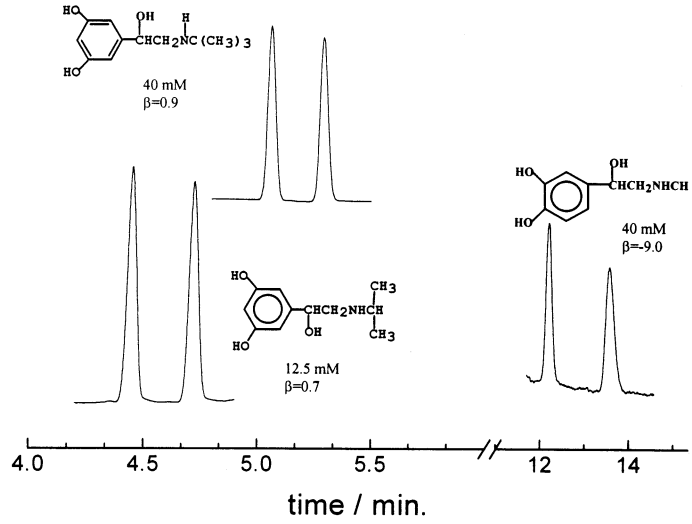

Fig. 4. Typical electropherograms of weak base analytes in the acidic, methanolic HDMS-βCD BEs. The numbers next to the structures indicate the HDAS-βCD concentrations (mM) and normalized electroosmotic flow mobilities (β). Detector sensitivities: between 1 and 5 mA U/full scale. Other conditions: see Section 2.

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