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Enantiomer separations by nonaqueous capillary electrophoresis using octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin

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Abstract

The newest member of the single-isomer isomer sulfated cyclodextrin family, octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin (ODAS-γ-CD) was used for the first time as a resolving agent for the nonaqueous capillary electrophoretic separation of the enantiomers of 26 weak base pharmaceuticals in an acidic methanol background electrolyte. The solubility limit of ODAS-γ-CD at room temperature proved to be 55 m*M* in this background electrolyte, which afforded good, fast enantiomer separations for most of the basic drugs tested. For all the bases studied, the effective mobilities and separation selectivities were found to follow the predictions of the charged resolving agent migration model of electrophoretic enantiomer separations. The effective mobilities of the weakly binding weak bases remained cationic throughout the entire 0 to 45 m*M* ODAS-γ-CD concentration range; separation selectivities increased as the ODAS-γ-CD concentration was increased. The effective mobilities of the moderately binding weak bases became anionic in the 2.5 to 45 m*M* ODAS-γ-CD concentration range; separation selectivities first increased as the effective mobilities approached zero, then decreased again as the ODAS-γ-CD concentration was increased further. The effective mobilities of the strongly binding weak bases became anionic in the 0 to 2.5 m*M* ODAS-γ-CD concentration range; separation selectivities decreased as the ODAS-γ-CD concentration was increased above 2.5 m*M*.

Keywords: Enantiomer separation; Nonaqueous capillary electrophoresis; Background electrolyte composition; Cyclodextrins; Basic drugs

1. Introduction

Over the last few years, charged cyclodextrins (CDs) became the most widely used chiral resolving agents in capillary electrophoresis (CE) [1–7]. Though both weak and strong electrolyte CDs were used successfully for the CE separation of enantiomers [5–7], the majority of recent papers utilized randomly substituted sulfobutyl ether CDs [8–10] or sulfated CDs [11]. In order to reduce the variability of CE enantiomer separations caused by compositional differences between different lots of the presumably identical resolving agent batches, a family of single-isomer β-CDs, fully sulfated on the primary hydroxy groups and uniformly substituted on the secondary hydroxy groups, were developed [12–14]. These single-isomer resolving agents were successfully used in aqueous [15–17], hydro–organic [18] and nonaqueous [19–21] background electrolytes (BGEs) for the separation of the enantiomers of neutral, weak base, weak acid and zwitterionic analytes. Recent efforts aimed at expanding the range of the available single-isomer sulfated CDs led to the synthesis, analytical characterization and use in aqueous BGEs of the first γ-CD derivative,

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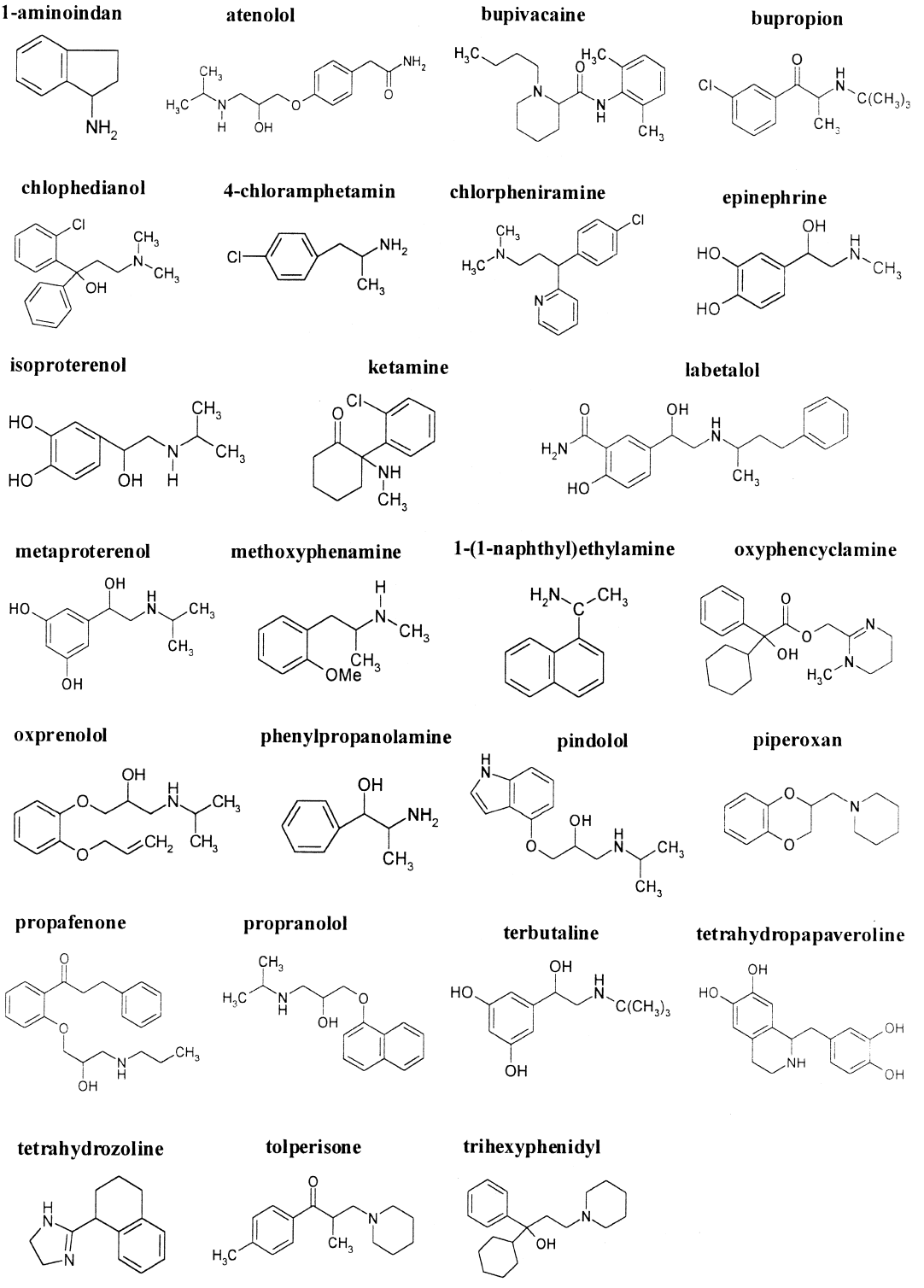

Fig. 1. Structures and names of the weak base analytes used in this study.

Table 1
Effective mobility, separation selectivity, normalized electroosmotic flow mobility and peak resolution data for the weakly binding bases (μ_2 is in 10^{-5} cm^2 V^{-1} s^{-1} units)

Name	ODAS-γ-CD																							
	0 m*M*	2.5 m*M*				5 m*M*				10 m*M*				20 m*M*				30 m*M*				45 m*M*			
	μ_2	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s
1-Aminoindan	25.93	7.7	1.01	1.2	<0.5	4.67	1.03	2.3	<0.5	2.59	1.068	1.2	0.6	1.97	1.077	2.1	0.5	1.65	1.079	2.6	0.6	1.4	1.1	2.5	1
2-Aminoindan	25.93					13.43	1	0.8	0	10.43	1.03	0.3	1.2	6.05	1.3	0.7	7.3	3.48	1.76	1.2	14.1	0.91	5.3	4	20.6
Bupivacaine	20.38	6.53	1.03	1.3	0.5	4.1	1.03	2.4	<0.5	3.8	1	1.1	0	3.3	1	1.8	0	2.82	1	1.5	0	2.3	1	1.6	0
Isoproterenol	17.14	6.9	1.07	1	1.9	4	1.07	2.2	0.8	2.38	1.1	1.2	1.2	1.39	1.17	2.9	0.6	0.99	1.416	5	1.2	3.1	1.84	5.3	2.8
Ketamine	23.96	10.25	1.03	1	<0.5	7.6	1.05	1.4	0.5	6.5	1.06	0.7	1.3	4.76	1.078	0.8	1.6	3.75	1.1	0.9	1.4	0.58	1.1	1.1	1.5
Methoxyphenamine	24.14	6.7	1	1.5	0	4.6	1	2.4	0	4	1	1.1	0	2.99	1.023	1.5	<0.5	2.28	1.07	1.9	1	1.7	1.1	2.2	1.2
Metaproterenol	17.2	6.53	1.07	1.4	1.3	4.2	1.08	2.7	<0.5	3.2	1.34	1.5	2.2	1.35	1.73	3.6	4.5	0.65	2.65	8.5	2.6	0.2	7.3	30	5.4
1-(1-Naphthyl)ethylamine	21.4	6.2	1.02	1.6	<0.5	3.5	1.06	3	<0.5	2.2	1.06	3	0.6	1.8	1.062	3.5	<0.5	1.5	1.07	3.6	<0.5	1.2	1.11	3.58	0.5
Phenylpropanolamine	21.42	5.83	1.036	1.7	0.6	3.3	1.04	3.3	<0.5	1.3	1.088	3.2	0.7	0.88	1.163	4.3	0.7	0.63	1.26	6.5	1.5	0.55	1.26	7.6	0.8
Oxprenolol	20.9	8.79	1.06	0.8	1.2	5.32	1.09	2.1	1.4	3.66	1.09	1.4	1.6	2.99	1.1	1.1	2	2.75	1.1	1.1	1.7	2.3	1.1	1.6	1.3
Pindolol	21	7.68	1.1	1.2	1.8	4.18	1.13	2.6	2.2	2.96	1.134	1.8	1.7	1.57	1.146	2.7	1.4	1.08	1.151	3.1	1	0.76	1.155	6	0.5
Terbutaline	18.4	8.53	1.09	0.9	1.5	5.73	1.1	1.9	1.7	4.57	1.1	1.2	1.9	3.49	1.1	1	1.09	2.79	1.11	1.2	1.4	2.2	1.12	1.4	1.7
Trihexyphenidyl	16.2	6.36	1.015	1.5	<0.5	4.97	1.03	2.1	<0.5	3.8	1.03	1.5	<0.5	3.3	1.032	1.4	<0.5	2.82	1.046	1.5	0.8	2.3	1.055	1.5	1.3
Tetrahydrazoline	31.4	12.67	1.04	0.8	1	9.33	1.066	1.1	0.9	6.74	1.08	0.9	1.3	5.1	1.09	0.8	2	4	1.1	1	1.9	1.93	1.1	1.2	1.9
Tolperisone	20.14	6.37	1.057	1.4	1	4.29	1.108	2.4	1.1	2.6	1.19	2.4	1.9	2.1	1.28	2.4	3.1	1.8	1.33	3.2	3.8	1.45	1.41	2.5	4.2

Table 2
Effective mobility, separation selectivity, normalized electroosmotic flow mobility and peak resolution data for the moderately binding bases (μ_2 is in 10^{-5} cm^2 V^{-1} s^{-1} units)

Name	ODAS-γ-CD																				
	0 m*M*	2.5 m*M*				5 m*M*				10 m*M*				20 m*M*				30 m*M*			
	μ_2	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s
Atenolol	15.9	4	1.42	1.8	3.8	1	1.9	10	3.3	−0.59	0.46	−6	3.9	−0.8	0.814	−4.7	1.1	−1	0.87	−4	1
Bupropion	22.4	7.6	1.42	1.4	6.8	4.29	1.72	1.6	10	2.18	2.847	1.9	20.1	0.6	8.06	7.4	26.3	−0.02	−330	382	29.2
Chlophedianol	15.5	2.11	1	4.4	0	−0.25	0.2	−41	2.3	−0.8	0.9	−5	<0.5	−0.9	0.91	−3.8	<0.5	−1	0.92	−4.1	<0.5
Epinephrine	16.74	0.7	−7	14.8	n/a	−2	0.628	−3	5.9	−3	0.795	−2.2	5.6	−3.5	0.82	−1.6	7	−3.1	0.885	−1.6	3.9
Oxyphencyclamine	21.44	3.13	1.16	3	1.5	0.71	2.14	13.8	2.1	−0.8	0.74	−3.9	3.1	−0.88	0.83	−4.1	1.3	−0.9	0.835	−4.7	1.1
Piperoxan	16.28	1.03	1.22	9.2	0.8	−0.4	0.61	−19	1.4	−0.6	0.725	−10	0.8	−1.5	0.894	−2.5	1.1	−1.6	0.93	−3.3	0.5
Propranolol	18.22	3.6	1.41	2.5	4	0.96	2.33	11	4.3	0.2	3.18	17.6	7	−0.2	−1	−35	3.8	−0.4	0.787	−11	0.6
Propafenone	16.91	3.93	1.29	2	3.1	1.02	2.76	10	3	−0.55	0.579	−6.9	3.6	−0.7	0.793	−6.9	1.4	−0.8	0.97	−5.2	<0.5
Tetrahydropapaveroline	15.7	3.02	4.83	2.8	8.2	1.22	−1.06	5.6	15.1	−0.2	0.082	−35	15	−0.62	0.25	−8.5	13	−1.1	0.46	−3.5	12.4

Table 3
Effective mobility, separation selectivity, normalized electroosmotic flow mobility and peak resolution data for the strongly binding bases (μ_2 is in 10^{-5} cm^2 V^{-1} s^{-1} units)

Name	ODAS-γ-CD																				
	0 m*M*	2.5 m*M*				5 m*M*				10 m*M*				20 m*M*				30 m*M*			
	μ_2	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s	μ_2	α	β	R_s
Chlorpheniramine	13.41	−3.59	0.916	−2.6	1.7	−4.68	0.95	−1.4	2.8	−5.2	0.95	−1.3	3.6	−4.3	0.96	−1.5	1.6	−3.5	0.96	−1.6	1.8
Labetalol-1	12.60	−1	0.627	−6.8	2.4	−1.88	0.729	−3	1.8	−2.4	0.883	−2.2	2.1	−2.35	0.937	−2.1	1.3	−2.19	0.965	−1.9	1.3
Labetalol-3	12.60	−2.72	0.647	−2.5	8.4	−4	0.789	−1.5	7.8	−3.4	0.822	−1.6	6.9	−2.9	0.888	−1.8	3.2	−2.5	0.915	−1.6	3.9

octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin (ODAS-γ-CD) [22].

Nonaqueous capillary electrophoresis (NACE) has extended the utility of CE for the analysis of poorly water-soluble analytes (for a recent review, see Ref. [1]). NACE has been used for the separation of enantiomers with neutral CDs, randomly sulfated β-CDs and single-isomer sulfated β-CDs [23–26]. The organic solvents used for NACE enantiomer separations included formamide, *N*-methylformamide, *N*,*N*-dimethylformamide, dimethyl sulfoxide (DMSO) [23–25], acetonitrile [26] and methanol [19–21]. Though the solubilities of sulfated CDs in acetonitrile and methanol are lower than in formamide, *N*-methylformamide, *N*,*N*-dimethylformamide and DMSO, the transparency of acetonitrile and methanol down to 200 nm in the UV range is an important advantage that more than compensates for the lower solubilities of single-isomer sulfated CDs. Therefore, it was important to test if the newly synthesized single-isomer sulfated γ-CD, ODAS-γ-CD [22], could also be used for the NACE separation of enantiomers in methanol as the BGE solvent. This paper presents the first results obtained with ODAS-γ-CD in acidic methanol BGEs.

2. Experimental

All NACE enantiomer separations were carried out with a P/ACE 2100 CE unit (Beckman–Coulter, Fullerton, CA, USA) using 26.5 cm (effective length 19.5 cm)×25 μm I.D.×150 μm O.D., untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). All electropherograms were recorded at 214 nm. The cartridge coolant of the CE unit was thermostated at 15°C. The samples were injected by 4 p.s.i. (approximately 0.27 bar) nitrogen, for 1 s.

All chemicals were obtained from Aldrich (Milwaukee, WI, USA) or Sigma (St. Louis, MO, USA), except ODAS-γ-CD (available from J&W Scientific, Folsom, CA, USA), which was synthesized in our laboratory as described in Ref. [22]. Twenty-six weak bases, mostly pharmaceuticals were used as test analytes. Their structures are shown in Fig. 1.

The acidic methanol stock buffer was prepared by adding 25 mmol phosphoric acid and 12.5 mmol sodium hydroxide to 1 l HPLC-grade methanol (EM Science, Gibbstown, NJ, USA). The calculated amounts of ODAS-γ-CD were weighed out and dissolved in this acidic methanol stock buffer to prepare the ODAS-γ-CD BGEs immediately prior to use. All BGEs and sample solutions were filtered through 0.45-μm PTFE membrane filters (Alltech, Deerfield, IL, USA). The solubility of ODAS-γ-CD in the acidic methanol buffer was found to be about 55 m*M*. Therefore, in order to remain on the safe side, the highest ODAS-γ-CD concentration used here was limited to 45 m*M*. With the field strength kept between 600 and 640 V cm^{-1}, the 0–45 m*M* ODAS-γ-CD BGEs resulted in electrophoretic currents of 2 to 5 μA. The low currents indicate that ODAS-γ-CD is only partially dissociated in the acidic methanol BGEs. Nevertheless, ODAS-γ-CD carried sufficient negative charge to confer anionic

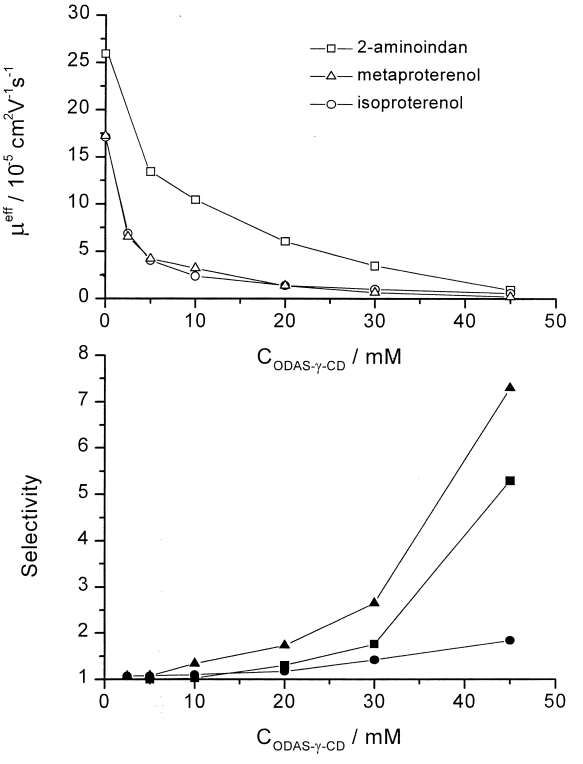

Fig. 2. Effective mobilities (top panel, open symbols) and separation selectivities (bottom panel, filled symbols) for a typical set of weakly binding bases as a function of the ODAS-γ-CD concentration. Symbols: square: 2-aminoindan, up-triangle: metaproterenol, circle: isoproterenol.

effective mobilities onto many of the weak base analytes studied.

Nitromethane was co-injected with each sample as electroosmotic flow (EOF) marker (neutral marker). The external EOF marker method [27] was used to ascertain that the effective mobility of nitromethane was zero within experimental error in all the BGEs used here. The effective mobilities of the enantiomers were calculated as $\mu^{eff}=\mu^{obs}-\mu^{eo}$, the separation selectivities as $\alpha=\mu_1^{eff}/\mu_2^{eff}$, where μ_2^{eff} was assigned arbitrarily to the enantiomer which was less mobile in the 2.5 m*M* ODAS-γ-CD BGE. The normalized EOF mobility [28] was calculated as $\beta=\mu^{eo}/\mu_2^{eff}$. Peak resolution, R_s, was 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

Based on the effective mobility measurements in the $0<c_{ODAS\text{-}\gamma\text{-}CD}<45$ m*M* concentration range, the weak bases were divided into three groups: weakly binding, moderately binding and strongly binding weak bases. The μ_2^{eff}, α, β and R_s values for the three families are listed in Tables 1–3. In the weakly binding group (Table 1), the effective mobilities of the weak bases remained cationic throughout the entire $0<c_{ODAS\text{-}\gamma\text{-}CD}<45$ m*M* concentration range tested. As expected from the predictions of the charged resolving agent migration model (CHARM model) of electrophoretic enantiomer separations [29], separation selectivities increased with increasing ODAS-γ-CD concentration. A set of typical μ_2^{eff} and α values are plotted in the top and bottom panels of Fig. 2, respectively.

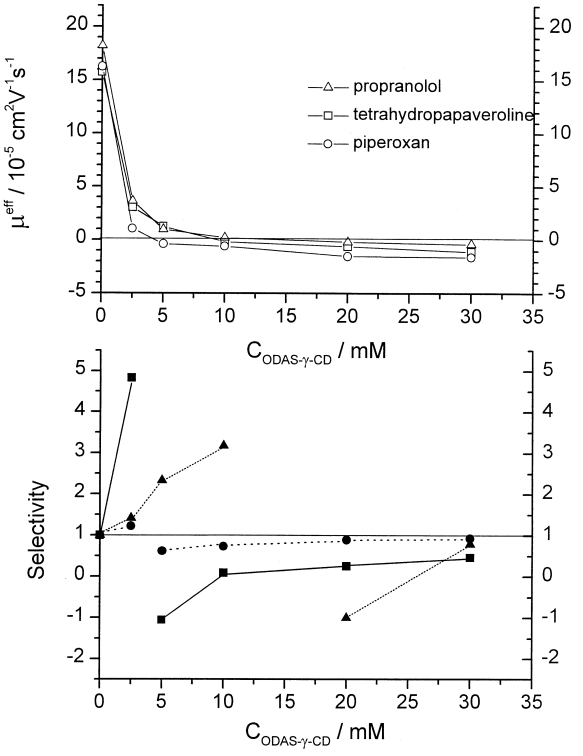

Fig. 3. Effective mobilities (top panel, open symbols) and separation selectivities (bottom panel, filled symbols) for a set of moderately binding bases as a function of the ODAS-γ-CD concentration. Symbols: up-triangle: propranolol, circle: piperoxan, square: tetrahydropapaveroline.

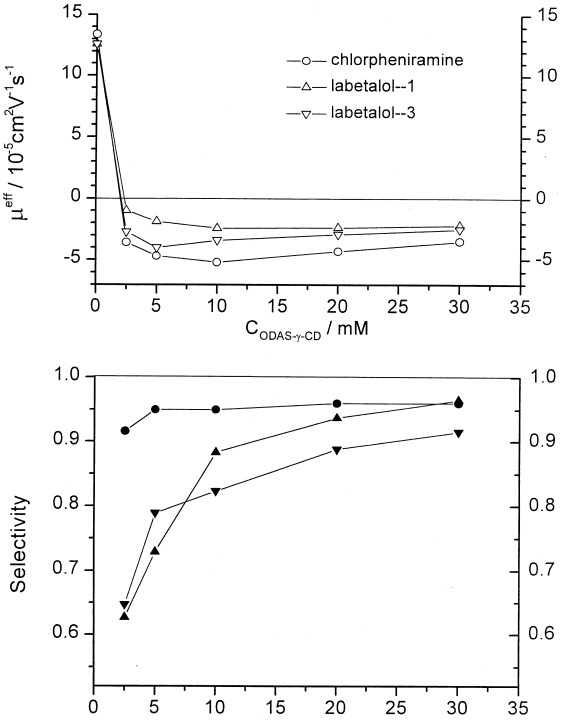

Fig. 4. Effective mobilities (top panel, open symbols) and separation selectivities (bottom panel, filled symbols) for a set of strongly binding bases as a function of the ODAS-γ-CD concentration. Symbols: circle: chlorpheniramine, up-triangle: labetalol (first-migrating enantiomer), down-triangle: labetalol (third-migrating enantiomer).

In the moderately binding group (Table 2), the initially cationic effective mobilities of the weak bases turned anionic in the 2.5 mM<$c_{\text{ODAS-γ-CD}}$<30 mM concentration range. A set of typical μ_2^{eff} and α values are plotted in the top and bottom panels of Fig. 3, respectively. In this group, as predicted by the CHARM model [29], separation selectivity increased (Fig. 3, bottom panel, filled symbols) as the cationic effective mobility of the less mobile enantiomer approached zero. Then, selectivity became a large negative value as the ODAS-γ-CD concentration was increased a little further. When the effective mobility of the other enantiomer became zero, separation selectivity also became zero. As $c_{\text{ODAS-γ-CD}}$ was increased further, selectivity became a positive value and went from zero toward its limiting, less than unity value.

For the strongly binding weak bases (Table 3), the initially cationic mobilities became anionic in the 0<$c_{\text{ODAS-γ-CD}}$<2.5 mM concentration range. A set of typical μ_2^{eff} and α values are plotted in the top and bottom panels of Fig. 4, respectively. As the ODAS-γ-CD concentration was increased, the anionic effective mobilities passed an extremum and began to decrease toward zero (Fig. 4, top panel, open symbols). The greater the anionic effective mobility of the weak base was at $c_{\text{ODAS-γ-CD}}$=2.5 mM, the sooner the analyte passed the anionic effective mobility extremum. As the concentration of ODAS-γ-CD was increased, the ionic strength of the BGE was also increased, which suppressed the mobilities of the analytes. Obviously, ionic strength did influence the effective mobility of every weak base (Figs. 2–4). The effective mobility distortions in

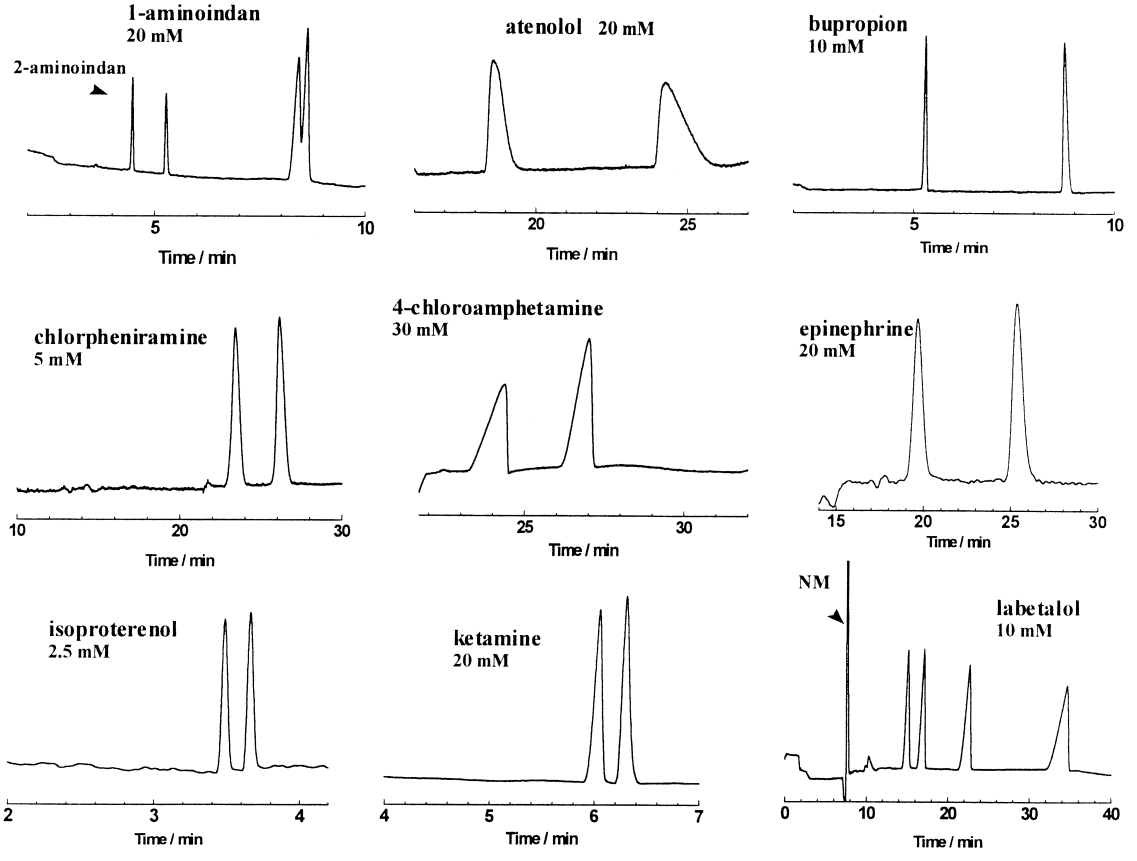

Fig. 5. Typical electropherograms of chiral weak base analytes. The concentrations next to the compound names indicate the concentrations of ODAS-γ-CD in the BGE (mM). Other conditions: see Experimental.

Figs. 2 and 3 are not as apparent though as in Fig. 4, because the mole fractions of the anionic complexes of the weak bases that were present were low due to weaker binding. Since the effective mobilities of all weak bases in Fig. 4 were anionic above $c_{\text{ODAS-γ-CD}}$=2.5 m*M*, separation selectivities approached their limiting $\alpha<1$ values as $c_{\text{ODAS-γ-CD}}$ was increased. This, again, is in agreement with the stipulations of the CHARM model [29].

Figs. 5–7 show typical NACE separations for the weak base enantiomers studied here. Most weak bases could be baseline-separated in the 2.5< $c_{\text{ODAS-γ-CD}}$<45 m*M* concentration range in less than 30 min. Note particularly the separation of the four enantiomers of labetalol that offers peak resolution values far in excess of those observed with the other sulfated cyclodextrins. Since all BGEs used in the present work contain two anions and two cations, non-comigrating system peaks [30–32] were often observed both for the cationically and anionically migrating weak base analytes. Due to the low boiling point of methanol and the limited hydrolytic stability of all acetylated cyclodextrins, the BGEs were replaced frequently to avoid undesirable changes in their compositions.

4. Conclusion

The newly synthesized, single-isomer octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin has been successfully used as chiral resolving agent for the NACE separation of the enantiomers of weak base analytes in acidic methanol BGEs. The migration behavior of the analytes and the separation selectivities followed the predictions of the CHARM model. ODAS-γ-CD

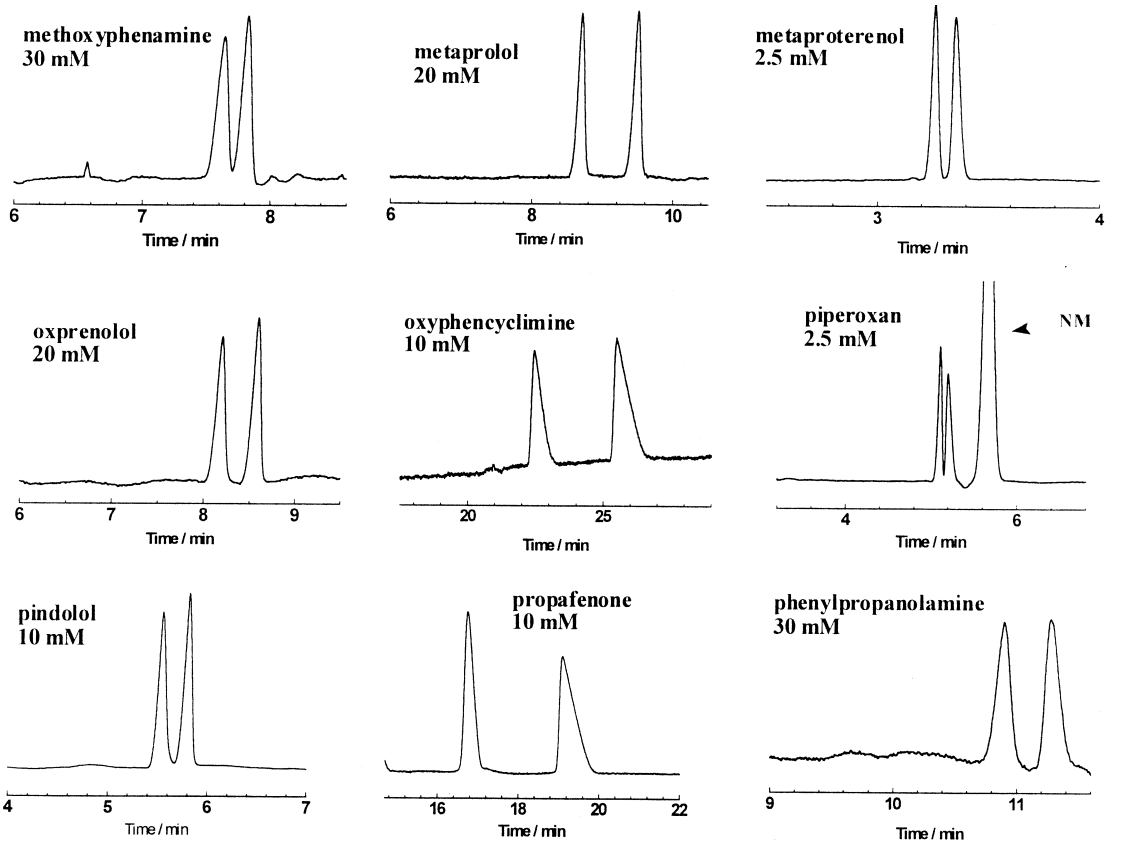

Fig. 6. Typical electropherograms of chiral weak base analytes. The concentrations next to the compound names indicate the concentrations of ODAS-γ-CD in the BGE (m*M*). Other conditions: see Experimental.

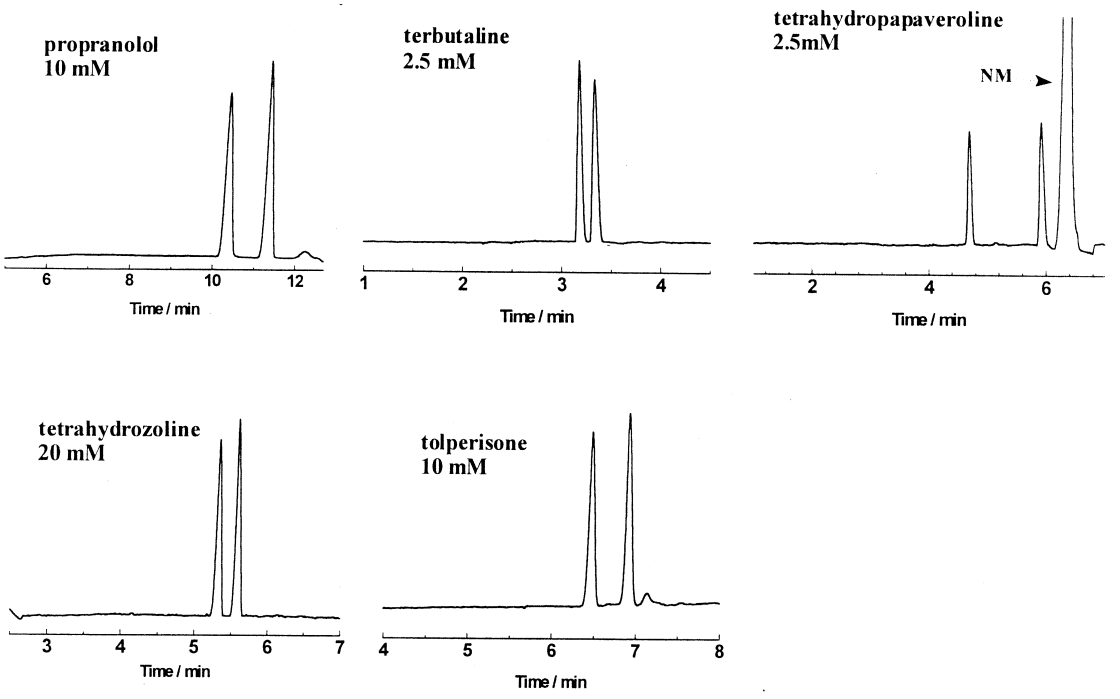

Fig. 7. Typical electropherograms of chiral weak base analytes. The concentrations next to the compound names indicate the concentrations of ODAS-γ-CD in the BGE (m*M*). Other conditions: see Experimental.

afforded fast separations, with good peak resolution values, for all 26 weak base analytes tested here.

Acknowledgements

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