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Capillary electrophoretic separation of enantiomers using the single isomer heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin as chiral resolving agent in methanol–water background electrolytes

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Abstract

The utility of the sodium salt of the single isomer heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin as chiral resolving agent in methanol–water background electrolytes was studied by capillary electrophoresis. The effective mobilities of neutral, weak acid and weak base enantiomers were measured as a function of the methanol concentration in the 0 to 50% (v/v) range, while the heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin concentration was simultaneously varied in the 0 to 100 m*M* range. The addition of methanol improved the solubility and decreased the effective mobility of the analytes. The methanol-induced reduction in the extent of binding of the analytes to the charged cyclodextrin could be compensated for by increasing the concentration of the charged cyclodextrin in the background electrolyte. Separation selectivities closely followed the theoretical predictions of the charged resolving agent migration model of enantiomer separations.

Keywords: Enantiomer separation; Background electrolyte composition; Cyclodextrins; Phenylbutyric acid; Benzoin; Tryptophan; Phenylpentanols; Propranolol

1. Introduction

Over the last few years, capillary electrophoresis has become an important tool for the separation of enantiomers; advances in the field have been extensively covered in recent reviews [1–3]. Though most electrophoretic separations occur in aqueous media, hydro–organic background electrolytes (BGEs) are beginning to appear in an increasing number of practical applications because organic solvents can improve the solubility of hydrophobic analytes and, thus, can significantly extend the scope of capillary electrophoretic (CE) separations [4]. Following the synthesis and first successful CE use of the single isomer sulfated cyclodextrin family in aqueous BGEs [5–10], the next logical step was to find out if the new resolving agents possessed sufficient solubility and retained enough of their aqueous separation selectivities in hydro–organic (methanol–water) BGEs. This paper summarizes our first experiences with the use of the single isomer charged cyclodextrin, heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin (HDMS-β-CD) in hydro–organic BGEs.

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2. Experimental

All CE separations were carried out with a P/ACE 5010 CE unit (Beckman Instruments, Fullerton, CA, USA) and 26 cm (effective length 19 cm)×25 μm I.D., 150 μm O.D. untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) thermostated at 15°C. The 214-nm filter of the UV detector was used to detect all analytes. The samples were pressure injected by 6 p.s.i. (1 p.s.i.=6894.76 Pa) nitrogen for 1 s. The applied potential was varied between 5 and 25 kV to maintain power dissipation below 500 mW/m.

All chemicals used in the BGE preparation were obtained from Aldrich (Milwaukee, WI, USA), except HDMS-β-CD (Regis Technologies, Morton Grove, IL, USA, Cat. No. 733402), which was synthesized as described in [7]. According to the dictates of the charged resolving agent migration model (CHARM model) of CE enantiomer separations [11], only a single acidic stock BGE was used. The acidic stock BGE was prepared by weighing into a 1-l volumetric flask 0.025 mol H_3PO_4, 0.0125 mol of triethylamine (TEA) and methanol (MeOH) to yield the required nominal percentage (v/v) concentration and, finally, filling the flask to the mark with water. Since phosphoric acid is a much stronger acid than most of the weak acid enantiomers studied, it can be expected that the H_3PO_4–$H_2PO_4^-$ (1:1) buffer will provide sufficiently acidic conditions at every MeOH concentration for each analyte studied, as dictated by the CHARM model. The 25, 50, 75 and 100 m*M* HDMS-β-CD BGEs were prepared by weighing 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 BGE solution.

Nitromethane (N, external mobility marker) samples (0.5 m*M*) were prepared with each HDMS-β-CD BGE. The effective mobility of N (μ_N^{eff}) was determined in each HDMS-β-CD BGE using the external electroosmotic flow (EOF) marker method [12]. μ_N^{eff} became zero (within experimental error) as soon as $c_{MeOH} \geq 15\%$ (v/v) and $c_{HDMS\text{-}\beta\text{-}CD} \leq 100$ m*M* indicating that, under these conditions, the binding of nitromethane to HDMS-β-CD is so weak that nitromethane can be used as a direct EOF marker ($\mu_N^{eff} = \mu_{EOF}$).

All analytes were obtained from Sigma (St. Louis, MO, USA) and were injected as 0.5 m*M* racemic solutions, dissolved in the respective HDMS-β-CD BGEs. As usual, the effective mobilities of the analyte enantiomers were calculated as $\mu_i^{eff} = \mu_i^{obs} - \mu_{EOF}$; separation selectivities, α, as $\alpha = \mu_1^{eff}/\mu_2^{eff}$ (subscript 1 arbitrarily refers to the enantiomer which had a higher μ^{eff} in the 10 m*M* HDMS-β-CD BGE); the normalized EOF mobility, β, as $\beta = \mu_{EOF}/\mu_2^{eff}$ [11]. Each analyte was tested at five different methanol concentrations with five HDMS-β-CD concentrations each, except when complexation was so weak that the analyte comigrated with nitromethane, or when separation time was excessive.

3. Results and discussion

3.1. Electroosmotic flow

As c_{MeOH} and $c_{HDMS\text{-}\beta\text{-}CD}$ were varied, μ_{EOF} varied greatly as shown in Fig. 1. In the absence of HDMS-β-CD, at $c_{MeOH} = 0\%$ (v/v), μ_{EOF} is initially anionic. Then, as c_{MeOH} is increased, the anionic EOF becomes weaker and weaker. Eventually, μ_{EOF} becomes cationic at $c_{MeOH} \geq 50\%$ (v/v). This behavior is understandable considering that protonated TEA adsorbs on the wall of the capillary. In water,

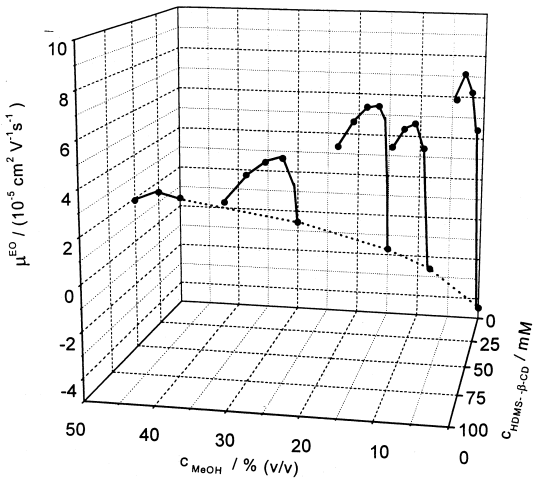

Fig. 1. Electroosmotic flow mobility (μ_{EOF}) as a function of the methanol and HDMS-β-CD concentration of the BGE. For other conditions see Section 2.

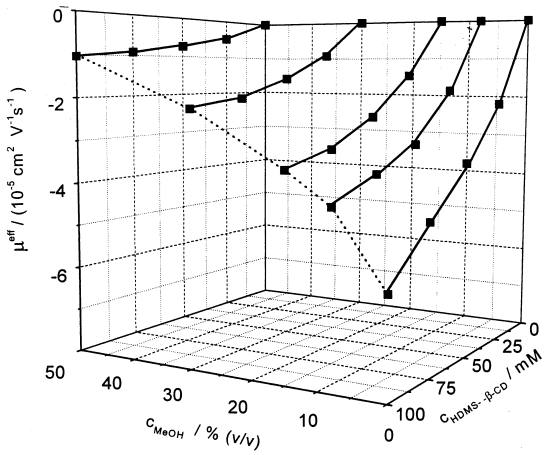

Fig. 2. Typical effective mobility (μ^{eff}) surface for weak acid (and neutral) analytes in the acidic BGE. Analyte: 2-phenylbutyric acid. For other conditions see Section 2.

$TEAH^+$ adsorbs strongly and creates a positively charged surface layer. As c_{MeOH} is increased, less and less $TEAH^+$ is adsorbed and the anionic EOF flow is weakened. Once HDMS-β-CD is added to the BGE, μ_{EOF} quickly becomes cationic. In fact, considering the acidity of the BGE, μ_{EOF} becomes quite strongly cationic indicating that HDMS-β-CD adsorbs strongly on the capillary wall and, even at $c_{HDMS\text{-}\beta\text{-}CD}$=25 m*M*, it readily overcomes the original positive surface charge of the wall that was created by $TEAH^+$. As $c_{HDMS\text{-}\beta\text{-}CD}$ is increased further, μ_{EOF} levels off until, finally, at around $c_{HDMS\text{-}\beta\text{-}CD}$=100 m*M*, it begins to decrease slightly due to the combined effects of greatly increased ionic strength and slightly increased BGE viscosity. The relative constancy of the μ_{EOF} values over a broad HDMS-β-CD concentration range can be utilized advantageously for the optimization of peak resolution and separation time [11].

3.2. Weak acid and neutral analytes

For these studies, 2-phenylbutyric acid was selected as weak acid analyte, 1-phenyl-1-pentanol, 2-

Table 1
Electrophoretic data for 2-phenylbutyric acid in the acidic HDMS-β-CD BGEs

C_{MeOH} (%, v/v)	$c_{HDMS\text{-}\beta\text{-}CD}$ (m*M*)	μ_{EOF} (10^{-5}cm^2 V^{-1} s^{-1})	μ_1^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	μ_2^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	α	β	U (kV)	R_s
0	0	−4.05	0.00	0.00	N/A	N/A	15.0	N/A
8	0	−2.76	0.00	0.00	N/A	N/A	15.0	N/A
15	0	−1.70	0.00	0.00	N/A	N/A	20.0	N/A
30	0	−0.67	0.00	0.00	N/A	N/A	25.0	N/A
50	0	0.36	0.00	0.00	N/A	N/A	30.0	N/A
0	25	11.53	−2.87	−2.14	1.340	−5.39	5.9	2.7
8	25	8.43	−2.38	−1.83	1.302	−4.62	7.0	2.4
15	25	6.10	−1.84	−1.47	1.253	−4.15	7.5	2.9
30	25	3.46	−1.09	−0.96	1.137	−3.59	3.0	0.9
50	25	1.52	−0.52	−0.48	1.063	−3.13	10.5	0.8
0	50	9.39	−4.44	−3.44	1.290	−2.73	5.2	4.2
8	50	9.02	−3.83	−3.06	1.251	−2.95	5.5	4.2
15	50	6.67	−2.97	−2.45	1.212	−2.72	6.0	4.2
30	50	4.06	−1.74	−1.57	1.111	−2.59	2.5	1.4
50	50	1.93	−0.77	−0.73	1.049	−2.63	9.0	0.8
0	75	11.03	−5.64	−4.57	1.235	−2.41	3.7	4.8
8	75	6.26	−4.31	−3.60	1.200	−1.74	4.5	5.6
15	75	6.10	−3.64	−3.11	1.173	−1.96	4.5	5.9
30	75	4.08	−2.23	−2.02	1.104	−2.03	6.0	3.2
50	75	2.13	−0.96	−0.92	1.040	−2.32	7.5	0.9
0	100	10.06	−7.09	−5.77	1.229	−1.74	3.7	4.1
8	100	6.26	−4.76	−4.11	1.157	−1.52	4.5	5.5
15	100	6.17	−3.87	−3.41	1.135	−1.81	4.5	5.4
30	100	3.71	−2.39	−2.21	1.082	−1.68	5.3	3.4
50	100	2.22	−1.07	−1.04	1.033	−2.14	7.5	0.8

phenyl-2-pentanol and benzoin (as well as warfarin, for a partial migration study at $c_{\text{HDMS-β-CD}}$=100 m*M*) as neutral analytes. In the acidic H_3PO_4–$H_2PO_4^-$ BGE, 2-phenylbutyric acid is almost completely protonated, so its migration behavior is expected to mimic that of the neutral analytes. The μ_{EOF}, μ_i^{eff}, α, β, applied potential and peak resolution, R_s, values for the four fully studied analytes are listed in Tables 1–4.

The doubly-curved μ^{eff} surface of 2-phenylbutyric acid is shown in Fig. 2. The μ^{eff} surfaces of the weak acid and neutral analytes (see Tables 1–4) are similar: as $c_{\text{HDMS-β-CD}}$ is increased, the analytes acquire increasingly anionic μ^{eff}. As MeOH is added to the BGE, the analytes bind less to HDMS-β-CD and their anionic μ^{eff} becomes slower. No limiting effective mobility has been reached in the range studied [up to c_{MeOH}=50% (v/v) and $c_{\text{HDMS-β-CD}}$= 100 m*M*].

Tables 1–4 and Figs. 3–5 show, in agreement with the predictions of the CHARM model [11], that for neutral analytes α decreases as $c_{\text{HDMS-β-CD}}$ is increased. The CHARM model [11] does not treat the methanol concentration of the BE as an explicit variable. Rather, the methanol effects are included in the changing K and μ^0 values. For neutral and weak acid analytes, two different separation selectivity dependencies were observed as a function of c_{MeOH}. For analytes which have one large, distinct hydrophobic portion, α decreases as c_{MeOH} is increased. This behavior is typified in Fig. 3 by 2-phenylbutyric acid. However, the opposite trend was found for benzoin and warfarin (structures shown in Fig. 5): in the range studied, α increases as c_{MeOH} is increased. For warfarin, the c_{MeOH} dependence was only studied at $c_{\text{HDMS-β-CD}}$=100 m*M* where, similarly to benzoin, separation selectivities increased with the methanol concentration: at c_{MeOH}=0% (v/v), α^{warfarin}=1.08;, at c_{MeOH}=8% (v/v), α^{warfarin}=1.10; at c_{MeOH}=15% (v/v), α^{warfarin}=1.11; at c_{MeOH}=30% (v/v),

Table 2
Electrophoretic data for 1-phenyl-1-pentanol in the acidic HDMS-β-CD BGEs

c_{MeOH} (%, v/v)	$c_{\text{HDMS-β-CD}}$ (m*M*)	μ_{EOF} (10^{-5} cm^2 V^{-1} s^{-1})	μ_1^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	μ_2^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	α	β	U (kV)	R_s
0	0	−4.47	0.00	0.00	1.000	N/A	15	N/A
8	0	−2.62	0.00	0.00	1.000	N/A	15	N/A
15	0	−1.71	0.00	0.00	1.000	N/A	20	N/A
30	0	−0.56	0.00	0.00	1.000	N/A	25	N/A
50	0	0.39	0.00	0.00	1.000	N/A	30	N/A
0	25	6.52	−2.15	−1.76	1.223	−3.71	2	1.3
8	25	4.46	−1.70	−1.43	1.191	−3.12	2.3	1.1
15	25	6.05	−1.26	−1.06	1.190	−5.72	7.5	1.4
30	25	3.34	−0.89	−0.79	1.120	−4.21	3	0.7
50	25	1.50	−0.41	−0.38	1.076	−3.93	10.5	0.7
0	50	11.87	−3.16	−2.58	1.224	−4.60	5.2	1.5
8	50	5.98	−2.88	−2.45	1.174	−2.44	1.8	1.1
15	50	6.63	−2.25	−1.93	1.167	−3.44	6	2.3
30	50	4.06	−1.54	−1.39	1.106	−2.91	2.5	1.0
50	50	1.90	−0.67	−0.63	1.065	−3.02	9	0.9
0	75	8.67	−4.27	−3.57	1.194	−2.43	3.7	2.9
8	75	6.26	−3.38	−2.90	1.169	−2.16	4.5	3.2
15	75	6.04	−2.94	−2.57	1.146	−2.35	4.5	3.2
30	75	4.08	−1.93	−1.76	1.099	−2.32	6	2.5
50	75	2.12	−0.86	−0.81	1.059	−2.62	7.5	1.1
0	100	9.98	−4.98	−4.26	1.167	−2.34	3.7	2.7
8	100	6.26	−3.98	−3.49	1.143	−1.80	4.5	4.3
15	100	6.17	−3.30	−2.94	1.123	−2.10	4.5	3.7
30	100	3.72	−2.13	−1.96	1.085	−1.90	5.3	2.9
50	100	2.17	−1.00	−0.95	1.053	−2.28	7.5	1.2

Table 3
Electrophoretic data for 2-phenyl-2-pentanol in the acidic HDMS-β-CD BGEs

c_{MeOH} % (v/v)	$c_{HDMS\text{-}\beta\text{-}CD}$ (m*M*)	μ_{EOF} 10^{-5} cm^2 V^{-1} s^{-1})	μ_1^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	μ_2^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	α	β	U (kV)	R_s
0	0	−4.47	0.00	0.00	N/A	N/A	15.0	N/A
8	0	−2.62	0.00	0.00	N/A	N/A	15.0	N/A
15	0	−1.71	0.00	0.00	N/A	N/A	20.0	N/A
30	0	−0.56	0.00	0.00	N/A	N/A	25.0	N/A
50	0	0.39	0.00	0.00	N/A	N/A	30.0	N/A
0	25	5.08	−1.13	−1.02	1.113	−4.99	6.0	0.8
8	25	3.16	−0.87	−0.78	1.115	−4.06	6.7	1.0
15	25	6.06	−0.74	−0.67	1.106	−9.07	7.5	0.5
30	25	3.38	−0.59	−0.54	1.097	−6.27	9.4	0.6
50	25	1.51	−0.26	−0.26	N/A	−5.90	10.5	N/A
0	50	10.18	−2.22	−2.01	1.106	−5.06	5.5	0.8
8	50	7.94	−1.74	−1.57	1.107	−5.06	5.5	0.8
15	50	6.66	−1.43	−1.29	1.104	−5.15	6.0	0.9
30	50	3.92	−1.12	−1.03	1.087	−3.79	2.5	0.9
50	50	1.90	−0.46	−0.44	1.063	−4.38	9.0	0.6
0	75	8.69	−2.79	−2.53	1.099	−3.43	3.7	1.0
8	75	6.29	−2.25	−2.05	1.099	−3.07	4.5	1.2
15	75	6.06	−1.98	−1.81	1.094	−3.35	4.5	1.4
30	75	4.08	−1.35	−1.25	1.077	−3.26	6.0	1.1
50	75	2.13	−0.62	−0.59	1.058	−3.60	7.5	0.7
0	100	10.05	−3.47	−3.18	1.091	−3.16	3.7	1.1
8	100	6.28	−2.77	−2.55	1.087	−2.46	4.5	1.6
15	100	6.15	−2.32	−2.15	1.082	−2.87	4.5	1.5
30	100	3.73	−1.56	−1.46	1.067	−2.55	5.3	1.4
50	100	2.22	−0.77	−0.73	1.052	−3.05	7.5	0.9

$\alpha^{warfarin}$=1.13, and at c_{MeOH}=50% (v/v), $\alpha^{warfarin}$= 1.16. One might speculate (subject to verification by high field NMR spectroscopic studies), that perhaps HDMS-β-CD can bind to benzoin (and warfarin) from either end, and that the parasitic (or at least less enantioselective) binding interactions are weakened by methanol to a greater extent than the more enantioselective binding interactions. Currently, it cannot be predicted a priori how separation selectivity will change as a function of c_{MeOH} for a particular neutral or weakly acidic analyte. Therefore, optimization of the CE separation requires the experimental study of the c_{MeOH} dependence.

3.3. Weak base and zwitterionic analytes

Propranolol was selected as a typical weak base analyte, tryptophan as a typical zwitterionic analyte. Since in the acidic H_3PO_4–$H_2PO_4^-$ BGE both of them are completely protonated, their migration behavior is expected to be similar. The observed μ_{EOF}, μ_i^{eff}, α, β and R_s values are listed in Tables 5 and 6.

The μ^{eff} surface for tryptophan is shown in Fig. 6; the μ^{eff} surface of propranolol is similar. At $c_{HDMS\text{-}\beta\text{-}CD}$=0 m*M*, tryptophan migrates as a cation and its cationic μ^{eff} decreases as c_{MeOH} is increased. When HDMS-β-CD is added to the BGE, μ^{eff} of tryptophan very rapidly becomes anionic and changes comparatively little once $c_{HDMS\text{-}\beta\text{-}CD}$>25 m*M*. Along the other axis, the anionic μ^{eff} becomes smaller as c_{MeOH} is increased, even at $c_{HDMS\text{-}\beta\text{-}CD}$= 100 m*M*.

The separation selectivity surfaces for the weak base and zwitterionic analytes are very interesting, as shown in Fig. 7. According to the CHARM model [11], α becomes infinitely large at a certain resolving agent concentration provided that (i) the analyte and the charged resolving agent are oppositely charged and (ii) the interactions between the analyte and the

Table 4
Electrophoretic data for benzoin in the acidic HDMS-β-CD BGEs

c_{MeOH} (m*M*)	$c_{HDMS\text{-}\beta\text{-}CD}$ (%, v/v)	μ_{EO} (10^{-5} cm^2 V^{-1} s^{-1})	μ_1^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	μ_2^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	α	β	U (kV)	R_s
0	0	−4.47	0.00	0.00	N/A	N/A	15.0	N/A
8	0	−2.62	0.00	0.00	N/A	N/A	15.0	N/A
15	0	−1.71	0.00	0.00	N/A	N/A	20.0	N/A
30	0	−0.56	0.00	0.00	N/A	N/A	25.0	N/A
50	0	0.39	0.00	0.00	N/A	N/A	30.0	N/A
0	25	5.84	−2.43	−2.10	1.159	−2.78	6.0	2.0
8	25	4.45	−2.16	−1.79	1.208	−2.49	2.3	2.0
15	25	6.06	−1.84	−1.46	1.260	−4.15	7.5	2.8
30	25	3.43	−1.11	−0.86	1.295	−4.01	3.0	1.7
50	25	1.38	−0.49	−0.35	1.370	−3.89	10.5	3.4
0	50	8.08	−3.87	−3.39	1.141	−2.38	5.2	2.5
8	50	6.00	−3.36	−2.83	1.185	−2.12	1.8	2.4
15	50	6.61	−2.88	−2.35	1.228	−2.82	6.0	4.5
30	50	4.01	−1.71	−1.36	1.258	−2.95	2.5	2.4
50	50	2.01	−0.78	−0.59	1.325	−3.41	9.0	4.1
0	75	8.64	−4.64	−4.14	1.121	−2.09	3.7	3.0
8	75	6.28	−3.94	−3.37	1.170	−1.86	4.5	4.9
15	75	6.05	−3.43	−2.87	1.194	−2.11	4.5	5.7
30	75	4.05	−2.14	−1.73	1.239	−2.34	6.0	6.1
50	75	2.08	−0.90	−0.69	1.292	−3.00	7.5	4.5
0	100	9.81	−5.15	−4.68	1.100	−2.10	3.7	3.2
8	100	6.28	−4.28	−3.74	1.142	−1.68	4.5	5.8
15	100	6.15	−3.56	−3.05	1.167	−2.02	4.5	5.9
30	100	3.68	−2.22	−1.83	1.208	−2.00	5.3	7.0
50	100	2.18	−0.98	−0.78	1.258	−2.79	7.5	4.5

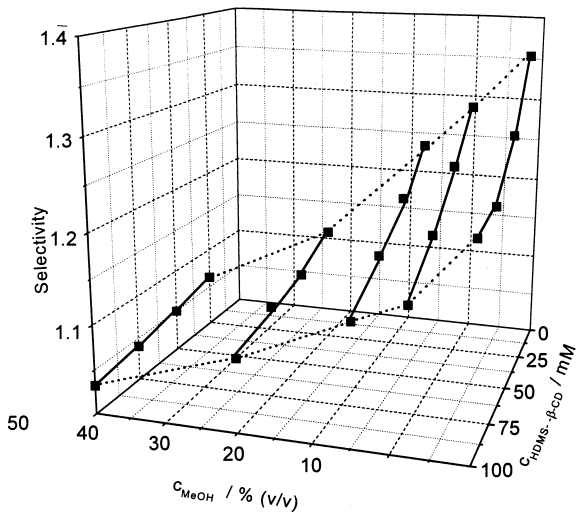

Fig. 3. Typical separation selectivity (α) surface for weak acid (and neutral) analytes in the acidic BGE. Analyte: 2-phenylbutyric acid. For other conditions see Section 2.

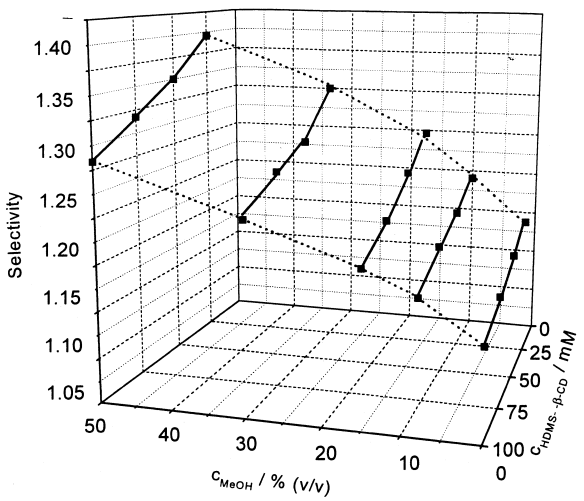

Fig. 4. Separation selectivity (α) surface for benzoin as neutral analyte in the acidic BGE. For other conditions see Section 2.

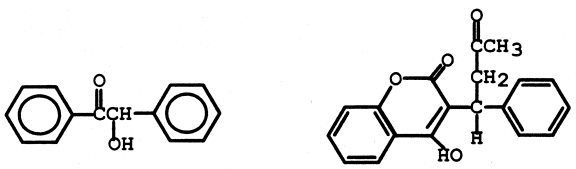

Fig. 5. Structure of benzoin and warfarin.

resolving agent are strong enough to change μ^{eff} of the analyte from cationic to anionic as the charged resolving agent concentration is increased. Thus, α increases towards infinity as μ^{eff} of the stronger binding enantiomer approaches zero. There is a discontinuity in α when μ^{eff} of the stronger binding enantiomer becomes exactly zero. Then, α changes sign and becomes a large negative value when μ^{eff} of the stronger binding enantiomer becomes negative. As μ^{eff} becomes increasingly negative, α first approaches, and then becomes zero. Finally, as the concentration of the charged resolving agent is increased further, α becomes positive and approaches its limiting, less-than-unity value.

These theoretical predictions are fully supported by the experimental data in Fig. 7. When c_{MeOH} of the BGE is zero, α is about -3 at $c_{HDMS\text{-}\beta\text{-}CD}=25$ m*M*. The discontinuity in α occurs close to this $c_{HDMS\text{-}\beta\text{-}CD}$ value. As c_{MeOH} is increased, the locus of the α discontinuity is shifted towards higher $c_{HDMS\text{-}\beta\text{-}CD}$ values because methanol weakens the interactions between the analyte and HDMS-β-CD: in the $c_{MeOH}=30\%$ (v/v) BGE the discontinuity occurs at $50<c_{HDMS\text{-}\beta CD}<75$ m*M*. This behavior offers an important tool for the analyst interested in the optimization of a difficult separation or the quantitation of a trace level minor enantiomer.

Table 5
Electrophoretic data for propranolol in the acidic HDMS-β-CD BGEs

c_{MeOH} (%, v/v)	$c_{HDMS\text{-}\beta\text{-}CD}$ (m*M*)	μ_{EOF} (10^{-5} cm^2 V^{-1} s^{-1})	μ_1^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	μ_2^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	α	β	U (kV)	R_s
0	0	−4.05	15.52	15.52	1.000	−0.26	15.0	N/A
8	0	−2.76	13.68	13.68	1.000	−0.20	15.0	N/A
15	0	−1.70	12.28	12.28	1.000	−0.14	20.0	N/A
30	0	−0.67	9.72	9.72	1.000	−0.07	25.0	N/A
50	0	0.00	N/A	N/A	N/A	N/A	30.0	N/A
0	25	5.76	−2.74	−2.25	1.217	−2.56	6.0	3.7
8	25	4.21	−1.97	−1.49	1.320	−2.82	6.7	5.1
15	25	6.04	−1.39	−0.97	1.432	−6.21	7.5	4.0
30	25	3.39	−0.10	0.10	−1.038	34.51	9.0	3.7
50	25	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	50	7.84	−3.09	−2.77	1.115	−2.83	5.2	2.1
8	50	5.95	−2.21	−1.90	1.167	−3.14	5.2	2.7
15	50	6.43	−1.62	−1.33	1.217	−4.82	6.0	2.7
30	50	4.12	−0.25	−0.07	3.734	−62.44	7.5	2.8
50	50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	75	8.60	−3.28	−3.06	1.071	−2.81	3.7	1.3
8	75	6.29	−2.36	−2.14	1.103	−2.94	4.5	1.9
15	75	5.89	−1.78	−1.58	1.128	−3.72	4.5	1.9
30	75	4.06	−0.36	−0.23	1.551	−17.45	6.0	1.9
50	75	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	100	8.16	−3.40	−3.26	1.044	−2.51	3.7	1.1
8	100	6.20	−2.45	−2.29	1.068	−2.70	4.5	1.6
15	100	6.12	−1.79	−1.64	1.089	−3.72	4.5	1.5
30	100	3.79	−0.49	−0.39	1.257	−9.74	5.3	1.5
50	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 6
Electrophoretic data for tryptophan in the acidic HDMS-β-CD BGEs

c_{MeOH} (%, v/v)	$c_{HDMS\text{-}\beta\text{-}CD}$ (m*M*)	μ_{EOF} (10^{-5} cm^2 V^{-1} s^{-1})	μ_1^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	μ_2^{eff} (10^{-5} cm^2 V^{-1} s^{-1})	α	β	U (kV)	R_s
0	0	−4.47	9.26	9.26	1.000	−0.48	15.0	N/A
8	0	−2.62	7.83	7.83	1.000	−0.33	15.0	N/A
15	0	−1.71	7.04	7.04	1.000	−0.24	20.0	N/A
30	0	−0.56	5.16	5.16	1.000	−0.11	25.0	N/A
50	0	0.39	3.80	3.80	1.000	0.10	30.0	N/A
0	25	5.05	−0.11	0.28	−2.530	18.20	6.0	2.4
8	25	4.10	0.26	0.21	0.787	19.62	6.7	<0.5
15	25	6.01	0.39	0.22	0.568	26.90	7.5	1.1
30	25	3.38	0.51	0.33	0.645	10.24	9.0	1.4
50	25	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	50	7.32	−0.92	−0.66	1.390	−11.04	5.2	1.1
8	50	5.88	−0.68	−0.46	1.480	−12.84	5.2	1.3
15	50	6.54	−0.32	−0.11	2.800	−56.89	6.0	1.4
30	50	3.92	−0.07	0.07	−1.050	56.12	7.5	1.8
50	50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	75	8.61	−1.35	−1.13	1.200	−7.65	3.7	0.9
8	75	6.28	−0.92	−0.73	1.260	−8.59	4.5	1.2
15	75	5.96	−0.64	−0.47	1.370	−12.79	4.5	1.2
30	75	4.07	−0.18	−0.05	3.820	−88.48	6.0	1.3
50	75	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	100	8.12	−1.63	−1.44	1.130	−5.63	3.7	1.0
8	100	6.20	−1.16	−1.00	1.160	−6.18	4.5	1.1
15	100	6.13	−0.85	−0.71	1.200	−8.64	4.5	1.0
30	100	3.71	−0.28	−0.18	1.530	−20.38	5.3	1.1
50	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A

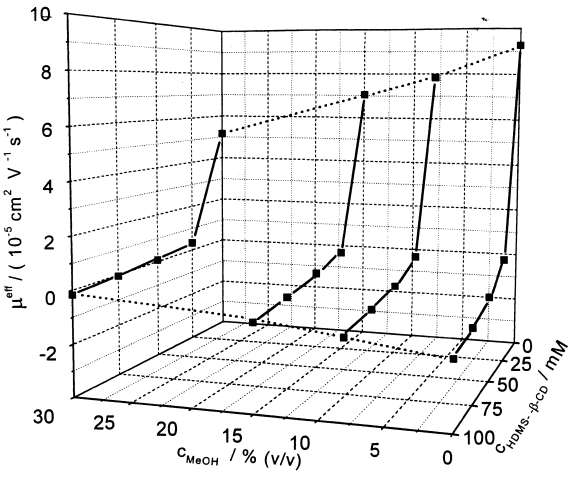

Fig. 6. Typical effective mobility (μ^{eff}) surface for weak base (or zwitterionic) analytes in the acidic BGE. Analyte: tryptophan. For other conditions see Section 2.

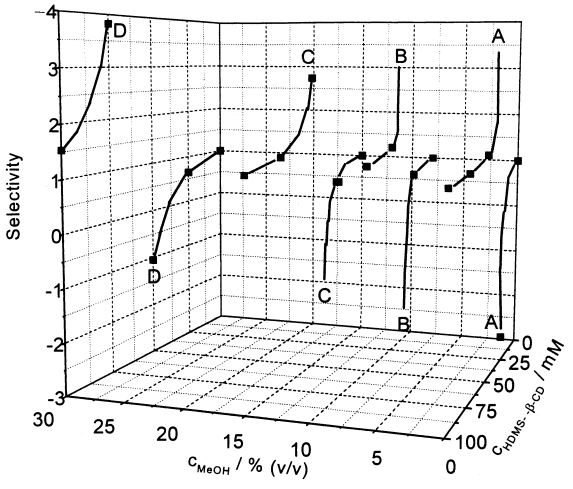

Fig. 7. Typical separation selectivity (α) surface for weak base (and zwitterionic) analytes in the acidic BGE. Analyte: tryptophan. (A) c_{MeOH}=0% (v/v); (B) c_{MeOH}=7.5% (v/v); (C) c_{MeOH}=15% (v/v); (D) c_{MeOH}=30% (v/v). For other conditions see Section 2.

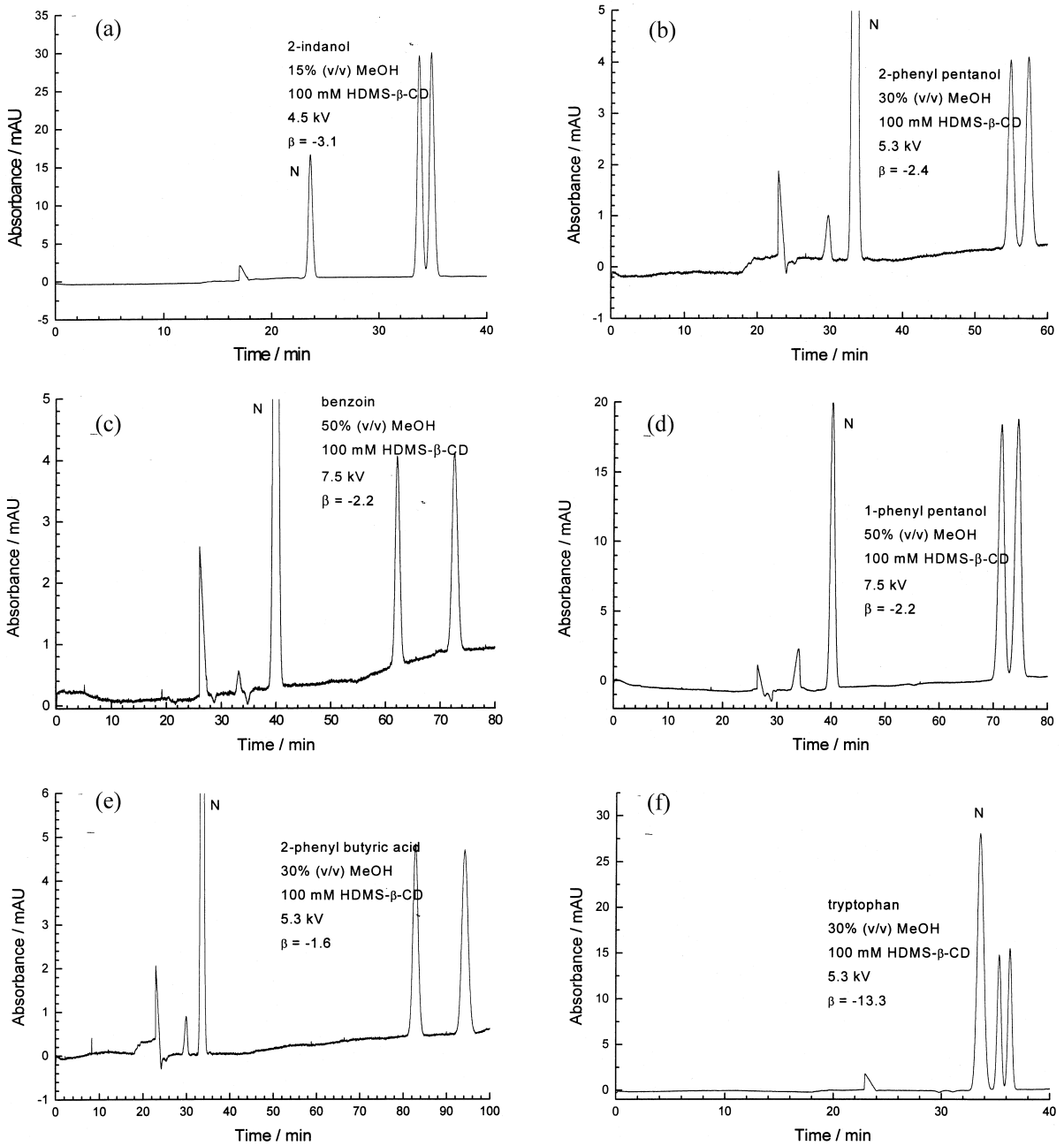

Fig. 8. Typical electropherograms of analytes which migrate anionically in the acidic HDMS-β-CD BGEs. The numbers next to the electropherograms indicate the c_{MeOH} (%, v/v), the $c_{HDAS\text{-}\beta\text{-}CD}$ (m*M*), the effective applied potential (kV) and the normalized EOF flow mobility (β) values. N, nitromethane marker. For other conditions see Section 2.

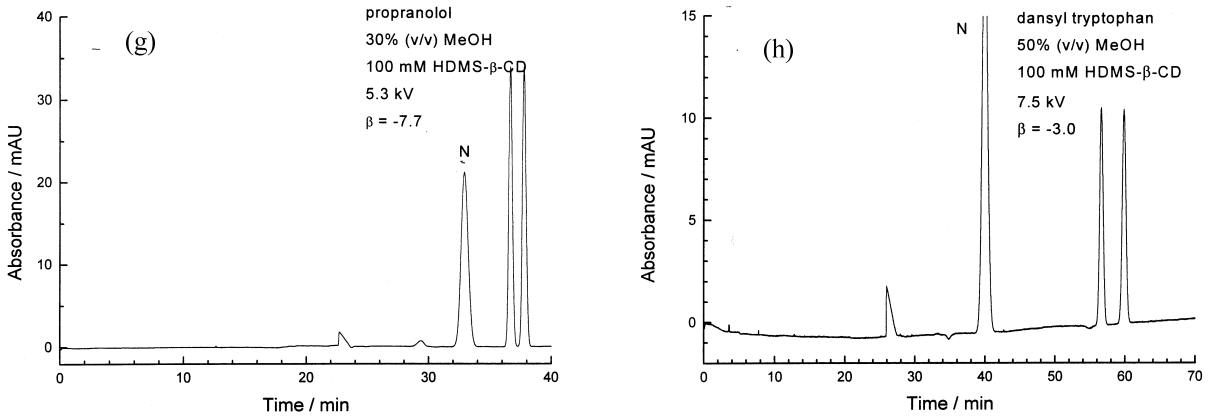

Fig. 8. (*continued*)

3.4. Enantiomer separations obtained in the methanol–water BGEs

A few representative electropherograms for moderately hydrophobic, anionically migrating neutral, weak acid, zwitterionic and cationic analytes are shown in Fig. 8. The respective c_{MeOH}, $c_{HDMS\text{-}\beta\text{-}CD}$, applied potential and β values are listed next to each electropherogram. Since methanol strongly decreases the extent of binding between the neutral analytes and HDMS-β-CD, fairly high (100 m*M*) HDMS-β-CD concentrations have to be used to confer sufficiently high effective charges upon the neutral analytes [11]. This limits the separation potential one can apply across the capillary if one wishes to remain within the linear region of Ohm's plot. Also, even at $c_{HDMS\text{-}\beta\text{-}CD}$=100 m*M*, μ_{EOF} is only between 4 and $8\cdot10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$ and the majority of the β values are around −2. Combined, these two factors lead to long separation times for neutral analytes.

A few representative electropherograms for the cationically migrating strong and weak base analytes are shown in Fig. 9. Since the β values are about 1, the separations are fast.

4. Conclusions

The CE separation of enantiomers was studied here in methanol–water background electrolytes using the new, hydrophobic, single isomer charged cyclodextrin, the sodium salt of heptakis-(2,3-di-methyl-6-sulfato)-β-cyclodextrin and varying, simultaneously, both c_{MeOH} and $c_{HDMS\text{-}\beta\text{-}CD}$. The μ^{eff} and α values for weak acid, neutral, weak base and zwitterionic enantiomers closely followed the theoretical predictions of the CHARM model in terms of the $c_{HDMS\text{-}\beta\text{-}CD}$-dependence. In general, at constant $c_{HDMS\text{-}\beta\text{-}CD}$, the addition of methanol decreased α, except for benzoin and warfarin-type structures, for which α increased significantly as c_{MeOH} was increased. For weak base and zwitterionic analytes, α — as a function of $c_{HDMS\text{-}\beta\text{-}CD}$ — displayed a discontinuity. The position of the α discontinuity shifted towards higher $c_{HDMS\text{-}\beta\text{-}CD}$ values as c_{MeOH} was increased. Good, fast enantiomer separations were obtained for weak bases which migrated cationically; good, though slow, separations were obtained for neutral, weak acid, zwitterionic and cationic analytes which migrated anionically.

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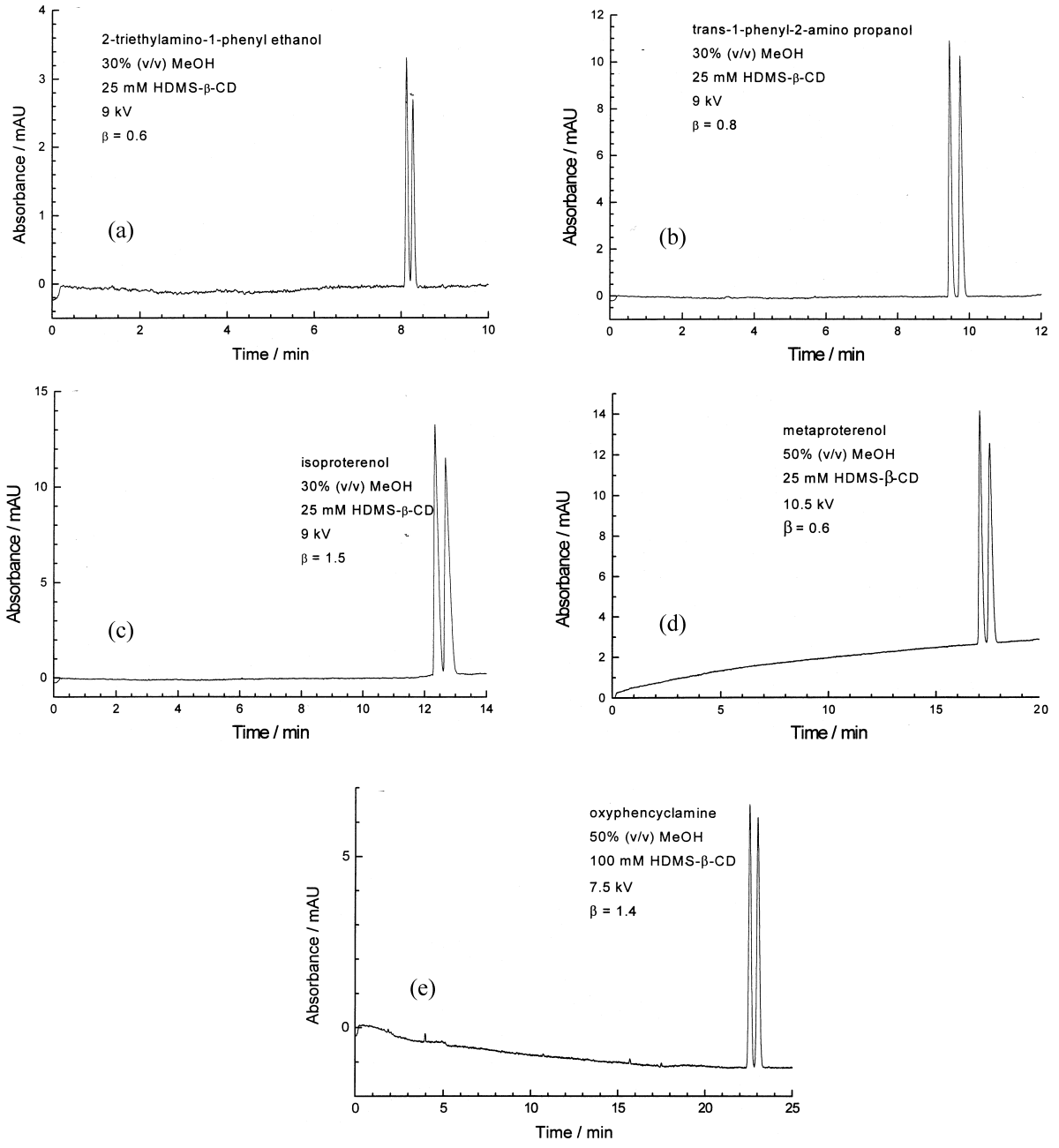

Fig. 9. Typical electropherograms of analytes which migrate cationically in the acidic HDMS-β-CD BGEs. The numbers next to the electropherograms indicate the c_{MeOH} (%, v/v), the $c_{HDAS\text{-}\beta\text{-}CD}$ (m*M*), the effective applied potential (kV) and the normalized EOF flow mobility (β) values. N, nitromethane marker. For other conditions see Section 2.

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