Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1051 (2004) 95–101
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Use of the new, single-isomer, hexakis(2,3-diacetyl-6-*O*-sulfo)-α-cyclodextrin in acidic methanol background electrolytes for nonaqueous capillary electrophoretic enantiomer separations

Shulan Li, Gyula Vigh*

Department of Chemistry, Texas A&M University, College Station, TX 77842-3012, USA

Abstract

The new, single-isomer, sulfated α-cyclodextrin, the sodium salt of hexakis(2,3-diacetyl-6-*O*-sulfo)-α-cyclodextrin (HxDAS), was used for the first time in acidic methanol background electrolytes (BGEs) to separate the enantiomers of weak base analytes by nonaqueous capillary electrophoresis (NACE). The concentration dependence of the effective mobilities and separation selectivities followed trends similar to those observed earlier in acidic methanol background electrolytes with heptakis(2,3-diacetyl-6-*O*-sulfo)-β-cyclodextrin (HDAS) and octakis(2,3-diacetyl-6-*O*-sulfo)-γ-cyclodextrin (ODAS). In general, interactions between the weak base analytes and HxDAS were weaker than with HDAS and ODAS. For some of the weak base analytes, separation selectivities observed in acidic aqueous and acidic methanol background electrolytes were complementary to each other, permitting the eventual separation of enantiomers that could not be achieved otherwise.

Keywords: Hexakis(2,3-diacetyl-6-*O*-sulfo)-α-cyclodextrin (HxDAS); Single-isomer sulfated cyclodextrin; Sulfated cyclodextrin; Nonaqueous capillary electrophoresis; Enantiomer

1. Introduction

During the last decade, enantiomer separations by capillary electrophoresis (CE) became very successful and widely used, a fact amply documented by the most recent, comprehensive review of the field [1]. Though most of these separations were achieved in aqueous background electrolytes (BGEs), there has been an increasing interest in the use of organic solvents as BGE components (for a good recent overview of the benefits of nonaqueous capillary electrophoresis (NACE), see, e.g. ref. [2]). NACE is a natural choice for resolving agents that have a high solubility in a variety of organic solvents, such as, e.g. native and derivatized cinchona alkaloids [3–11], and derivatized amino acids and other acids [12,13]. Polar, albeit UV absorbing organic solvents, such as formamide, *N*-methyl formamide, *N*,*N*-dimethyl formamide, permitted the use of noncharged cyclodextrins (CDs) [14,15], a randomly substituted quaternary ammonium CD [16] and a randomly substituted sulfated CD [17] as chiral resolving agents in NACE. A few years ago, single-isomer sulfated CDs became available [18–23] as a result of efforts to provide well-characterized chiral resolving agents that can be used not only for the CE separation of enantiomers [24], but also for the molecular level NMR studies of the interactions between the analytes and the CDs (see, e.g. refs. [25–29]). Of the single-isomer sulfated CDs, the 2,3-diacetyl-6-sulfo CDs [18,21] and the 2,3-dimethyl-6-sulfo CDs [20,23] are adequately soluble in methanol. Since methanol is UV-transparent, the 2,3-diacetyl-6-sulfo CDs [18,21] and the 2,3-dimethyl-6-sulfo CDs [20,23] became successful resolving agents in NACE [30–35]. In order to increase the structural variety of the available single-isomer sulfated cyclodextrins, the large-scale synthesis of the sodium salt of hexakis(2,3-diacetyl-6-*O*-sulfo)-α-cyclodextrin (HxDAS) was recently completed [36]. In this brief paper, we

* Corresponding author. Tel.: +1 979 845 2456; fax: +1 979 845 4719.
E-mail address: vigh@mail.chem.tamu.edu (G. Vigh).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.08.043

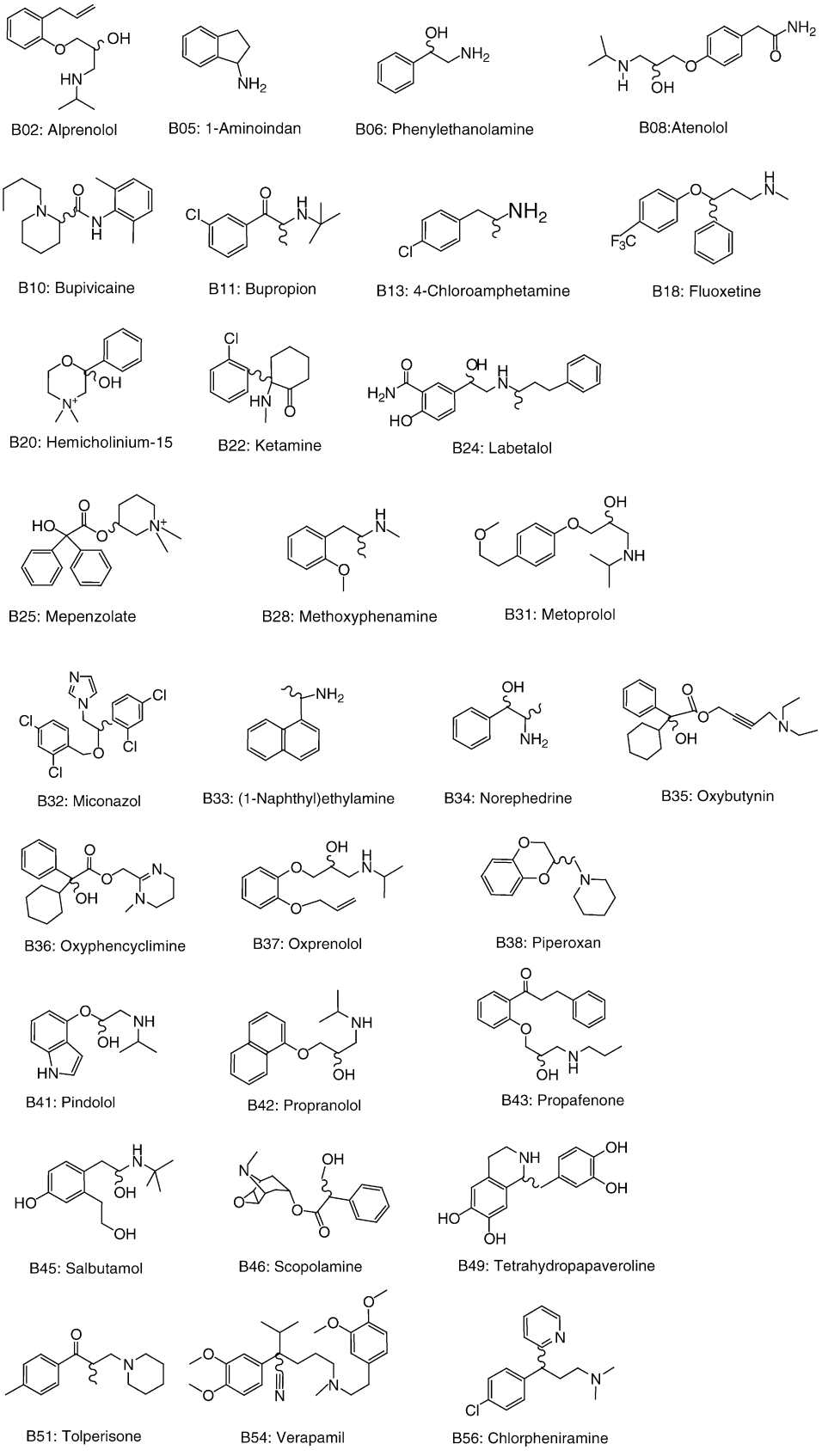

Fig. 1. Structures of the weak base analytes used in this study.

report the results of the first NACE use of HxDAS in acidic methanolic background electrolytes.

2. Experimental

2.1. Materials

Phosphoric acid, lithium hydroxide and HPLC grade methanol, used for the preparation of the BGEs, were purchased from Aldrich (Milwaukee, WI, USA). HxDAS was synthesized and analytically characterized in our laboratory as described in Ref. [36]. Thirty chiral weak base analytes that are part of our standard sulfated CD evaluation kit [3], listed in Fig. 1, were obtained from Aldrich, Sigma (St. Louis, MO, USA), Wiley Organics (Coshocton, OH, USA) and Research Diagnostics (Rockdale, MD, USA) as racemic mixtures. The acidic methanolic stock buffer, prepared by adding 25 mmol H_3PO_4 and 12.5 mmol LiOH to a 1 L volumetric flask and filling the flask with methanol, was used to dissolve the calculated amounts of HxDAS to make the 0–30 mM HxDAS BGEs for the NACE separations. The test analytes were dissolved in the respective BGEs at an approximate concentration of 0.5 mM.

2.2. Methods

Separations were carried out with a P/ACE 2100 CE system (Beckman-Coulter, Fullerton, CA, USA), at 214 nm and 20 °C, using 25 μm i.d., 150 μm o.d., 26.4 cm total length (L_t), 19.6 cm injector-to-detector length (L_d), uncoated, fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The samples were pressure injected in triplicate separations. Since the external mobility marker method [37] indicated that DMSO did not complex measurably with HxDAS even in acidic aqueous BGEs [36], DMSO was used as the electroosmotic flow (EOF) marker in the present study as well, and was co-injected in each run with the analyte to determine the EOF mobility, μ_{EOF}. Since the Ohm's plots were linear up to an applied potential of 16 kV even in the 30 mM HxDAS BGE, all experiments were carried out at 16 kV. The effective mobilities of the enantiomers (μ_1^{eff} and μ_2^{eff}) were obtained as $\mu_1^{eff} = \mu_1^{obs} - \mu_{EOF}$, the separation selectivities, α, as $\alpha = \mu_1^{eff}/\mu_2^{eff}$ (where subscript 2 arbitrarily refers to the enantiomer whose effective mobility in the 5 mM HxDAS BGE was smaller), and the normalized electroosmotic flow mobility values, β, as $\beta = \mu_{EOF}/\mu_2^{eff}$ [38]. Typically, the μ_{EOF} values were less than $+10 \times 10^{-5}$ cm^2 V^{-1} s^{-1} in the 5 mM HxDAS BGE and decreased to about $+5 \times 10^{-5}$ cm^2 V^{-1} s^{-1} in the 30 mM HxDAS BGE.

3. Results and discussion

Since the solubility of the sodium salt of HxDAS in acidic methanol is at least as high as 40 mM, similarly to what

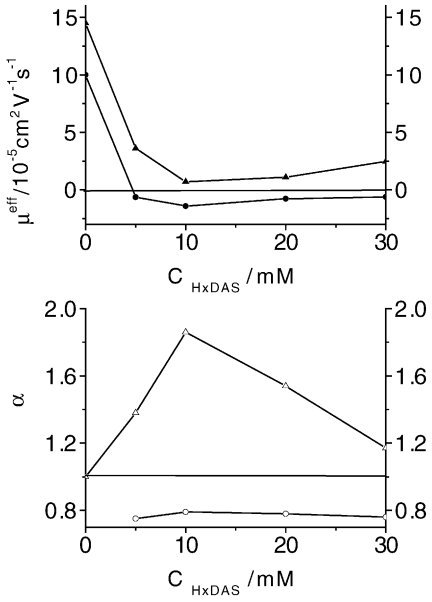

Fig. 2. Effective mobilities (top panel, full symbols) and separation selectivities (bottom panel, open symbols) for fluoxetin, B18 (symbol triangle) and verapamil, B54 (symbol circle) as a function of the concentration of HxDAS.

was observed for the sodium salts of heptakis(2,3-diacetyl-6-*O*-sulfo)-β-CD (HDAS) [30] and octakis(2,3-diacetyl-6-*O*-sulfo)-γ-CD (ODAS) [32], it made sense to test the behavior of HxDAS as a resolving agent in NACE. Table 1 lists values of μ, the effective mobility of the less mobile enantiomer, α, the separation selectivity, β, the corresponding normalized electroosmotic flow mobility, R_s, the peak resolution, and U, the injector-to-detector potential drop ($U = U_{appl}\ L_d/L_t$, in kV units) for the 30 weak bases tested. At least partial peak resolution was achieved for the enantiomers of 27 of the 30 weak bases tested.

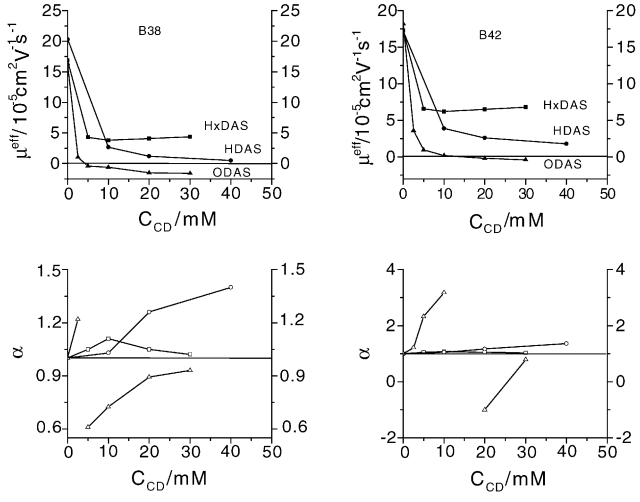

Fig. 3. Effective mobilities (full symbols) and separation selectivities (open symbols) for piperoxan, B38 (left panels) and propranolol, B42 (right panels) as a function of the concentration of the 2,3-diacetyl-6-*O*-sulfo CDs. Symbol square: HxDAS, symbol circle: HDAS [30] and symbol triangle: ODAS [32].

Table 1
Effective mobilities of the less mobile enantiomer (μ, in 10^{-5} cm^2 V^{-1} s^{-1} units), separation selectivities (α), measured peak resolution values (R_s), dimensionless EOF mobility values (β) and the injector-to-detector effective potential drop (U, in kV units) in acidic methanolic HxDAS BGEs for the weak base analytes

Analyte	HxDAS (mM)															
	5[a]				10[a]				20[a]				30[a]			
	μ	α	β	R_s	μ	α	β	R_s	μ	α	β	R_s	μ	α	β	R_s
B02	9.3	1.02	0.5	<0.6	7.2	1.04	0.6	1.1	7.9	1.03	0.4	1.0	8.6	1.01	0.2	<0.6
B05	15.3	1.16	0.5	8.9	10.7	1.22	0.3	6.3	8.0	1.24	0.2	3.8	7.3	1.26	0.2	4.7
B06	7.8	1.03	0.7	1.0	7.4	1.05	0.6	1.3	7.1	1.06	0.4	0.7	6.7	1.07	0.6	1.0
B08	6.3	1.03	0.7	<0.6	4.1	1.07	1.2	0.7	4.6	1.04	0.5	<0.6	4.9	1.02	0.2	<0.6
B10	9.7	1.03	0.6	<0.6	7.5	1.05	0.6	1.4	8.5	1.04	0.3	0.8	8.6	1.03	0.2	0.9
B11	13.2	1.00	2.7	0	11.9	1.02	0.4	<0.6	12.9	1.00	0.2	0	13.4	1.00	0.2	0
B13	6.7	1.03	0.7	<0.6	3.7	1.08	1.4	1.7	4.0	1.07	0.6	1.5	4.3	1.04	0.2	1.5
B18	4.1	1.20	0.9	1.2	0.7	1.86	6.8	2.6	1.1	1.54	2.0	1.7	2.5	1.17	0.4	3.8
B20	11.0	1.02	0.4	<0.6	7.3	1.04	0.6	1.1	7.8	1.03	0.3	<0.6	8.0	1.02	0.2	<0.6
B22	12.9	1.00	0.3	0	10.6	1.01	0.4	<0.6	9.1	1.02	0.3	<0.6	8.6	1.03	0.2	<0.6
B24	3.2	1.03	41	0.7	2.3	1.04	3.3	1.0	1.6	1.06	2.4	1.3	1.8	1.05	3.8	1.4
	2.9	1.10		1.8	2.0	1.15		0.7	1.5	1.07		0.6	1.7	1.06		0.6
	1.4	2.07		3.6	1.2	1.37		2.1	1.1	1.36		1.4	1.5	1.13		1.4
B25	5.0	1.02	0.8	<0.6	0.7	1.16	5.9	0.9	1.0	1.12	3.0	0.7	1.3	1.06	0.6	<0.6
B28	12.4	1.00	0.3	0	8.9	1.02	0.4	<0.6	9.2	1.01	0.3	<0.6	9.4	1.00	0.2	0
B31	7.0	1.03	1.0	<0.6	5.8	1.05	1.2	1.1	6.1	1.03	0.3	<0.6	6.4	1.02	0.2	<0.6
B32	6.5	1.03	1.1	<0.6	5.6	1.04	0.7	1.3	5.8	1.04	0.4	1.0	6.1	1.03	0.3	1.1
B33	8.9	1.04	0.8	<0.6	6.8	1.06	0.5	0.6	7.5	1.04	0.3	1.3	8.1	1.02	0.2	0.9
B34	7.4	1.03	1.0	<0.6	5.7	1.05	0.6	1.2	6.6	1.03	0.4	0.9	7.1	1.02	0.3	1.1
B35	4.7	1.00	1.6	0	4.2	1.00	1.1	0	4.4	1.00	1.0	0	4.5	1.00	0.9	0
B36	7.9	1.00	0.9	0	7.1	1.00	0.7	0	6.6	1.00	0.4	0	6.4	1.00	0.4	0
B37	10.1	1.02	0.7	<0.6	9.7	1.03	0.5	<0.6	9.3	1.04	0.3	<0.6	9.0	1.05	0.2	<0.6
B38	4.3	1.05	1.7	<0.6	3.8	1.11	1.4	<0.6	4.1	1.05	0.7	1.6	4.4	1.02	0.5	1.4
B41	9.2	1.02	0.8	<0.6	8.8	1.03	0.6	<0.6	8.4	1.04	0.3	<0.6	8.3	1.04	0.1	0.9
B42	6.6	1.05	1.1	<0.6	6.2	1.08	0.8	0.8	6.5	1.05	0.4	1.1	6.8	1.03	0.2	0.8
B43	5.1	1.06	1.4	<0.6	4.7	1.07	1.1	1.3	4.4	1.09	0.7	1.2	4.8	1.05	0.3	1.0
B45	9.6	1.00	0.7	0	9.0	1.00	0.5	0	8.5	1.00	0.3	0	8.3	1.00	0.4	0
B46	2.5	1.04	2.8	<0.6	2.5	1.00	2.0	0	2.9	1.00	0.9	0	3.1	1.00	0.8	0
B49	4.7	1.02	1.5	<0.6	4.1	1.04	1.0	<0.6	3.8	1.05	0.8	<0.6	3.6	1.06	0.3	<0.6
B51	9.7	1.02	0.8	<0.6	9.1	1.03	0.5	0.9	8.7	1.04	0.4	1.1	8.5	1.04	0.2	0.7
B54	−0.6	0.75	−11	<0.6	−1.4	0.79	−3.2	1.2	−0.8	0.78	−5.4	1.0	−0.6	0.76	−2.0	1.0
B56	8.6	1.03	0.8	<0.6	8.0	1.04	0.5	1.0	7.4	1.05	0.4	0.8	7.2	1.06	0.3	0.9

[a] U (kv): 16.

Similarly to what was observed with the other sulfated CDs in NACE [30–35], the weak bases could be classified into two groups according to the migration pattern observed: the group of weakly binding weak bases and the group of strongly binding weak bases [39]. The majority of the weak bases, 29 of the 30 studied, fell into the first group, the group of weakly binding bases. Only one base (verapamil, B54) fell into the second group, the group of strongly binding bases. Fig. 2 shows the two migration patterns using fluoxetin, B18 and verapamil, B54 as examples.

In the group of weakly binding weak bases that display the first migration pattern (typified by fluoxetin, B18), the effective mobilities of the analytes (top panel of Fig. 2) remain cationic throughout the entire HxDAS concentration range studied: the cationic effective mobilities of fluoxetin decrease toward a minimum as the HxDAS concentration is increased to 10 mM, then the effective mobilities increase slightly as the HxDAS concentration is increased to 30 mM. The effective mobility minimum is believed to be caused by the interplay of increasing complexation of the analyte and the differential depression of the mobility of the uncomplexed weak base and the complexed weak base by the increased ionic strength of the BGE that occurs as the concentration of HxDAS is increased [39]. Initially, even the small amount of the negatively charged complexed form of the weak base causes a significant reduction in the cationic effective mobility of the analyte band. Since ionic strength at low HxDAS concentrations is still low, mobility depression is not yet severe. Due to weak binding, the mole fraction of the complexed form of the analyte does not increase significantly as the HxDAS concentration is increased to 30 mM, but the ionic strength of the BGE does. This depresses the anionic effective mobility of the complexed form of the analyte (five negative charges) much more than that of the free form of the analyte (one positive charge). Thus, the effective mobility of the band becomes a higher cationic value than at the minimum, at an

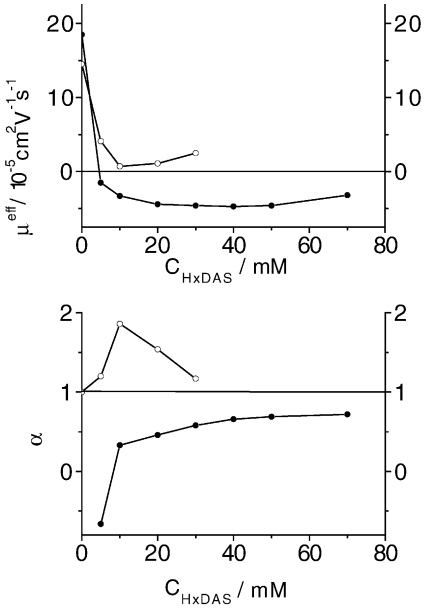

Fig. 4. Effective mobilities (top panel) and separation selectivities (bottom panel) for fluoxetine, B18 (symbol circle) in aqueous BGE [37] (full symbols) and methanolic BGE (open symbols) as a function of the concentration of HxDAS.

HxDAS concentration of 10 mM. The concentration dependence of the separation selectivity (bottom panel of Fig. 2) also follows the pattern that was reported [33] for the weakly binding bases: a separation selectivity maximum occurs at the HxDAS concentration where there is a minimum in the effective mobilities.

In the group of strongly binding weak bases that display the second migration pattern (verapamil, B54), the effective mobilities of the analyte become anionic at a low HxDAS concentration (below 5 mM), then decrease toward 0 (though remain anionic) as the HxDAS concentration is increased to 30 mM. Once again, the observed anionic mobility maximum is believed to be caused by the increased ionic strength of the BGE that results from the increased concentration of HxDAS [39]: strong complexation causes the mole fraction of the anionic complex (and thus the anionic effective mobility of the band) to be high at low HxDAS concentrations where the ionic strength, and the resulting mobility depression, are still relatively low. A further increase in the concentration of HxDAS cannot increase the already high mole fraction of the anionic complex significantly, but it does increase the ionic strength which, in turn, depresses the anionic effective mobility of the analyte band. Though the CHARM model of enantiomer separations [40] predicts a discontinuity in separation selectivity at the point where cationic effective mobility becomes anionic (mobility cross-over), the discontinuity was not observed experimentally for verapamil (bottom panel of Fig. 2), because no measurements were made below the 5 mM HxDAS concentration level.

In order to gain a sense about the relative strength of interactions between the weak bases and HxDAS, HDAS [30] and ODAS [32], Fig. 3 shows the effective mobilities (top

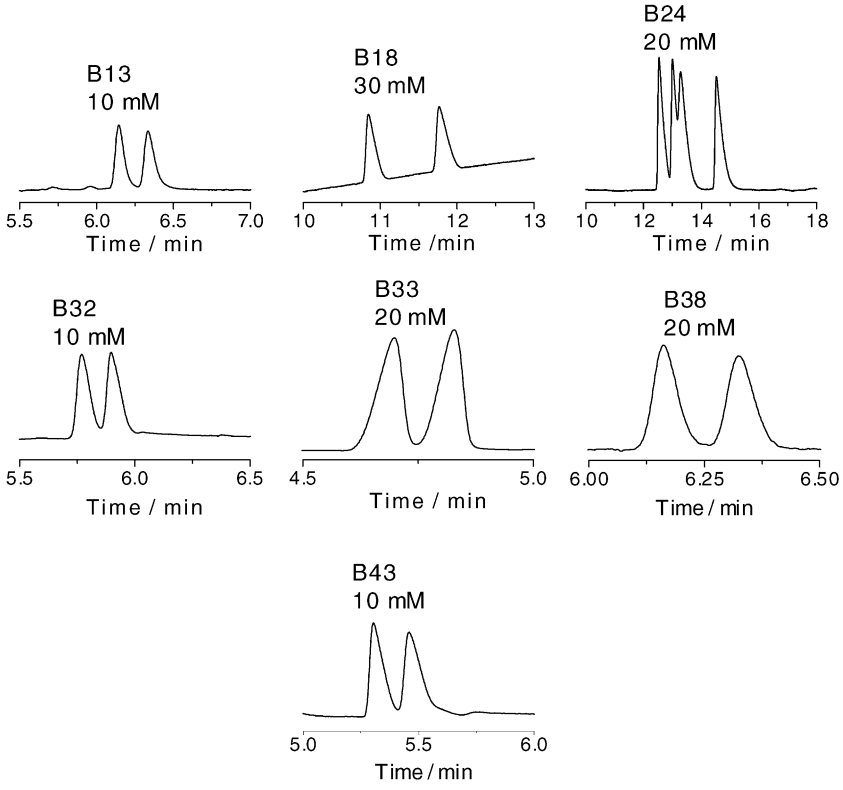

Fig. 5. Typical electropherograms of the weak base analyte enantiomers. The numbers next to the compound codes (see Fig. 1) indicate the HxDAS concentrations (mM) used. Capillary: 25 μm i.d., 19/26 cm effective/total length, uncoated, fused silica. Other conditions: see Section 2.

panel) and separation selectivities (bottom panel) for two analytes, piperoxane, B38 (left panels) and propranolol, B42 (right panels). For both analytes, the interaction strength increases in the HxDAS < HDAS < ODAS order. The separation selectivites are completely different with the three acetylated CDs. For example, both weak bases behave as weakly binding bases with HxDAS and HDAS (no mobility cross-over [33]), but as strongly binding bases with ODAS (mobility cross-over [33]) indicating that all three single isomer sulfated CDs have unique utilities for the separation of enantiomers.

A similar utility difference can be observed when one compares the use of HxDAS for the separation of weak bases in acidic aqueous [36] and acidic methanol BGEs. Fig. 4 shows the effective mobilities (top panel) and separation selectivities (bottom panel) for fluoxetine, B18. In methanol, fluoxetine behaves as a weakly binding base, in water it behaves as a strongly binding base. Separation selectivities for the enantiomers of fluoxetine are also significantly different in the two solvents. Furthermore, the enantiomers of four weak bases, 1-aminoindan, B05, hemicholinium-15, B20, propafenone, B43 and scopolamine, B46 that could not be resolved in acidic aqueous HxDAS BGEs [36], were separated in acidic methanol HxDAS BGEs. On the other hand, the enantiomers of pindolol, B41, and propranolol, B42, which could be baseline separated in acidic aqueous HxDAS BGEs [36], could not be baseline separated in acidic methanol HxDAS BGEs. Thus, depending on the analyte, water and methanol as BGE solvents have complementary roles in the separation of weak base enantiomers with HxDAS.

Finally, Fig. 5 shows a few representative electropherograms for some of the weak base analytes. The numbers next to the compound codes (Fig. 1) indicate the HxDAS concentrations used.

4. Conclusions

The use of the new, single-isomer, regioselectively acetylated and sulfated α-CD, the sodium salt of hexakis(2,3-diacetyl-6-*O*-sulfo) α-cyclodextrin has been studied in acidic methanol BGEs. Interactions between HxDAS and most of the weak base analytes studied were weaker than those observed with the other single-isomer, regioselectively acetylated and sulfated CDs, HDAS and ODAS. Also, HxDAS interacted with the weak bases less strongly in the acidic methanolic BGEs than in the acidic aqueous BGEs. For some of the weak base analytes studied, enantiomer resolution could only be achieved in methanolic or aqueous acidic BGEs, but not in both. For other weak bases, baseline separations could be achieved in either solvent. At our current level of understanding, we cannot predict a priori which analyte will require which solvent. Thus, for a new analyte, one may have to explore both aqueous and NACE systems to achieve adequate enantiomer resolution.

Acknowledgments

Partial financial support of this work by the Texas Coordination Board of Higher Education ARP-TDT program (Project Number 010366-016 and 010366-0152-1999), ANTEK Instruments Inc. (Houston, TX, USA), Beckman-Coulter (Fullerton, CA, USA) and the Texas A&M University Gradipore Chair in Separation Science Endowment is gratefully acknowledged.

References

[1] G.K.E. Scriba, Electrophoresis 24 (2003) 2409.
[2] M.L. Riekkola, M. Jussila, S.P. Porras, I.E. Valko, J. Chromatogr. A 892 (2000) 155.
[3] V. Piette, M. Lammerhofer, W. Lindner, J. Crommen, Chirality 11 (1999) 622.
[4] V. Piette, M. Fillet, W. Lindner, J. Crommen, J. Chromatogr. A 875 (2000) 353.
[5] M. Lammerhofer, E. Zarbl, W. Lindner, J. Chromatogr. A 892 (2000) 509.
[6] V. Piette, W. Lindner, J. Crommen, J. Chromatogr. A 894 (2000) 63.
[7] M. Lammerhofer, E. Zarbl, V. Piette, J. Crommen, W. Lindner, J. Sep. Sci. 24 (2001) 706.
[8] E. Zarbl, M. Lammerhofer, P. Franco, M. Petracs, W. Lindner, Electrophoresis 22 (2001) 3297.
[9] V. Piette, W. Lindner, J. Crommen, J. Chromatogr. A 948 (2002) 295.
[10] C. Czerwenka, M. Lammerhofer, W. Lindner, Electrophoresis 23 (2002) 1887.
[11] V. Piette, M. Lammerhofer, W. Lindner, J. Crommen, J. Chromatogr. A 987 (2003) 421.
[12] Y. Hedeland, M. Hedeland, U. Bondesson, C. Pettersson, J. Chromatogr. A 984 (2003) 261.
[13] H. Loden, Y. Hedeland, M. Hedeland, U. Bondesson, C. Pettersson, J. Chromatogr. A 986 (2003) 143.
[14] F. Wang, M.G. Khaledi, Anal. Chem. 68 (1996) 3460.
[15] K. Eder, F. Sinner, M. Mupa, C.G. Huber, M.R. Buchmeister, Electrophoresis 22 (2001) 109.
[16] F. Wang, M.G. Khaledi, J. Chromatogr. A 817 (1998) 121.
[17] F. Wang, M.G. Khaledi, J. Chromatogr. B 731 (1999) 187.
[18] J.B. Vincent, A.D. Sokolowski, T.V. Nguyen, G. Vigh, Anal. Chem. 69 (1997) 4226.
[19] J.B. Vincent, D. Kirby, T.V. Nguyen, G. Vigh, Anal. Chem. 69 (1997) 4419.
[20] H. Cai, T.V. Nguyen, G. Vigh, Anal. Chem. 70 (1998) 580.
[21] W. Zhu, G. Vigh, Anal. Chem. 72 (2000) 310.
[22] W. Zhu, G. Vigh, Electrophoresis 24 (2003) 130.
[23] M.B. Busby, P. Lim, G. Vigh, Electrophoresis 24 (2003) 351.
[24] A. Rizzi, Electrophoresis 22 (2001) 3079.
[25] B. Chankvetadze, N. Burjanadze, D.M. Maynard, K. Bergander, D. Bergenthal, G. Blaschke, Electrophoresis 23 (2002) 3027.
[26] B. Chankvetadze, N. Burjanadze, J. Breitkreutz, K. Bergander, D. Bergenthal, O. Kataeva, R. Fröhlich, H. Luftmann, G. Blaschke, J. Sep. Sci. 25 (2002) 1155.
[27] M. Wedig, S. Laug, T. Christians, M. Thunhorst, U. Holzgrabe, J. Pharm. Biomed. Anal. 27 (2002) 531.
[28] F. Süß, C. Kahle, U. Holzgrabe, G.K.E. Scriba, Electrophoresis 23 (2002) 1301.
[29] L. Zhou, R. Thompson, R.A. Reamer, C. Miller, C. Welch, D.K. Ellison, J.M. Wyvratt, J. Chromatogr. A 987 (2003) 409.
[30] J.B. Vincent, G. Vigh, J. Chromatogr. A 816 (1998) 233.
[31] M. Tacker, P. Glukhovskiy, H. Cai, G. Vigh, Electrophoresis 20 (1999) 2794.

[32] W. Zhu, G. Vigh, J. Chromatogr. A 892 (2000) 499.
[33] W. Zhu, G. Vigh, Electrophoresis 21 (2000) 2016.
[34] M.B. Busby, O. Maldonado, G. Vigh, Electrophoresis 23 (2002) 456.
[35] A.C. Servais, M. Fillet, A.M. Abushoffa, P. Hubert, J. Crommen, Electrophoresis 24 (2003) 363.
[36] S.L. Li, G. Vigh, Electrophoresis 24 (2003) 2487.
[37] B.A. Williams, G. Vigh, Anal. Chem. 69 (1997) 4445.
[38] Y.Y. Rawjee, G. Vigh, Anal. Chem. 66 (1994) 619.
[39] D.K. Maynard, G. Vigh, Electrophoresis 22 (2001) 3212.
[40] B.A. Williams, G. Vigh, J. Chromatogr. A 777 (1997) 295.