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Non-aqueous capillary electrophoretic enantiomer separations using the tetrabutylammonium salt of heptakis(2,3-*O*-diacetyl-6-*O*-sulfo)-cyclomaltoheptaose, a single-isomer sulfated β-cyclodextrin highly-soluble in organic solvents

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Abstract

The tetrabutylammonium salt of heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-cyclomaltoheptaose, a single-isomer sulfated β-cyclodextrin that is adequately soluble in a number of protic and aprotic polar solvents was synthesized on the large scale and used for the capillary electrophoretic separation of the enantiomers of weak bases in acidic acetonitrile background electrolytes. The effective mobilities and separation selectivities observed for these analytes followed trends similar to those found with other single-isomer sulfated cyclodextrins in acidic methanol background electrolytes. Enantiomer separations obtained with the tetrabutylammonium and sodium salts of heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-cyclomaltoheptaose were different indicating, for the first time, that selection of the counter ion of the single-isomer sulfated cyclodextrin is also of importance for the separation of enantiomers.

Keywords: Non-aqueous capillary electrophoresis; Enantiomer separations; Cyclodextrins; Sulfated cyclodextrins; Single-isomer sulfated β-cyclodextrin; Heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-cyclomaltoheptaose; Tetrabutylammonium salt of heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-cyclomaltoheptaose

1. Introduction

Currently, aqueous background electrolytes (BGEs) are used in the majority of capillary electrophoretic (CE) separations because (i) the acid–base chemistry of aqueous solutions is comparatively well understood, (ii) the dielectric constant of water is high and (iii) the volatility of water is low. However, CE separations can also be carried out in protic and aprotic organic solvents, provided that the solvents have (i) sufficiently high dielectric constants, (ii) relatively low viscosities and (iii) low vapor pressures. Since the physical characteristics and acid–base chemistries of water and organic solvents are very different, aqueous CE and non-aqueous CE (NACE) frequently offer different separation selectivities (for a recent, comprehensive review of NACE see, e.g. [1]).

Enantiomer separations by NACE using such chiral selectors as camphorsulfonate (e.g. [2,3]), cyclodextrins (e.g. [4–14]), quinine, quinidine and their derivatives (e.g. [15–19]) and such solvents as methanol (e.g. [11–13]), ethanol (e.g. [15,18]), acetonitrile (e.g. [17,19]), formamide (e.g. [7,9,10]), *N*-methylformamide (e.g. [4–6,9]), and *N*,*N*-dimethylformamide (e.g. [4]) were described in the last decade. The solvent–resolving agent combinations selected for these enantiomer separations were often dictated by solubility constraints one can expect to encounter in NACE. Of the cyclodextrins (CDs), native CDs (e.g. [4–8]), quaternary ammonium CDs [9], randomly sulfated CDs (e.g. [4,5]) and single-isomer sulfated CDs (e.g. [11–14]) have all been used in NACE with formamide, *N*-methylformamide, *N*,*N*-dimethylformamide and methanol as solvents.

The dielectric constant to viscosity ratio of acetonitrile (ACN) is quite favorable [1]. Acetonitrile cannot act as proton donor in hydrogen-bonds: this often reduces the strength of solvent-ion interactions, promotes the formation of het-

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eroconjugates and ion pairs, and drastically changes the acid–base chemistry of the dissolved species [1,20]. Consequently, competition between the solvent and the analyte enantiomers for the chiral resolving agents, including CDs, is expected to be weaker in acetonitrile than in protic solvents. However, the currently available single-isomer sulfated CDs [21–30] are not sufficiently soluble in acetonitrile to permit their use as resolving agents.

We have observed during the preparation of different single-isomer sulfated cyclodextrins that some of the synthetic intermediates, specifically, the pyridinium salts of the 2,3-di-*O*-acetyl-6-*O*-sulfo CDs and 2,3-di-*O*-methyl-6-*O*-sulfo CDs were quite soluble in organic solvents (unpublished results). Thus, we hypothesized that the poor solubilities of the single-isomer sulfated cyclodextrins in acetonitrile were caused not so much by the CD anions, rather by their sodium counter ion, and that a more hydrophobic cation, such as tetrabutylammonium, TBA^+, might increase their solubility in acetonitrile sufficiently to permit their effective use for NACE enantiomer separations. This paper describes the synthesis of the first acetonitrile-soluble single-isomer sulfated β-cyclodextrin, the tetrabutylammonium salt of heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-cyclomaltoheptaose, TBA_7HDAS, and its use for the NACE separation of the enan-

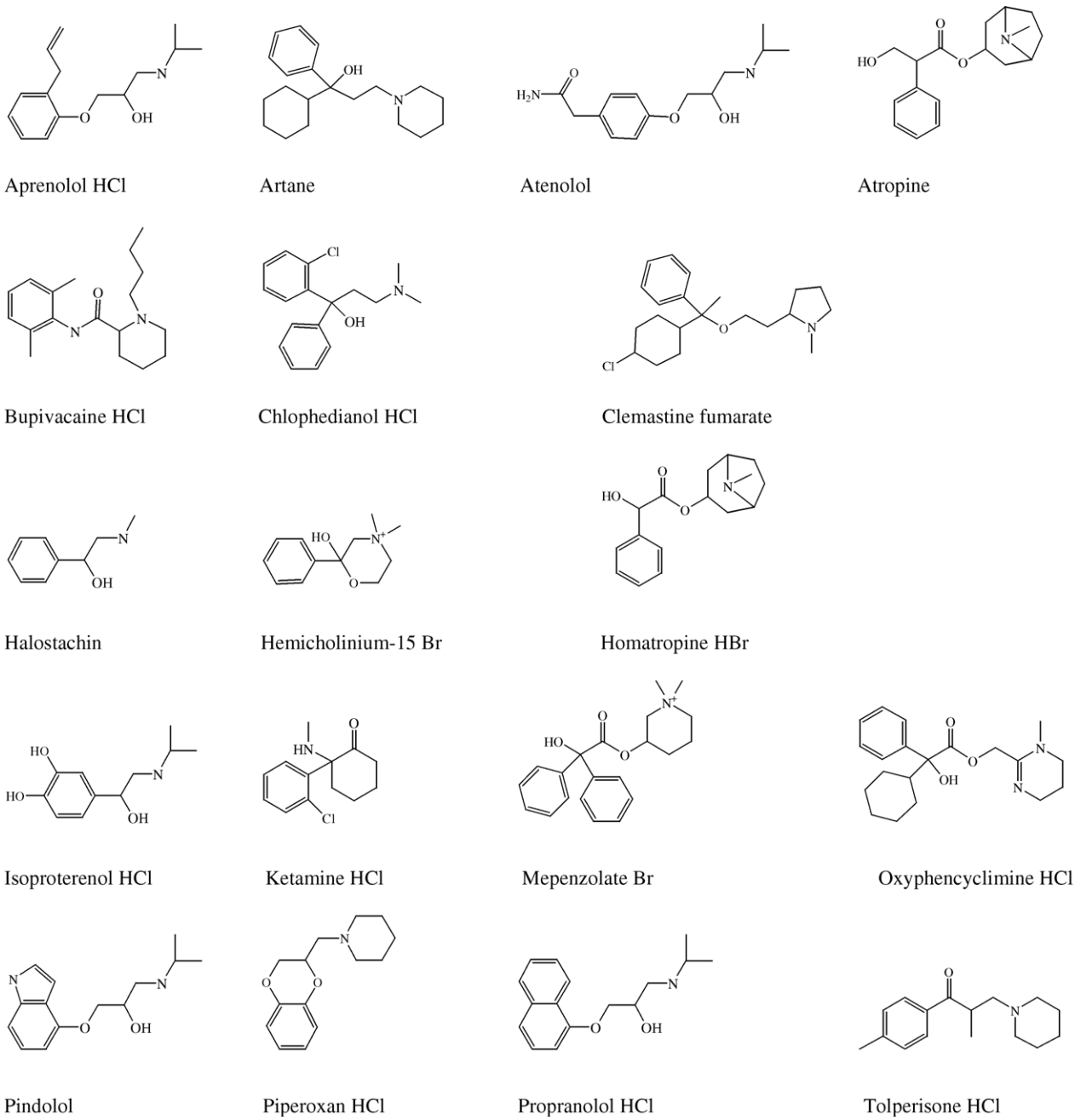

Fig. 1. Structures of the chiral bases used in this study.

tiomers of a few weak bases in acidic acetonitrile background electrolytes.

2. Experimental

HPLC-grade methanol, ethanol, iso-propanol, acetonitrile, acetone, ethyl acetate, dichloromethane, chloroform, carbon tetrachloride, benzene, toluene, *tert*-butyl methyl ether and hexanes were purchased from EM Science (Gibbstown, NJ, USA) and used as received. Nitromethane, methanesulfonic acid, α-naphthalenesulfonic acid, picric acid, triethylamine, tetrabutylammonium chloride and benzyltriethylammonium chloride were obtained from Aldrich (Milwaukee, WI, USA). The chiral analytes listed in Fig. 1 were from Sigma (St. Louis, MO, USA).

TBA_7HDAS was synthesized in our laboratory by first preparing, with an isomeric purity of greater than 99.5% (mol/mol), the sodium salt of heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-cyclodextrin, Na_7HDAS, according to the procedure described in ref. [21]. Next, an aqueous solution containing Na_7HDAS and tetrabutylammonium chloride was prepared and TBA_7HDAS was extracted into dichloromethane. The aqueous and organic phases were separated and dichloromethane was rotovaped at room temperature. The crude solid product obtained was purified by repeated crystallization until neither Na^+ nor Cl^- could be detected in the final product by indirect UV detection CE analysis (Fig. 2). One- and two-dimensional NMR analysis of TBA_7HDAS proved that the material had the desired structure (the ^1H NMR spectrum is shown in Fig. 3). The chemical shift and coupling constant values for TBA_7HDAS and Na_7HDAS in D_2O and $CDCl_3$ as solvents are compared in Table 1.

The stock buffer for the NACE studies was prepared by weighing 50 mmol of methanesulfonic acid (MSA) and 21 mmol triethylamine (TEA) into a 1 L volumetric flask. The

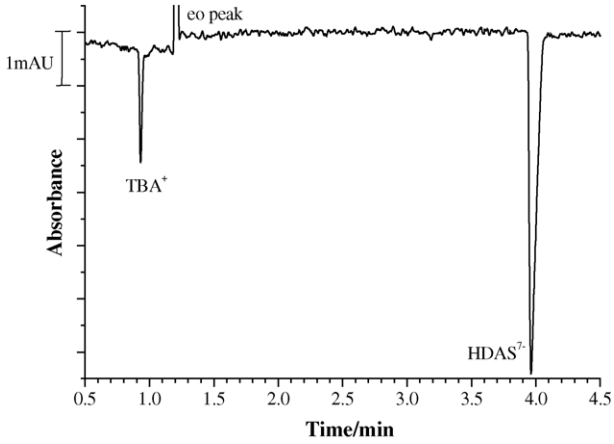

Fig. 2. Indirect-UV detection capillary electropherogram of TBA_7HDAS. For conditions, see Section 2.

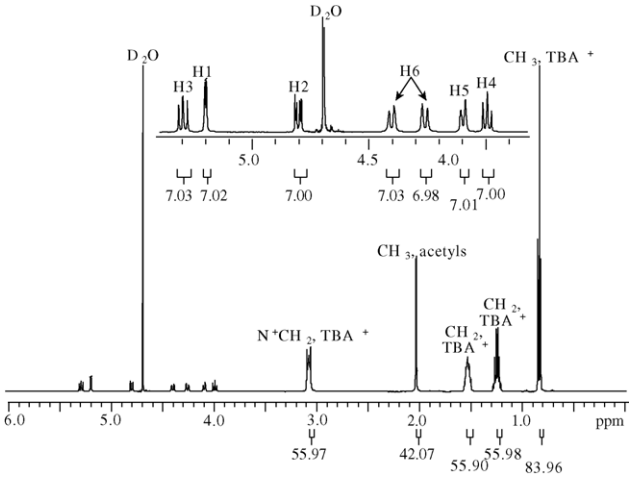

Fig. 3. Assigned 400 MHz ^1H NMR spectrum of TBA_7HDAS. Solvent: D_2O.

Table 1
Chemical shift and coupling constant, *J*, values for the protons in Na_7HDAS and TBA_7HDAS

Proton	Coupling constant (Hz)	Na_7HDAS in D_2O		TBA_7HDAS in D_2O		TBA_7HDAS in $CDCl_3$	
		Chemical shift (ppm)	Coupling constant (Hz)	Chemical shift (ppm)	Coupling constant (Hz)	Chemical shift (ppm)	Coupling constant (Hz)
1		5.20		5.20		5.52	
	J_{12}		3.1		2.9		3.0
2		4.82		4.80		5.00	
	J_{23}		10.0		10.0		10.2
3		5.30		5.30		5.60	
	J_{34}		8.6		8.6		8.4
4		4.00		4.00		4.20–4.40	
	J_{45}		10.0		9.3		–
5		4.12		4.10		4.20–4.40	
	J_{56}		10.7		10.7		–
6		4.25, 4.40		4.25, 4.40		4.6	
CH_3 in acetyl		2.004, 2.008		2.004, 2.008		2.23, 2.26	

flask was then filled to the mark with HPLC grade acetonitrile. The TBA_7HDAS-containing background electrolytes were prepared freshly, every 3 h, by weighing the required amount of TBA_7HDAS into a 10 mL volumetric flask and filling the flask to the mark with the stock buffer. The background electrolyte-containing flask was kept in an ice water bath to minimize the loss of the acetyl groups from TBA_7HDAS: the portion of the background electrolyte used for sample preparation and for the NACE separation was brought to room temperature 5 min prior to the experiment.

The method described in ref. [25] was used to determine that nitromethane (NM) did not interact measurably with TBA_7HDAS in the respective background electrolytes and could be used for the determination of the electroosmotic flow (EOF) mobility, μ^{EOF}. The effective mobilities, μ^{eff} of benzyltriethylammonium ($BzTEA^+$) and α-naphthalenesulfonate (NSA^-) were determined in each background electrolyte using pressure-mediated capillary electrophoresis (PreMCE) [31]. Either $BzTEA^+$ or NSA^- was then added to each sample solution to serve as an external mobility marker [21] and facilitate the calculation of μ^{eff} for each analyte in each background electrolyte.

Samples were prepared immediately before their analysis as follows. Separate 2 mM stock solutions of the EOF marker (N) and external mobility markers ($BzTEA^+$ and NSA^-) and a 1 mM stock solution of the analyte (chiral base) were prepared using pure acetonitrile. Next, 50 μL of the selected marker solution and 100–150 μL of the analyte solution were added to a 0.5 mL volumetric flask. The flask was then filled to the mark with the respective background electrolyte to produce the final sample solution that was injected for 1 s by 0.5 psi nitrogen (1 psi = 6894.76 Pa) into the capillary. Each separation was carried out in triplicate.

The pH^* values of the background electrolytes were determined by a Corning Model 150 pH meter and a combination glass electrode (Corning, Medfield, MA, USA) in the potential reading mode. The glass electrode was calibrated with acetonitrile solutions of picric acid: the pH^* values of these solutions were known to be relatively uneffected by the presence of low concentrations of protic solvent contaminants [20].

All NACE measurements were carried out with a P/ACE 2100 system (Beckman-Coulter, Fullerton, CA, USA). The UV detector was set at 210 nm, the thermostating liquid temperature at 20 °C. Positive polarity was used at the injection side of the 26 μm I.D. bare fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) that had an injector-to-detector length of 19 cm and total length of 26 cm. The applied potentials were kept within the linear segment of the respective Ohm's plots.

Separation selectivities (α) were calculated as $\alpha = \mu_1^{eff}/\mu_2^{eff}$ (where subscript 2 arbitrarily refers to the enantiomer whose effective mobility in the 2 mM TBA_7HDAS background electrolyte had a smaller absolute value [21]). The normalized EOF mobility values (β) were calculated as $\beta = \mu^{EOF}/\mu_2^{eff}$ [32].

3. Results and discussion

3.1. Solubility of TBA_7HDAS in organic solvents

Solubility tests (Table 2) revealed that the solubility of TBA_7HDAS was low (<0.1 g/mL) in non-polar solvents, such as benzene, toluene, carbon tetrachloride and *tert*-butyl methyl ether. TBA_7HDAS was very soluble in water (1.7 g/mL), similarly to Na_7HDAS. TBA_7HDAS was much more soluble in polar protic organic solvents, such as methanol (2.5 g/mL), than Na_7HDAS. More importantly for this work, TBA_7HDAS was also quite soluble in moderately and strongly polar aprotic solvents, such as acetonitrile, acetone, chloroform and ethyl acetate (Table 2), unlike the analogous sodium salt, Na_7HDAS which was practically insoluble in these solvents. In fact, the solubility of TBA_7HDAS in acetonitrile (1.5 g/mL) was almost as high as in water (1.7 g/mL) indicating that TBA_7HDAS might serve as a useful chiral resolving agent in acetonitrile background electrolytes.

3.2. Buffer selection for acetonitrile background electrolytes

Previously, NACE separations with Na_7HDAS in methanol (MeOH) were carried out in 25 mM dichloroacetic acid (DCAA): 12.5 mM triethylamine buffers [11,12]. In acetonitrile, dichloroacetic acid behaves as a weak acid with a pK_a value of 15, while triethylamine acts as a sufficiently strong base [20]. Thus, the pH^* value of the dichloroacetic acid:triethylamine buffer may not be low enough to completely protonate all the weak base analytes selected for this study, especially the secondary amines. Therefore, methanesulfonic acid with a pK_a of 10 [20] was selected to form the

Table 2
Solubility of TBA_7HDAS at 22 °C

Non-polar solvent	Solubility (g/mL)	Polar aprotic solvent	Solubility (g/mL)	Polar protic solvent	Solubility (g/mL)
Benzene	0.5	Acetonitrile	1.5	Methanol	2.5
Toluene	<0.1	Acetone	0.9	Ethanol	2.3
Carbon tetrachloride	<0.1	Chloroform	0.4	Water	1.7
tert-Butyl methyl ether	<0.1	Ethyl acetate	0.2	Isopropanol	0.9

Table 3
Measured effective mobilities (μ^{eff}, in 10^{-5} $cm^2/V\,s$ units) of the basic analytes in different acidic background electrolytes

Analyte	Buffer		
	μ^{eff} water	μ^{eff} MeOH	μ^{eff} ACN
	25 mM H_3PO_4 12.5 mM LiOH[a]	25 mM H_3PO_4 12.5 mM NaOH[b]	50 mM MSA 21 mM TEA[c]
Hemicholinium-15 Br	19.7	30.9	48.8
Mepenzolate Br^-	15.3	23.9	41.5
Tolperisone·HCl	20.6	20.1	31.7
Oxyphencyclimine	21.8	21.4	30.4
Bupivacaine·HCl	19.8	20.7	29.1
Piperoxan·HCl	27.2	16.3	28.6
Chlophedianol·HCl	24.0	15.5	25.6
Clemastine fumarate	17.0		24.4
Homatropine·HBr	17.9	14.0	22.6
Atropine	16.5		22.7
Artane·HCl	18.1		20.8
Halostachin	33.2	23.0	25.1
Isoproterenol·HCl	24.1	17.1	21.8
Ketamine·HCl	25.6	23.9	30.8
Pindolol	23.2	21	19.7
Alprenolol·HCl	24.7	21.6	18.9
Propanolol·HCl	24.3	18.2	18.2
Atenolol	21.1	15.9	14.9

[a] Refs. [25,27].
[b] Ref. [41].
[c] Ref. [42].

buffer system. Homoconjugation of methanesulfonic acid

$$CH_3SO_3H + CH_3SO_3^- \leftrightarrows CH_3SO_3^-HO_3SCH_3$$

lowers the apparent pK_a value of methanesulfonic acid in acetonitrile to about 8.5 [20]. Since triethylamine acts as a strong base in acetonitrile [20], the reaction

$$CH_3SO_3H + (CH_3CH_2)_3N \rightarrow CH_3SO_3^- + (CH_3CH_2)_3NH^+$$

is expected to be quite complete, permitting the creation of a buffer system based on methanesulfonic acid and its conjugate base, MSA^-. However, due to heteroconjugation, homoconjugation and, possibly, ion pairing [20], the maximum buffering capacity in acetonitrile may not occur at the 2–1 mol ratio of methanesulfonic acid and triethylamine, as it is in water. Therefore, the buffering capacity of the acetonitrile stock buffer as a function of the buffer composition (mole ratio of methanesulfonic acid to triethylamine) was determined experimentally. The maximum buffering capacity was found to be at the mole ratio of 2.38. Accordingly, a 50 mM methanesulfonic acid–21 mM triethylamine buffer was selected for the rest of the measurements; this buffer had a pH^* of 8.4. At this pH^*, most primary, secondary and tertiary amines are expected to be fully protonated [33].

The measured effective mobilities of the test analytes in acidic aqueous, methanolic and acetonitrile background electrolytes are compared in Table 3. The mobility values of the quaternary ammonium compounds (hemicholinium and mepenzolate) in the different background electrolytes reflect, primarily, the combined effects of solvent viscosity and ionic strength, while those of the primary, secondary and tertiary amines reflect, in addition to the effects of viscosity and ionic strength, the differences in their degrees of protonation in the different background electrolytes. The last column in Table 3 shows that the effective mobilities of the amines studied are the highest in the methanesulfonic acid–triethylamine–acetonitrile background electrolytes, indicating that (i) the viscosity of the methanesulfonic acid–triethylamine–acetonitrile background electrolyte is low (2.96×10^{-4} Pa s, measured using the P/ACE as a capillary viscometer [34]) and (ii) the amines carry a relatively high positive effective charge in the methanesulfonic acid–triethylamine–acetonitrile background electrolyte.

3.3. *Effective mobilities of the analytes in the TBA_7HDAS–methanesulfonic acid–triethylamine–acetonitrile background electrolytes*

The effective mobility, separation selectivity, normalized electroosmotic flow mobility and peak resolution values for the enantiomers of the weak bases studied in the TBA_7HDAS–methanesulfonic acid–triethylamine–acetonitrile background electrolytes are listed in Table 4.

Depending on how their effective mobilities vary with the concentration of TBA_7HDAS [25,40], the analytes studied can be assigned to one of three groups. In the first group (5 of the 20 analytes tested: hemicholinium, mepenzolate, tolperison, bupivacaine and artane), the effective mobilities remain cationic even at a TBA_7HDAS concentration as high as 5 mM indicating that the interactions between the analyte and TBA_7HDAS are weak. Interestingly, all analytes in this group have the positively charged nitrogen atom in a six-member, saturated ring.

The effective mobilities for the rest of the analytes become anionic by the time the TBA_7HDAS concentration is increased to 5 mM. For the second group, consisting of alprenolol, halostachin, isoproterenol, oxyphencyclimine and pindolol, the anionic effective mobility maximum brought about by the interplay of increasing complexation, increasing background electrolyte viscosity (3.89×10^{-4} Pa s at a TBA_7HDAS concentration of 10 mM) and increasing ionic strength [25] is clearly visible: these analytes interact with TBA_7HDAS most strongly. While the viscosity effects are similar for the analytes that are expected to have similar solvated ion radii (such as most of the analytes in Table 4), the ionic strength effects depend on the magnitude of the binding constants and the concomitant mole fractions of the free and complexed species: the location of the anionic mobility maximum can shift from 5 to 10 or 15 mM with a mere 10% change in the respective binding constant [25].

Table 4
Effective mobility of the less mobile enantiomer (μ in 10^{-5} cm^2 V^{-1} s^{-1} units), separation selectivity (α), normalized EOF mobility values (β), measured peak resolution value (R_s) in 50 mM methanesulfonic acid–21 mM triethylamine–acetonitrile background electrolyte, at an applied potential of 15 kV

Analyte	TBA_7HDAS (mM)											
	2.0				5.0				10.0			
	μ	α	β	R_s	μ	α	β	R_s	μ	α	β	R_s
Hemicholinium-15 Br	20.9	1.00	1.6	0.0	5.8	1.00	2.7	0.0	−5.2	0.83	−3.7	4.5
Mepenzolate Br^-	12.8	1.03	2.7	<0.6	1.3	1.36	16.7	1.7	−7.9	0.97	−2.2	1.1
Tolperisone·HCl	7.4	1.09	4.7	0.3	4.5	1.21	5.2	2.8	−0.8	−0.69	−30	3.9
Oxyphencyclimine	6.1	1.00	5.8	0.0	−4.0	0.96	−6.4	0.61	−3.2	0.96	−5.0	<0.6
Bupivacaine·HCl	8.1	1.00	4.2	0.0	4.1	1.00	5.4	0.0	−8.6			
Piperoxan·HCl	7.1	1.00	3.3	0.0	−2.2	0.87	−9.3	1.2	−7.6	0.97	−2.3	1.6
Chlophedianol·HCl	5.4	1.00	4.1	0.0	−8.5	0.93	−3.1	2.6	−8.9	0.93	−1.9	4.4
Clemastine fumarate	5.2	1.00	4.8	0.0	−4.2	1.00	−6.6	0.0	−4.8	1.00	−3.5	0.0
Homatropine·HBr	−6.6	1.00	−4.3	0.0	−11.2	1.00	−2.3	0.0	−11.5	1.00	−1.8	0.0
Atropine	−5.2	1.00	−6.6	0.0	−5.4	0.98	−3.9	<0.6	−19.4	0.99	−1.5	<0.6
Artane·HCl	4.3	1.00	6.0	0.0	4.4	1.00	5.0	0.0	−10.1	1.00	−3.1	0.0
Halostachin	−16.6	0.99	−2.0	0.9	−14.6	0.99	−1.6	<0.6	−13.7			
Isoproterenol·HCl	−14.6	0.95	−1.7	2.2	−18.6	0.99	−1.5	1.4	−16.4	0.98	−1.4	2.6
Ketamine·HCl	−5.2	1.00	−6.2	0.0	−10.3	1.00	−2.6	0.0	−16.4			
Pindolol	−13.1	0.95	−2.7	1.3	−14.6	0.97	−1.9	1.4	−13.7	0.99	−1.8	0.6
Alprenolol·HCl	−11.2	0.96	−3.1	1.2	−15.7	0.97	−1.8	2.0	−15.0	0.99	−1.5	0.9
Propanolol·HCl	−7.1	0.91	−4.2	1.3	−11.0	0.96	−2.0	2.6	−14.0	0.98	−1.6	1.2
Atenolol	−5.2	0.93	−1.9	3.3	−20.1	0.97	−1.4	4.3	−25.4	0.99	−1.2	3.3

The third group is formed by the analytes whose anionic effective mobility maximum must be located at a TBA_7HDAS concentration higher than 10 mM: atenolol, atropine, chlophendianol, clemastine, homatropine, ketamine, piperoxan, and propranolol. These analytes must interact with TBA_7HDAS more strongly than the analytes in the first group, but less strongly than those in the second group. Since no mobility measurements were made at TBA_7HDAS concentrations higher than 10 mM, the predicted anionic effective mobility maxima were not observed experimentally for the analytes assigned to the third group.

Considering the close similarities of the structures of the analytes involved, the molecular level reasons for these apparently different effective mobility trends are not obvious. Furthermore, though all amines interacted with TBA_7HDAS (Table 4), the enantiomers of five of the 20 analytes (artane, bupivacaine, clemastine, homatropine and ketamine) could not be separated under the conditions used. It is impossible to rationalize why the enantiomers of structurally very closely related analytes (such as atropine and homatropine) could and could not be separated. Only detailed NMR spectroscopic studies, such as those in refs. [35–39] could shed light on the actual interactions of the analytes and TBA_7HDAS. Fortunately, since TBA_7HDAS can now be synthesized on the large scale, and since TBA_7HDAS is highly soluble in deuterated acetonitrile, such NMR studies will become possible in the future.

As an example, Fig. 4 shows the separation of the enantiomers of four of the weak base analytes using an acidic acetonitrile background electrolyte that contained 5 mM of TBA_7HDAS.

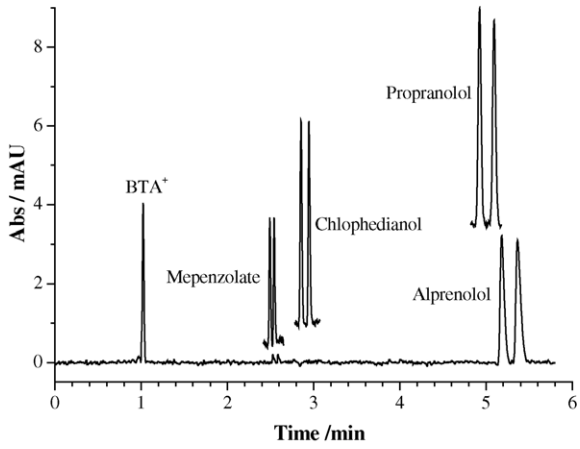

Fig. 4. Electropherograms for a few analytes resolved with a background electrolyte that contained 5 mM TBA_7HDAS in the 50 mM methanesulfonic acid–21 mM triethylamine–acetonitrile stock buffer. For other conditions, see Section 2.

3.4. Comparison of enantiomer separations obtained with Na_7HDAS and TBA_7HDAS in methanesulfonic acid–triethylamine–acetonitrile background electrolytes

Because of the very low solubility of Na_7HDAS in acetonitrile, measurements could not be made at a Na_7HDAS concentration higher than 2 mM. The effective mobility, separation selectivity, normalized electroosmotic flow mobility and peak resolution values for the enantiomers of the weak bases studied in the Na_7HDAS–methanesulfonic acid–triethylamine–acetonitrile background electrolytes are listed in Table 5.

Table 5
Effective mobility of the less mobile enantiomer (μ in 10^{-5} cm^2 V^{-1} s^{-1} units), separation selectivity (α), normalized EOF mobility values (β), measured peak resolution values (R_s) in 50 mM methanesulfonic acid–21 mM triethylamine–acetonitrile background electrolyte with 2 mM Na_7HDAS, at an applied potential of 15 kV

Analyte	μ	α	β	R_s
Hemicholinium-15 Br	38.0	1.00	0.6	0
Mepenzolate Br^-	31.4	1.05	0.7	1.2
Tolperisone·HCl	17.0	1.00	1.3	0
Oxyphencyclimine	23.0	1.00	1.0	0
Bupivacaine·HCl	16.0	1.00	2.1	0
Piperoxan·HCl	13.0	1.00	2.8	0
Chlophedianol·HCl	12.0	1.00	1.0	0
Clemastine fumarate	14.0	1.00	3.0	0
Homatropine·HBr	9.0	1.09	2.7	0.9
Atropine	7.9	1.00	2.2	0
Artane·HCl	16.0	1.00	1.0	0
Halostachin	0.5	3.50	54	1.5
Isoproterenol·HCl	15.0	1.04	1.9	<0.6
Ketamine·HCl	5.9	1.00	6.2	0
Pindolol	2.5	1.00	11	0
Alprenolol·HCl	3.2	1.00	6.0	0
Propanolol·HCl	0.3	1.00	66	0

With Na_7HDAS, the effective mobilities of all amines studied remained cationic indicating either weaker complexation or lower effective anionic charge on the amine:cyclodextrin complex. Both effects might be caused by incomplete dissociation of Na_7HDAS: for the first, the lower negative charge could lead to weaker ion-pairing, for the second, even for identical binding strength, lower anionic mobility for the complex. Sodium ion is a hard ion that might be stabilized in acetonitrile [20] by ion-paring with HDAS. It is interesting to note that the enantiomers of homatropine that could not be separated with TBA_7HDAS, are separated with Na_7HDAS. This observation might mean that the enantiorecognition mechanism in the Na_7HDAS-containing background electrolyte is different from the one that acts in the TBA_7HDAS-containing background electrolyte. However, only detailed NMR spectroscopic studies can determine if this explanation is correct [35–39].

4. Conclusions

An organic-solvent soluble, anionic, single-isomer β-CD was synthesized on the large scale by extracting TBA_7HDAS from an aqueous mixture of TBACl and Na_7HDAS with dichloromethane. The replacement of sodium ions with the more hydrophobic TBA^+ cation increased the solubility of the single-isomer sulfated CD to about 150 mM in methanol and 100 mM in acetonitrile. The existence of a single-isomer sulfated CD so highly soluble in commonly used NACE solvents could broaden the range of applications of anionic CDs to compounds that are non-soluble in aqueous media.

Separations obtained with the sodium salt and the tetrabutylammonium salt of HDAS at 2 mM concentrations demonstrate that the counter ion of the CD significantly affects the separation of enantiomers.

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