SYNTHESIS OF CHARGED CYCLODEXTRIN HIGHLY SOLUBLE IN ORGANIC SOLVENTS FOR ENANTIOMER SEPARATIONS IN CAPILLARY ELECTROPHORESIS

A Thesis

by

OMAR MALDONADO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Chemistry

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Approved by:

Chair of Committee, Gyula Vigh
Committee Members, Manuel Soriaga
Suryakant Waghela
Head of Department, Emile Schweikert

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ABSTRACT

Synthesis of Charged Cyclodextrin Highly Soluble in Organic Solvents for Enantiomer Separations in Capillary Electrophoresis. (August 2005)

Omar Maldonado, B.S., University of Puerto Rico

Chair of Advisory Committee: Dr. Gyula Vigh

Synthesis of charged cyclodextrin highly soluble in organic solvents was made by exchanging the inorganic counter ion (Na^+) of heptakis (2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD (Na_7HDMS) with tetrabutylammonium (TBA^+), to produce TBA_7HDMS. The same ion exchange was used to synthesize the TBA salts of the analogous CDs TBA_6HxDMS and TBA_8ODMS. Indirect-UV detection capillary electrophoresis (CE) and ^{1}H NMR were used as the characterization methods.

Separations of thirteen pharmaceuticals were made using TBA_7HDMS as the chiral resolving agent in aqueous CE. On the other hand, a set of twenty pharmaceuticals was used for the enantiomer separations in non-aqueous CE (NACE). Comparison between the results obtained with TBA_7HDMS in aqueous and non-aqueous CE were made. In addition, comparison between the results obtained with TBA_7HDMS and Na_7HDMS in aqueous and non-aqueous CE were made as well.

To my beloved family Oscar Maldonado, Julia E. Oquendo, Oscar Maldonado Jr. and Omayra Maldonado who deserve only the best from me.

ACKNOWLEDGMENTS

First, I give thanks to God for everything that He gives me and for everything that He takes away from me.

I would like to thank everyone that helped me to complete this work. My special thanks to my family and the Pescadores members that always kept me focused on the important things in life. In addition, I greatly appreciate all the ideas and help obtained from former and present members of the separation science group.

Finally, I give thanks to my advisor Dr. Gyula Vigh, for all the time he dedicated to me and my project.

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CHAPTER I

INTRODUCTION

1.1 Enantiomeric separations

The separation of enantiomers is vital in the pharmaceutical, chemical and biological fields. The stereochemistry of such compounds can affect their biological activities, therefore method development for their purification and separation is of continued interest. In order to achieve enantiomeric separation, a chiral resolving agent must be used. Some common techniques for the separation of enantiomers are gas chromatography (GC) which uses the chiral resolving agent in the stationary phase [1-3], high performance liquid chromatography (HPLC) which uses the chiral resolving agent either in the stationary phase or in the mobile phase or both [4], and capillary electrophoresis (CE) which uses a background electrolyte (BGE) that contains the chiral resolving agent dissolved in a buffer solution [5-7]. From all the techniques, CE stands out due to its short analysis time, high efficiency, simplicity and low material consumption. Since CE does not use a stationary phase, band broadening problems related to mass transfer to the stationary phase are minimal compared to HPLC and GC.

In order to achieve enantiomeric separation in CE, each enantiomer has

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This thesis follows the style and format of *Electrophoresis*.

to complex differently with the chiral resolving agent to form two different diasteromeric complexes with different hydrodynamic volumes. Therefore, the diasteromeric complexes will be separated by their differences in the electrophoretic velocities when the electric field is applied along the capillary [7-14]. The observed electrophoretic velocity (v^{obs}) of the enantiomer is defined in equation 1 [15].

$$v^{obs} = E^{appl} \times \mu^{obs} \quad (1)$$

where E^{appl} is the applied electric field strength and μ^{obs} is the observed electrophoretic mobility of the enantiomer which can be further described by equation 2 as the sum of the effective electrophoretic mobility (μ^{eff}) of the enantiomer and the electroosmotic flow mobility (μ^{eo}).

$$\mu^{obs} = \mu^{eff} + \mu^{eo} \quad (2)$$

Separation selectivity (α) for the enantiomers is defined as the ratio of the effective mobility of the faster enantiomer (μ_1^{eff}) over the effective mobility of the slower enantiomer (μ_2^{eff}) (equation 3).

$$\alpha = \mu_1^{eff} / \mu_2^{eff} \quad (3)$$

An important parameter in the separation of enantiomers is the normalized electroosmotic flow (β) which is defined as the ratio between the electroosmotic flow and the effective mobility of the slower enantiomer (equation 4).

$$\beta = \mu^{eo} / \mu_2^{eff} \quad (4)$$

Optimization of the separation selectivity can be accomplished by varying the pH, temperature, concentration of the organic modifier and the concentration

of the chiral resolving agent in the BGE [7-14,16-19]. On the other hand, optimization of resolution can be obtained by adjusting the electroosmotic flow mobility to be closer, but opposite in sign to the magnitude of the effective mobility of the slower enantiomer giving a β value closer to negative one [20-22]. This can be done by using coated capillaries and/or by manipulating the viscosity of the BGE with the addition of hydroxymethylcellulose or other viscosity modifiers.

Resolution is described by equation 5

$$R_s = \sqrt{\frac{E l e_o}{8kT}} * \frac{\text{abs}(\alpha - 1)\sqrt{\text{abs}(\alpha + \beta)}\sqrt{\text{abs}(1 + \beta)}\sqrt{z_1^{\text{eff}}}\sqrt{z_2^{\text{eff}}}}{\sqrt{\text{abs}((\alpha + \beta)^3) z_1^{\text{eff}}} + \sqrt{\alpha \cdot \text{abs}((1 + \beta)^3) z_2^{\text{eff}}}} \qquad (5)$$

e_o is the fundamental charge, k is the Boltzman constant and z^{eff} is the effective charge of the analyte. Having a β value equal to negative one will increase the resolution to an infinitely high value. This is true only if the separation selectivity (α) is not unity. This idea was first discussed in what is known as the chiral resolving agent migration model (CHARM) [5, 9, 18-20]. According to the predictions of the CHARM model, resolution can be increased by increasing the applied potential (the multiple of the capillary length (l) and the increasing applied electric field, E) and decreasing temperature (T) which will increase complexation between the chiral resolving agent and the enantiomer. In addition, resolution is dependent on the charge state of the enantiomers, the concentration and charge state of the cyclodextrin and the electroosmotic flow.

Since the interaction between the chiral resolving agent and the analyte is crucial to the separation of the enantiomers, choosing the right chiral resolving agent may be the difference between separation and no separation. Some chiral resolving agents that are available are: crown ethers, macrocyclic antibiotics, chiral micelles, proteins, oligo- and polysaccharides and the most popular of all, cyclodextrins (CDs) [5, 7, 23-26].

1.2 CDs as chiral resolving agents for CE

Native CDs are cyclic oligosaccharides formed by glucopyranose units attached to each other by α-1,4 glycosidic bonds creating a truncated cone shape (Figure I-1). Depending on the number of glucopyranose units in the ring, the CD will be called alpha CD (α-CD, 6 units), beta CD (β-CD, 7 units) or gamma CD (γ-CD, 8 units). CDs offer three major sites to interact with the analyte: i) the internal cavity which is made of the carbons in the glucose units (hydrophobic site), ii) the achiral face where the primary alcohol groups are located (C6 in the glucose unit, hydrophilic sites), iii) the chiral face where the secondary alcohol groups are located (C2 and C3 in the glucose unit, hydrophilic sites). Some disadvantages of native CD are its poor solubility and limited types of intermolecular interactions (hydrogen-bonding and Van der Waals). In order to overcome these disadvantages, researchers have been interested in the

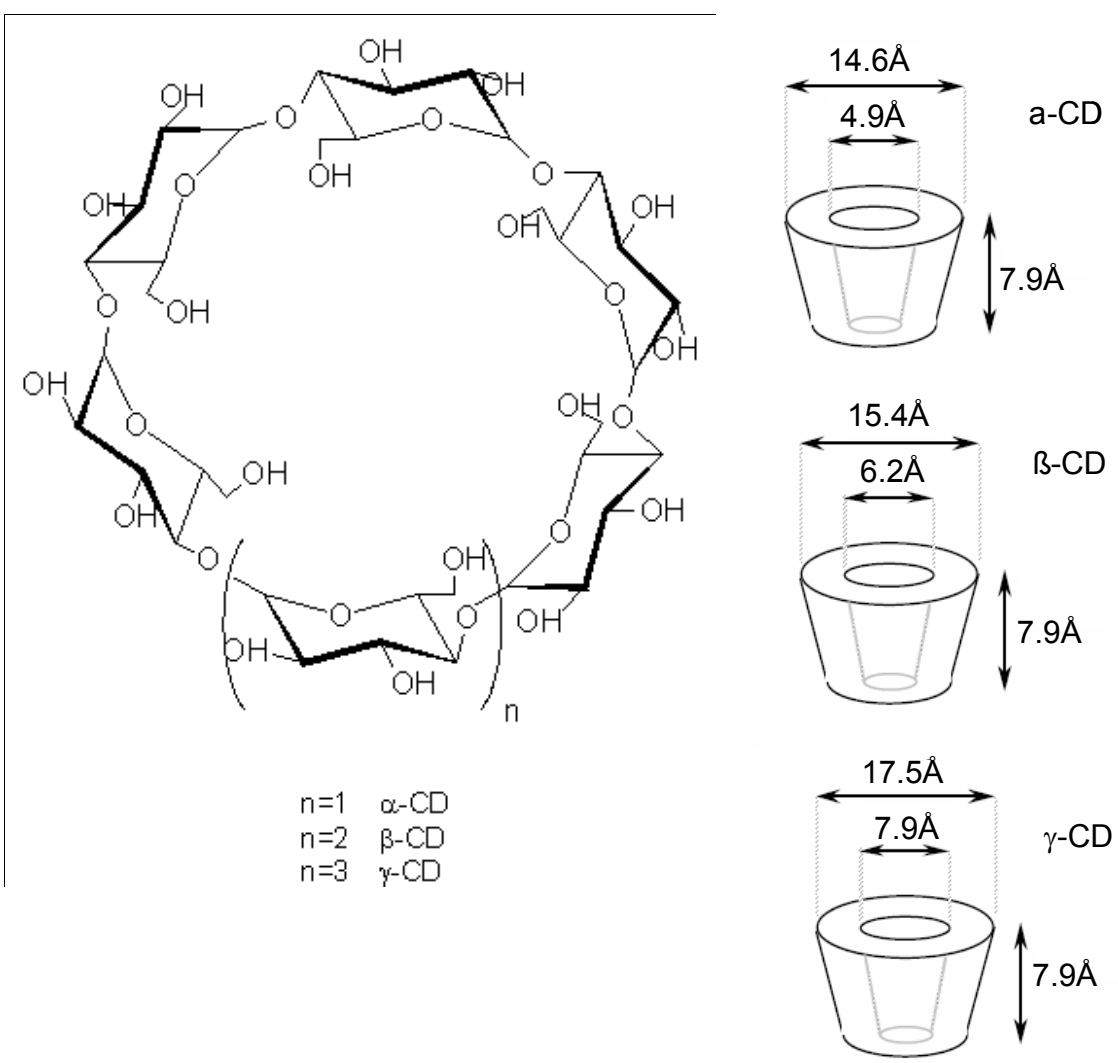


Figure I-1. Molecular structure of native CDs [27].

modification of CDs by substituting the hydroxyl groups attached to C2, C3 and C6 with different functional groups. Since β-CD offers, lower cost and greater commercial availability compared to α-CD and γ-CD, its derivatives are more popular. A wide variety of CD derivatives are available and are generally classified into neutral and charged CDs.

Some neutral CD derivatives have been synthesized by substituting acetyl, benzyl, hydroxypropyl or methyl groups for the hydroxyl groups at the C2, C3 and/or C6 positions of the CDs [28, 29]. These CD derivatives offer different types of intermolecular interactions which lead to different stereoselectivities; however, they can only separate charged enantiomers and are not suitable for the separation of neutral enantiomers.

Charged CD derivatives were introduced in order to expand their applicability to the separation of neutral enantiomers. These ionic CDs can be made by substituting the hydroxyl groups in the C2, C3 and C6 positions with weakly or strongly acidic or basic functional groups. Some examples of weakly acidic and basic functional groups are carboxylic acid groups and alkylamino groups. Since the charge states of these functional groups are pH dependent, method development for the separation of enantiomers is more complicated as separation selectivity is pH dependent as well.

On the other hand, strongly acidic and basic functional groups, such as sulfate and quaternary ammonium groups are pH independent since they are permanently charged through the entire working pH range (pH 2-12). Sulfated

CD derivatives are preferred over the quaternary ammonium CD derivatives because positively charged CDs have been shown to bind to the wall of the commonly used fused silica capillaries which contain dissociated silanol groups [30]. This will lead to an increment in band broadening due chromatographic retention. Capillary coating (e.g., polyacrylamide) had been successfully used to overcome this problem [31]; however, this is not required when negatively charged CD derivatives are used [32].

In agreement with the information mentioned above, sulfated β-CD derivatives are the cyclodextrins of choice of those which are commercially available. Unfortunately, most of these derivatives are found as isomeric mixtures with a certain average degree of substitution. There are several problems with using isomeric mixtures including: i) the isomeric mixture composition can change from batch to batch, which requires optimization of the separation every time a new batch is synthesized or purchased; ii) since the chiral selectivity of the CD depends on how many substituents are attached to the CD and where they are located, the separation selectivity can be increased, reduced, or in the worst case, eliminated by having the same selectivity for both enantiomers [33]; iii) due to the different isomers, kinetic band broadening may happen when differences in the complexation rates are presents [34]; iv) it's almost impossible to characterize the complexes and to perform studies at the molecular level with an isomeric CD mixture; v) it is impossible to predict any result *a priori* with such complex mixtures.

On the other hand, single-isomer sulfated β-CD derivatives have proven to be the solution for all the problems that come with the use of randomly substituted materials. Characterization and applicability in the pharmaceutical field have been reported for single-isomer sulfated β-CD [34-43]. Enantiomer separations using single-isomer sulfated β-CD derivatives follow the behavior predicted by the CHARM model. This model predicts that there are three types of enantiomer separations for the weak electrolytes: i) ionoselective; ii) desionoselective and iii) duoselective separations. In the ionoselective separation (Figure I-2), the chiral resolving agent complexes selectively with the ionic form of the enantiomer. On the other hand, desionoselective separation (Figure I-3) occurs when the chiral resolving agent complexes selectively with the neutral form of the enantiomer. Finally, duoselective separation (Figure I-4) happens when the chiral resolving agent complexes selectively with both the ionic and nonionic forms of the enantiomers [8, 9]. This clearly indicates that the best separation selectivity can be found by performing the enantiomer separations using only a low pH BGE and a high pH BGE.

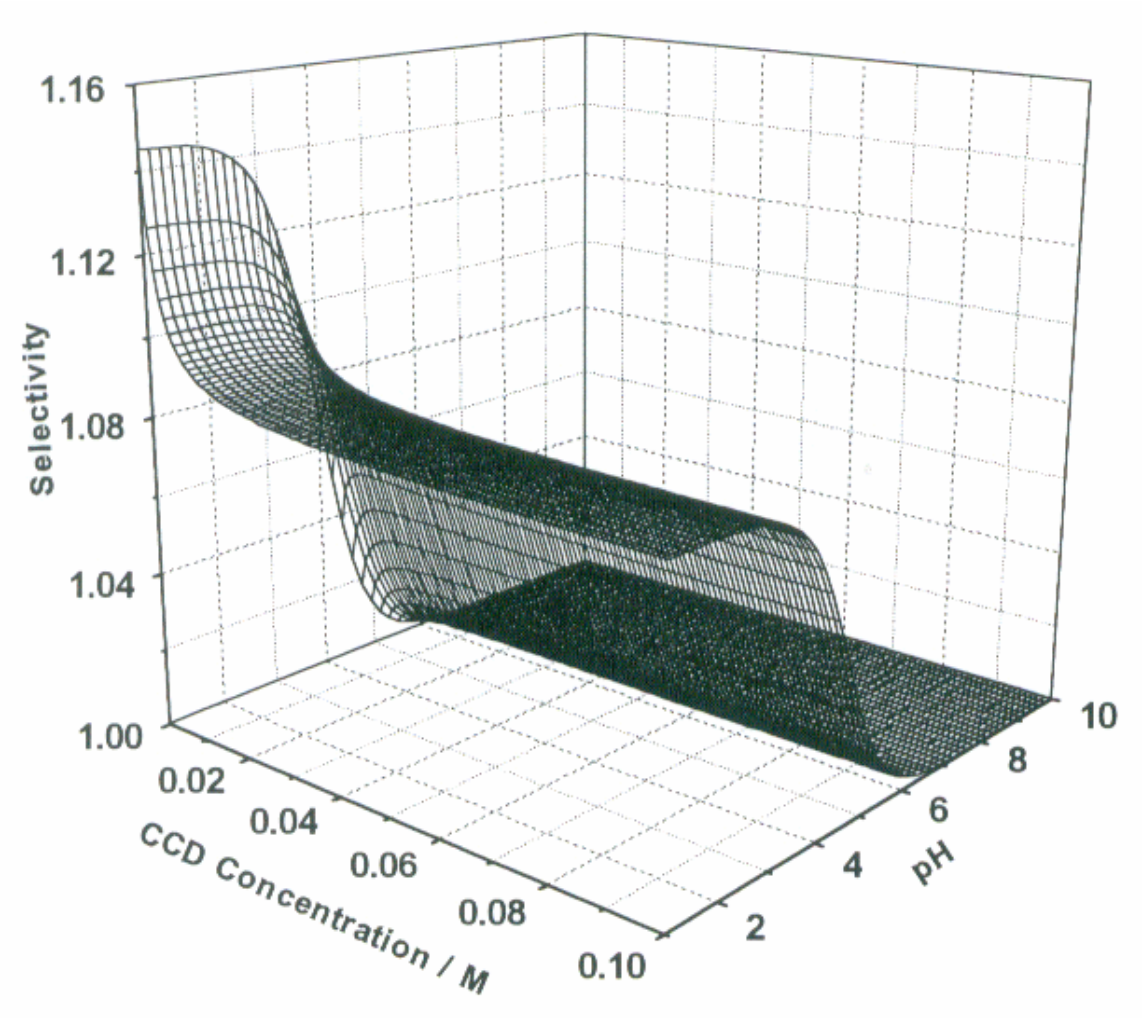


Figure I-2. Ionoselective separation of a weak base: Selectivity surface as a function of charged CD concentration and the pH of the BGE [9].

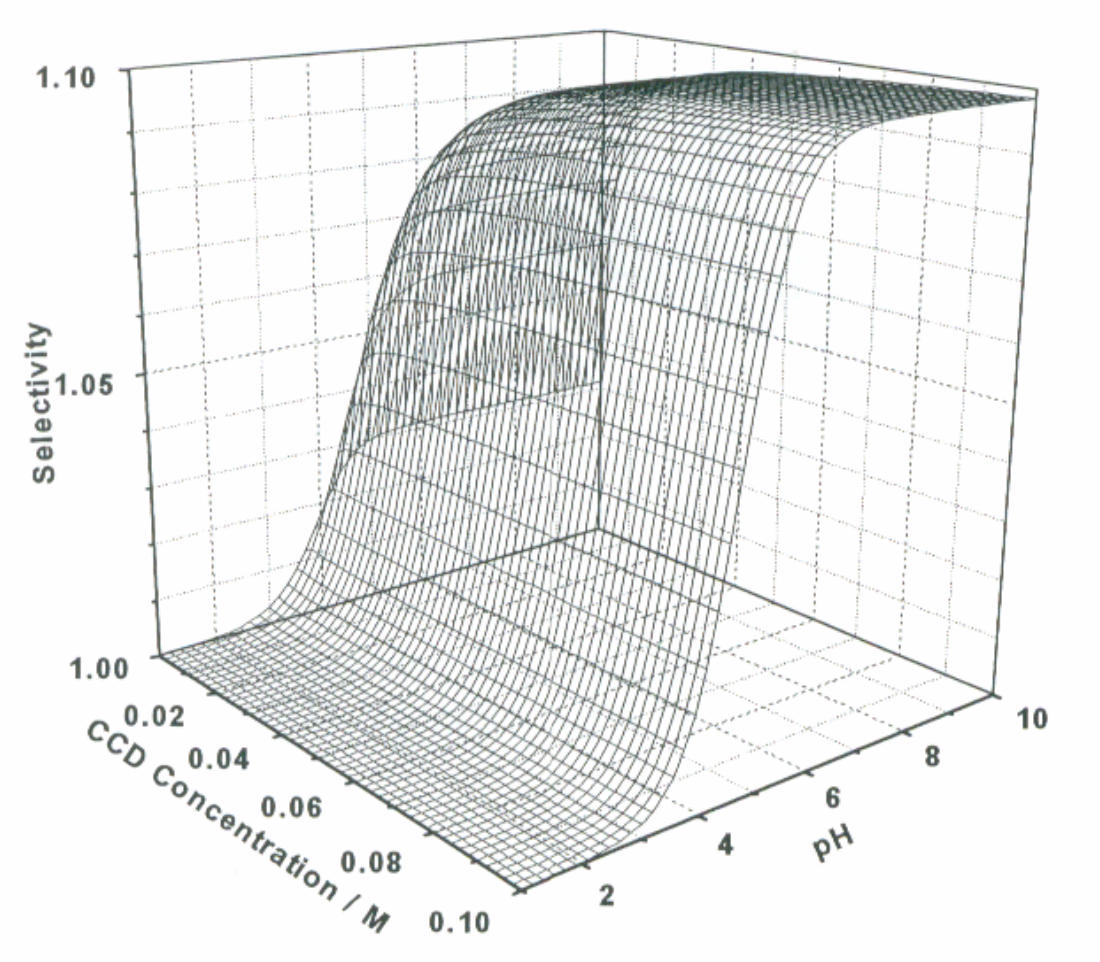


Figure I-3. Desionoselective separation of a weak base: Selectivity surface as a function of charged CD concentration and the pH of the BGE [9].

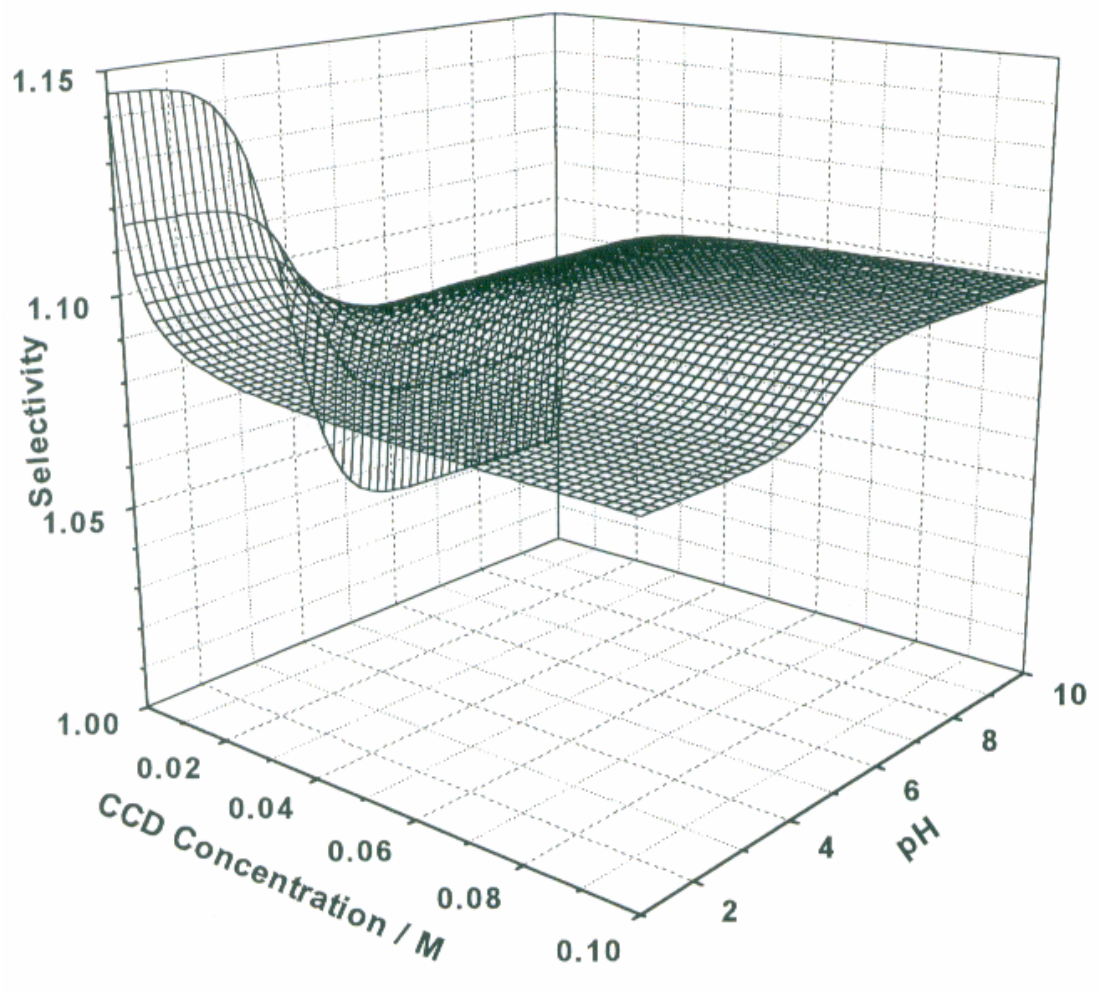


Figure I-4. Duoselective separation of a weak base: Selectivity surface as a function of charged CD concentration and the pH of the BGE [9].

1.3 Non-aqueous CE enantiomer separation

A wide variety of solvents have been reported to be suitable as the main components of BGEs in CE. Aqueous BGEs have been extensively used since water has low viscosity, high relative permittivity, low volatility, low UV-cutoff and dissolves most analytes. On the other hand, there are many other solvents (organic solvents) that meet these criteria such as: i) acetonitrile [44-46]; ii) amides such as formamide [47-50], N-methylformamide [51, 49, 52, 53], N, N-dimethylformamide [49]; and, most commonly used, methanol [41, 45, 53-64]. Non-aqueous CE (NACE) separation has been of interest since it complements and sometimes improves separations obtained in aqueous CE. For example, NACE offers a low conductivity media which means that a higher potential can be used, therefore faster analysis with higher resolution can be achieved. In addition, solubility is obtained for the water-insoluble analytes. Moreover, separation selectivity may improve due to the lower dielectric constant of the solvent which provides a different media where intermolecular interactions that were not present or were weak (negligible) in aqueous media can be observed. On the other hand, interactions between the analyte and the chiral resolving agent are usually weakened in NACE, lowering the formation constant of the complex. Finally, NACE extends the applicability of CE by coupling it to mass spectrometry (CE-MS) which has become a very powerful technique [49, 65-69].

Although NACE (hydro-organic) enantiomer separations with CDs have been used for more than a decade, the interest of using pure non-aqueous

solvents as BGEs has been growing recently. However, progress has been limited due to the poor solubility of charged cyclodextrins in an organic media.

1.4 Purpose of this thesis project

Enantiomer separations by CE have been very interesting to separation scientists. So far there is no way to know *a priori* what is the right chiral resolving agent for a particular enantiomer. Therefore, more studies concerning chiral selectivity are needed in order to understand such systems more and to be able make predictions efficiently.

As mentioned before, single-isomer sulfated β-CD has been used successfully for enantiomer separations and it is preferred over the randomly substituted sulfated β-CDs, since the single-isomer simplifies the system studied. Due to the poor solubility of the single-isomer sulfated β-CD in organic solvents, the synthesis of a charged CD soluble in organic solvent is needed. It has been proved that the solubility of the charged CD in an organic solvent increases significantly by exchanging the inorganic counter ion of the charged CD with an organic counter ion.

A very stable and well characterized single-isomer sulfated β-CD has been chosen for further derivatization. It is the sodium salt of the single-isomer heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD (Na_7HDMS). This particular CD has been successfully used for the separation of the enantiomers of pharmaceuticals in aqueous and non-aqueous BGEs. It is the aim of this project to investigate

the effect on the chiral selectivity and the solubility of the new derivative TBA_7HDMS by exchanging the sodium counter ion of Na_7HDMS with the tetrabutylammonium counter ion. In order to be able to make any comparison of the results obtained with TBA_7HDMS, the chiral drugs that were chosen in this project were previously analyzed by Na_7HDMS using aqueous BGE and non-aqueous BGE (methanol as the solvent).

CHAPTER II

SYNTHESIS OF THE TETRABUTYLAMMONIUM SALT OF HEPTAKIS (2,3-DI-*O*-METHYL-6-*O*-SULFO)-β-CYCLODEXTRIN

2.1 Introduction

The synthesis of the tetrabutylammonium salt of heptakis (2,3-di-*O*-methyl-6-*O*-sulfo)-β-cyclodextrin (TBA_7HDMS) was synthesized by adding one more step (ion exchange) to the reported [35] synthesis of the sodium salt of heptakis (2,3-di-*O*-methyl-6-*O*-sulfo)-β-cyclodextrin (Na_7HDMS). To obtain the final product several intermediates have been synthesized and purified using modified procedures of the synthesis reported by Takeo [27] and well characterized by reverse phase-HPLC (RP-HPLC), CE, nuclear magnetic resonance (NMR) spectroscopy and MS. The whole synthesis scheme consists of five steps as shown in Figure II-1. Once TBA_7HDMS was successfully made, the same procedure was used for the synthesis of TBA_6HxDMS (α-CD) and TBA_8ODMS (γ-CD) derivatives (Figure II-2). The first four steps of Figure II-1 are explained below which show the preparation of the sodium salt of heptakis (2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD (Na_7HDMS) which were previously made in our laboratories and was utilized as the starting material.

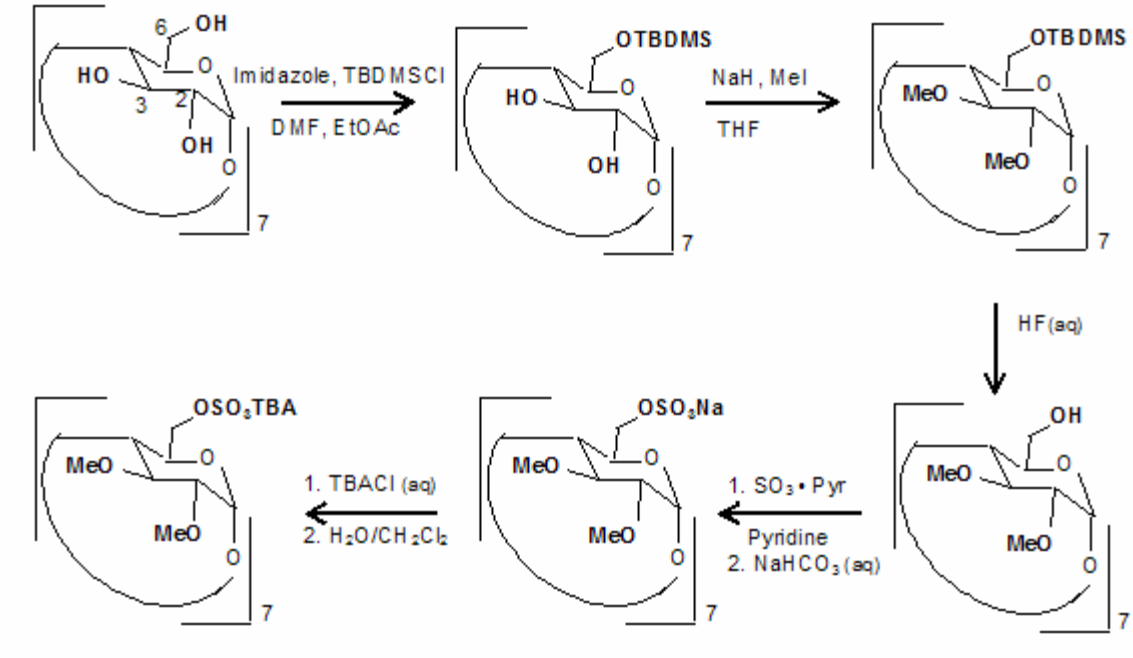


Figure II-1. Synthetic scheme of TBA_7HDMS.

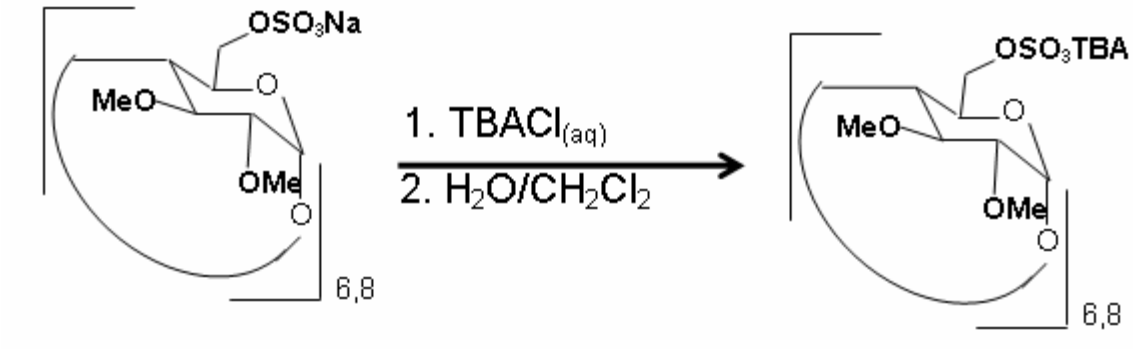


Figure II-2. Synthesis of the tetrabutylammonium salts of hexakis(2,3-di-*O*-methyl-6-*O*-sulfo)-α-CD and octakis(2,3-di-*O*-methyl-6-*O*-sulfo)-γ-CD.

2.1.1 Synthesis of intermediate I, heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-CD

The first step consists of the protection of the primary alcohol groups at the C6 position. This is done by reacting native β-CD dissolved in dimethyl formamide (DMF) with the silylating agent, *tert*-butyldimethylchlorosilane (TBDMSi-Cl) dissolved in ethyl acetate (EtOAc). Although protection of the hydroxyl group at C6 is selective, undesirable isomeric impurities were formed. They were successfully removed by a series of recrystallizations using a ternary solvent mixture (DMF/acetone/water) and dried in vacuo. This first intermediate can be obtained with an isomeric purity higher than 99%.

2.1.2 Synthesis of intermediate II, heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD

The second step consists of the methylation of the hydroxyl groups at the C2 and C3 positions. This is made by reacting intermediate I with iodomethane (CH_3I) and sodium hydride (NaH) using anhydrous tetrahydrofuran (THF) as the reaction solvent. Once the reaction is completed, sodium iodide (NaI) is removed by precipitating it with n-butylacetate (BuOAc) and eventually filtering it off. This intermediate can be obtained with a purity higher than 99%.

2.1.3 Synthesis of intermediate III, heptakis(2,3-di-*O*-methyl)-β-CD

The third step consists of the desilylation of intermediate III by removing the TBDMSi group from the hydroxyl group at C6. This is done by dissolving

intermediate III in alcohol and hydrofluoric acid (HF). After the reaction is completed, TBDMSi-F is removed and the final product can obtained with a purity higher than 99%.

2.1.4 Synthesis of intermediate IV, the sodium salt of heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD

In the fourth step, the hydroxyl group at C6 is sulfated producing a negatively charged cyclodextrin. This is made by dissolving intermediate IV in DMF and reacting it with sulfur trioxide pyridine complex (SO_3·Pyr). In order to produce the sodium salt of the product, a slurry of sodium bicarbonate ($NaHCO_3/H_2O$) is added to the reaction mixture after the reaction is completed. Finally, sodium sulfate (Na_2SO_4) is removed by precipitating it with isopropanol (IPA). This intermediate (Na_7HDMS) has been obtained with a purity higher than 99%.

2.2 Materials and methods

The starting material, Na_7HDMS was made in our laboratories using the synthetic route reported in Ref. 35. It was dissolved in a solution that was previously made by titrating tetrabutylammonium hydroxide (TBAOH), (Acros Organics, Pittsburgh, PA) to pH 7.2 with a solution of 1M hydrochloric acid (HCl), (EM Science, Gibbstown, NJ). After dissolving Na_7HDMS in the solution mentioned above, the ion exchange was made by several extractions using

dichloromethane (CH_2Cl_2), (EM Science, Gibbstown, NJ) and deionized (DI) water obtained from Milli-Q unit (Millipore, Milford, MA).

Indirect-UV detection CE (P/ACE 2100 CE system, Beckman-Coulter, Fullerton, CA) was used to monitor the removal of the salt (sodium chloride, (NaCl)) and to obtain the purity of the final product. The capillary used was a 26.4cm total length fused-silica capillary with 27μm internal diameter (Polymicro Technologies, Phoenix, AZ) and the detection window at 19.6cm (wavelength at 214nm) thermostated at 20 ^{o}C and 10kV applied potential (positive polarity). The BGE used was made by titrating 20mM tris(hydroxymethyl)aminomethane (THAM), (EM Science, Gibbstown, NJ) to pH 8.3 with p-toluenesulfonic acid (pTSA), (Aldrich Chemical Company, Milwaukee, WI) using DI water as solvent. Before using the solutions, they were filtered with a 0.45μm Nalgene nylon membrane filter (VWR, South Plaintfield, NJ).

For further characterization of the material, ^{1}H NMR spectroscopy was used to confirm the completion of ion exchange. The ^{1}H NMR spectra were collected on a Varian Inova 300MHz spectrometer, UNIX based (Varian Assoc., Walnut Creek, CA), with a Quad probe for ^{1}H, ^{13}C, ^{31}P and ^{19}F and Solaris software (version 2.4) using a SUN workstation. The sample contained 30mg of the final product dissolved in deuterium oxide (D_2O), (Cambridge Isotope, Andover, MA).

The synthesis was first tried on a small scale (1g batch) and after the optimum conditions were found, the synthesis was scaled up to 10g and 25g

batches. The same synthesis procedure was also tried with the alpha and the gamma CD derivatives on a small scale (1g batch).

2.3 Results and discussion

2.3.1 Characterization of TBA_7HDMS

Since the starting material (Na_7HDMS) was well characterized by ^1H NMR, ^{13}C NMR, indirect-UV detection CE and ESI-MS, the characterization of the final product (TBA_7HDMS) consisted of monitoring the removal of the salt (NaCl) produced in the process and monitoring the incorporation of the TBA counter ion into the CD. Using indirect-UV detection CE, one can determine the purity of the final product and detect the presence of Na^+ and TBA^+ using positive polarity (positive to negative), and the presence of Cl^-, using negative polarity (negative to positive). Once sodium is removed (previous counter ion), ^1H NMR was used to confirm that TBA^+ was the counter ion of the sulfate group in the CD. This was possible by calculating the ratio of either the protons on the methyl group as the C2 or C3 positions of the $HDMS^{7-}$ with protons on the TBA group or the ratio between the anomeric protons with the protons on the TBA counter ion.

The indirect-UV electropherograms and ^1H NMR spectra for the three different batches (1g, 5g and 25g) are shown in Figures II-3 to II-8. The electropherograms show the EO peak and the presence of TBA^+ (no sodium) and the presence of $HDMS^{7-}$ in positive mode. They also show the absence of

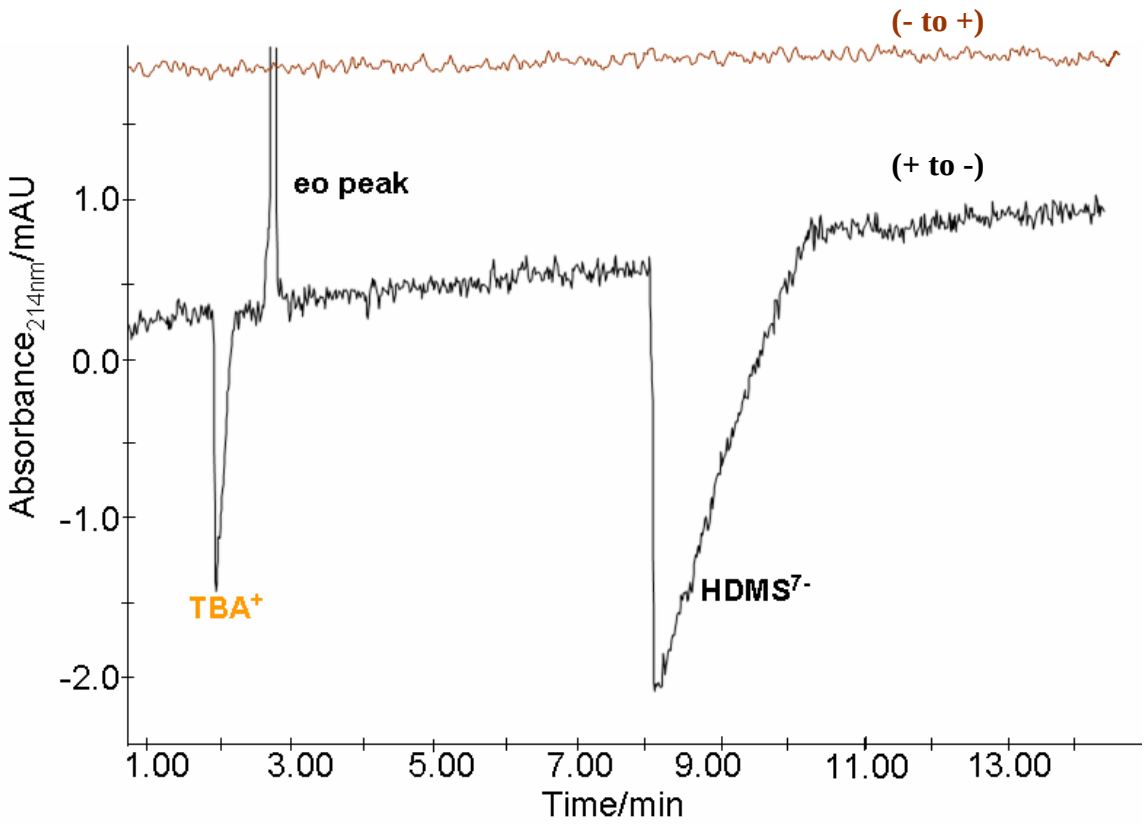


Figure II-3. Indirect-UV detection CE electropherograms of TBA_7HDMS (1g batch).

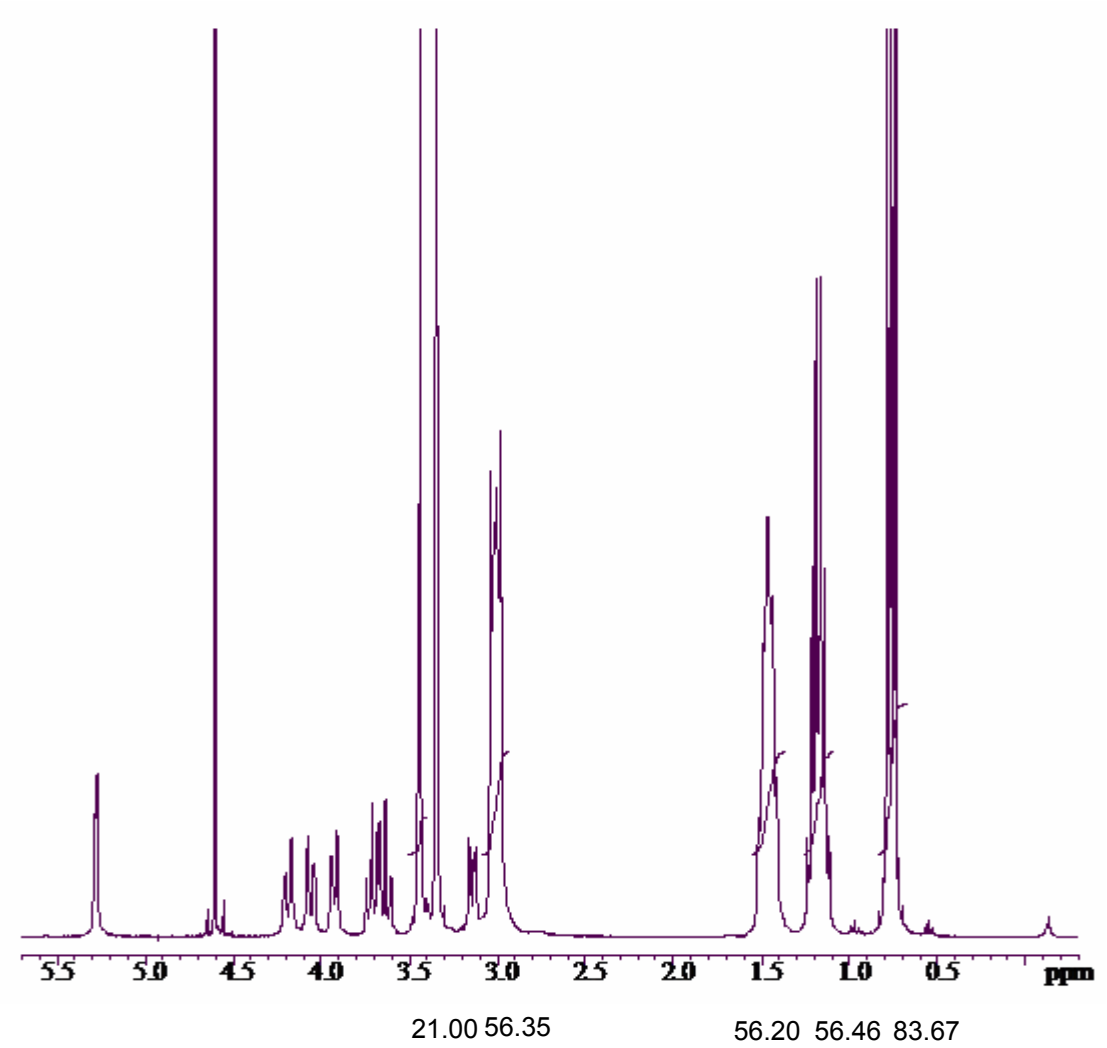


Figure II-4. ^{1}H NMR in D_2O of TBA_7HDMS (1g batch).

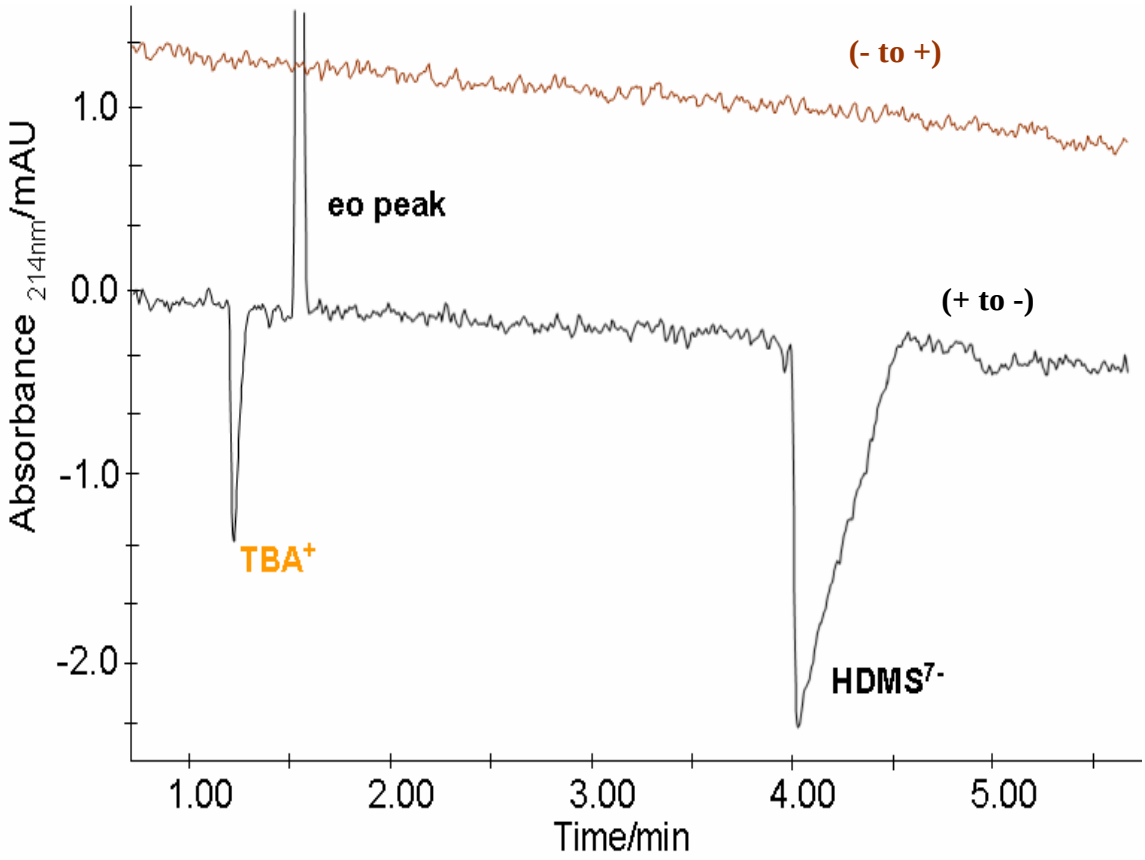


Figure II-5. Indirect-UV detection CE electropherograms of TBA_7HDMS (10g batch).

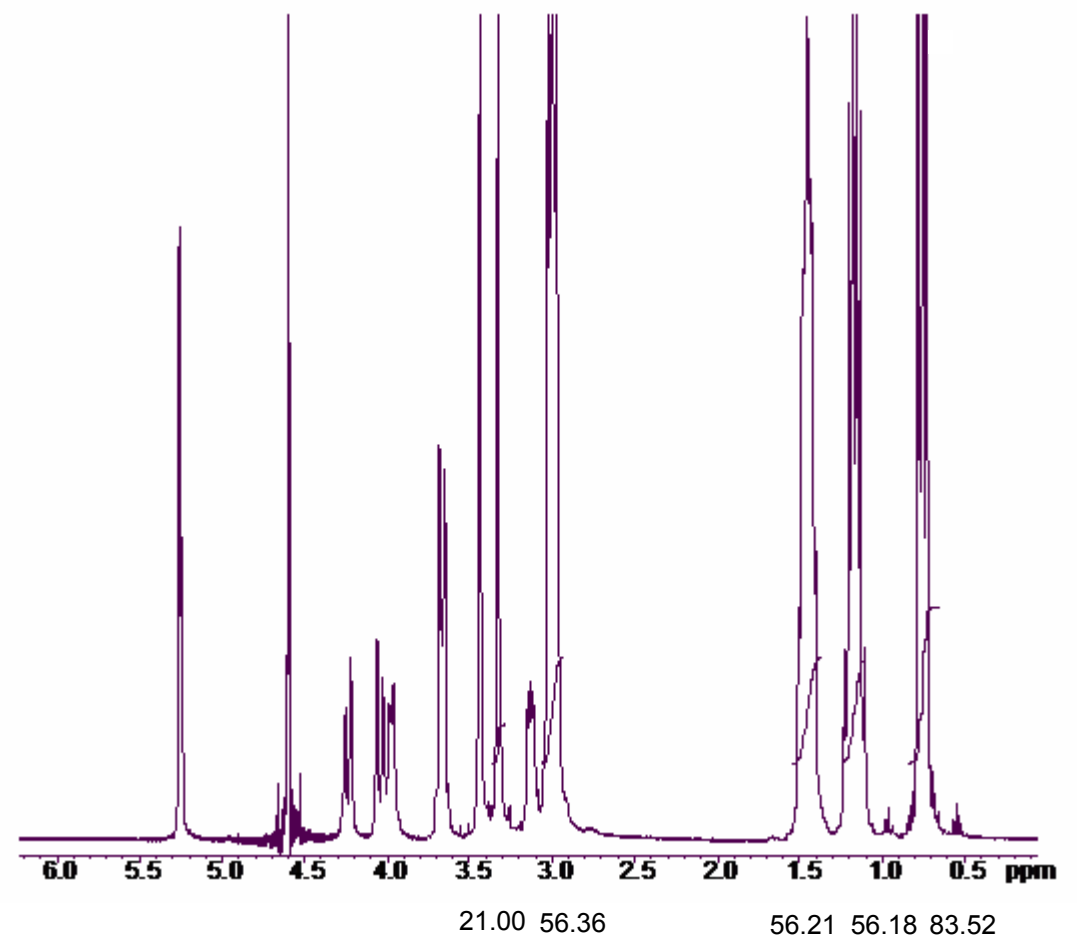


Figure II-6. ^{1}H NMR in D_2O of TBA_7HDMS (10g batch).

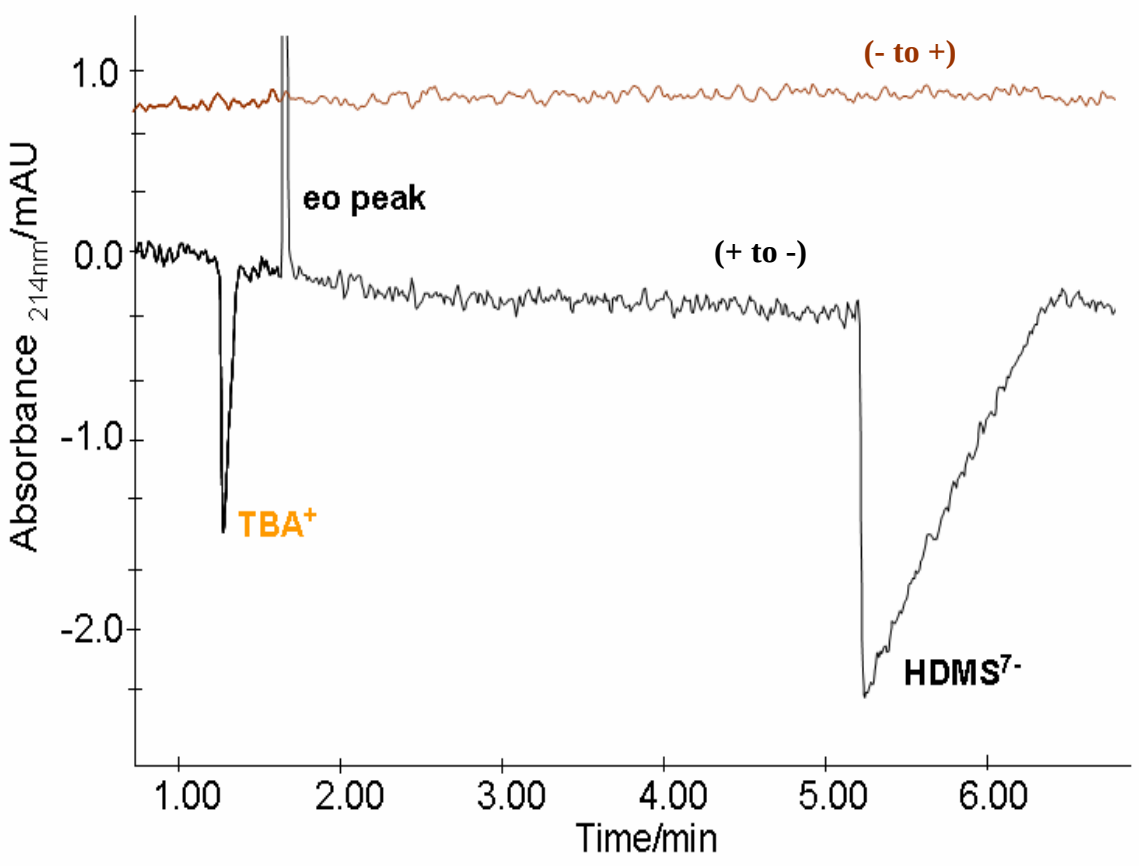


Figure II-7. Indirect-UV detection CE electropherograms of TBA_7HDMS (25g batch).

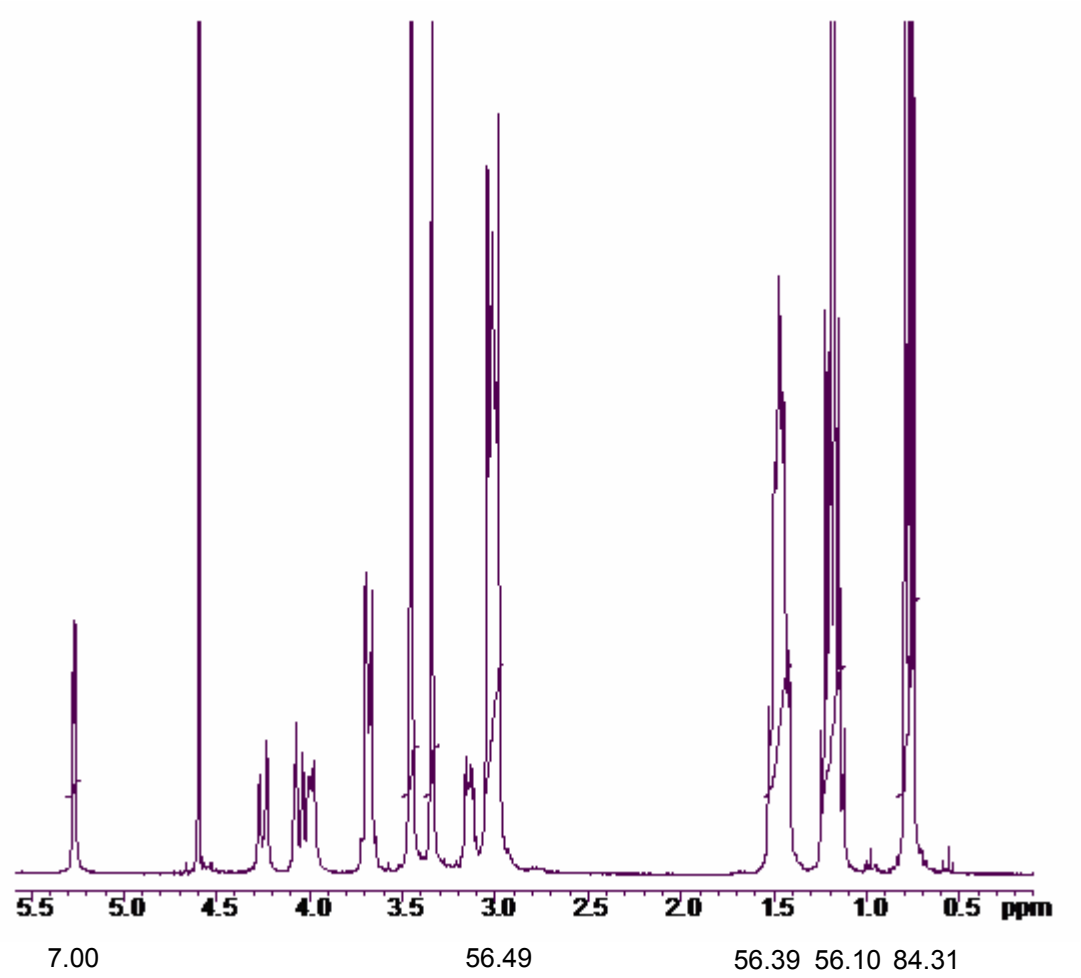


Figure II-8. ^{1}H NMR in D_2O of TBA_7HDMS (25g batch).

chloride ions in the final product (negative mode). On the other hand, the ^{1}H NMR spectra show that for every $HDMS^{7-}$ there are seven TBA^+ molecules. In TBA^+, there are 36 protons which produce four sets of peaks in the ^{1}H NMR spectra, three from the $–CH_2$ and one from the $–CH_3$ (which integrate as 8, 8, 8 and 12 protons, respectively). Since $HDMS^{7-}$ has seven TBA^+ counter ions the 1H NMR spectra show integration values of 56, 56, 56 and 84 protons, respectively.

2.3.2 Characterization of the tetrabutylammonium salt of hexakis(2,3-di-*O*-methyl-6-*O*-sulfo)-α-CD

The same characterization methods were used for the synthesis of 1g of TBA_6HxDMS. The indirect-UV detection CE electropherograms in Figure II-9 show no traces of sodium chloride, but the presence of TBA^+ and $HxDMS^{6-}$. The integration values in the ^{1}H NMR spectra (Figure II-10) for TBA^+ are 48, 48, 48 and 72, which agree with the structure of TBA_6HxDMS ($HxDMS^{6-}$ has six TBA^+).

2.3.3 Characterization of the tetrabutylammonium salt of octakis(2,3-di-*O*-methyl-6-*O*-sulfo)-γ-CD

The synthesis of 1g of TBA_8ODMS was also characterized by indirect-UV detection CE and ^{1}H NMR spectroscopy. The electropherograms of the final

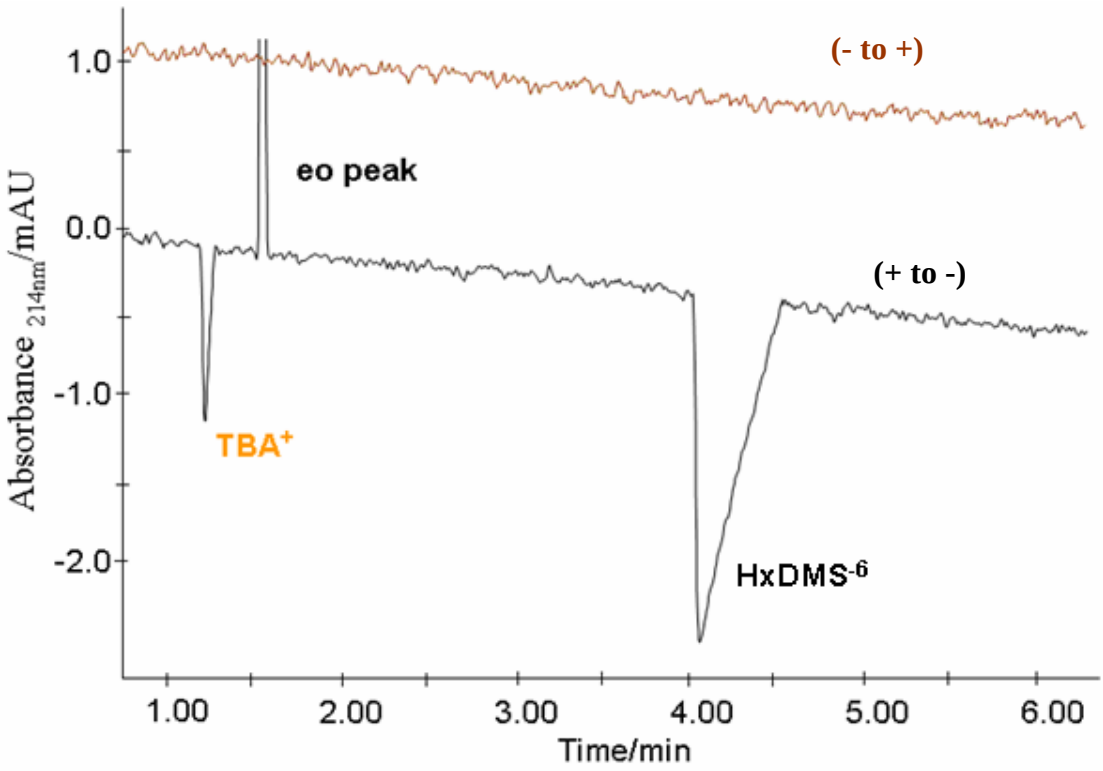


Figure II-9. Indirect-UV detection CE electropherograms of TBA_6HxDMS (1g batch).

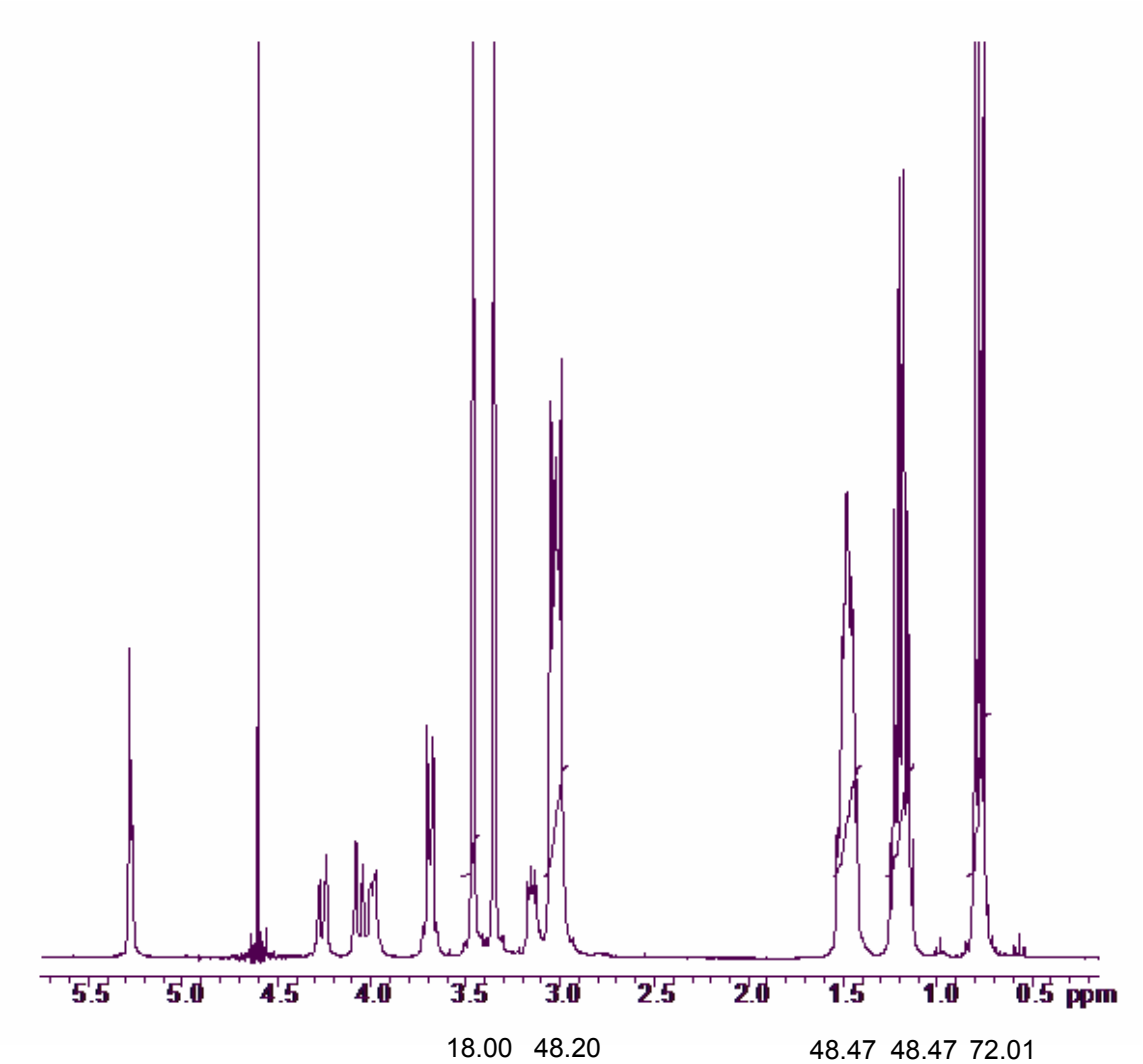


Figure II-10. ^{1}H NMR in D_2O of TBA_6HxDMS (1g batch).

product are shown in Figure II-11 which show the presence of TBA^+ and $ODMS^{8-}$ with no traces of sodium chloride. The integration values in the ^1H NMR spectra (Figure II-12) for the TBA^+ are 64, 64, 64 and 96 which confirm the incorporation of eight TBA^+ counter ions into $ODMS^{8-}$.

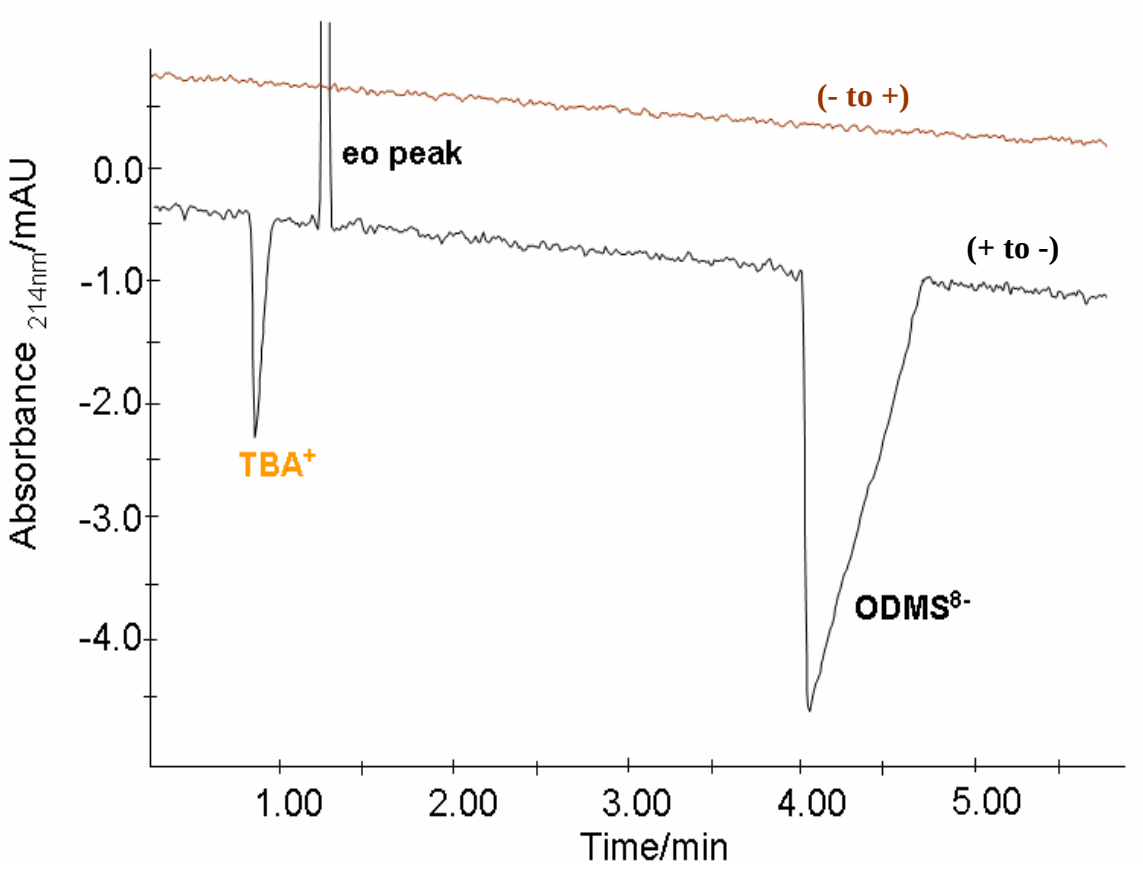


Figure II-11. Indirect-UV detection CE electropherograms of TBA_8ODMS (1g batch).

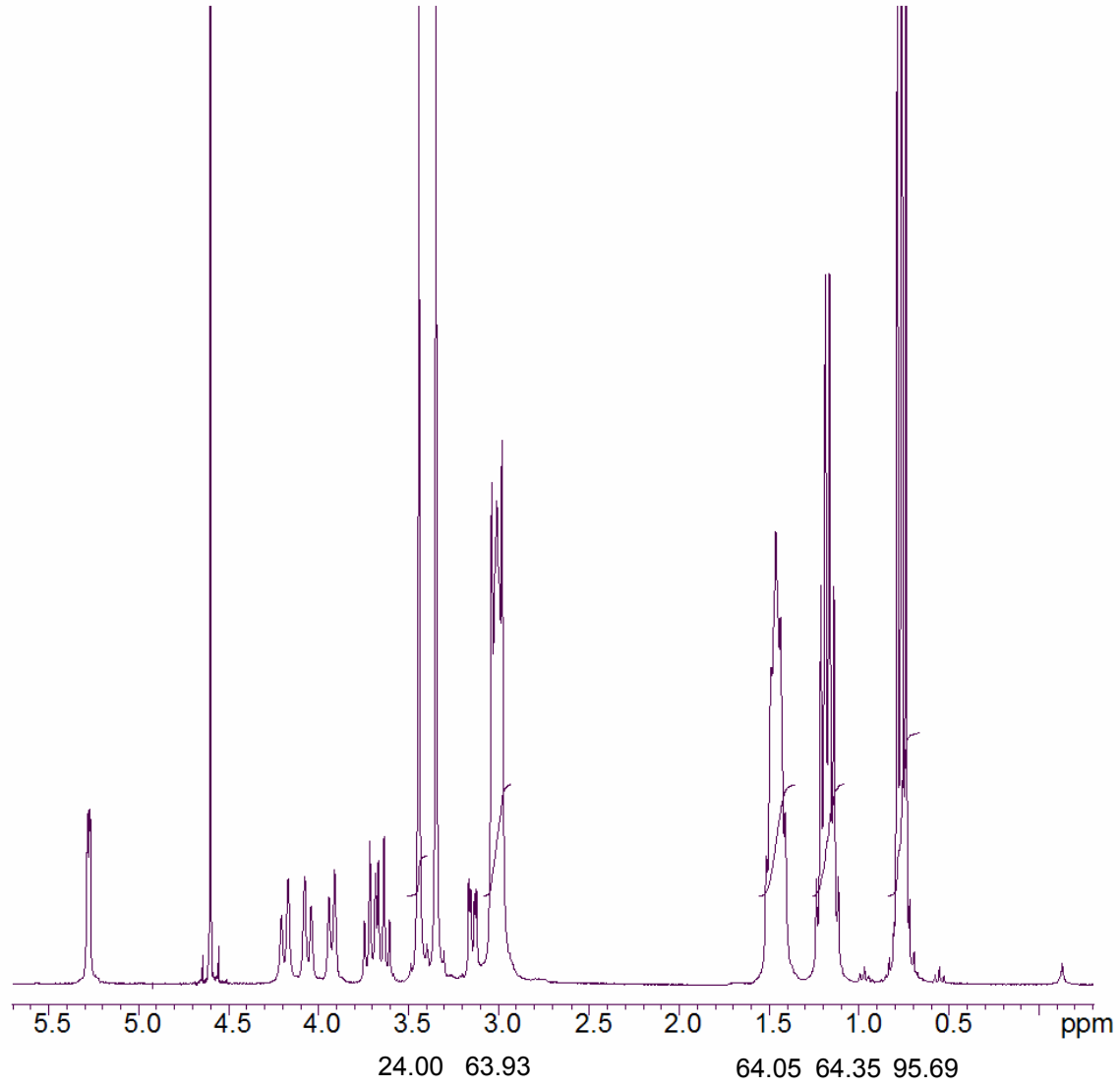


Figure II-12. ^{1}H NMR in D_2O of TBA_8ODMS (1g batch).

CHAPTER III

ENANTIOMER SEPARATIONS IN AQUEOUS CAPILLARY ELECTROPHORESIS WITH HEPTAKIS (2,3-DI-*O*-METHYL-6-*O*-SULFO)-β-CYCLODEXTRIN TETRABUTYLAMMONIUM SALT

3.1 Introduction

Separations of thirteen selected pharmaceuticals were achieved by CE using an aqueous BGE with TBA_7HDMS as the chiral resolving agent. These enantiomers can be classified as weak bases, neutral compounds and ampholytic compounds (Table III-1). As mentioned in Chapter I, according to the CHARM model, only a low pH and a high pH BGE are needed to obtain the best enantiomer separations. Since single-isomer sulfated CD derivatives have been shown to be very successful in the separation of cationic enantiomers, a low pH BGE was chosen for the analysis of these particular analytes. Therefore ampholytics, the zwitterions and the weak bases were in their cationic forms as protonated species. Consequently, they had cationic mobilities (positive μ^{eff}) at zero chiral resolving agent concentration. The concentration of the chiral resolving agent (TBA_7HDMS) was increased from 0mM to 50mM. The effect of the concentration of TBA_7HDMS on the effective mobility (μ^{eff}) of the enantiomer and the separation selectivity (α) were studied. It was observed that the

Table III-1. Enantiomers analyzed in aqueous BGE

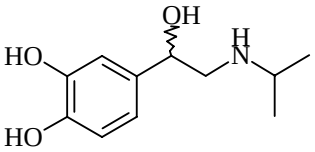
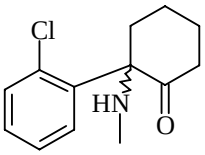
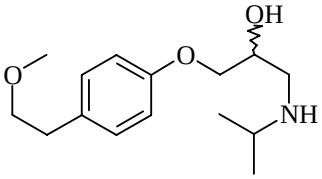
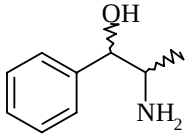
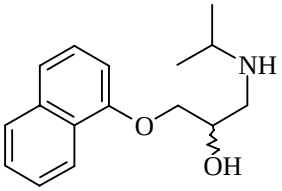
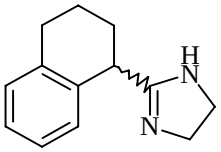
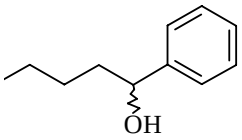
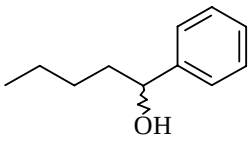
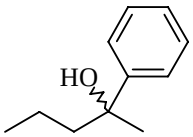
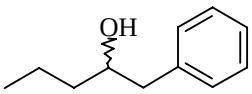
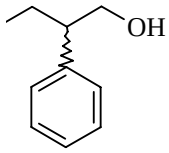
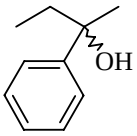
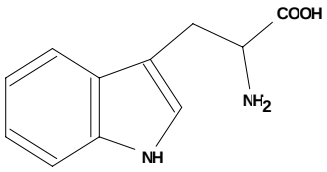
Identifier	Compound Name	Structure	Biological Activity
Bases			
B21	Isoproterenol		β-adrenergic agonist
B22	Ketamine		Anesthetic
B31	Metoprolol		β-blocker
B34	Norephedrine		Sympathetic nerve-stimulant
B42	Propranolol		β-blocker
B58	Tetrahydrozoline		Sympathomimetic agent with α-adrenergic activity

Table III-1. Continued.

Identifier	Compound Name	Structure	Biological Activity
Neutrals			
N21	1-Phenylbutanol		Percutaneous enhancer for transdermal drug delivery
N25	1-Phenylpentanol		Percutaneous enhancer for transdermal drug delivery
N26	2-Phenyl-2-pentanol		Percutaneous enhancer for transdermal drug delivery
N27	1-Phenyl-2-pentanol		Percutaneous enhancer for transdermal drug delivery
N28	2-Phenyl-1-propanol		Percutaneous enhancer for transdermal drug delivery
N38	2-Phenyl-2-butanol		Percutaneous enhancer for transdermal drug delivery
Ampholyte			
Tryp	Tryptophan		Neurotransmitter biosynthesis precursor

effective mobilities were decreasing as the concentration of the chiral resolving agent increased. This is expected since the viscosity and the ionic strength of the BGE increase as the concentration of TBA_7HDMS increases, suppressing the effective mobilities. In addition, the complexation constant between the analyte and the chiral resolving agent (which carries 7 negative charges) is increased as well making the enantiomer less cationic to the point that its migration becomes anionic in most of the cases. In order to find the best separation conditions, the effective mobilities and the separation selectivities were plotted as a function of the chiral resolving agent concentration.

3.2 Materials and methods

The pharmaceuticals used for the enantiomer separation were purchased from either Aldrich Chemical Co. (Milwaukee, WI), Sigma (St. Louis, MO), Research Diagnostics (Rockdale, MD) or Wiley Organics (Coshocton, OH). The aqueous BGE solutions were made using deionized water obtained from a Milli-Q unit (Millipore, Milford, MA). The buffer components were phosphoric acid (H_3PO_4), obtained from Aldrich Chemical Co. (Milwaukee, WI), and tetrabutyl ammonium hydroxide (TBAOH), obtained from Acros Organics, Pittsburgh, PA. The low pH BGE was prepared by titrating 25mM H_3PO_4 with TBAOH to pH 2.5. Dimethylsulfoxide (DMSO) was used as the EOF marker and it was obtained from EM Science (Gibbstown, NJ). The chiral resolving agent used for the enantiomer separation was TBA_7HDMS which was made in our

laboratories as explained in Chapter II. The stock buffer solution was used to prepare the seven different BGEs by varying the concentration of TBA_7HDMS from 0mM to 50mM. Before using the solutions, they were filtered with a 0.45μm Nalgene nylon membrane filter (VWR, South Plaintfield, NJ).

The enantiomer separations were performed using a P/ACE 2100 CE instrument indirect-UV detection mode at 214nm wavelength. The capillary used was a 26.4cm total length (19.6cm to detector) fused-silica capillary with 27μm internal diameter, thermostated at 20 ^{o}C. The pharmaceuticals as well as the EOF marker were dissolved in the BGE solution and were co-injected for 1 second by 5psi nitrogen gas from a solution where both were around 0.5mM in concentration. The potential used was chosen from the linear region of Ohm's plot. The electroosmotic flow mobility was calculated using equation 1 and the DMSO peak as the EOF peak. On the other hand, the effective mobilities of the enantiomers were calculated using equation 2. In addition, the three-band PreMCE method [70] was used when needed, providing the necessary information in a shorter analysis time.

3.3 Enantiomer separations in low pH aqueous BGE using TBA_7HDMS as the chiral resolving agent

The results for the separations of the thirteen pharmaceuticals are shown in Table III-2. This table shows the effective mobility (μ^{eff}, $10^{-5}cm^2/Vs$ units) of the slower enantiomer, the separation selectivity (α) which was calculated using

Table III-2 Enantiomer separations data in low pH aqueous BGE

Compound Name	5mM TBA_7HDMS (15kV)			
	μ^{eff}	α	β	Rs
Bases				
Isoproterenol (B21)	1.62	1.89	6.9	2.1
Ketamine (B22)	7.13	1.07	2.0	0.7
Metoprolol (B31)	-4.70	0.92	-2.9	0.5
Norephedrine (B34)	5.02	1.22	2.6	1.2
Propranolol (B42)	-6.53	0.85	-2.0	2.1
Tetrahydrozoline (B58)	4.69	1.04	2.8	0.3
Ampholyte				
Tryptophan	0.42	0.00	0.00	0.0
Neutrals				
1-Phenylbutanol (N21)	N/A	N/A	N/A	N/A
1-Phenylpentanol (N25)	N/A	N/A	N/A	N/A
2-Phenyl-2-pentanol (N26)	N/A	N/A	N/A	N/A
1-Phenyl-2-pentanol (N27)	N/A	N/A	N/A	N/A
2-Phenyl-1-propanol (N28)	N/A	N/A	N/A	N/A
2-Phenyl-2-butanol (N38)	N/A	N/A	N/A	N/A

Table III-2 Continued.

Compound Name	10mM TBA_7HDMS (21kV)			
	μ^{eff}	α	β	Rs
Bases				
Isoproterenol (B21)	-1.47	0.39	-3.2	2.6
Ketamine (B22)	7.67	1.08	0.6	2.1
Metoprolol (B31)	-7.60	0.96	-2.5	1.8
Norephedrine (B34)	1.31	2.24	3.6	3.4
Propranolol (B42)	-8.56	0.91	-2.3	4.7
Tetrahydrozoline (B58)	1.16	1.00	16.9	0.0
Ampholyte				
Tryptophan	-0.70	0.76	-11.7	1.5
Neutrals				
1-Phenylbutanol (N21)	-1.31	0.90	-7.5	0.5
1-Phenylpentanol (N25)	-0.94	1.00	-9.5	0.0
2-Phenyl-2-pentanol (N26)	-3.61	1.00	-3.1	0.0
1-Phenyl-2-pentanol (N27)	N/A	N/A	N/A	0.6
2-Phenyl-1-propanol (N28)	-0.55	1.00	-17.3	0.0
2-Phenyl-2-butanol (N38)	-0.36	1.00	-24.6	0.0

Table III-2 Continued.

Compound Name	15mM TBA_7HDMS (17kV)			
	μ^{eff}	α	β	Rs
Bases				
Isoproterenol (B21)	-3.44	0.73	-2.5	10.0
Ketamine (B22)	3.19	1.17	2.7	3.1
Metoprolol (B31)	-10.08	N/A	-1.0	N/A
Norephedrine (B34)	1.11	1.95	6.7	6.8
Propranolol (B42)	-7.06	N/A	-1.0	N/A
Tetrahydrozoline (B58)	1.02	1.13	7.2	1.0
Ampholyte				
Tryptophan	-2.21	0.92	-4.0	1.6
Neutrals				
1-Phenylbutanol (N21)	-0.72	0.71	-12.4	0.6
1-Phenylpentanol (N25)	-1.31	1.00	-6.7	0.0
2-Phenyl-2-pentanol (N26)	-0.49	1.00	-17.5	0.0
1-Phenyl-2-pentanol (N27)	-0.50	0.68	-17.4	0.8
2-Phenyl-1-propanol (N28)	-0.56	1.00	-15.9	0.0
2-Phenyl-2-butanol (N38)	-0.34	1.00	-24.7	0.0

Table III-2 Continued.

Compound Name	30mM TBA_7HDMS (13kV)			
	μ^{eff}	α	β	Rs
Bases				
Isoproterenol (B21)	-3.33	0.79	-3.4	5.3
Ketamine (B22)	1.89	1.24	3.7	3.8
Metoprolol (B31)	-7.89	0.97	-1.5	4.0
Norephedrine (B34)	0.11	9.25	6.2	8.5
Propranolol (B42)	-7.07	0.93	-1.6	7.8
Tetrahydrozoline (B58)	-1.01	0.90	-11.6	0.8
Ampholyte				
Tryptophan	-2.09	0.95	-5.4	0.9
Neutrals				
1-Phenylbutanol (N21)	-1.13	0.68	-10.3	1.4
1-Phenylpentanol (N25)	-2.78	0.82	-2.4	4.3
2-Phenyl-2-pentanol (N26)	-0.52	1.00	-21.8	0.0
1-Phenyl-2-pentanol (N27)	-0.83	0.74	-13.6	1.4
2-Phenyl-1-propanol (N28)	-0.90	1.00	-12.5	0.0
2-Phenyl-2-butanol (N38)	-0.52	1.00	-21.8	0.0

Table III-2 Continued.

Compound Name	40mM TBA_7HDMS (10kV)			
	μ^{eff}	α	β	Rs
Bases				
Isoproterenol (B21)	-3.14	0.79	-3.4	6.5
Ketamine (B22)	1.76	1.29	6.4	2.9
Metoprolol (B31)	-6.24	0.97	-1.8	2.8
Norephedrine (B34)	-0.82	1.00	-13.8	0.0
Propranolol (B42)	-5.54	0.93	-2.0	4.8
Tetrahydrozoline (B58)	-0.74	1.00	-15.2	0.0
Ampholyte				
Tryptophan	-1.86	0.95	-4.7	1.0
Neutrals				
1-Phenylbutanol (N21)	-0.99	0.70	-11.3	1.3
1-Phenylpentanol (N25)	-1.37	0.76	-8.2	0.7
2-Phenyl-2-pentanol (N26)	-0.57	1.00	-16.2	0.0
1-Phenyl-2-pentanol (N27)	-0.90	0.74	-10.4	1.5
2-Phenyl-1-propanol (N28)	-0.93	1.00	-10.1	0.0
2-Phenyl-2-butanol (N38)	-0.55	1.00	-17.3	0.0

Table III-2 Continued.

Compound Name	50mM TBA_7HDMS (10kV)			
	μ^{eff}	α	β	Rs
Bases				
Isoproterenol (B21)	-2.60	0.79	-1.9	10.2
Ketamine (B22)	1.17	1.31	4.2	4.2
Metoprolol (B31)	-2.85	0.97	-1.6	3.5
Norephedrine (B34)	-0.98	0.34	-5.0	9.5
Propranolol (B42)	-2.10	0.94	-1.7	4.3
Tetrahydrozoline (B58)	-0.82	1.00	-5.9	0.0
Ampholyte				
Tryptophan	-1.49	0.95	-5.2	0.6
Neutrals				
1-Phenylbutanol (N21)	-1.20	0.75	-3.8	2.8
1-Phenylpentanol (N25)	-1.66	0.81	-2.8	2.0
2-Phenyl-2-pentanol (N26)	-0.56	0.85	-13.8	0.7
1-Phenyl-2-pentanol (N27)	-0.94	0.76	-8.3	2.0
2-Phenyl-1-propanol (N28)	-1.02	0.96	-3.4	0.6
2-Phenyl-2-butanol (N38)	-0.68	0.95	-5.1	0.5

equation 3, the normalized electroosmotic flow (β) which was calculated using equation 4 and the resolution (Rs) which was calculated by dividing the difference in peak times in seconds (Δt) with the average of the half height peak width of the two enantiomers multiplied by 1.699 ($1.699(w^{R}_{1/2}+ w^{S}_{1/2})/2$) at seven different TBA_7HDMS concentrations (0, 5, 10, 15, 30, 40 and 50mM) with its respective applied voltage. If the enantiomer was not analyzed at a particular concentration or a value could not be calculated accurately, an entry of N/A was used.

Since all the BGEs that were prepared had a pH of 2.5, the six neutral enantiomers behaved as neutrals and the six weak bases as well as the ampholytic compound behaved as cations at zero TBA_7HDMS concentration. At the conditions specified in section 3.1, all thirteen chiral drugs showed some separation selectivity. The best results were obtained for the cationic analytes compared to the neutral one. It was expected, since previous work has shown that negatively charged single-isomer sulfated β-CD derivatives work better for the positively charged analytes than the neutral or negatively charged compounds.

The weak bases and the ampholytic compound had positive effective mobilities (migrated toward the cathode) at 0mM TBA_7HDMS. As the concentration of the chiral resolving agent was increased, the effective mobility decreased to a value of zero mobility units. Moreover, further increases in the TBA_7HDMS concentration resulted in an anionic mobility (migration toward the

anode) of the enantiomer. This may be due to different factors associated with the increasing TBA_7HDMS concentration such as: i) the ionic strength increases causing a decrease in the effective mobilities of the analytes; ii) the viscosity of the BGE, which is inversely proportional to the ionic mobility, increases almost twice in the 0mM to 50mM range (Figure III-1 and III-2); and iii) complexation between the chiral resolving agent and the chiral compound increases making the cationic compound to behave more as anionic. On the other hand, in the case of the neutral compounds, they migrated toward the cathode with the electroosmotic flow since their effective mobilities are zero in the presence of an electric field. As soon as complexation occurred between TBA_7HDMS and the enantiomer, the analyte migrated toward the anode ($\mu^{eff}<0$) since the new diastereomer that was formed was negatively charged.

Moreover, the data revealed that there are three different separation behaviors that can be observed when separating weak bases and ampholytics. The first one is that of the weakly binding analytes which are enantiomers whose migration never becomes anionic over the entire TBA_7HDMS concentration range used. It is the case of the weak base ketamine (B22) (Figure III-3). While the effective mobility of ketamine decreases approaching a value of zero, the separation selectivity increases to a non-limiting value as the concentration of TBA_7HDMS increases. The second type is moderately strongly binding analytes that are enantiomers that migrate as cations at low chiral resolving agent concentrations and migrate as anions at middle to high TBA_7HDMS

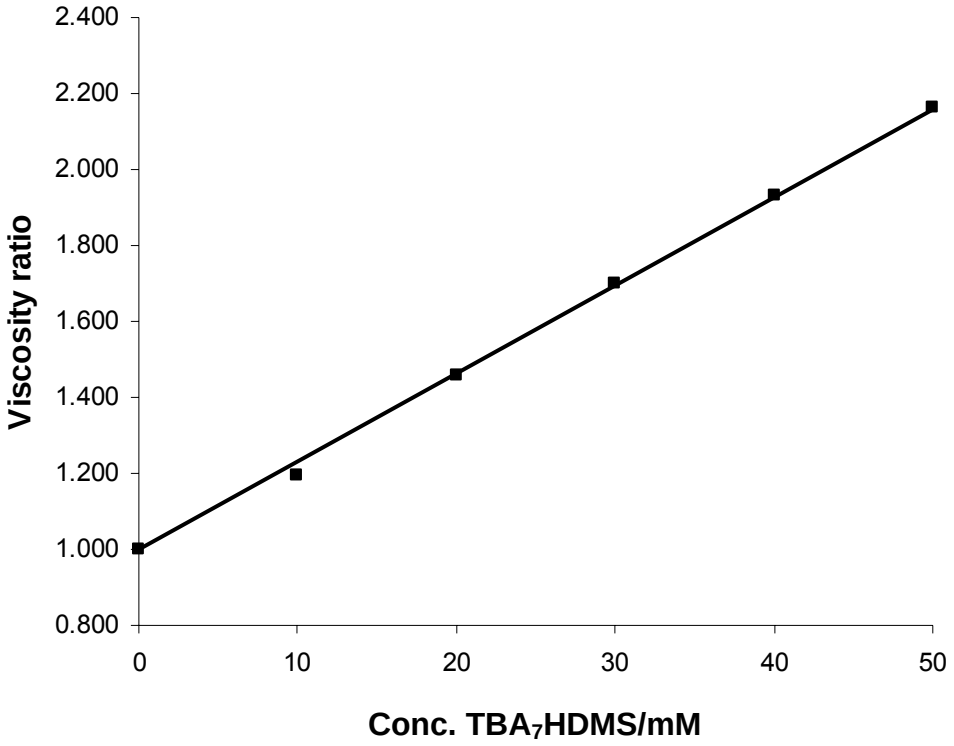


Figure III-1 Contribution of the TBA_7HDMS concentration to the viscosity of the BGE.

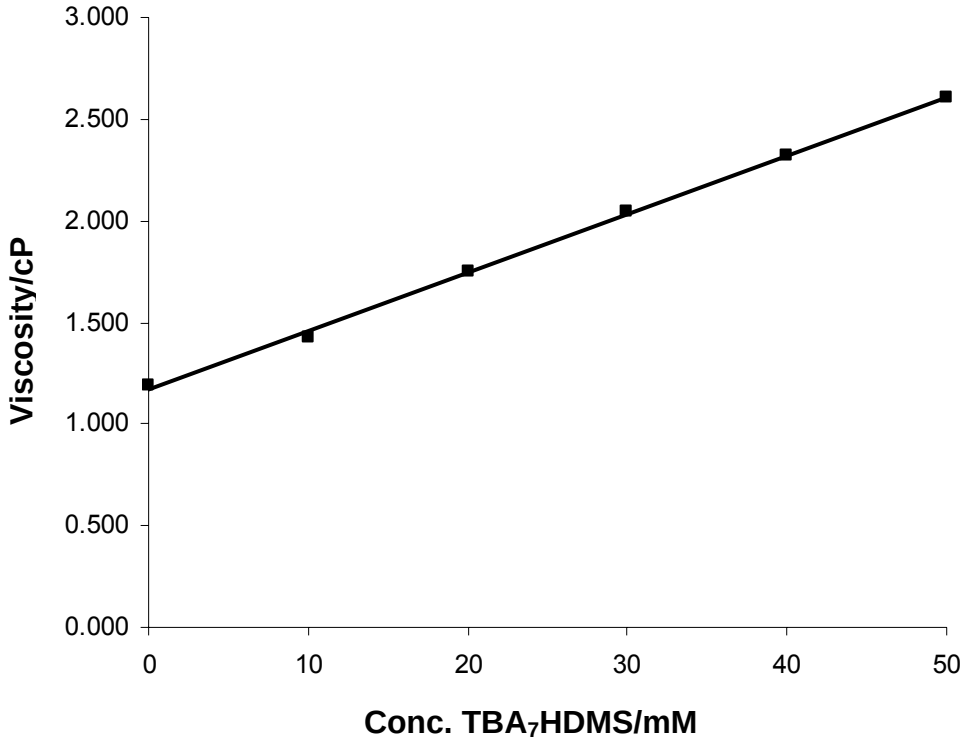


Figure III-2. Absolute viscosity of the BGEs at different TBA_7HDMS concentrations.

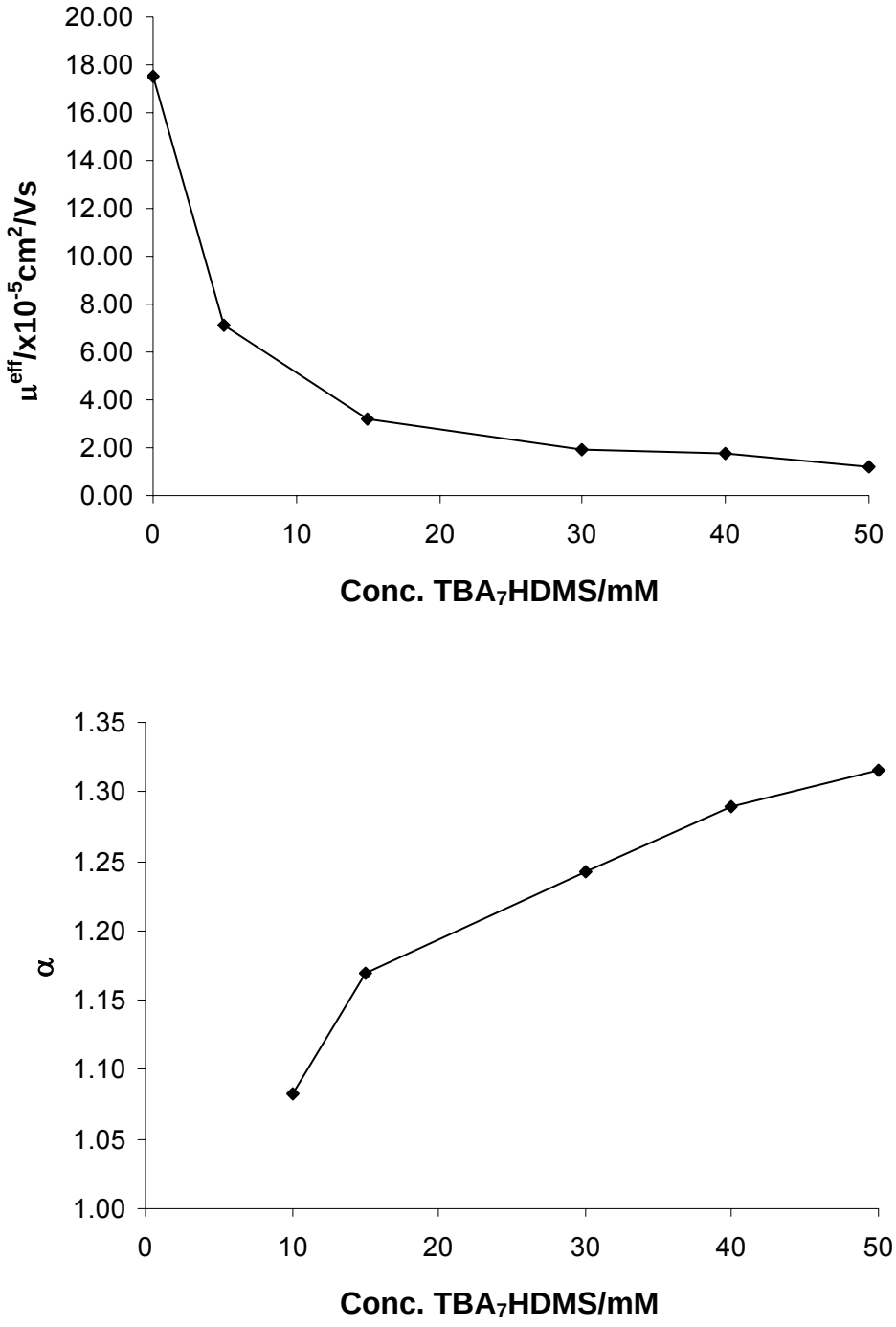


Figure III-3. Effective mobility (top panel) and separation selectivity plots (bottom panel) for the weakly binding analyte ketamine (B22) in low pH aqueous BGE.

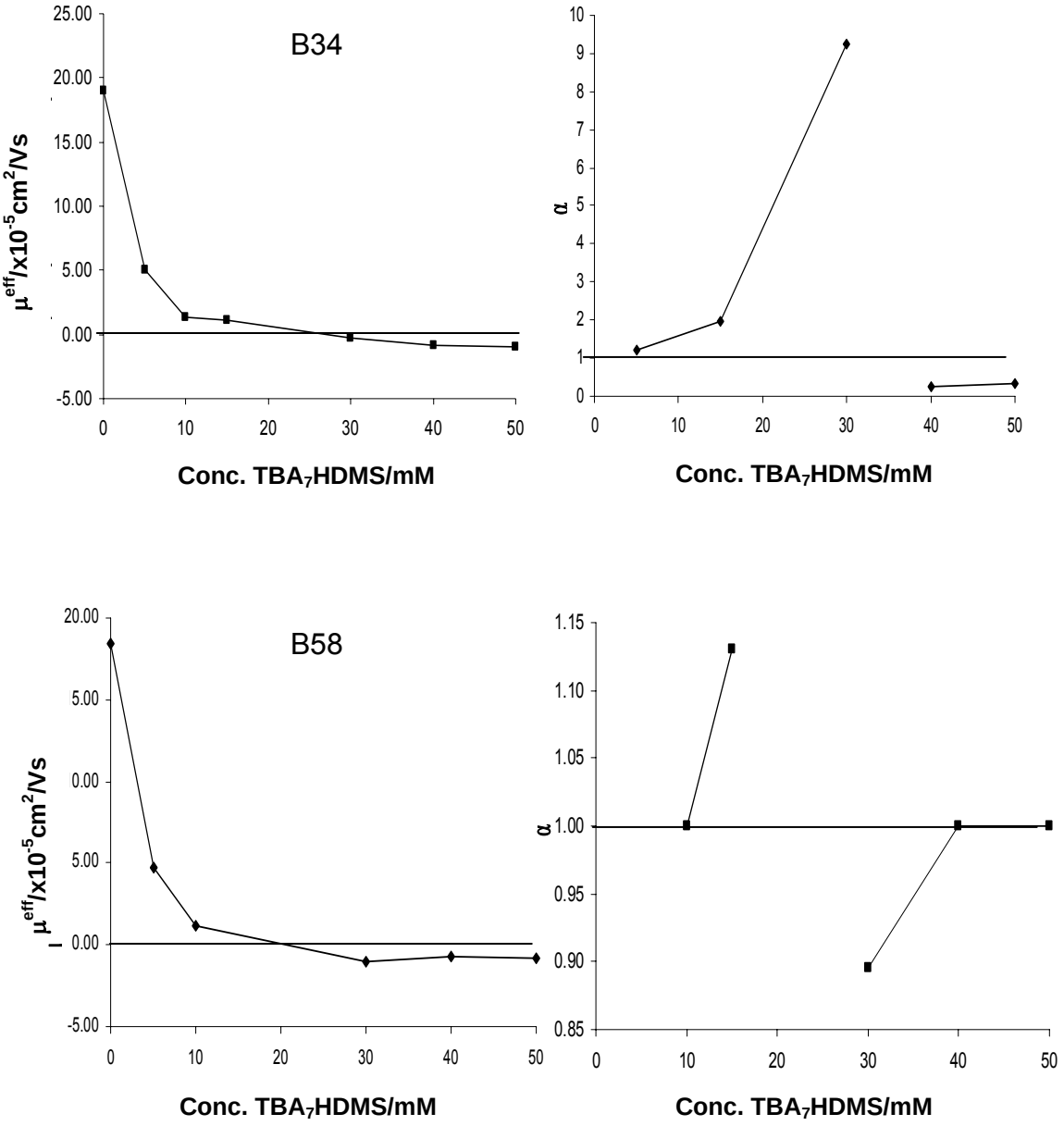


Figure III-4. Effective mobility (left panels) and separation selectivity plots (right panels) for the moderately strongly binding analytes B34and B58 in low pH aqueous BGE.

concentrations. This is the case for norephedrine (B34) and tetrahydrozoline (B58) (Figure III-4). These enantiomers initially migrate as cations and become anionic at the 20-25mM TBA_7HDMS concentrations. As a result, their separation selectivities increase to an infinitely high value and show a discontinuity at a concentration where the enantiomers migrate in the opposite directions. After that point, the separation selectivities increase from negative infinite value become positive again and approach unity. The third type of separation is that of the strongly binding analytes which are enantiomers that complex with the chiral resolving agent so strongly that they become anionic even at a very low TBA_7HDMS concentration. This is the case for three weak bases: isoproterenol (B21), metoprolol (B31) and propranolol (B42) and an ampholytic compound, tryptophan (Tryp) (Figure III-5). These enantiomers became anionic at a concentration around 5mM and remain anionic through the entire TBA_7HDMS concentration range. Only isoproterenol (B21) and tryptophan (Tryp) had a cationic mobility at 5mM which translates to a separation selectivity equal or higher than one at that particular TBA_7HDMS concentration or below. After that point, in the separation selectivity pattern showed a discontinuity right where the enantiomers started migrating to the opposite direction and then the separation selectivity increased from negative infinite to unity. On the other hand, metoprolol (B31) and propranolol (B42) did not have cationic mobility through the entire TBA_7HDMS concentration range,

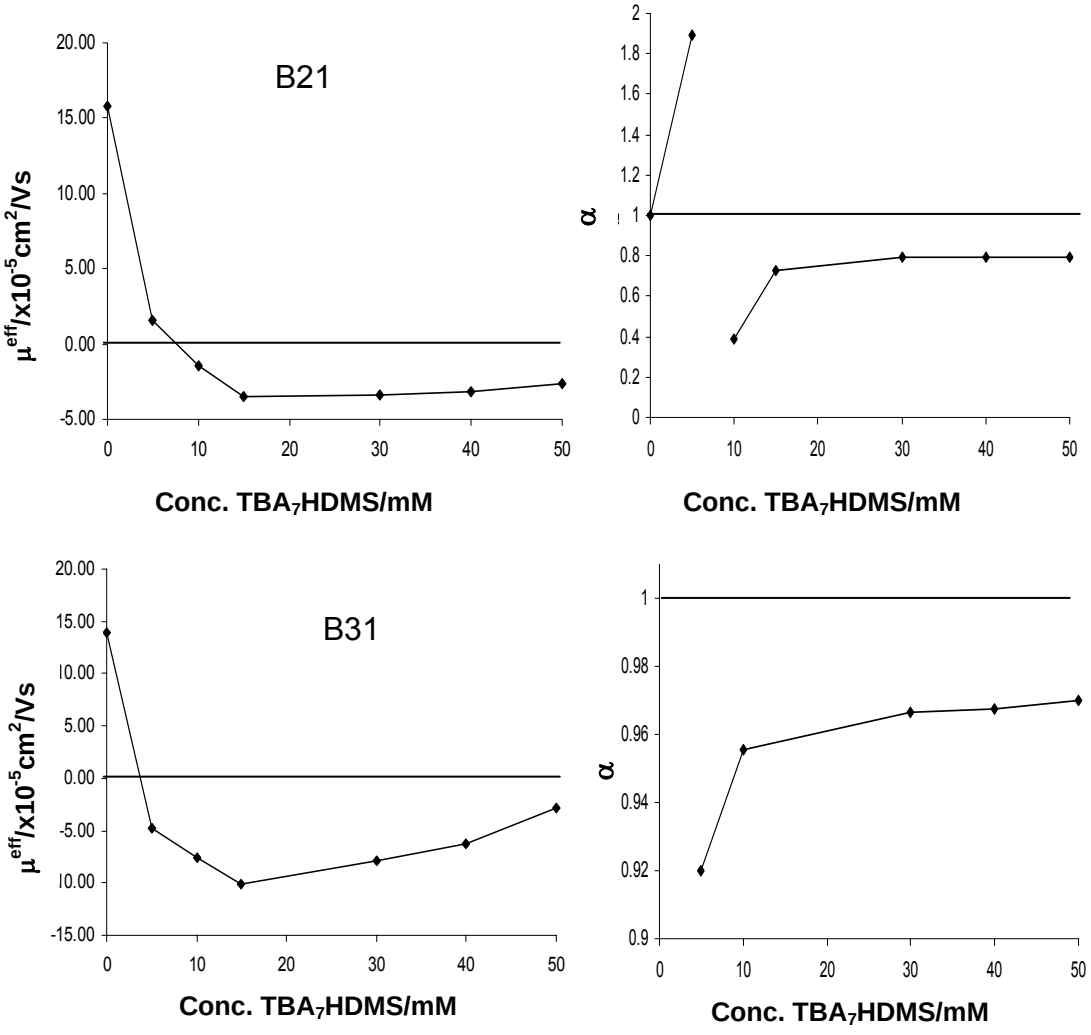


Figure III-5. Effective mobility (left panels) and separation selectivity plots (right panels) for the strongly binding analytes B21, B31, B42 and Tryp in low pH aqueous BGE.

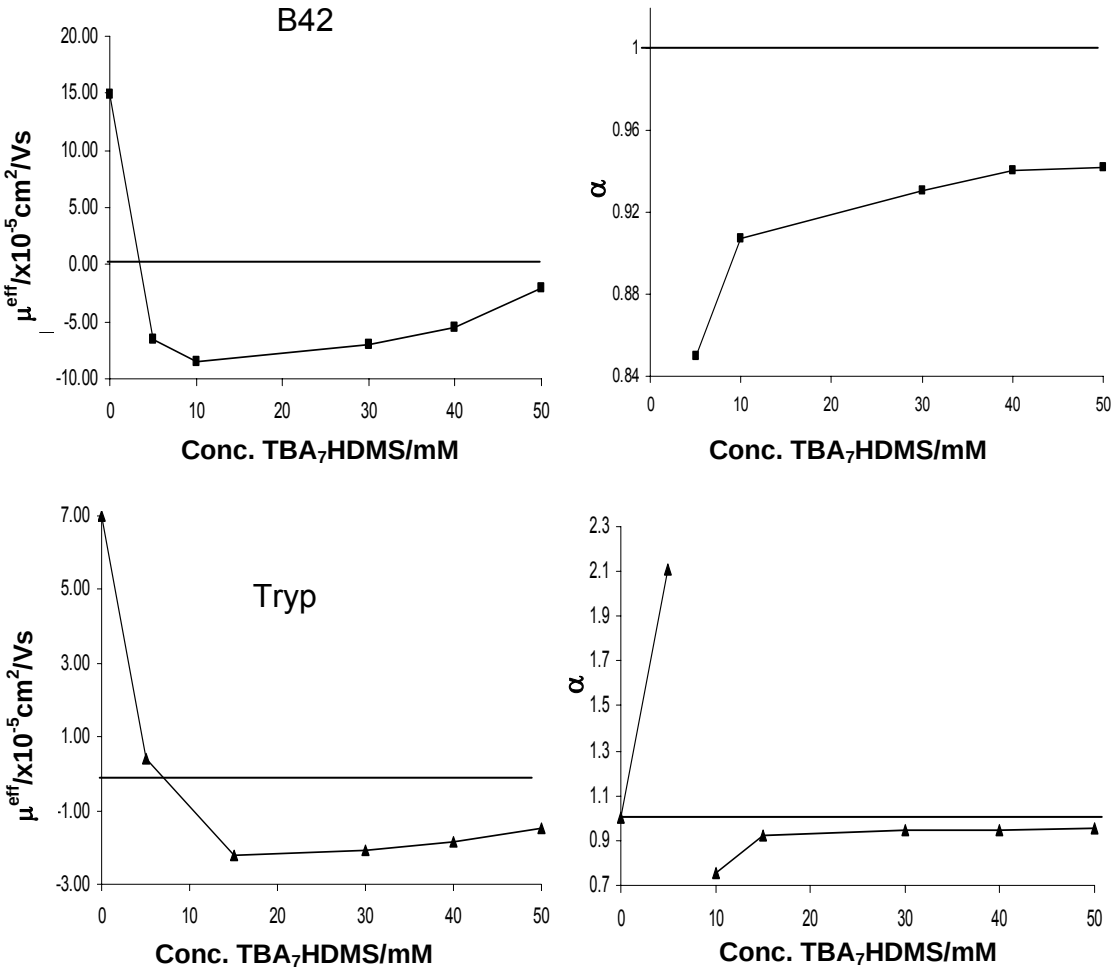


Figure III-5. Continued.

therefore their separation selectivity did not show discontinuity and increased from negative infinity to unity.

In the case of neutral enantiomers, the separation selectivity pattern is completely different. Based on the CHARM model [21], separation selectivity of non-charged analytes can be described as follow,

$$\alpha = \frac{\mu^{o}_{RCD} K_{RCD}}{\mu^{o}_{SCD} K_{SCD}} \cdot \frac{1 + K_{SCD}[CD]}{1 + K_{RCD}[CD]} \qquad (6)$$

where μ^{0}_{RCD} and μ^{0}_{SCD} are the ionic mobilities and K_{RCD} and K_{SCD} are the complexation constants of the enantiomers. According to the CHARM model, if the concentration of the free enantiomer is much smaller than the concentration of the complexed enantiomer, separation selectivity decreases as the concentration of the free charged cyclodextrin [CD] increases. This can be observed in Figure III-6, where separation selectivity for 1-phenylbutanol (N25) and 1-phenylpentanol (N26) decreases as TBA_7HDMS concentration increases.

Electropherograms of the separations of the thirteen enantiomers are shown in Figure III-7 and Figure III-8. Resolutions greater than 1.5 (baseline separation) were obtained for nine of the thirteen enantiomers and the other four were only partially separated. Each electropherogram shows the structure of the analyte, the applied potential (kV) and the TBA_7HDMS concentration used for that separation. In some cases the EOF marker (DMSO) appears in the electropherogram as well. In the case of the weak bases and the ampholytic

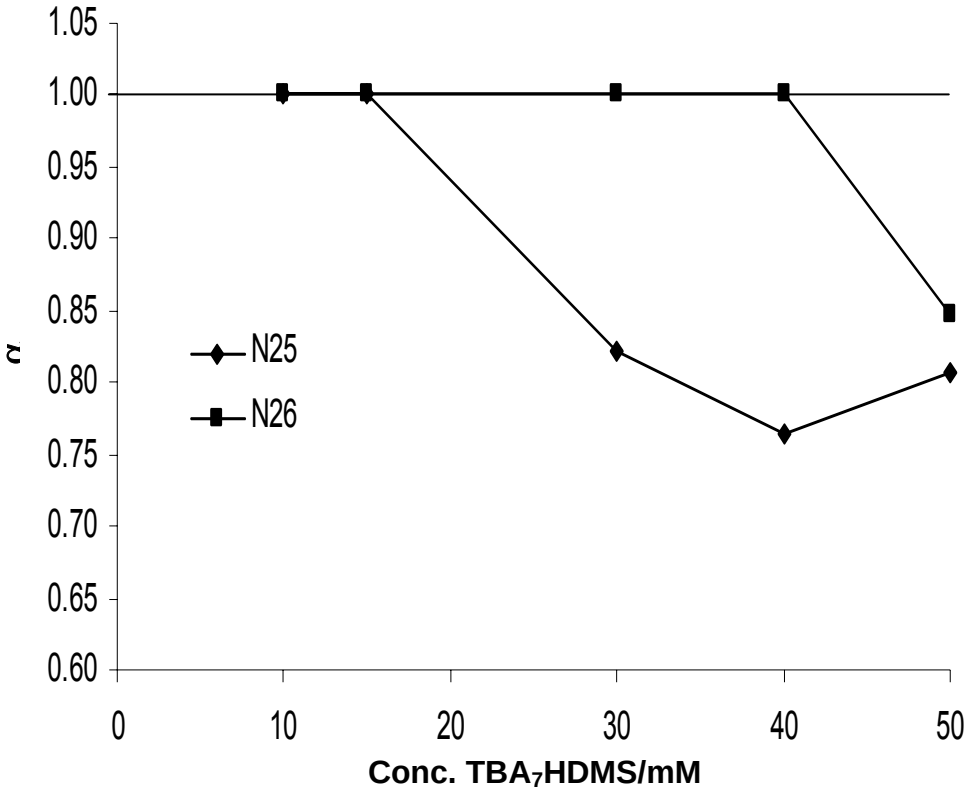


Figure III-6. Separation selectivity plots of N25 and N26 using TBA_7HDMS as the chiral resolving agent in low pH aqueous BGE.

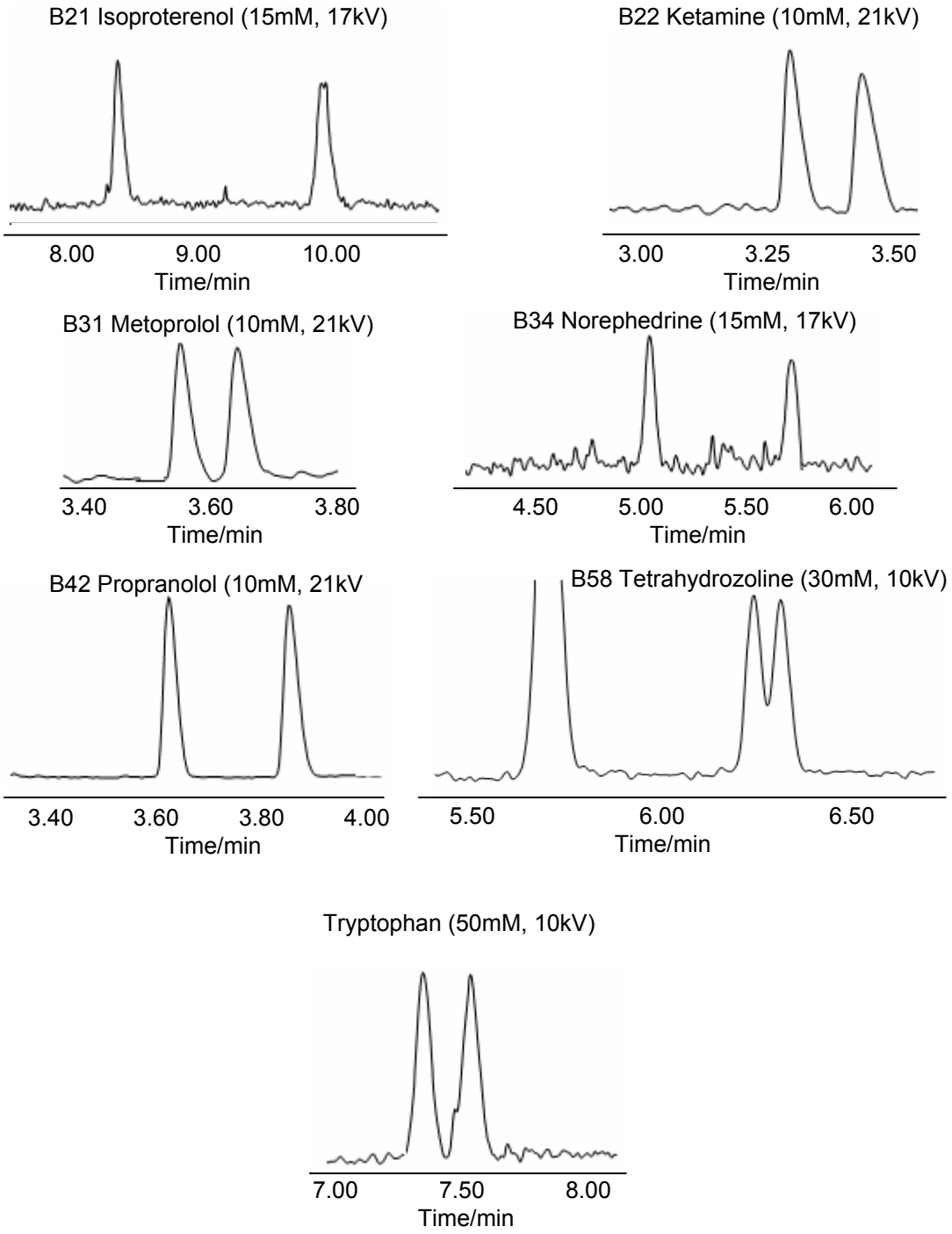


Figure III-7. Electropherograms of weak basic and ampholytic enantiomers at low pH aqueous BGE using TBA_7HDMS as the chiral resolving agent.

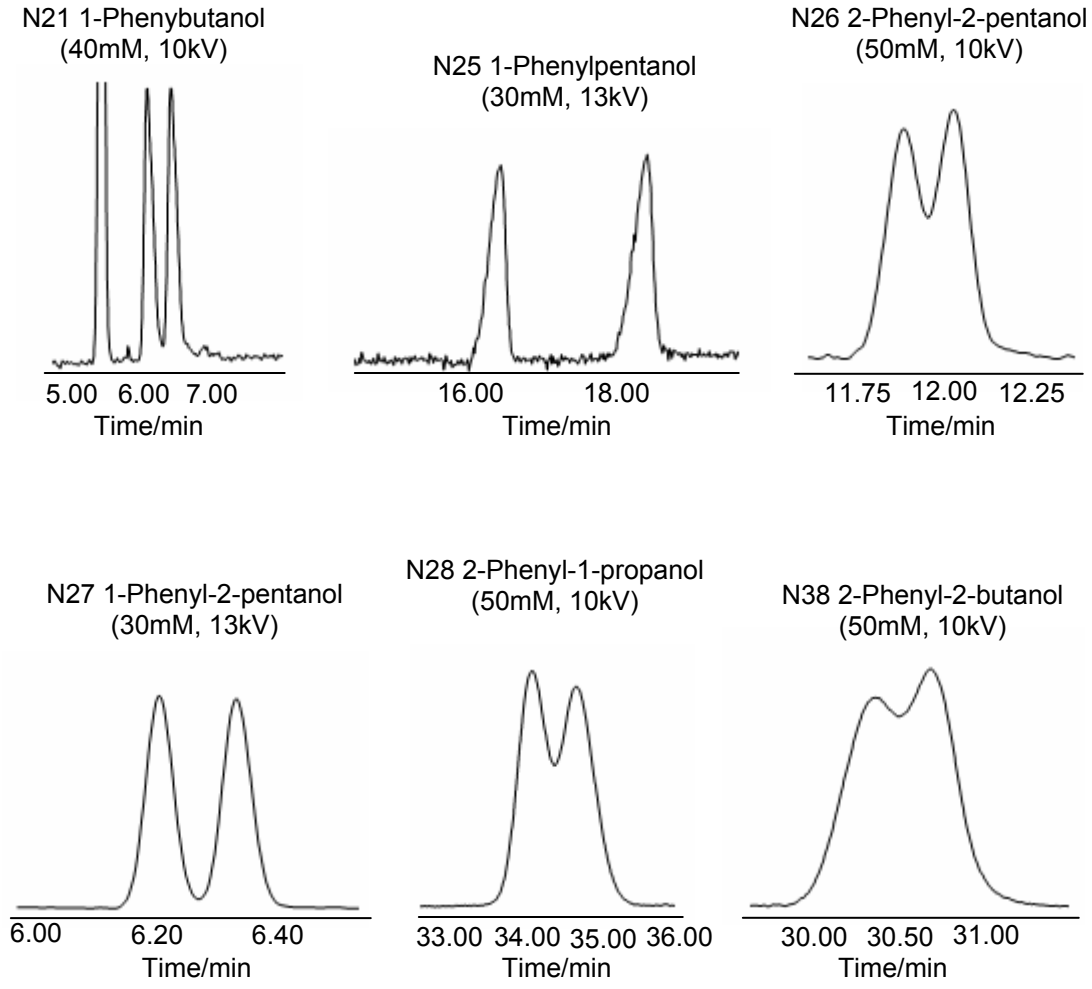


Figure III-8. Electropherograms of neutral enantiomers at low pH aqueous BGE using TBA_7HDMS as the chiral resolving agent.

compound, the run time was in the range of three to six minutes with the shortest run time at three and a half minutes for the weakly binding ketamine (B22) and the longest run time at six minutes for the moderately binding tetrahydrozoline (B58).

On the other hand, the separation time for the neutral enantiomers was in the range of six to thirty one minutes with the shortest run time at six minutes for 1-phenylbutanol (N21) and the longest run time at thirty one minutes for 2-phenyl-2-butanol (N38).

3.4 Comparison of enantiomer separations using TBA_7HDMS and Na_7HDMS as the chiral resolving agent in low pH aqueous BGEs

All the thirteen enantiomers separated using TBA_7HDMS were previously separated using Na_7HDMS under the same conditions. Although, the term same conditions refer to the same buffer concentration, same pH, same ionic strength and same temperature, the contributions of the two cyclodextrins to the viscosity of the BGE were different. The viscosity of the aqueous BGE for TBA_7HDMS was twice higher than the viscosity of the aqueous BGE with Na_7HDMS (Figure III-9 and III-10). Therefore, all analytes experienced a different environment (media) even though both were in aqueous BGEs. Figure III-9 and Figure III-10 show the contributions of the chiral resolving agent to the viscosity of the BGE and the absolute viscosity of the BGE for both chiral resolving agents, respectively.

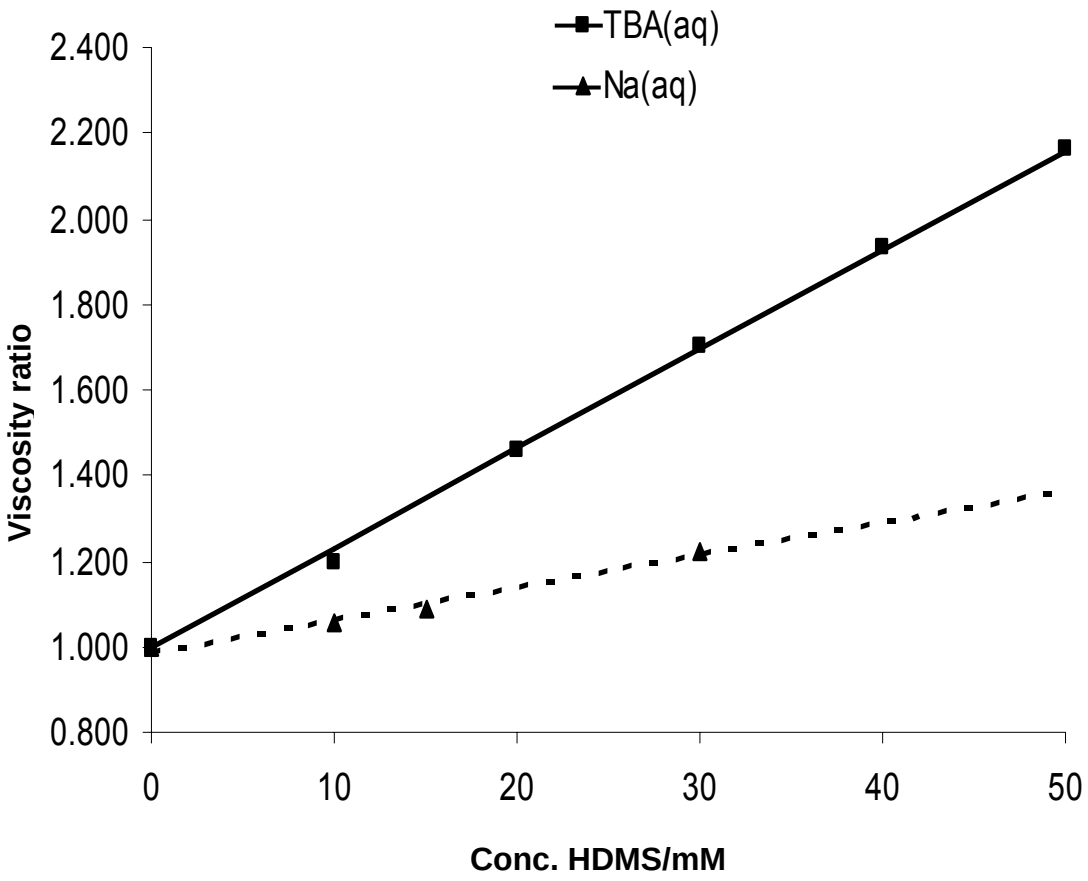


Figure III-9. Comparison between the contribution of TBA_7HDMS and Na_7HDMS concentration to the viscosity of the BGE.

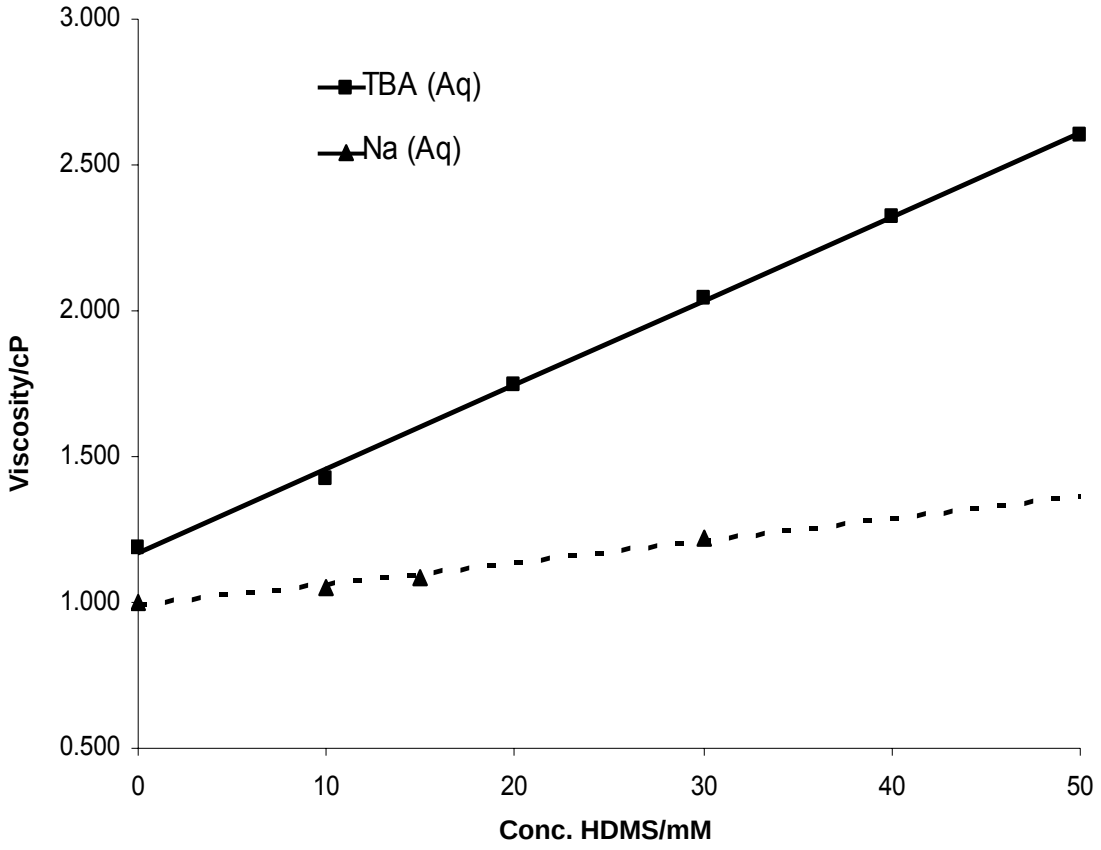


Figure III-10. Comparison of the absolute viscosity of the BGEs at different TBA_7HDMS and Na_7HDMS concentrations.

Based on the higher viscosity of the TBA_7HDMS BGE, the effective mobilities of the enantiomers are expected to be lower ($\mu^{eff} \alpha 1/\eta$). This is the case for the separations of neutral enantiomers; however the effective mobilities of the weak bases were slightly higher in the TBA_7HDMS BGEs than in Na_7HDMS BGEs (Figure III-11 and III-12). Figure III-11 shows the effective mobilities and the separation selectivities of some of the neutral enantiomers using TBA_7HDMS and Na_7HDMS as the chiral resolving agent under the same conditions. Figure III-12 shows the effective mobilities and the separation selectivities of the ampholytic compound and some of the weak base enantiomers using TBA_7HDMS and Na_7HDMS as the chiral resolving agent under the same conditions. Something interesting can be noticed in Figure III-12 and it is the change in binding strength only by changing the counter ion of $HDMS^{-7}$. For example, isoproterenol (B21) was a weakly binding analyte with Na_7HDMS, but it became strongly binding analyte with TBA_7HDMS. In addition, tryptophan (Tryp) which was a moderately binding analyte with Na_7HDMS, became a strongly binding analyte with TBA_7HDMS. Another two enantiomers, norephedrine (B34) and tetrahydrozoline (B58), were weakly binding analytes with Na_7HDMS and became moderately binding analyte with respect to TBA_7HDMS. In general, a trend of increasing binding strength can be observed for the seven cationic compounds when a more hydrophobic counter ion is used for in the chiral resolving agent. The opposite was observed for the case of the neutral enantiomers.

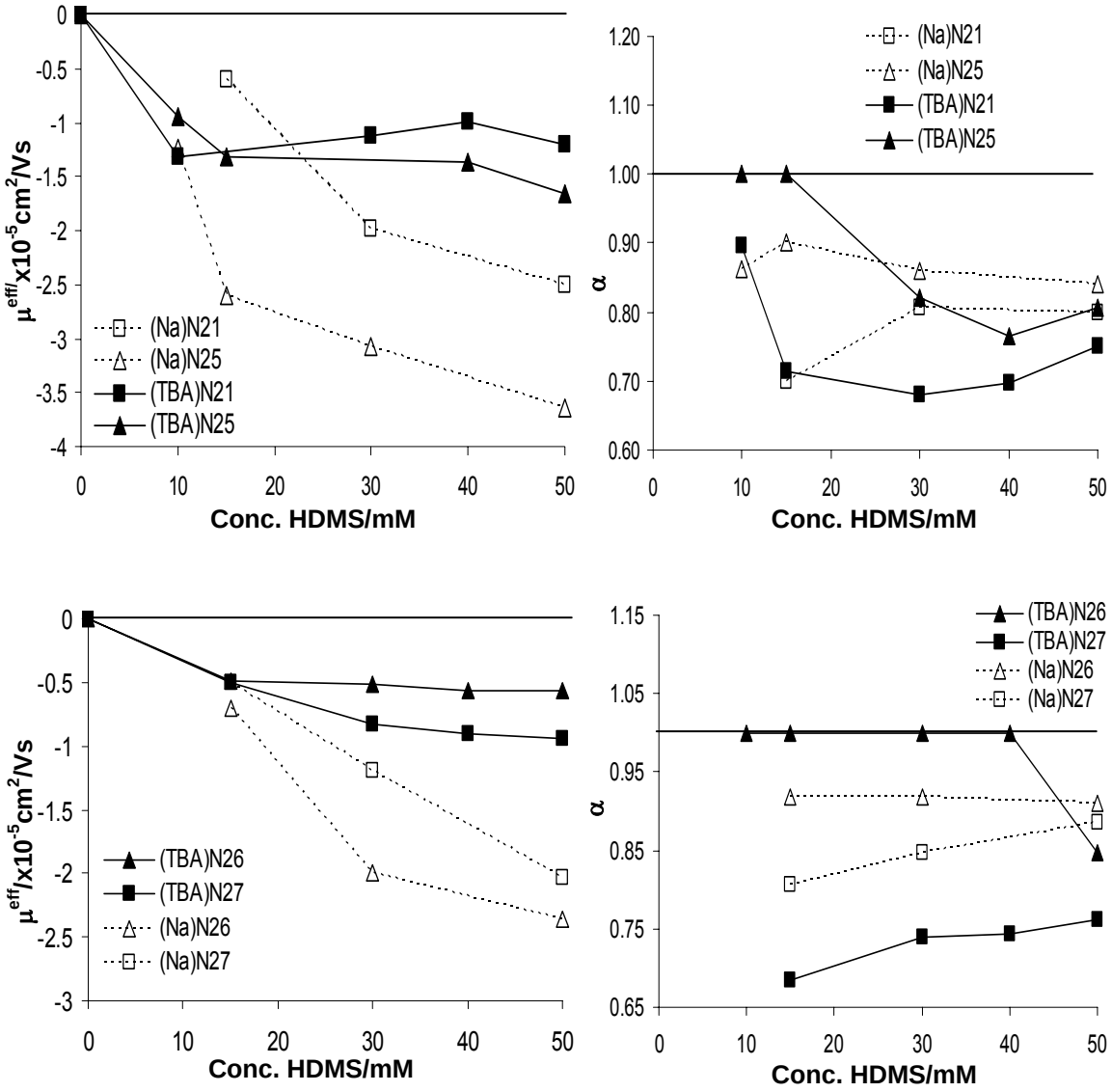


Figure III-11. Comparison of the mobility (left panel) and separation selectivity plots (right panel) for N21, (N25), (N26) and (N27) when TBA_7HDMS and Na_7HDMS were used in low pH aqueous BGE.

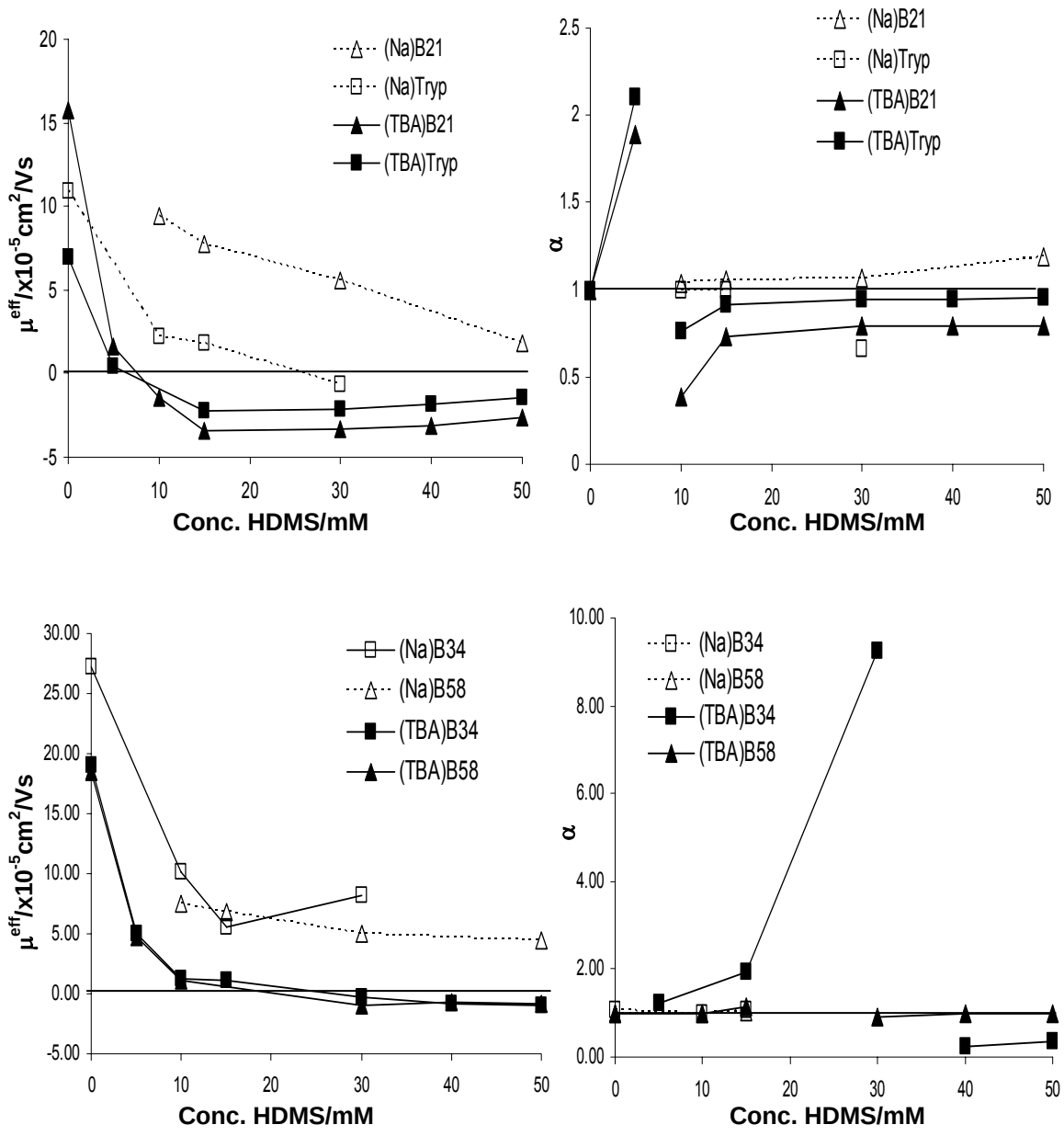


Figure III-12. Comparison of the mobility (left panel) and separation selectivity plots (right panel) for isoproterenol (B21), tryptophan (Tryp), norephedrine (B34) and tetrahydrozoline (B54) when TBA_7HDMS and Na_7HDMS were used in low pH aqueous BGE.

CHAPTER IV

ENANTIOMER SEPARATIONS IN NON-AQUEOUS CAPILLARY ELECTROPHORESIS WITH HEPTAKIS (2,3-DI-*O*-METHYL-6-*O*-SULFO)-β-CYCLODEXTRIN TETRABUTYLAMMONIUM SALT

4.1 Introduction

In CE, the solvent used in the BGE plays a very important role in the separation of enantiomers. Different solvents may promote different intermolecular interactions that may enhance separation selectivity for the enantiomers. The interest in using solvents other than water has been growing in the past few years. Non-aqueous solvents provide a low conductivity which allows the use of higher potential for a faster separation and higher resolution. It also helps in the solubility of the water-insoluble compounds.

Since Na_7HDMS was successfully used for the separation of enantiomers in acidic methanolic BGE, the same conditions were used as well with TBA_7HDMS in order to be able to make comparisons. Twenty pharmaceuticals (Table IV-1) were chosen which include nineteen weak bases and an ampholytic compound. Due to protonation in the acidic methanolic BGE, these enantiomers were positively charged at zero chiral resolving agent concentration. Their effective mobilities and separation selectivities as a function of TBA_7HDMS concentration were studied.

Table IV-1 Enantiomers analyzed in non-aqueous BGE.

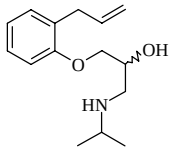
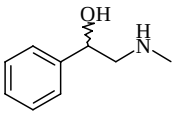
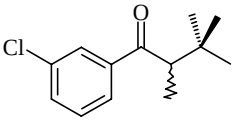
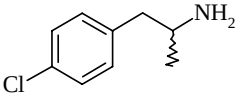
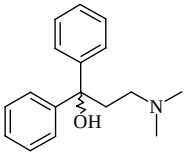
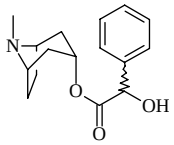
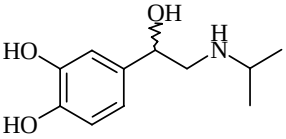
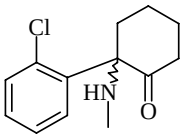
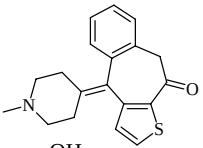
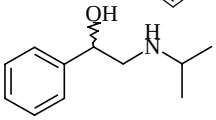
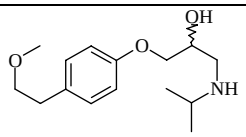
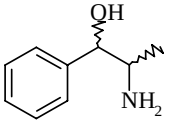
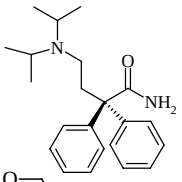
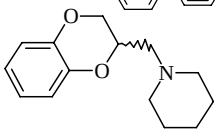
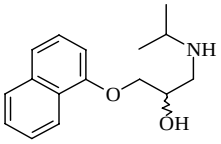
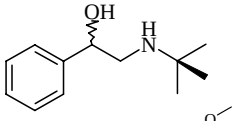
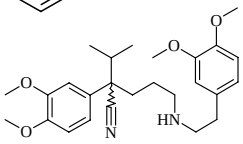
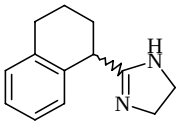
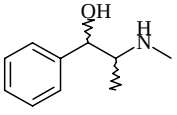
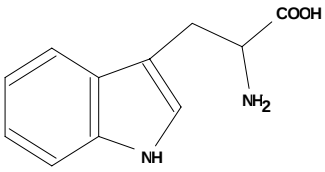
Identifier	Compound Name	Structure	Biological Activity
Bases			
B02	Alprenolol		β-blocker
B04	Halostachine		β-adrenergic agonist
B11	Bupropion		Antidepressant
B13	4-Chloroamphetamine		Brain serotonin depletion
B14	Chlophedianol		Antitussive
B19	Homatropine		Anti-muscarinic
B21	Isoproterenol		β-adrenergic agonist
B22	Ketamine		Anesthetic
B23	Ketotifen		Antihistamine
B30	Metaproterenol		β-adrenergic agonist

Table IV-1. Continued.

Identifier	Compound Name	Structure	Biological Activity
Bases			
B31	Metoprolol		β-blocker
B34	Norephedrine		Sympathetic nerve-stimulant
B36	Oxyphencyclimine		Anticholinergic
B38	Piperoxan		α-blocker
B42	Propranolol		β-blocker
B47	Terbutaline		β-adrenergic agonist
B54	Verapamil		Antihypertensive
B58	Tetrahydrozoline		Sympathomimetic agent with α-adrenergic activity
B60	Ephedrine		α&β-adrenergic agonist
Ampholyte			
Tryp	Tryptophan		Neurotransmitter biosynthesis precursor

4.2 Materials and methods

All pharmaceuticals were purchased from either Aldrich Chemical Co. (Milwaukee, WI), Sigma (St. Louis, MO), Research Diagnostics (Rockdale, MD) or Wiley Organics (Coshocton, OH). The acidic methanolic buffer was made by preparing a solution of 25mM H_3PO_4 and 12.5mM TBAOH using HPLC grade methanol (EM Science (Gibbstown, NJ)) as the solvent. The stock buffer solution was used to prepare eight different BGEs containing 0mM, 0.25mM, 0.5mM, 1mM, 5mM, 10mM, 25mM and 40mM of TBA_7HDMS. The chiral resolving agent, TBA_7HDMS was synthesized as explained in Chapter II. All the solutions were filtered with a .45μm Nalgene nylon membrane filter prior to use. The enantiomer separations were performed using the same system and methodology as explained in Chapter III, with the same capillary specification. For the calculation of the electroosmotic flow mobility values (μ^{eo}), DMSO was used as an EOF marker and naphthalenesulfonic acid (NSA) (Aldrich Chemical Co.) was used as an anionic mobility marker, when needed. The effective mobility of NSA was obtained using the three-band PreMCE method [70]. Ohm's plot was used to choose the highest possible potential to be used which is within the linear region. The effective mobility (μ^{eff}), separation selectivity (α), normalized electroosmotic flow (β) and resolution (Rs) values were calculated for each enantiomer as explained in Chapter III.

4.3 Enantiomer separations in acidic methanolic BGE using TBA_7HDMS as chiral resolving agent

The results for the separations of the twenty pharmaceuticals are shown in Table IV-2. This table shows the effective mobility (μ^{eff}, $10^{-5}cm^2/Vs$) of the slower enantiomer, the separation selectivity (α), the normalized electroosmotic flow (β) and the resolution (Rs) values for each enantiomer at each concentration with its respective applied potential. If the enantiomer was not analyzed at a particular concentration or a value could not be calculated accurately, an entry of N/A was used.

All the enantiomers showed some separation selectivity with the exception of two; homatropine (B19) and isoproterenol (B21). Nine of the eighteen enantiomers that were separated had baseline separation (Rs>1.5). Typical electropherograms of some of the enantiomers that were separated are shown in Figure IV-1. This figure shows the molecular structure of the enantiomer with its identifier, the concentration of TBA_7HDMS and the potential applied (kV) for that particular electropherogram. In some cases the EOF marker (DMSO) or the anionic mobility marker (NSA) appear in the electropherograms as well. The shortest separation time achieved was one minute and a half for the strongly binding ketamine (B22) which occurred at 0.25mM TBA_7HDMS and 25kV applied voltage. As the concentration of the chiral resolving agent is increased, the ionic strength also increases which causes a higher conductivity producing more Joule heating. To avoid this,

Table IV-2. Enantiomer separation data in acidic methanolic BGE

Compound Name	0.25mM HDMSTBA (25kV)			
	μ^{eff}	α	β	Rs
Bases				
Alprenolol (B02)	N/A	N/A	N/A	N/A
Halostachine (B04)	N/A	N/A	N/A	N/A
Bupropion (B11)	N/A	N/A	N/A	N/A
4-Chloroamphetamine (B13)	3.66	1.00	1.9	0.0
Chlophedianol (B14)	N/A	N/A	N/A	N/A
Homatropine (B19)	2.89	1.00	2.6	0.0
Isoproterenol (B21)	N/A	N/A	N/A	N/A
Ketamine (B22)	15.04	1.03	0.4	0.4
Ketotifen (B23)	-3.37	0.94	-1.9	0.5
Metaproterenol (B30)	N/A	N/A	N/A	N/A
Metoprolol (B31)	N/A	N/A	N/A	N/A
Norephedrine (B34)	N/A	N/A	N/A	N/A
Oxyphencyclimine (B36)	N/A	N/A	N/A	N/A
Piperoxan (B38)	6.49	1.00	0.9	0.0
Propranolol (B42)	N/A	N/A	N/A	N/A
Terbutaline (B47)	N/A	N/A	N/A	N/A
Verapamil (B54)	0.17	2.15	29.8	0.4
Tetrahydrozoline (B58)	N/A	N/A	N/A	N/A
Ephedrine (B60)	N/A	N/A	N/A	N/A
Ampholyte				
Tryptophan	2.52	1.00	2.3	0.0

Table IV-2. Continued

Compound Name	0.5mM HDMSTBA (25kV)			
	μ^{eff}	α	β	Rs
Bases				
Alprenolol (B02)	4.28	1.00	2.3	0.0
Halostachine (B04)	2.49	1.00	4.1	0.0
Bupropion (B11)	12.30	1.04	0.8	0.4
4-Chloroamphetamine (B13)	-6.04	0.95	-1.7	1.4
Chlophedianol (B14)	2.00	1.00	5.1	0.0
Homatropine (B19)	-4.25	1.00	-2.4	0.0
Isoproterenol (B21)	3.35	1.00	3.1	0.0
Ketamine (B22)	N/A	N/A	N/A	N/A
Ketotifen (B23)	-6.22	1.00	-1.7	0.0
Metaproterenol (B30)	1.60	1.00	6.8	0.0
Metoprolol (B31)	2.54	1.00	4.2	0.0
Norephedrine (B34)	1.36	1.00	4.4	0.0
Oxyphencyclimine (B36)	4.85	1.00	2.1	0.0
Piperoxan (B38)	N/A	N/A	N/A	N/A
Propranolol (B42)	0.96	1.25	6.6	1.0
Terbutaline (B47)	2.30	1.06	2.5	0.6
Verapamil (B54)	N/A	N/A	N/A	N/A
Tetrahydrozoline (B58)	15.19	1.00	0.4	0.0
Ephedrine (B60)	2.86	1.00	2.2	0.0
Ampholyte				
Tryptophan	N/A	N/A	N/A	N/A

Table IV-2. Continued

Compound Name	1mM HDMSTBA (15kV)			
	μ^{eff}	α	β	Rs
Bases				
Alprenolol (B02)	-10.85	0.96	-0.4	0.6
Halostachine (B04)	-14.17	0.97	-0.3	0.7
Bupropion (B11)	3.44	1.26	1.2	2.4
4-Chloroamphetamine (B13)	-15.52	1.00	-0.3	0.0
Chlophedianol (B14)	-13.46	0.97	-0.3	0.4
Homatropine (B19)	-15.46	1.00	-0.3	0.0
Isoproterenol (B21)	-10.82	1.00	-0.4	0.0
Ketamine (B22)	-17.52	0.98	-0.3	0.0
Ketotifen (B23)	-16.25	1.00	-0.3	0.0
Metaproterenol (B30)	-10.74	1.00	-0.3	0.0
Metoprolol (B31)	-11.15	0.97	-0.5	0.6
Norephedrine (B34)	-14.49	1.00	-0.4	0.0
Oxyphencyclimine (B36)	-8.96	0.99	-0.6	0.4
Piperoxan (B38)	-13.73	0.96	-0.5	2.9
Propranolol (B42)	-11.79	0.96	-0.5	2.5
Terbutaline (B47)	0.51	1.00	9.1	0.0
Verapamil (B54)	-14.01	0.99	-0.5	1.6
Tetrahydrozoline (B58)	9.06	1.00	0.5	0.0
Ephedrine (B60)	-11.67	0.96	-0.4	0.7
Ampholyte				
Tryptophan	-5.94	1.00	-0.4	0.0

Table IV-2. Continued

Compound Name	5mM HDMSTBA (15kV)			
	μ^{eff}	α	β	Rs
Bases				
Alprenolol (B02)	-21.94	0.99	-0.6	0.9
Halostachine (B04)	-23.49	0.99	-0.6	0.7
Bupropion (B11)	-18.61	1.00	-0.8	0.0
4-Chloroamphetamine (B13)	-24.75	1.00	-0.6	0.0
Chlophedianol (B14)	-22.26	1.00	-0.6	0.0
Homatropine (B19)	-24.15	1.00	-0.6	0.0
Isoproterenol (B21)	-22.04	1.00	-0.6	0.0
Ketamine (B22)	-17.97	0.99	-0.7	1.8
Ketotifen (B23)	-24.13	1.00	-0.5	0.0
Metaproterenol (B30)	-21.71	1.00	-0.6	0.0
Metoprolol (B31)	-21.90	0.99	-0.5	0.6
Norephedrine (B34)	-23.29	1.00	-0.5	0.0
Oxyphencyclimine (B36)	-20.96	1.00	-0.6	0.0
Piperoxan (B38)	-23.16	0.99	-0.6	1.3
Propranolol (B42)	-22.45	0.99	-0.6	0.9
Terbutaline (B47)	-21.51	0.99	-0.6	1.2
Verapamil (B54)	-23.02	1.00	-0.6	0.0
Tetrahydrozoline (B58)	-19.07	0.93	-0.6	3.9
Ephedrine (B60)	-23.00	1.00	-0.6	0.0
Ampholyte				
Tryptophan	-18.85	0.99	-0.7	1.8

Table IV-2. Continued

Compound Name	10mM HDMSTBA (15kV)			
	μ^{eff}	α	β	Rs
Bases				
Alprenolol (B02)	-14.49	0.99	-0.4	0.7
Halostachine (B04)	-15.71	0.99	-0.3	0.5
Bupropion (B11)	-11.40	0.95	-0.5	6.0
4-Chloroamphetamine (B13)	-16.51	1.00	-0.3	0.0
Chlophedianol (B14)	-14.60	1.00	-0.4	0.0
Homatropine (B19)	-15.84	1.00	-0.3	0.0
Isoproterenol (B21)	-14.47	1.00	-0.4	0.0
Ketamine (B22)	-11.97	0.99	-0.5	1.4
Ketotifen (B23)	-15.92	1.00	-0.3	0.0
Metaproterenol (B30)	-14.44	1.00	-0.3	0.0
Metoprolol (B31)	-14.70	0.99	-0.4	0.8
Norephedrine (B34)	-15.68	0.99	-0.4	0.7
Oxyphencyclimine (B36)	-13.82	1.00	-0.4	0.0
Piperoxan (B38)	-15.51	0.99	-0.4	1.5
Propranolol (B42)	-14.77	0.99	-0.4	1.5
Terbutaline (B47)	-14.14	0.99	-0.4	1.9
Verapamil (B54)	-14.97	1.00	-0.4	0.0
Tetrahydrozoline (B58)	-13.31	0.92	-0.4	10.8
Ephedrine (B60)	-15.47	1.00	-0.4	0.5
Ampholyte				
Tryptophan	-13.09	0.99	-0.5	2.1

Table IV-2. Continued

Compound Name	25mM HDMSTBA (15kV)			
	μ^{eff}	α	β	Rs
Bases				
Alprenolol (B02)	-10.05	0.99	-0.6	0.9
Halostachine (B04)	-10.81	0.99	-0.5	0.7
Bupropion (B11)	-8.30	0.96	-0.7	6.6
4-Chloroamphetamine (B13)	-11.37	1.00	-0.5	0.0
Chlophedianol (B14)	-10.08	1.00	-0.6	0.0
Homatropine (B19)	-10.95	1.00	-0.5	0.0
Isoproterenol (B21)	-9.99	1.00	-0.6	0.0
Ketamine (B22)	-8.52	0.99	-0.7	1.6
Ketotifen (B23)	-11.01	1.00	-0.6	0.6
Metaproterenol (B30)	-10.16	1.00	-0.6	0.0
Metoprolol (B31)	-9.74	0.99	-0.5	0.7
Norephedrine (B34)	-10.40	0.99	-0.5	0.9
Oxyphencyclimine (B36)	-9.31	1.00	-0.5	0.0
Piperoxan (B38)	-10.22	0.99	-0.5	1.5
Propranolol (B42)	-10.30	0.99	-0.5	1.2
Terbutaline (B47)	-10.50	1.00	-0.5	0.0
Verapamil (B54)	-10.60	1.00	-0.5	0.0
Tetrahydrozoline (B58)	-9.32	0.92	-0.6	8.2
Ephedrine (B60)	-10.83	0.99	-0.5	0.6
Ampholyte				
Tryptophan	-9.23	0.99	-0.6	1.7

Table IV-2. Continued

Compound Name	40mM HDMSTBA (15kV)			
	μ^{eff}	α	β	Rs
Bases				
Alprenolol (B02)	-6.35	0.99	-0.5	1.2
Halostachine (B04)	-6.95	0.99	-0.4	0.9
Bupropion (B11)	-4.88	0.94	-0.6	7.7
4-Chloroamphetamine (B13)	-7.46	1.00	-0.4	0.0
Chlophedianol (B14)	-6.40	1.00	-0.5	0.0
Homatropine (B19)	-7.03	1.00	-0.5	0.0
Isoproterenol (B21)	-6.31	1.00	-0.6	0.0
Ketamine (B22)	-4.92	0.99	-0.7	2.4
Ketotifen (B23)	-7.33	1.00	-0.7	1.3
Metaproterenol (B30)	-6.42	1.00	-0.6	0.6
Metoprolol (B31)	-6.51	0.99	-0.7	1.2
Norephedrine (B34)	-7.14	0.99	-0.6	1.8
Oxyphencyclimine (B36)	-5.70	1.00	-0.5	0.0
Piperoxan (B38)	-6.90	0.99	-0.7	2.4
Propranolol (B42)	-6.61	0.99	-0.7	2.1
Terbutaline (B47)	-6.06	0.98	-0.5	2.2
Verapamil (B54)	-6.55	1.00	-0.5	0.0
Tetrahydrozoline (B58)	-5.55	0.88	-0.5	15.1
Ephedrine (B60)	-6.79	0.99	-0.5	1.2
Ampholyte				
Tryptophan	-5.76	0.99	-0.6	1.7

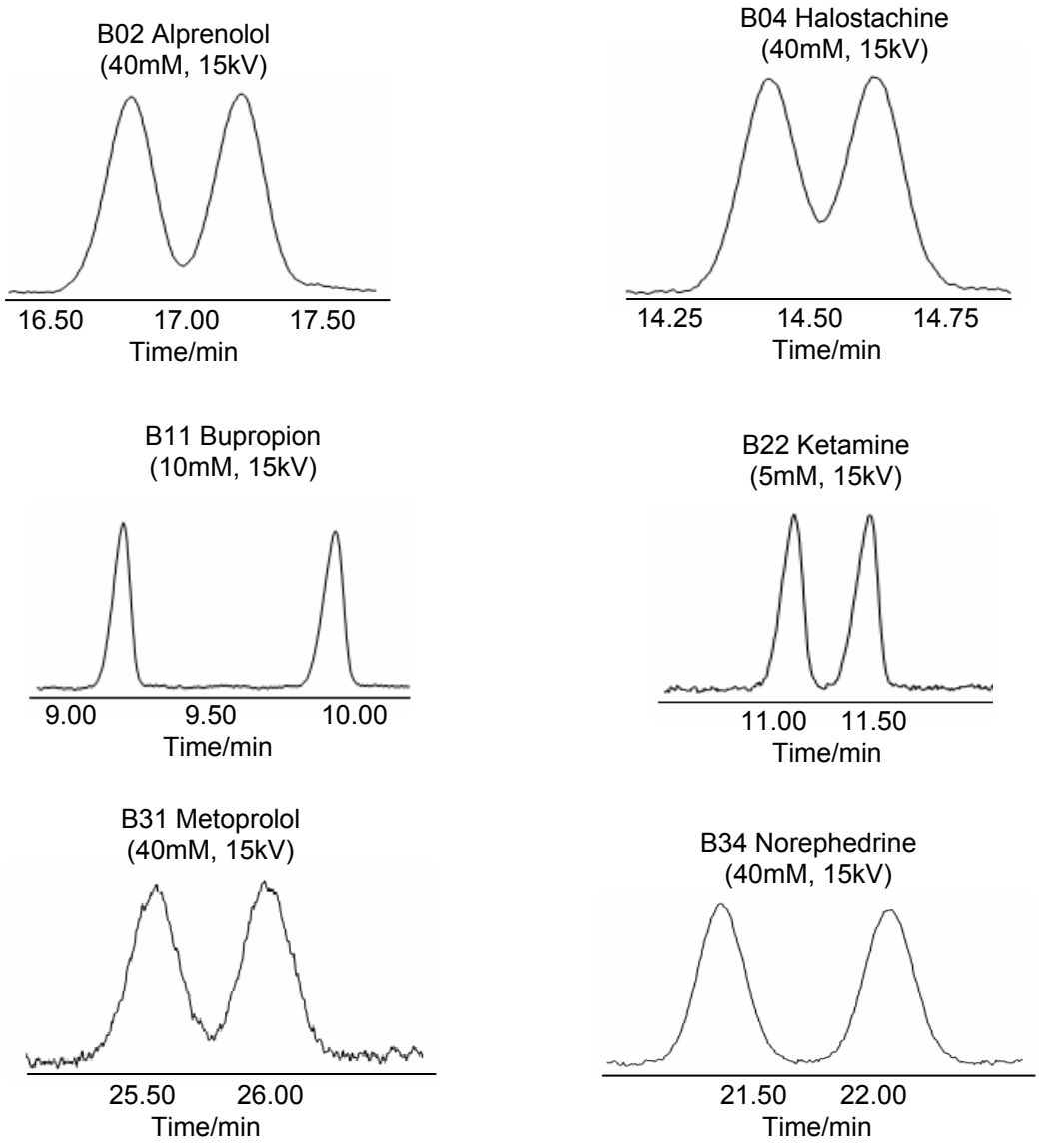


Figure IV-1. Electropherograms of some weak basic and ampholytic enantiomers at acidic methanolic BGE using TBA_7HDMS as the chiral resolving agent

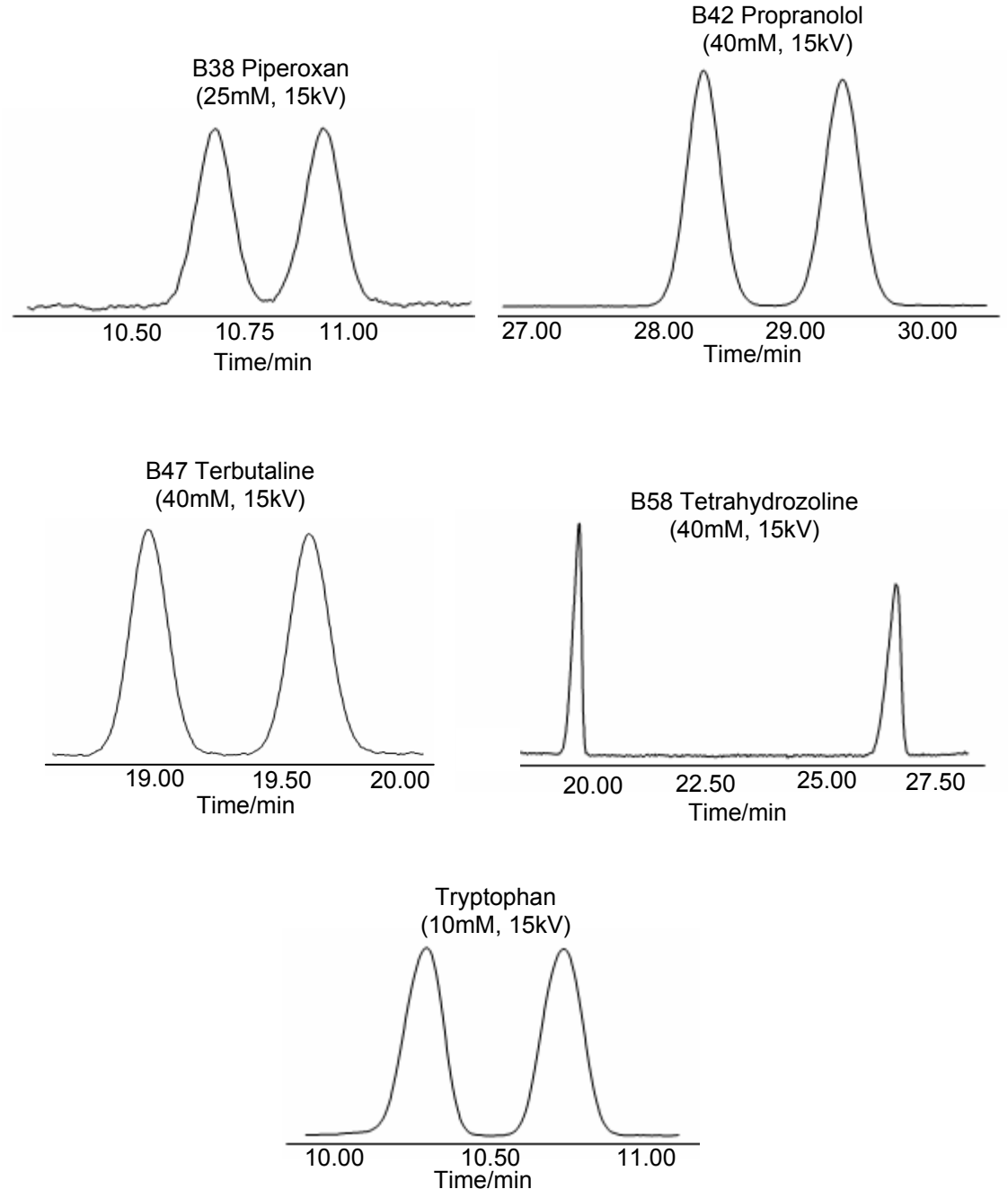


Figure IV-1. Continued

a lower potential need to be used to be able to stay in the linear region of the Ohm's plot so accurate measurements of effective mobilities can be made. In addition, the viscosity of the BGE also increases if the concentration of the chiral resolving agent is increased. Figure IV-2 and IV-3 show the contribution of the cyclodextrin concentration to the viscosity of the BGE and the absolute viscosity of the BGE, respectively. All of these factors (high ionic strength and high TBA_7HDMS concentration) lead to a lower ionic mobility of the enantiomer. In Figure IV-4, the effective mobility of the enantiomer was plotted as a function of TBA_7HDMS concentration. Since all analytes are positively charged at 0mM TBA_7HDMS, the cationic effective mobility (analyte migrates toward the cathode) decreases rapidly as the concentration of TBA_7HDMS is increased. Further increase in the TBA_7HDMS concentration increases the complexation between the enantiomer and the chiral resolving agent which results in the reverse migration of the enantiomer: the complex migrates towards the anode (anionic mobility) and migration remains anionic through the entire TBA_7HDMS concentration range. Although the enantiomer stays anionic after a concentration of 3mM TBA_7HDMS, the effective mobility of the enantiomer decreases with increasing TBA_7HDMS concentration. This may be due to the higher viscosity of the BGE ($\mu^{eff} \alpha 1/\eta$), and more importantly, to the higher ionic strength. At high TBA_7HDMS concentration the ionic strength may suppress the dissociation of the tetrabutylammonium counter ion (TBA^+) from the CD. This will cause a decrease in the ionic mobility of TBA_7HDMS. In addition, at high

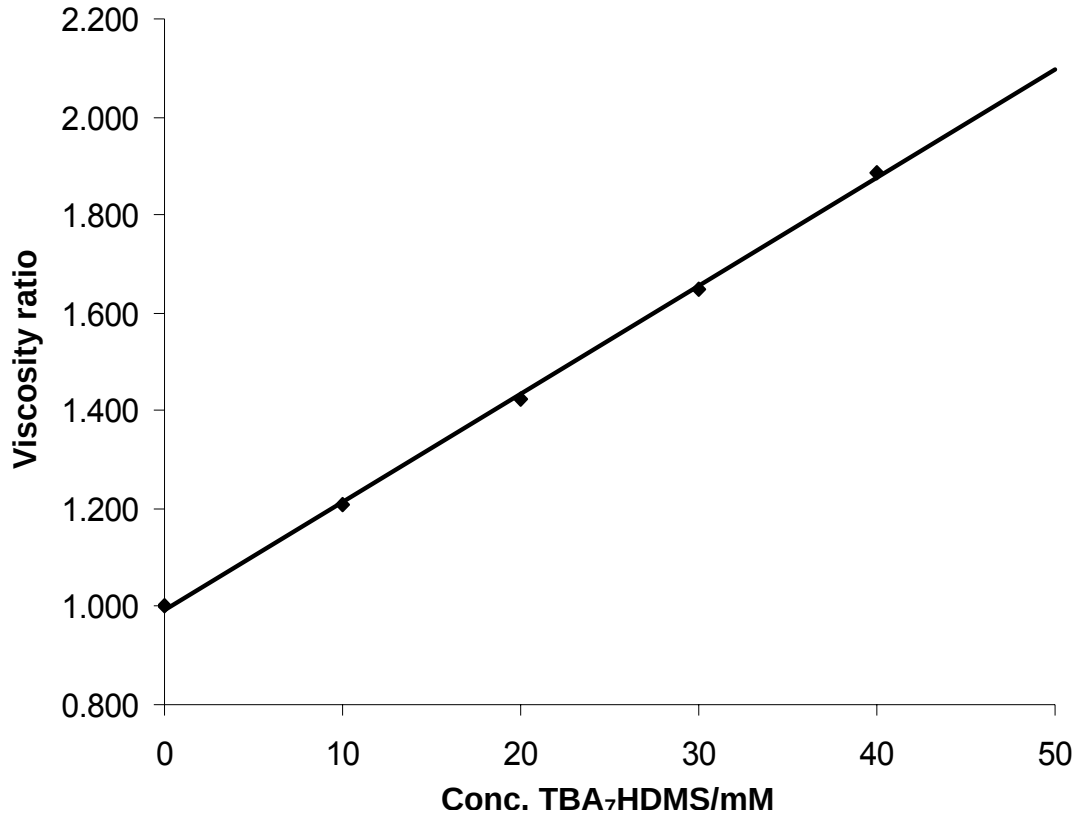


Figure IV-2. Contribution of the TBA_7HDMS concentration to the viscosity of the acidic methanolic BGE.

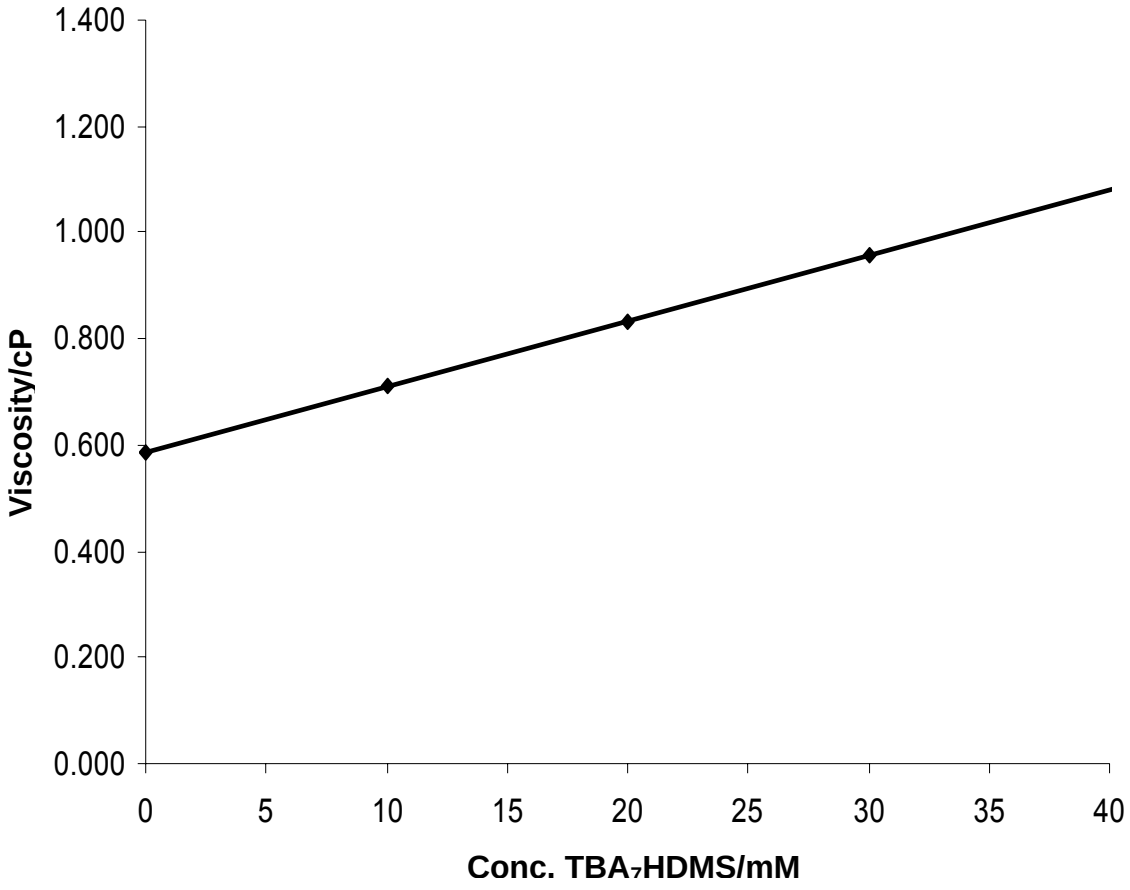


Figure IV-3. Absolute viscosity of the acidic methanolic BGE at different TBA_7HDMS concentrations.

TBA_7HDMS concentration the protonated analyte may compete with the counter ion of the CD (TBA^+) to ion-pair with the CD which is promoted in a non-aqueous solvent.

Optimization of the separation of a strongly binding analyte (in aqueous BGE) can be done in NACE since it is typical that non-aqueous BGE weakens the binding strength between the analyte and the enantiomer. However, something interesting to be noticed in Figure IV-4 is that all the twenty enantiomers studied are strongly binding analytes. These cationic analytes became anionic (migrate toward the anode) at a very low TBA_7HDMS concentration (as low as 1mM TBA_7HDMS) and remain anionic afterward. The effective mobility and the separation selectivity as a function of the TBA_7HDMS concentration for some of the enantiomers are shown in Figure IV-5 and Figure IV-6. Figure IV-5 shows the effective mobility and separation selectivity as a function of TBA_7HDMS concentration for two analytes that had cationic mobility at a very low TBA_7HDMS concentration and became anionic afterward. The separation selectivity increases to an infinitely high value and shows a discontinuity when the analyte becomes anionic. After that discontinuity, separation selectivity goes through zero, becomes a positive value and finally, approaches unity. On the other hand, Figure IV-6 shows the effective mobility and separation selectivity as a function of the TBA_7HDMS concentration for two enantiomers that were anionic through the entire TBA_7HDMS concentration range studied. In this case, only the second part of the separation selectivity

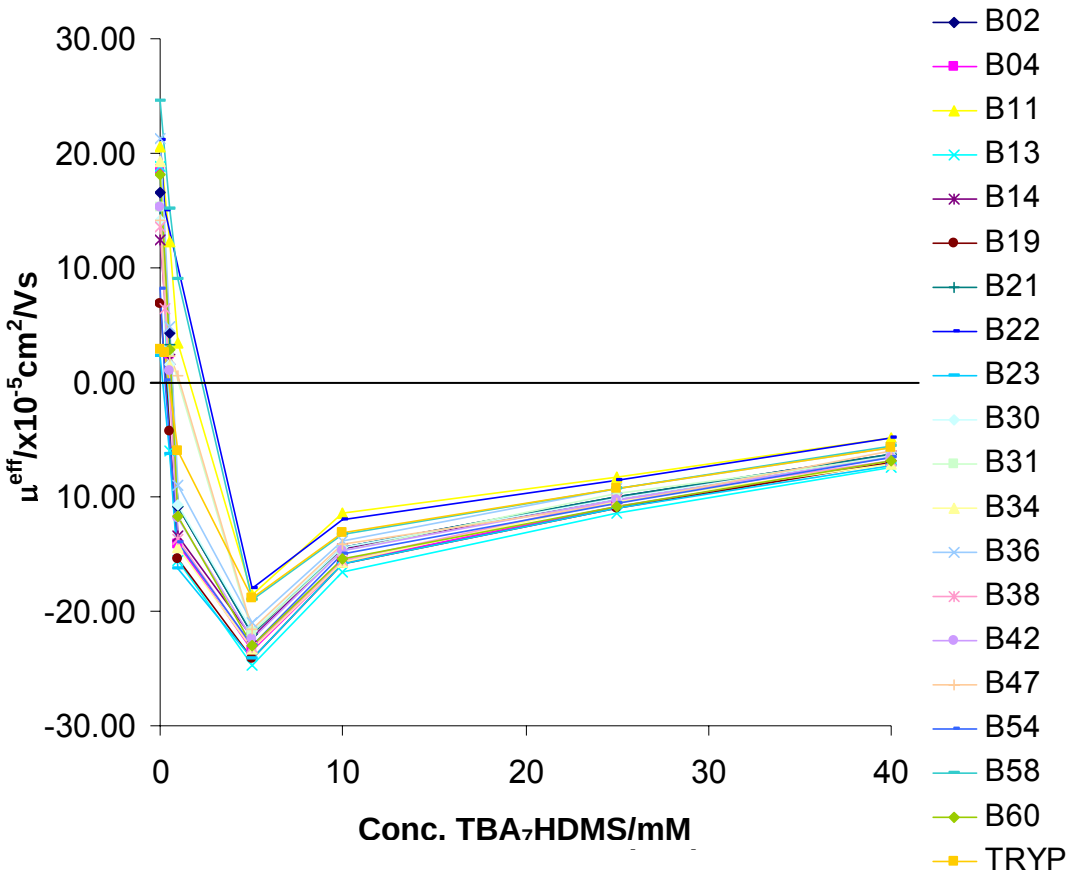


Figure IV-4. Effective mobilities as a function of TBA_7HDMS concentration for all the twenty enantiomers which are strongly binding analytes.

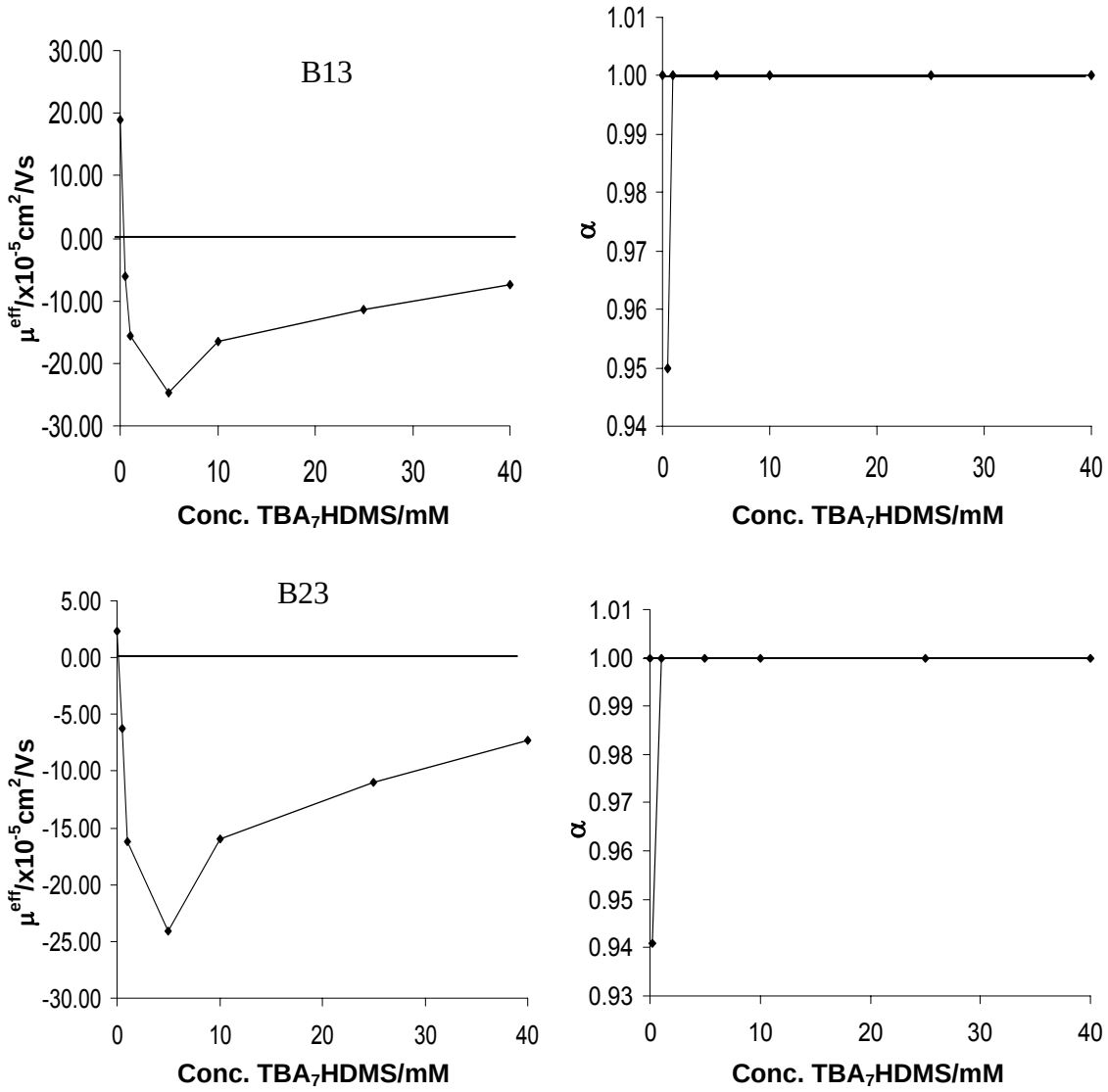


Figure IV-5. Effective mobility (left panels) and separation selectivity plots (right panels) of 4-chloroamphetamine (B13) and ketotifen (B23) when TBA_7HDMS was used in acidic methanolic BGE.

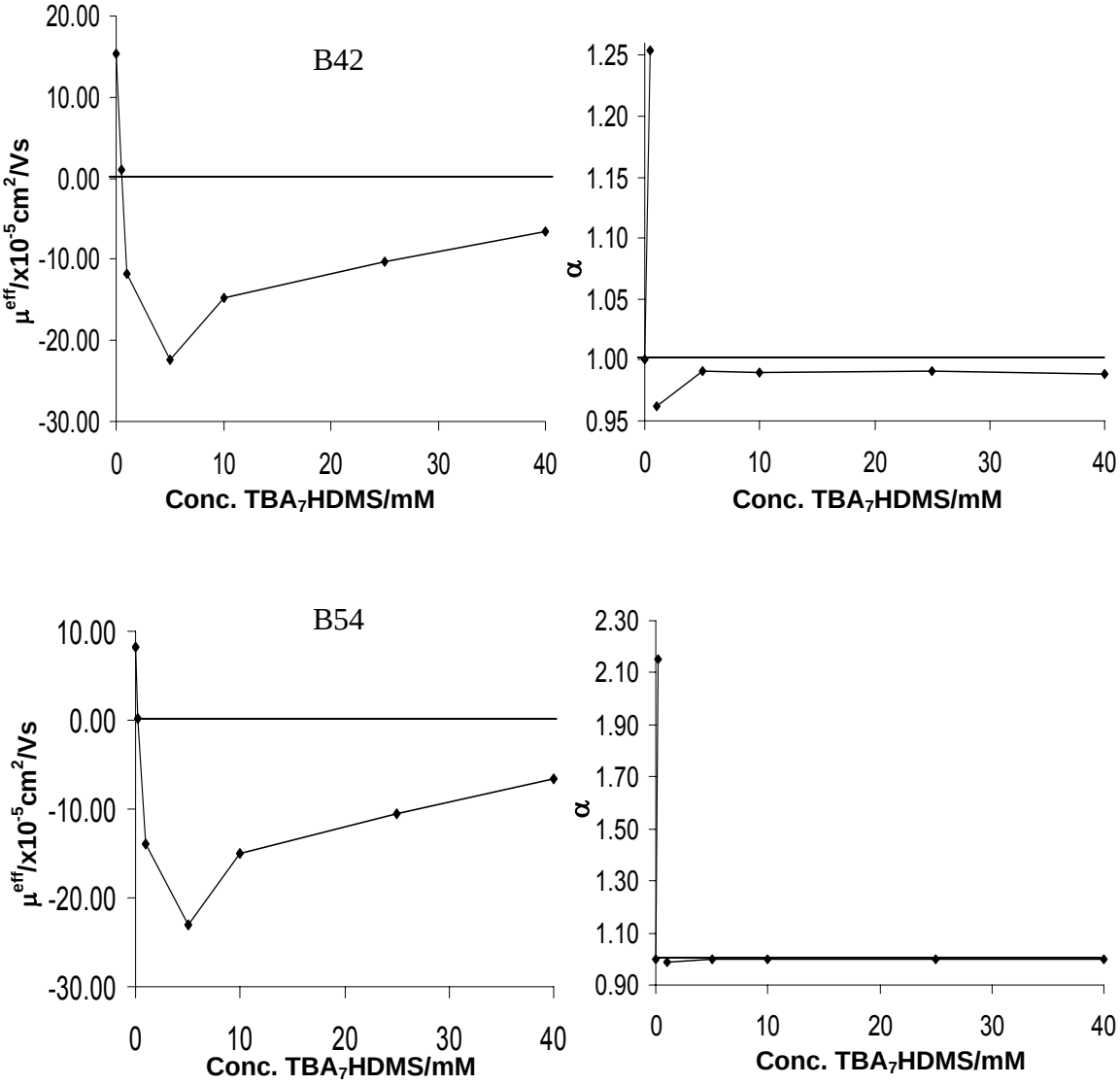


Figure IV-6. Effective mobility (left panels) and separation selectivity plots (right panels) of propranolol (B42) and verapamil (B54) when TBA_7HDMS was used in acidic methanolic BGE.

curve is seen, indicating the change for negative infinity to a positive value that approaches unity.

4.4 Comparison of enantiomer separations using TBA_7HDMS as chiral resolving agent in aqueous and acidic methanolic BGEs

Seven of the twenty enantiomers that were analyzed in acidic methanolic BGE using TBA_7HDMS as the chiral resolving agent were also separated in aqueous BGE. Weaker binding strength in NACE is typical; however all the enantiomers analyzed in the acidic methanolic BGE were strongly binding analytes. With the exception of tetrahydrozoline (B58) (Rs=1, β=7.2), all the analytes were baseline separated (Rs>1.5) in the aqueous BGEs. On the other hand, B58 had the highest resolution (Rs=15.1) in NACE where the normalized electroosmotic flow was much better (β=-0.5). Moreover, isoproterenol (B21) which was baseline separated at all TBA_7HDMS concentrations in aqueous BGE, did not show any separation selectivity in NACE, even with a better β value. In general, NACE provided a better β and in most cases a better α; however the best separation results (highest Rs) were obtained using aqueous BGE with the exception of B58.

Effective mobility and separation selectivity as a function of the TBA_7HDMS concentration for ketamine (B22) in NACE and in aqueous BGE are shown in Figure IV-7. The analyte that is weakly binding in aqueous BGE became a strongly binding analyte in NACE. Although the β value in NACE was

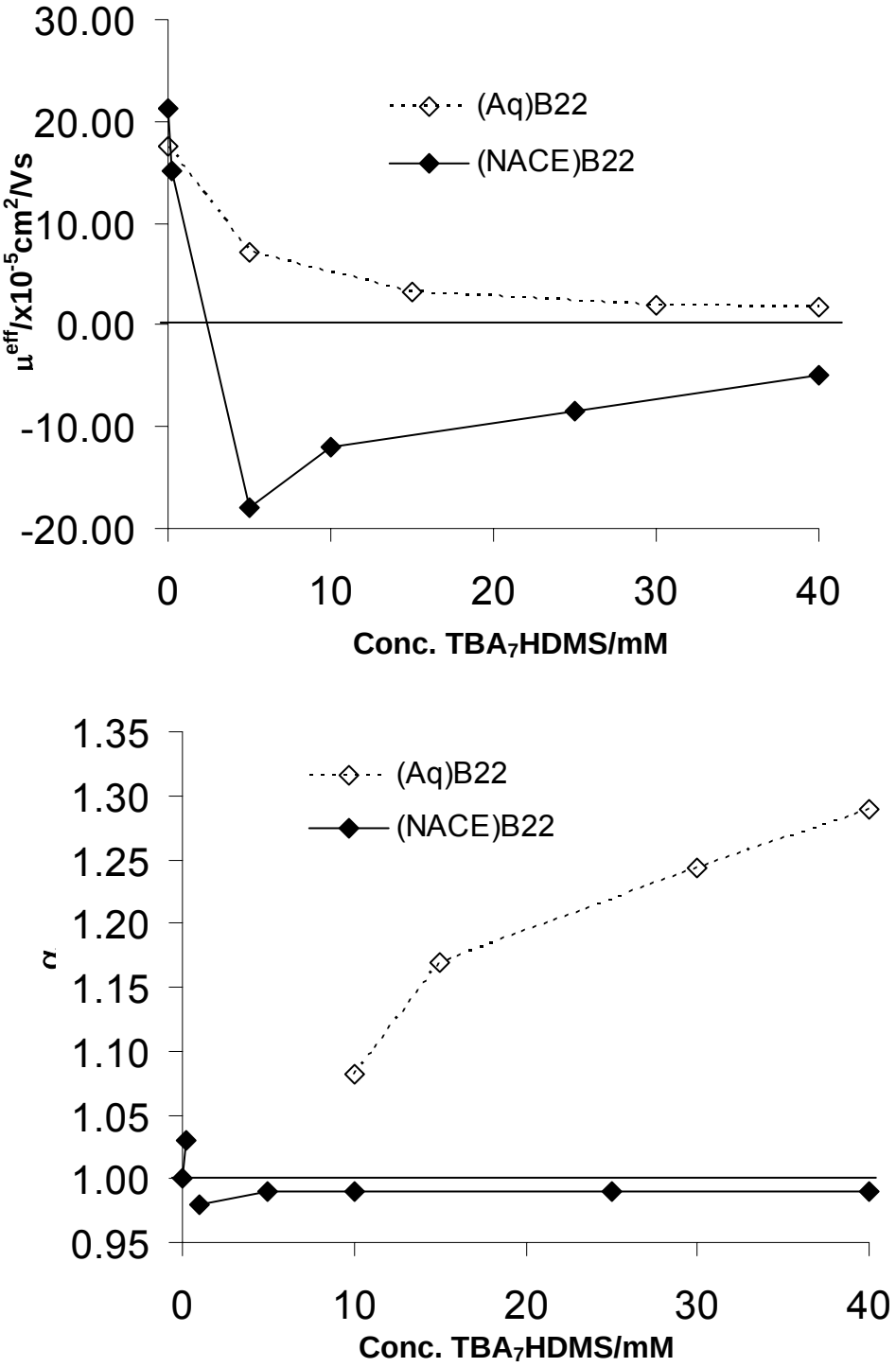


Figure IV-7. Comparison of the mobility plots (top panel) and separation selectivity (bottom panel) for ketamine (B22) when TBA_7HDMS was used in aqueous and non-aqueous BGEs.

more favorable, the separation selectivity in aqueous BGE was so high that it lead to a higher resolution compared to NACE.

Norephedrine (B34) and tetrahydrozoline (B58), which were moderately binding analytes in aqueous BGE, became strongly binding analytes in NACE (Figure IV-8). It is clear that the separation selectivity for B34 in aqueous BGE was more favorable in comparison to NACE. Even with a β of negative five, the resolution was nine and a half in the aqueous BGE in comparison to a very good β of negative one half and a resolution of almost two in NACE. On the other hand, the separation selectivity for B58 was almost the same in the aqueous BGE and the acidic methanolic BGE, but the better β value (β=-0.5) in NACE made the difference with a resolution of fifteen in comparison to a resolution of one in aqueous BGE (β=7.2).

Finally, the last four compounds to be discussed were the ones that were strongly binding analytes in both the aqueous and non-aqueous BGEs. Even though all the analytes are strongly binding analytes in both BGEs, they became anionic at a lower TBA_7HDMS concentration (1mM) in NACE than in aqueous BGE (5-7mM TBA_7HDMS) (Figure IV-9). Figure IV-9 clearly shows how the separation selectivity for all four enantiomers is better in aqueous BGE than in NACE. Only two enantiomers were baseline separated in both the aqueous and non-aqueous BGEs (propranolol (B42) and tryptophan (Tryp)). That was not the case for metoprolol (B31) which was baseline separated in the aqueous BGE,

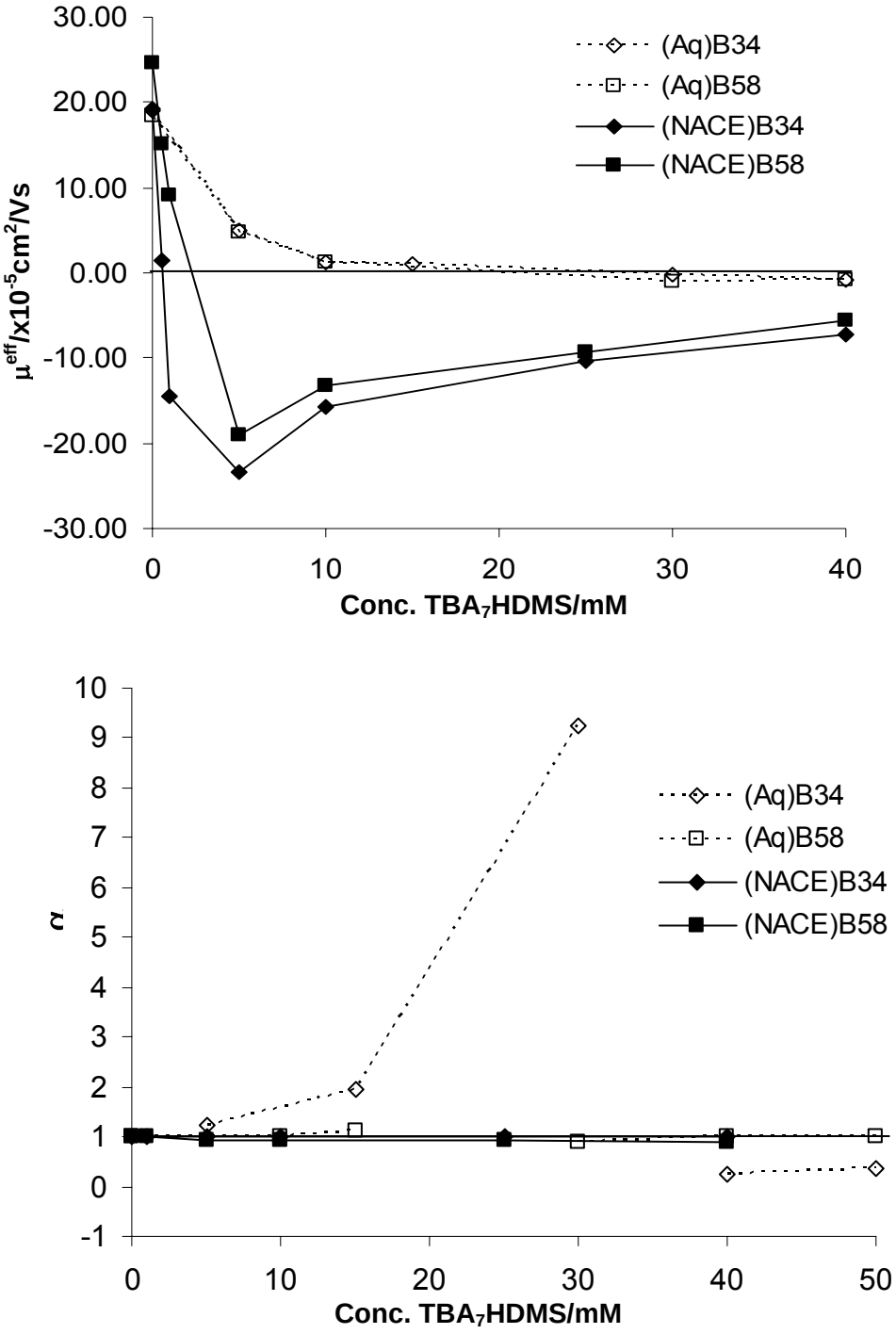


Figure IV-8. Comparison of the mobility (top panel) and separation selectivity plots (bottom panel) for norephedrine (B34) and tetrahydrozoline (B58) when TBA_7HDMS was used in aqueous and non-aqueous BGEs.

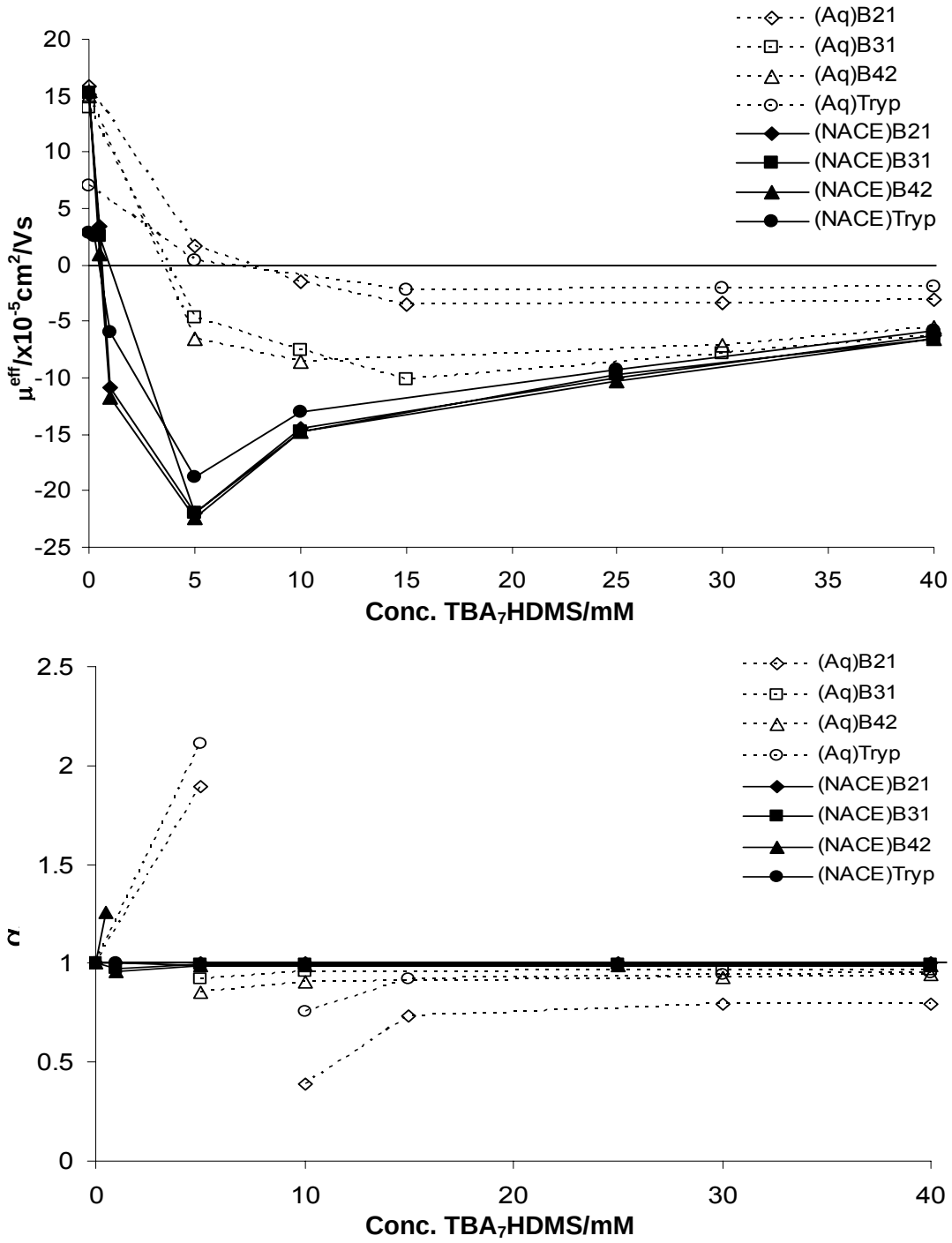


Figure IV-9. Comparison of the mobility (top panel) and separation selectivity plots (bottom panel) for isoproterenol (B21), metoprolol (B31), propranolol (B42) and tryptophan (Tryp) when TBA_7HDMS was used in aqueous BGE and non-aqueous BGEs.

but only partially separated in NACE despite the very good β value (β=-0.7). A totally opposite scenario was obtained for isoproterenol (B21) which was baseline separated at all TBA_7HDMS concentrations in the aqueous BGE and did not show any separation selectivity at any of the TBA_7HDMS concentrations in NACE.

4.5 Comparison of enantiomer separations using TBA_7HDMS and Na_7HDMS as chiral resolving agents in acidic methanolic BGE

Six of the twenty pharmaceuticals separated using TBA_7HDMS were previously separated using Na_7HDMS. To be able to make any comparison, both enantiomer separations were performed at the same temperature, buffer concentration, ionic strength and pH. However, the contribution of the CD to the viscosity of the BGE is different, which provides two different media for the separation to take place. Figure IV-10 and Figure IV-11 show the contribution of both CDs to the viscosity of the non-aqueous BGE and the absolute viscosity of the non-aqueous BGE with both CDs, respectively. The TBA_7HDMS BGE showed a higher viscosity than the Na_7HDMS BGE as expected since that was the case in the aqueous BGE. A higher viscosity is expected to lead to a lower effective mobility in that BGE; however the highest effective mobilities for the six enantiomers with TBA_7HDMS are in the range of -18 to -25 mobility units in comparison to 8 to -4 mobility units with Na_7HDMS.

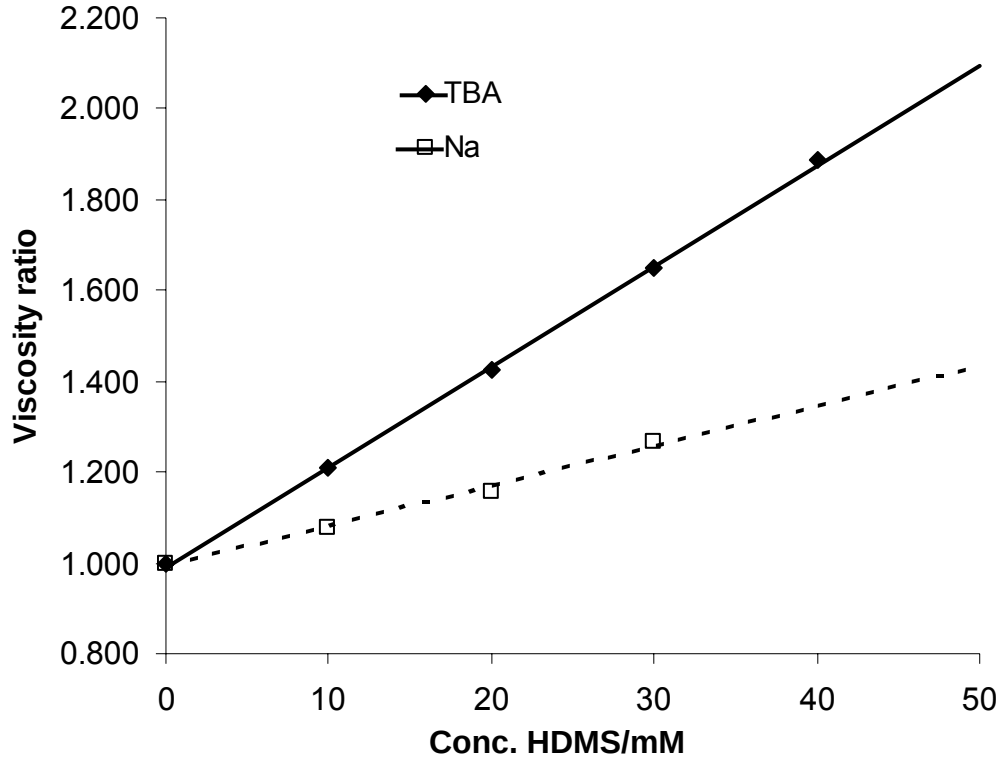


Figure IV-10. Comparison between the contribution of TBA_7HDMS and Na_7HDMS concentration to the viscosity of the non-aqueous BGE.

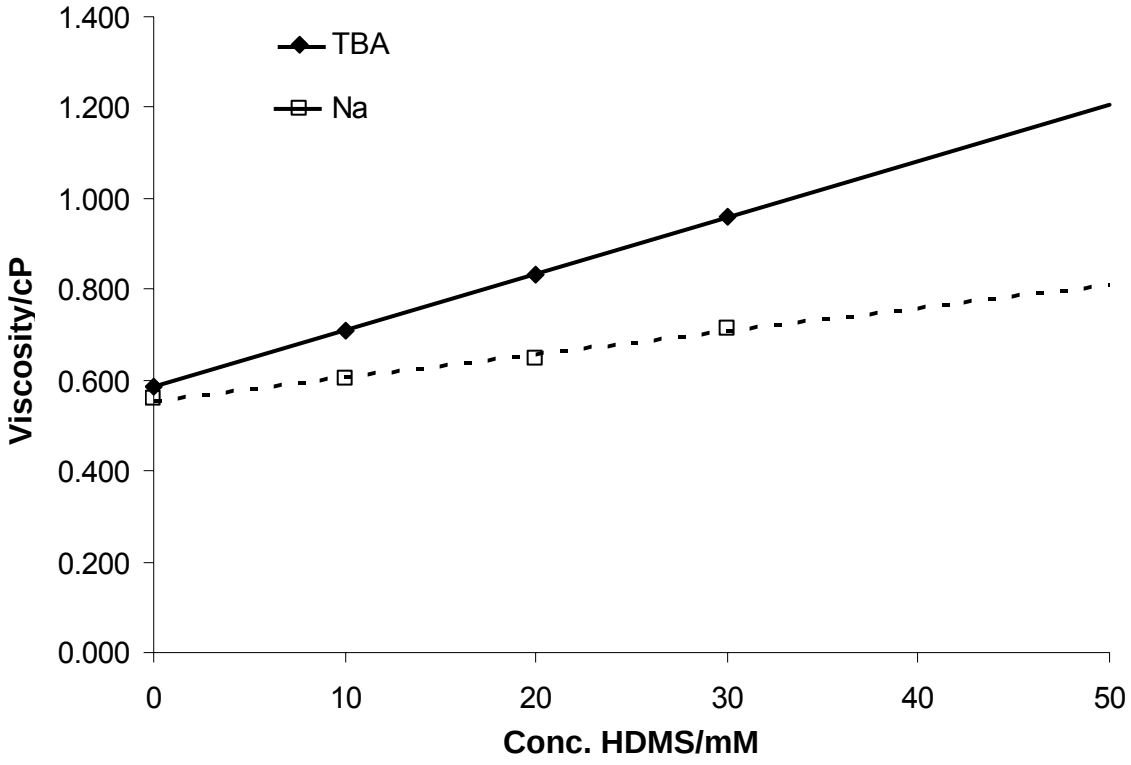


Figure IV-11. Comparison of the absolute viscosity of the non-aqueous BGEs at different TBA_7HDMS and Na_7HDMS concentrations.

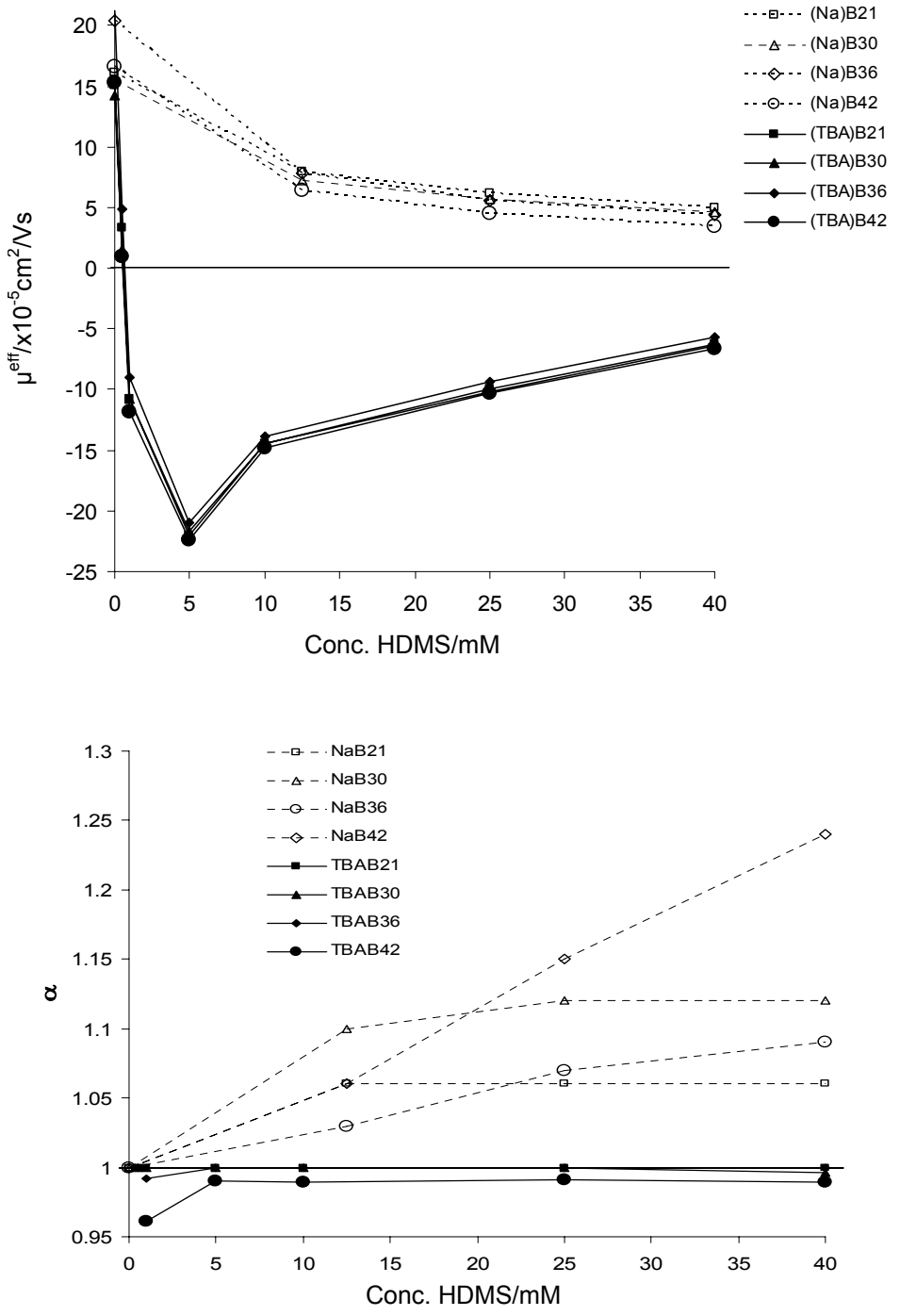


Figure IV-12. Comparison of the mobility (top panel) and separation selectivity plots (bottom panel) for isoproterenol (B21), metaproterenol (B30), oxyphencyclimine (B36) and propranolol (B42) when TBA_7HDMS and Na_7HDMS were used in NACE.

On the other hand, four of the six enantiomers were weakly binding analytes using Na_7HDMS and became strongly binding analytes when TBA_7HDMS was used (Figure IV-12). Figure IV-12 shows the effective mobilities of isoproterenol (B21), metaproterenol (B30), oxyphencyclimine (B36) and propranolol (B42) as a function of the concentration of the chiral resolving agent. When Na_7HDMS was used all analytes had a cationic mobility (migrated toward the cathode), through the entire Na_7HDMS concentration range. On the other hand, when TBA_7HDMS was used, the cationic mobility of the analytes became anionic at a very low TBA_7HDMS concentration (1mM TBA_7HDMS) and remained anionic with further increase in the TBA_7HDMS concentration.

Since all twenty enantiomers became strongly binding analytes when TBA_7HDMS was used, the separation selectivity may not be favorable in comparison with the use of Na_7HDMS that led to some weakly binding analytes, some moderately binding analytes and no strongly binding analytes. Separation selectivity may not be favorable for strongly binding analytes because the chiral resolving agent may complex so strongly with both enantiomers (R and S) that chiral recognition may not be observed (no selectivity, $\alpha=1$) and the mobility differences between the new diastereomers may not be significant. The separation selectivity as a function of the CD concentration for the four analytes that were weakly binding to Na_7HDMS and strongly binding to TBA_7HDMS are shown in Figure IV-12. It is very clear that separation selectivities for the enantiomers separated using Na_7HDMS are much better than those obtained

using TBA_7HDMS which only shows separation selectivity different from one for two enantiomers out of the four. Those enantiomers that did not show any separation selectivity using TBA_7HDMS were isoproterenol (B21) and metaproterenol (B30), which were baseline separated at all Na_7HDMS concentrations ($Rs>1.5$).

In the case of the two enantiomers that were moderately binding to Na_7HDMS and strongly binding to TBA_7HDMS, the separation selectivity was also favorable using Na_7HDMS (Figure IV-13). Both enantiomers were baseline separated at all Na_7HDMS concentrations in contrast with the results obtained by TBA_7HDMS where both were only partially separated ($Rs<1.5$).

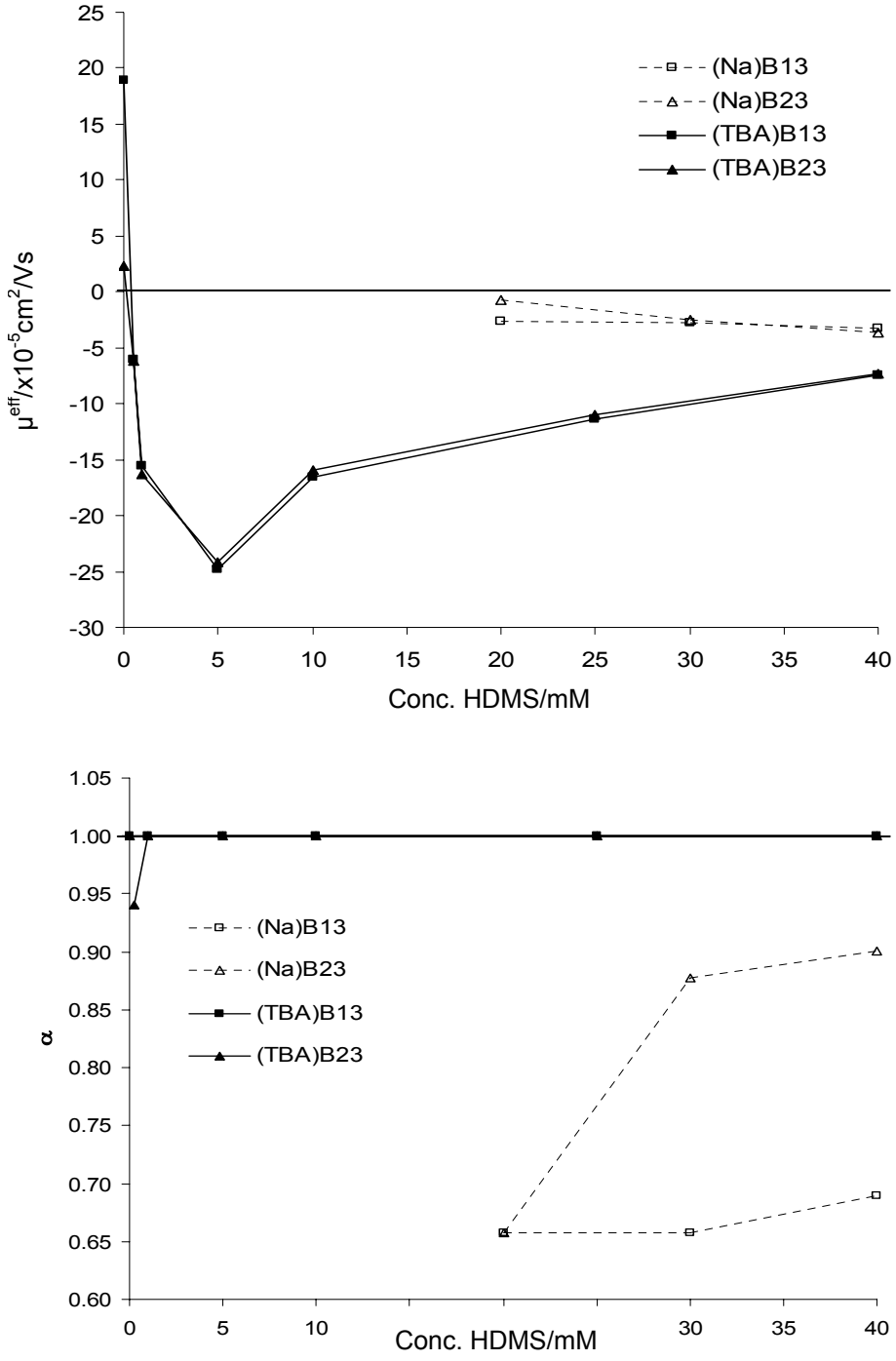


Figure IV-13. Comparison of the mobility (top panel) and separation selectivity plots (bottom panel) for 4-chloroamphetamine (B13) and ketotifen (B23) when TBA_7HDMS and Na_7HDMS were used in NACE.

CHAPTER V

CONCLUSIONS

Synthesis of a sulfated CD (TBA_7HDMS) highly soluble in organic solvent was obtained by adding one more step to the reported synthetic route of Na_7HDMS. This step consisted of an extraction of TBA_7HDMS with dichloromethane from an aqueous solution containing Na_7HDMS and TBACl. The synthesis of TBA_7HDMS was scaled up from 1g batch to 25g batch using the well characterized single-isomer Na_7HDMS as the starting material. The procedure turned out to be fairly easy, so it was also tried with the sodium salt of hexakis(2,3-di-*O*-methyl-6-*O*-sulfo)-α-cyclodextrin and the sodium salt of octakis (2,3-di-*O*-methyl-6-*O*-sulfo)-γ-cyclodextrin yielding the expected results as TBA_7HDMS. All three single-isomers sulfated CDs were characterized by indirect-UV detection CE and NMR spectroscopy which provided the necessary information to confirm the proposed structures with purities higher than ninety seven percent.

Separations of thirteen pharmaceuticals were achieved using CE as the separation technique in an aqueous BGE. The low pH aqueous BGE contained 25mM phosphoric acid buffered to pH 2.5 using TBAOH. It also contained TBA_7HDMS which was the chiral resolving agent. The potential used was chosen to be within the linear region of Ohm's law plots. Effective mobilities and separation selectivities as a function of the TBA_7HDMS concentration were

plotted. The effective mobility and the separation selectivity trends for the weak bases and the neutrals enantiomers followed the predictions of the CHARM model. Based on these trends, the weak bases and the ampholytic compound were categorized into three binding strength groups: i) strongly binding analytes; ii) moderately strongly binding analytes and; iii) weakly binding analytes. These enantiomers were baseline separated with the exception of tetrahydrozoline (B58) which was only partially separated. In the case of the neutral enantiomers, only three of the six were baseline separated. In most cases, the separation selectivities appeared at high TBA_7HDMS concentrations (30mM) in contrast to the weak bases and the ampholytic compound which were separated at 5mM TBA_7HDMS.

Comparisons between chiral resolving agents (TBA_7HDMS and Na_7HDMS) were made in aqueous BGE. The binding strength of the weak bases and the zwitterion increased when TBA_7HDMS was used. In addition, TBA_7HDMS provided better separation selectivities for the weak bases and the zwitterion in comparison to the one obtained with Na_7HDMS. On the other hand, comparable results in the separation selectivities of the neutral enantiomers were achieved for both chiral resolving agents.

TBA_7HDMS was also used for the separation of twenty pharmaceuticals in acidic methanolic BGEs. The effective mobilities and separation selectivities as a function of TBA_7HDMS were also plotted. Surprisingly, all the enantiomers became strongly binding analytes with very similar mobility trends. This may

suggest that the binding strength is independent of the molecular structure of the enantiomer under these particular conditions. Nine of the eighteen analytes were baseline separated and the rest were partially separated with the exception of homatropine (B19) and isoproterenol (B21). The highest resolution (Rs=15.1) was obtained for tetrahydrozoline (B58) which was only partially separated (Rs=1) in aqueous BGE. Overall, separation selectivities in the aqueous BGEs were more favorable than in the acidic methanolic BGEs using TBA_7HDMS in both.

Comparison between TBA_7HDMS and Na_7HDMS were made for the separations in non-aqueous BGEs. Four enantiomers (isoproterenol (B21), metaproterenol (B30), oxyphencyclimine (B36) and propranolol (B42)) that were weakly binding analytes using Na_7HDMS became strongly binding analytes using TBA_7HDMS. The separation selectivities for these analytes using Na_7HDMS were more favorable than the ones obtained using TBA_7HDMS. In addition, two moderately binding analytes (4-chloroamphetamine (B13) and ketotifen (B23)) when Na_7HDMS was used, became strongly binding analytes when TBA_7HDMS was used. More favorable separation selectivities were obtained when Na_7HDMS was used allowing baseline separation at all Na_7HDMS concentrations. On the other hand, only partial separation was obtained for these two enantiomers when TBA_7HDMS was used.

In conclusion, changing the inorganic counter ion of sodium in Na_7HDMS to a hydrophobic counter ion tetrabutylammonium (TBA^+), not only improved the

solubility of the $HDMS^{7-}$ in the non-aqueous solvent, but it also resulted in an increased binding strength when comparison of the same analytes using TBA_7HDMS and Na_7HDMS were made. Therefore, different separation selectivity patterns were obtained which were in some cases more favorable for the separation of enantiomers using TBA_7HDMS (i.e., aqueous BGEs) and in other cases, more favorable when Na_7HDMS was used (i.e., NACE).

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APPENDIX A

SYNTHESIS PROTOCOL FOR TBA_7HDMS

Synthesis of the tetrabutylammonium salt of heptakis (2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD (TBA_7HDMS) from the sodium salt of heptakis (2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD (Na_7HDMS).

Materials needed for 25g scale:

Na_7HDMS:

$m_{Na7HDMS}$ = 25g Na_7HDMS ($MW_{Na7HDMS}$ = 2045.69g/mol)

TBAOH 40%w/w in water (MW_{TBAOH} = 259.46g/mol; ρ_{TBAOH} = 0.99g/mL):

$V_{TBAOH} = n_{NA7HDMS} \times 7 \times 1.05 \times MW_{TBAOH} \times (100/40\%)/\rho_{TBAOH}$ = 59mL

Deionized (DI) water

Hydrochloric acid (HCl)

Dichloromethane (CH_2Cl_2)

Procedure:

1. Add the 59mL of 40%w/w TBAOH into a 250mL beaker with a 2” flat-shape teflon coated stri-bar. Place the beaker onto a stir-plate.

2. Start stirring the solution. Using a calibrated pH-meter, add the necessary amount of HCl to adjust the pH to 7.0-7.2 (pH can not be lower than 7.0).

3. Add 25g of Na_7HDMS into the beaker while stirring the TBACl solution. It may take fifteen to thirty minutes to dissolve the solid.

4. Once a homogeneous solution is obtained, measure the volume. Pour the reaction mixture into a 1L separatory funnel. Add the same volume of CH_2Cl_2. Shake the separatory funnel and wait until the organic phase (at the bottom) and aqueous phase (at the top) get separated. Dispose the aqueous phase (containing NaCl).

5. Measure the volume of the organic phase. Add the organic phase back to the separatory funnel with an equal volume of DI water. Shake the separatory funnel and wait until the phases get separated. Dispose the aqueous phase.

6. Transfer the organic phase to a 1L round-bottom flask with 2" football-shaped teflon coated stir-bar. Using a rotovap, remove the solvent (CH_2Cl_2) at 50°C.

7. To confirm the removal of NaCl, use an indirect-UV detection CE. Prepare the background electrolyte (BGE) with 25mM TRIS and 12.5mM pTSA (pH 8.3). Weigh 20mg of the final product and dissolve it in 5mL of BGE. All the solution must be filter with 0.45µm nylon-membrane before use. Use a fused-silica capillary 26cm total length and detection window at 19cm (wavelength 214nm). Carry out the analysis at 20°C using potential of 10kV. Use a negative polarity at the detector to check for the removal of Na^+, the presence of TBA^+ and the purity of the $HDMS^{7-}$. To check for the removal of Cl^- use a positive polarity at the detector. If NaCl is detected, repeat step 5.

8. To look for the ratio of TBA^+ and $HDMS^{7-}$ (7:1) use ^1H NMR. Dissolve 30mg of the final product in D_2O. Pour the solution in an NMR tube until 5cm of the tube are filled with the sample.

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