STUDIES OF ENANTIOMERIC SEPARATIONS

USING CAPILLARY ELECTROPHORESIS

by

MAN-YUNG BENJAMIN TONG

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE IN CHEMISTRY

THE UNIVERSITY OF TEXAS AT ARLINGTON

AUGUST 2009

ACKNOWLEDGEMENTS

I would like to give thanks to my Heavenly Father, who gives me a chance to study in the United States of America and leads me in my whole life.

I would also like to thank to my research advisor, Prof. Daniel W. Armstrong for giving me guidance and this great opportunity to work in his laboratory. His broad knowledge, scientific attitude, creative thinking and wonderful presentation and interpretation skills have inspired me. I am very grateful for all his advice and patience he has given me. Also, I would also like to express my gratitude to my thesis committee and all teachers who have taught me during my academic studies and shaped me into a scientist.

I would like to take this opportunity to express my appreciation especially to Ye Bao and Violet Jiang and all other group members for their assistance on my projects.

I would like to dedicate this thesis to my parents, Mr. Choi-Shun, Tong and Mrs. Yuet-Ngor, Chiu for their constant support on my academic studies in the United States. At last but not least, I would like to express my gratitiude to some of my friends in Hong Kong especially Teresa, Suet-Ching, Lam.

July 20, 2009

ABSTRACT

STUDIES OF ENANTIOMERIC SEPARATIONS

USING CAPILLARY ELECTROPHORESIS

Man-Yung Benjamin Tong, M.S.

The University of Texas at Arlington, 2009

Supervising Professor: Daniel W. Armstrong

The focus of this thesis is on using state-of-the-art capillary electrophoresis (CE) in enantiomeric separations. Chapter 1 provides a brief introduction to enantiomeric separations using CE. Chapters 2 through 5 presents the use of different novel chiral selectors in CE. The selector properties and strategies for developing enantiomeric separations are considered. Chapter 2 presents an overview on the most broadly useful chiral selectors for CE enantiomeric separations, sulfated cyclodextrins. Chapter 3 presents a new chiral selector, sodium arsenyl L-(+) tartrate. This chiral selector shows enantioselectivity for many amine-containing compounds and ruthenium (II) polypyridyl complexes and produces electropherograms with short to moderate migration times. Chapter 4 examines the CE enantiomeric separations of ten ruthenium (II) polypyridyl complexes using different types of cyclodextrin chiral selectors. Micellar capillary electrophoresis (MCE) was uilitized in this study. A comparison of the results using MCE to capillary zone electrophoresis (CZE) was discussed. Chapter 5 shows enantiomeric separations of almost 100 different amine-containing compounds using a new

chiral selector, sulfated cyclofructan. Separations using the normal and reversed polarity mode were utilized in this study. Results from both separation modes were compared and discussed.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 General Introduction

With extensive growth in the number of chiral synthetic pharmaceutical compounds, analytical separation techniques for enantiomerics continue to attract considerable attention [1-3]. Due to the fact that enantiomers of racemic drugs often exhibit different pharmacological and toxicological properties, [4] the United States Food and Drug Admisitration (FDA) issued guidelines in 1992 concerning the development of stereoisomeric drugs. Since then, the determination of enantiomeric purities of chiral compounds has become an important issue. As a result, the demand for analytical separation methods possessing high resolution and high efficiency with short analysis times has continued to escalate.

Several analytical and chromatographic methods such as high-performance liquid chromatography (HPLC), gas chromatography (GC) and thin layer chromatography (TLC) are the most commonly used chromatograhic approaches for enantioseparations. Capillary electrophoresis, in the last two decades, has been established as an alternative technique to chromatography for the separation of enantiomers and the determination of enantiomeric purities.

1.2 Enantiomeric Separations Using Capillary Electrophoresis

Capillary electrophoresis (CE) is suitable for enantiomeric separations due to its high efficiency, short analysis times and low consumption of both the chiral selectors and samples. In general, separation by electrophoresis is based on differences in solute velocity in an electric field. In other words, molecular separations in CE are based on differences in their charge-to-size ratio that determine the electrophoretic migration times of analytes under an electric field [5]. Since the pioneering publication of first paper on the application of CE for the separation of dansyl amino acid enantiomers in 1985 by Gassmann *et al.* [6], CE has become one of the more attractive approaches in agrochemical enantiomeric separation science. It is now used in many different scientific areas including pharmaceutical, medical, and environmental endeavors [7-8]. Capillary zone electrophoresis (CZE) is the most widely used mode due to its versatility and simplicity. This method involves an addition of one or more chiral selectors into the achiral background eletrolyte (BGE). Enantiomers are separated based on the charge-to-size ratio of the complexes and differential interactions with chiral selectors. A large number of applications that take advantage of different chiral selectors such as cyclodextin-modified micellar capillary electrophoresis (CD-MCE), have been developed [9]. CD-MCE is an alternative method for enantiomeric separations using achiral micelles, such as SDS, in the running buffer with chiral selectors as a two-pseudophase system. Enantiomers in this condition are separated based on partition in and out of the micelle formed by the surfactants and differential interactions with chiral selectors. For example, the more hydrophobic compounds interact more strongly with the micelle and are retained longer. Cyclodextrins (CD) and their derivatives are the most widely used chiral selectors in CE even though they were originally developed for HPLC and TLC [10-12].

Cyclodextrins are cyclic oligosaccharides and are commercially available. There are three types: α-, β-, and γ-CD which have 6, 7 and 8 glucose units, respectivity [2,10,13]. Because of its truncated cone shape with a hydrophobic cavity and a hydrophilic exterior of hydroxy groups, the most common mechanism for enantioseparations with a native cyclodextrin involves the inclusion of a hydrophobic analyte into its hydrophobic cavity [10]. Native cyclodextrins can also be modified to obtain different CD derivatives such as carboxymethyl-, hydroxypropyl- and sulfated-CDs in order to increases their solubility in aqueous buffer and in the latter case allows analysis of uncharged molecules [14]. Among all derivatives, sulfated CDs show the broadest enantiomeric selectivity especially when separating oppositely charged analytes because of their strong electrostatic interactions. Also, compared to the neutral CDs, a higher separation selectivity can be achieved due to a large “separation window” when using charged cyclodextrins [13,15]. In general, uncharged or native CDs are useful for the enantioseparation of charged molecules while charged CDs are useful for the enantioseparation of uncharged or oppositely charged enantiomers. Even though CDs and their derivatives are the dominant chiral selectors in CE enantioseparations, as the complexity of synthetic pharmaceutical compounds increases, the continuing exploration and development of new chiral selectors is necessary.

CHAPTER 2

REVIEW: ENANTIOMERIC SEPARATIONS USING SULFATED CYCLODEXTRINS IN CAPILLARY ELECTROPHORESIS

2.1 Introduction

Enantiomeric separations in the area of pharmaceutical, environmental and biological science have been extensively explored over the last three decades. With extensive growth in the number of chiral synthetic compounds and samples of greater complexity, the demand for and development of analytical separation methods possessing high efficiency, high resolution capabilities, short analysis times and low cost has become increasingly important. Due to the fact that each enantiomer of a chiral compound can be transported and interact differently with biological and biochemical entities, thereby exhibiting different pharmacological and toxicological effects [16], the health regulatory authorities (e.g., Food and Drug Administration), have issued guidelines concerning the development of chiral drugs and chemicals [4]. As a result, separation-based techniques are essential for determining enantiomeric purities of enantiomeric compounds made *via* any and/or all procedures.

Although early advances in enantioselective HPLC provided the impetus for the 1992 FDA regulations, capillary electrophoresis (CE) was soon found to be an effective and efficient alternative for analytical enantiomeric separations. Indeed, the dominant chiral selectors in CE are cyclodextrins and their derivatives, many of which were originally developed for HPLC and thin layer chromatography (TLC) [10-12,17]. Cyclodextrins eventually became the dominant chiral selectors in GC as well [18-22]. Non-inclusion mechanisms for enantiomeric separations by cyclodextrins were first found by Armstrong *et. al*. in GC and HPLC [23-25]. Interestingly, they also may occur in CE especially for negatively charged chiral selectors with some

oppositely charged analytes. Negatively charged chiral selectors (like sulfated cyclodextrins) are particularly useful for separating oppositely charged analytes (for example, amine-containing compounds) because of their strong electrostatic interactions. They also effectively separate a variety of neutral molecules.

It is well known that the difference in mobility between free positively-charged analytes and the complexed analytes is increased when using negatively-charged chiral selectors [13-15]. In this case, a higher separation selectivity can be achieved due to a larger “separation window” when using charged cyclodextrins (see Figure 1). For example, the negatively-charged sulfated cyclodextrins migrate in the direction opposite to that of the EOF. When the positively charged analyte complexes with the sulfated cyclodextins, the effective negatively-charged complex also travels in a direction opposite to that of the EOF, thereby providing the analyte with a slower apparent electrophoretic mobility. As a result, the “separation window” is enhanced due to the difference between the eletrophoretic mobility of the free analyte and the effective eletrophoretic mobility of the sulfated cyclodextrins. Figure 1 illustrates an idealized comparison of electrophreograms for enantiomeric separations using sulfated cyclodextrins and neutral cyclodextrins. Also note that the difference in the binding constants between the two enantiomers and sulfated cyclodextrin are the most important factor in enantiomeric separations.

The advantages of CE include high efficiency, high resolving power due to its flat flow profile [26], short analysis times, low consumption of chiral selectors and the possibility of using dual chiral selector or pseudophase systems [27-36]. In general, there are two types of separation approaches for enantiomeric separations in CE. One is called direct chiral separation in which reversible diasteroisomeric complexes are formed by weak bonds between the enantiomers and the chiral selectors [37]. The other type is called indirect chiral separation in which enantiomeric separation is based on the formation of stable diastereoisomers using a

chiral optically-pure derivatizing agent [38]. In this review, we only focus on the direct separation method using high-performance capillary electrophoretic separations with sulfated cyclodextrins as the enantioselective complexing agent.

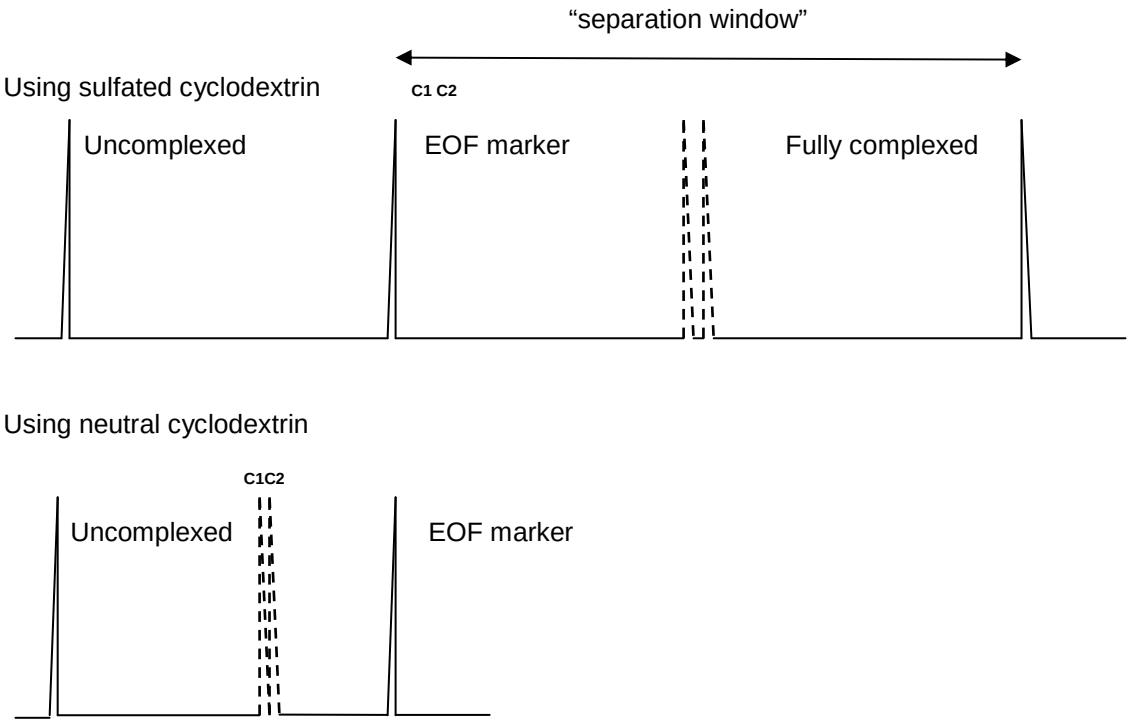


Figure 1. A theoretical comparing the mechanism of enantiomeric separations of cationic analytes using native cyclodextrin and sulfated cyclodextrin. For enantiomeric separation, different association constants between the enantiomers and the cyclodextrin are necessary.

2.2 Introduction to Cyclodextrins and Their Use in CE

Cyclodextrins are cyclic oligosaccharides composed of D-glucopyranose units (Figure 2). There are 3 types of readily available cyclodextrins: α-, β- and γ-CD which have 6, 7 and 8

glucose units, respectively [2,13,17]. Cyclodextrins and their derivatives are the most frequently used chiral selectors in CE [13,39-41], and their range of applications has grown rapidly over the last decade [42]. In aqueous or hydrooragnic solvents, the most common mechanism for enantioresolution with a cyclodextrin involves the inclusion of the analyte into the cavity of the chiral selector and the secondary interactions between the various groups on the rim of the cyclodextrin and the analyte [10-11]. Several favorable features of cyclodextrins make them a common choice as chiral selectors in CE enantiomeric separations. First, they are transparent to UV light. Second, they have good solubility in aqueous solution. Third, they are environmentally friendly and relatively inexpensive. Finally, they have very broad applicability. In general, cyclodextrins can be classified into four different groups: neutral cyclodextrins, positively-charged cyclodextrins, negatively-charged cyclodextrins and amphoteric cyclodextrins. Among these groups, negatively-charged sulfated cyclodextrins exhibit the broadest success in enantiomeric separations.

The hydroxyl groups present on the rim of cyclodextrins can easily be modified by chemical reactions to obtain cyclodextin derivatives with different degrees of substitution. For example, hydroxylpropyl-derivatized β-cyclodextrin is among the most useful uncharged chiral selector for CE. Of course, it can only be used to separate charged enantiomers. Conversely, charged cyclodextrin derivatives can be used to separate both neutral and oppositely-charged enantiomers. Several different charged cyclodextrins are now commercially available and used in CE for enantiomeric separations. A number of review papers document the characteristics and properties of various types of native and charged cyclodextins [2,13,42-45]. In general, negatively-charged cyclodextrins are more useful than positively charged cyclodextrins. For instance, positively-charged cyclodextrins (containing an amine group) tend to adsorb to the wall of the capillary due to electrostatic interaction between the negatively charged silanol groups on the capillary wall and the positively charged amine groups on the cyclodextrins

resulting in reduction or reversal of eletro-osmotic flow (EOF) and possible peak broadening. Sometimes dynamic or static modification of the capillary wall is necessary [46-47]. Conversely, this does not occur when using negatively-charged cyclodextins. Also, the separation window is much smaller when using positively-charged chiral selectors as opposed to the negatively-charged ones. Several theoretical and mathematical models have been developed to describe separations with charged cyclodextrins in CE enantiomeric separations. For instance, Stalcup *et. al.* utilized Wren and Rowe's model to describe the behavior using randomly-substituted charged cyclodextrins in enantiomeric separation [48]. Vigh *et al.* derived a set of mathematical guidelines called "Charged Resolving Agent Migration Model" (CHARM) to optimize enantiomeric separations using negatively-charged single isomeric cyclodextrins [49]. Surapaneni *et al.* employed a combination of charged and neutral cyclodextrins as a dual selector system to develop a theoretical model for separation of enantiomers of neutral species [29]. In this model, neutral cyclodextrins are added to the buffer solution to affect the selectivity of the enantiomers. This results in the reduction of complexation in the free fraction of neutral analyte with the charged cyclodextrins.

2.3 Sulfated Cyclodextrins

Sulfated native cyclodextrins were first introduced as enantioseletive agents in CE in 1995 by Stalcup and co-workers [50-51]. In comparison with neutral cyclodextrins, the solubility of sulfated cyclodextrins in water (>100 g/ 100 mL) is higher than that of neutral cyclodextrins (~14 g /100 mL for α-CD; ~1.8 g/100 mL for β-CD and ~23 g /100 mL for γ-CD), thereby increasing their possible optimization range for use at high concentrations. In CE, these cyclodextrin derivatives possess a permanent negative charge over the entire pH range and are pH-independent. Sulfated cyclodextrins offer not only inclusion complexation interaction, but

also strong electrostatic interaction between negatively-charged sulfated groups and positively-charged analytes (Figure 1 and Introduction). Therefore, they are particularly useful in the enantiomeric separation of cationic species [52-53]. In addition, sulfated cyclodextins can be used in both normal polarity mode (counter-EOF) and reversed polarity mode (co-EOF) in CE. The concentration of sulfated cyclodextrins used in enantioseparation is usually much lower than that of neutral cyclodextrins when used in the normal polarity mode [13,43,51]. Conversely, when used in the reversed polarity mode, the concentration of sulfated cyclodextrins should be increased to generate a negative mobility for analyte-selector complexes [46].

Two terms commonly used in the literature to describe the types of sulfated cyclodextrins include “single isomer” and “random”. “Single isomer” refers to a chiral selector that consists of only one single molecular species with a given degree of substitution, while “random” is used to describe sulfated cyclodextrins randomly sulfated at various hydroxyl groups with different degrees of sulfation. Synthesis of different single isomeric sulfated cyclodextrins and randomly highly-sulfated cyclodextrins have been documented in the literature [47,54-55]. Sulfated cyclodextrins with more than one-half of the possible sites sulfated are referred to “highly sulfated”. Highly-sulfated cyclodextrins with average degrees of sulfation of 11, 12, 13 for α-, β-, γ-CD, respectively, with a narrow range of heterogeneity and low batch-to-batch variations were synthesized and used in CE by Evangelista and Chen of Beckman Coulter [56].

Three hydroxyl sites that could potentially be sulfated on a cyclodextrin (Figure 2) and the potential degree of complete sulfation per cyclodextrin, therefore, are 18, 21 and 24 for α-, β- and γ-CDs, respectively. However, complete sulfation of cyclodextrins is not achievable, probably due to steric hindrance and electrostatic repulsion of the charged groups. Chen *et al.* employed ^{13}C nuclear magnetic resonance (NMR), negative-ion electrospray ionization mass spectrometry (ESI-MS) and indirect CE analysis to characterize highly sulfated cyclodextrins

[55]. Results showed that among the three hydroxyl positions, the C-6 position hydroxyl groups were readily sulfated followed by the C-2 positions and the C-3 positions. The C-6 primary hydroxyls were nearly completely sulfated, the C-2 secondary hydroxyls were more than 70% partially sulfated, and the C-3 secondary hydroxyls remained largely intact. This is probably due to the high reactivity of primary alcohols at the C-6 hydroxyls and the increased acidity of the C-2 secondary hydroxyls compared to the C-3 secondary hydroxyls [57]. These result also showed that HS β-CDs consist of an average of 12.2 sulfates per molecule, as determined by elemental analysis. On the other hand, the ^{13}C NMR indicated 6.7 sulfate esters per HS β-CD molecule at the C-6 position and 5.4 sulfate esters per molecule at the C-2 position. Therefore, the total number of sulfate esters per HS β-CD molecule, 12.1, was comparable to the 12.2 sulfates per molecule obtained by elemental analysis. Furthermore, the sulfation of the C-2 equatorial hydroxyls leaves the regiospecific C-3 hydroxyls available to form hydrogen bonding with guest molecules. These relative positions of the C-2 sulfated and C-3 hydroxyls provide regiospecific interaction with the analytes for enantiomeric separations.

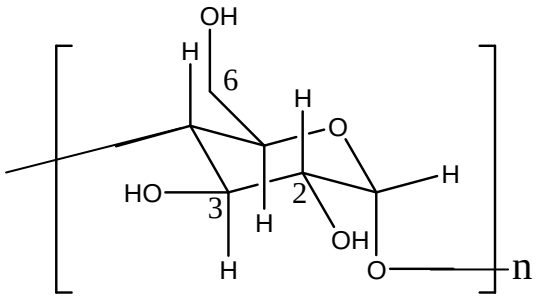


Figure 2. The α-1,4-linked glucose structural unit of cyclodextrin. n = 6 for α-cyclodextrin ; n = 7 for β-cyclodextrin; n = 8 for γ-cyclodextrin.

2.4 Mode of Separations

Enantiomeric separations using sulfated cyclodextrins in CE are commonly accomplished in three different modes: (i) normal polarity mode, (ii) reversed polarity mode and (iii) electro-osmotic flow suppressed mode. In the normal polarity mode, the cathode is located at the detector end of the capillary. Since the EOF velocity is high enough to overcome the countercurrent movement of the analyte–sulfated cyclodextrin complexes, the net movement of both free analytes and analyte-sulfated cyclodextrin complexes move towards the detector (Figure 1). The negative migration of the sulfated cyclodextrins in this mode can increase the "separation window", thereby enhancing the enantioseparation, especially for analytes with a greater affinity for the sulfated cyclodextrins. In the reversed polarity mode, the positions of the anode and the cathode are reversed, relative to the normal polarity mode where the anode is placed at the detector end of the capillary. In this mode, only highly negatively-charged solutes (like highly-sulfated cyclodextrins) can overcome the EOF, thus eventually reaching the detector. Analytes that are extensively associated with sulfated cyclodextrins will move toward the detector and will be eluted first. Analytes that are less strongly associated will migrate at a slower rate since they spend a greater portion of their time as free, uncomplexed species. Non-complexing or weakly complexed analytes would not be detected as they migrate in the opposite direction. In the "suppressed EOF mode", the EOF is greatly suppressed by modification, either statically or dynamically, of the capillary wall. Several polymers have been introduced for coating capillary walls to suppress the EOF in CE [58]. In this mode, analyte-sulfated cyclodextrin complexes possessing strong anionic character would quickly migrate towards the detector located at the anode, thereby producing shorter migration times. Cationic analytes would not be detected as they migrate in the opposite direction.

2.5 Range and Applications and Selectivity

In order to be considered a broadly-useful separation strategy, a chiral selector must be able to separate a wide range of enantiomers. Examples of the range of applications using sulfated cyclodextrins are truly impressive, as indicated in Table 1. Sulfated β-cyclodextrins have been reported to separate a great number of compounds with a wide range of structures. In more recent studies, the number of applications using sulfated α- and γ-cyclodextrins in enantiomeric separation has been increased [54-55,59-61]. Also, most successful enantiomeric separations were performed under acidic conditions (~pH 2.5) using "the suppressed EOF mode" to achieve baseline separations.

The types of separated enantiomers, including those with acidic, basic, neutral and zwitterionic character, encompass a broad range of pharmaceuticals [3,51,62,69], plant extracts [70], alkaloids [71-72], fungicides [73-74] amino acids and derivatives [75-76], metal complexes [77], compounds of geochemical importance and chiral synthetic catalysts [53,78]. For example, all 9 enantiomers of amphetamine-type stimulants were baseline separated within 30 minutes using the reversed EOF separation mode with highly sulfated γ-cyclodextrins by Iwata *et. al.* [60]. Fourteen triazole fungicides were successfully separated using randomly sulfated β-cyclodextrins at pH 3.0 in the reverse polarity mode [74]. Zhou *et al.* employed different single isomer sulfated cyclodextrins and different randomly sulfated cyclodextrins to separate 17 basic drugs. The authors also reported that using randomly sulfated cyclodextrins for enantiomeric separation is superior than using single isomer sulfated cyclodextrins. Baseline separations were obtained for all compounds using either one of the sulfated cyclodextrins under acidic conditions at pH 2.5 [67]. Two alkaloids, Littorine and atropine, were baseline separated within 5 minutes at pH 7.0 and were evaluated the enantiomeric purity using sulfated β-cyclodextrins [70]. Highly sulfated cyclodextrins also have been employed in separation of a chiral synthetic catalyst. Armstrong *et. al.* successfully separated and determined the enantiomeric impurities in

Table 1. Examples of Applications Using Sulfated Cyclodextrins in Capillary Electrophoresis*

Compounds	Selector	Buffer	pH	Single/ Random	Avg. DS	Ref.
Glutethimide N-benzoyl methyl prperazine BMS compounds	Highly Sulfated β-CDs Heptakis (6-sulfato) β-CDs	Phosphate-triethanolamine	2.5	R S	7-10 7	3
Pharmaceutical drugs	Sulfated β-CDs	Phosphate	2.5	R	12	62
Butorphanol Cycloamine	Highly sulfated γ-CDs	TRIS-phosphate	2.0-4.0	R	NG	78
Aromatic amino acids	Highly Sulfated α-CDs Highly Sulfated β-CDs Highly Sulfated γ-CDs	Phosphate-triethanolamine	2.5	R	NG	75
14 Triazole fungicides	Highly Sulfated β-CDs	Phosphate	3.0	R	7-11	76
Cyanobenz[f]isoindole amino acids	Highly Sulfated β-CDs	Phosphate	2.0	R	NG	77
Indan, Tetralin, Benzosuberan derivatives	Sulfated β-CDs with γ-CDs	Phosphate	3.1-7.0	R	4	30
6 neutral, cyclic and bicyclic monoterpenes	Sulfated β-CDs with γ-CDs	Phosphate	3.3	R	4	31
Methadone Fluoxetine Venlafaxine Tramadol	Highly Sulfated γ-CDs	Ammonium formate	2.5	R	NG	59
Ruthenium (II) polypyridyl Complexes	Highly sulfated γ-CDs Highly sulfated β-CDs	phosphate	7.5	R R	14 7-11	53
Amino acids derivatives Phenylamines	Highly Sulfated α-CDs Highly Sulfated β-CDs Highly Sulfated γ-CDs	Phosphate-triethanol-amine	2.5	R	NG	88

Table 1 – *Continued.*

Compounds	Selector	Buffer	pH	Single/ Random	Avg. DS	Ref.
Tertrahydronapthalene derivatives	Highly Sulfated α-CDs Highly Sulfated β-CDs Highly Sulfated γ-CDs	Phosphate-triethanolamine	2.5	R	NG	61
7 Antihistamines, 2 β-blockers, 2 antimalarials, 3 β-agonsits	Sulfated CDs Heptakis-6-sulfato-β-CDs	phosphate	2.5	R S	7-11 NG	63
16 pharmaceutical drugs	Sulfated β-CDs Sulfated γ-CDs Heptakis(2,3-dimethyl-6-sulfato)-β-CDs Heptakis(2,3-diacetyl-6-sulfato)-β-CDs Heptakis-6-sulfato-β-CDs Octakis(2,3-diacetyl-6-sulfato)-γ-CDs	phosphate	5.0	R R S S S S	7-11 NG 4-5 4-5 4-5 4-5	67
5 antihistamines & antimalarials	Sulfated β-CDs	Phosphate	3.8	R	NG	64
25 basic pharmaceutical drugs	Highly sulfated α-CDs Highly sulfated α-CDs Highly sulfated α-CDs with neutral CDs	Phosphate-triethylamine	2.5	R	NG	35
Eticlopride Sulpiride	Sulfated β-CDs	Citrate	2.9	R	7-11	65
Glutethimide	Sulfobutyl-β-CDs	phosphate	5.0	R	4	34
E-6006 antidepressant	Highly Sulfated α-CDs Highly Sulfated β-CDs Highly Sulfated γ-CDs	Phosphate-triethanolamine	3.0	R	11 7-11 13	86
4 pheniramine derivatives chloroquine	Sulfated β-CDs	Phosphate	3.8	R	7-10	32
Terbutaline	Sulfated β-CDs	Phosphate	3.0	R	7-11	48

Table 1 – *Continued.*

Compounds	Selector	Buffer	pH	Single/ Random	Avg. DS	Ref.
25 basic, acid and neutral pharmaceutical compounds	Highly Sulfated α-, β-, γ- CDs with neutral CDs	Phosphate-triethanolamine	2.5	R	NG	35
15 basic pharmaceutical compounds	Neutral CDs with Sulfated β-CDs	Phosphate-triethylamine	3.0	R	NG	36
Littorine enantiomers, Atropine enantiomers Alkaloids	Sulfated β-CDs	Phosphate	7.0	R	13-16	70
Butorphanol Cycloamine	Highly Sulfated γ-CDs	Tris-phosphate	2.0-4.0	R	NG	83
9 amphetamine-type stimulants	Highly sulfated γ-CDs or with neutral β-CDs	phosphate	2.6	R	NG	60
Aminoindanol	Sulfated β-CDs Highly Sulfated β-CDs Single Isomer Sulfated β-CDs	TRIS-phosphate/ Phosphate-triethylamine	2.5	R S	4 7-10 NG	82
Triadimefon Triadimenol	Sulfated β-CDs	Phosphate	2.5	R	7-11	73
3 benzoin and its derivatives	Sulfated β-CDs with neutral CDs	Borate / Phosphate	9.0	R	NG	33
4 alkaloids	Sulfated β-CDs Heptakis(2,3-di-*O*-diacetyl-6-*O*-sulfo)- β-CDs Heptakis(2,3-di-*O*-dimethyl-6-*O*-sulfo)-β-CDs	Sodium tetraborate	9.2	R S S	NG NG NG	71

* Avg. DS indicates the average of degree of sulfation substitution; NG: Not given; single/random represents whether the selector is randomly sulfated (R) or single isomer (S); Ref. shows the cited reference number. TRIS: Tris(hydroxymethyl)aminomethane

chiral catalysts, auxiliaries and synthons using sulfated β-cyclodextrins [78]. Recently, we have also successfully separated 7 ruthenium polypyridyl complexes using sulfated β-, γ-cyclodextrins and other cyclodextrin derivatives using capillary zone electrophoresis (CZE) mode and micellar capillary electrophoresis (MCE) (Figure 3) [53]. Baseline separation were obtained for the complexes using either one of the cyclodextrin derivatives.

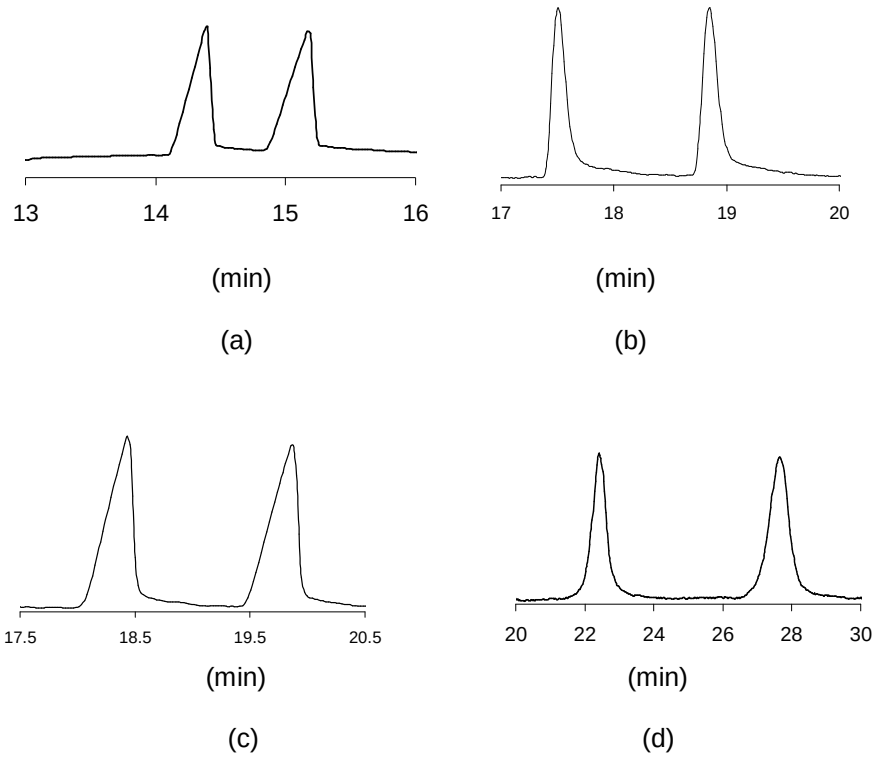


Figure 3. Representative electropherograms of ruthenium (II) complexes. Separation condition: 10 -30 mg/mL of sulfated γ-CD (DS=14), 60mM phosphate buffer at pH 7.5, 5kV, UV detection at 214nm. a) ruthenium (II) tris-phenanthroline, b) ruthenium (II) nitrophendiphenanthroline, c) ruthenium (II) aminophendiphenanthroline, d) ruthenium (II) phendionediphenanthroline.

The broad enantiorecognition capabilities of sulfated cyclodextins with different degree of sulfation are also shown in Table 1. According to these studies, degrees of sulfation between seven and eleven for sulfated cyclodextrins are optimal for separating a large number of structurally-diverse compounds. In a further extension of the application range, separations using non-aqueous mobile phases, such as methanol or formamide, with sulfated cyclodextrins also were reported [79]. Wren and Khaledi showed that the ion-pair effect between the charged cyclodextrins and the oppsitely-charged analyte were even stronger in non-aqueous media than in aqueous solutions. Consequently, a lower concentration of charged cyclodextrins can be used to achieve baseline separations.

In addition to the use of a single type of additive in CE enantiomeric separations, several studies also reported successful separations using multiselector combination systems including sulfated cyclodextrins with other selectors [30-36]. For instance, 25 basic compounds were separated using a combination of highly sulfated cyclodextrins plus neutral cyclodextrins in a dual selector based system (89% of which were better separated with a dual system than with a single highly sulfated cyclodextrin system) [35]. Nishi *et al.* used a dual cyclodextrin system to separate 11 drug analytes with best resolution and determine 0.5 % of the minor enantiomer [36]. Successful enantioseparation of a wide variety of neutral and anionic derivatives of indan, tetralin and benzosuberan, as well as monoterpenes, using the reversed polarity mode with a mixture of sulfated β-cycldoextrins and neutral native γ-cyclodextrins or hydroxylpropyl β-cyclodextrins was demonstrated by Armstrong and co-workers [30-31].

Cyclodextrin-modified micellar capillary electrophoresis (CD-MCE) is an alternative method for enantiomeric separations using achiral micelles (for example, SDS, CTAB or Brij35) with native or charged cyclodextins as a two-pseudophase system (Figure 4). Since micelles can increase the solubility of hydrophobic compounds in aqueous media, this method is particularly useful for separations of less water-soluble analytes. The use of sulfated

cyclodextins in CD-MCE technique for enantioseparations and enantiopurity tests have been reported [9,71,80]. For instance, Bitar *el. al.* used sulfated β-cyclodextrins with SDS to separate 4 different alkaloids within 7 minutes [71]. Four imidazole derivatives were successfully baseline separated using highly sulfated cyclodextrins at acidic condition in CD-MCE mode by Vaccher and co-workers as well. All separations were obtained in less than 6 minutes [80].

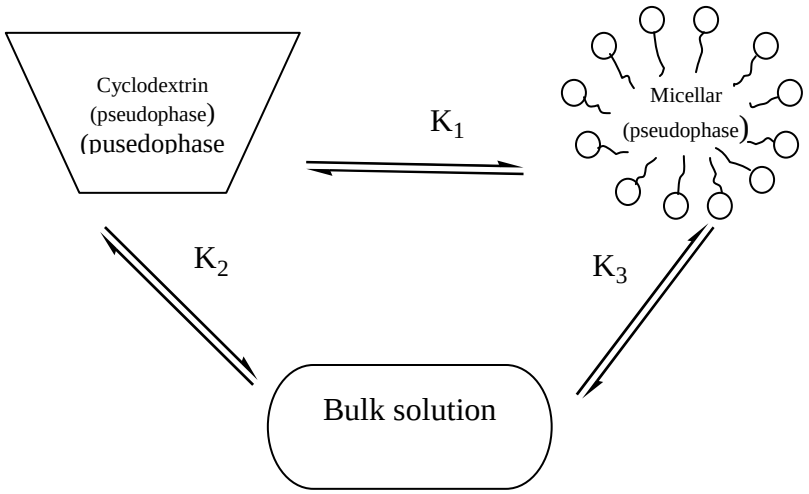


Figure 4. Representation of the three phase model in the CD-MCE mode. "K" is the binding or partition constant of a solute between the indicated phases.

2.6 Rapid Screening and Enantiopurity Determination

Sulfated cyclodextins have been used for rapid analyte screening and enantiopurity applications in the pharmaceutical industry. Rapid analyte screening requires both applicability to a broad range of analyte structures and fast separation times. These modified cyclodextrins successfully separated 131 out of 135 drugs and nearly all of the reported separations were accomplished in less than 20 minutes [81]. Massart *et al.* used highly sulfated cyclodextrins to

screen two series of phenylamines and amino acid derivatives. They were able to baseline separate 62 out of 67 compounds under acidic condition at pH 2.5 using sulfated α-, β- and γ-cyclodextins in the reversed polarity mode. Good separations were obtained for most compounds with separation times of less than 10 minutes [68]. The results also showed that highly sulfated cyclodextrins do not always follow the classic rules of enantiomeric separations using native CDs: α-CDs are used for enantioseparations of non-aromatic or one aromatic ring compounds; β-CDs are used for enantioseparations of one substituted ring or 2 aromatic ring compounds and γ-CDs are used for enantioseparations of 2 or more aromatic ring compounds [10-12,17]. In this study, for instance, most phenylamines contained one aromatic ring. Highly sulfated-γ-cyclodextrins produced the best separations (54% of the total compounds) followed by highly sulfated-β-cyclodextrins (33% of total separation). This provides some circumstantial evidence that an inclusion complex between the analyte and cyclodextrins may not always be required, especially when strong electrostatic interactions are dominant.

Enantiopurity determinations by CE require the separation of small impurities from the major enantiomer. The concentration of the major enantiomer can be a hundred- to a thousand-times higher than that of the impurity. As a result, the separation method should accommodate both high and low sample loading and provide high resolution capabilities. Several articles reported that CE using sulfated cyclodextrins were optimal for meeting these requirements [82-83]. In the clinical environment, enantiopurity determinations play an important role, especially for *in vitro* and *in vivo* determination of inversion between enantiomers. For example, Ensing *et al.* used sulfated β-cyclodextrins to determine the *in vivo* enantioconversion of the anti-microbial agent, ofloxacin. 2μg/mL of the R-(+)-enantiomer of ofloxacin was separated and quantified in 100 μg/mL of the S-(-)-enantiomer of ofloxacin in urine [69]. Heyden *et al.* used highly sulfated cyclodextrins to develop a mathematical resolution (Rs) for determining distomers (relative to the presence of 99% eutomers) at the 0.1% impurity level in four basic drugs (propranolol,

atenolol, chropheniramine and tryptophan) [84]. Peak asymmetry, efficiency and peak and height ratios between the eutomer and the distomer were taken into an account for calculation of Rs. The authors suggested that Rs = 3.0 should be used instead of the classic Rs = 1.5 in cases where overloading leads to asymmetric peaks.

2.7 Robustness of Separation

Variations in enantiomeric separations may occur when a method is performed under different conditions. These may include measurements conducted on different days, with different instrument types, by different analysts, and in different laboratory locations. The degree of sulfation of the cyclodextrin may also has a significant effect on separation robustness. Due to the large number of possible homologous isomers in randomly sulfated cyclodextrins, batch-to-batch variations can yield different selectivities. Therefore, it is necessary to assess the robustness of an enantiomeric separation method using sulfated cyclodextrins by investigating a large number of variables.

Heyden *et al.* performed a robustness study for enantiomeric separations using highly sulfated cyclodextins in phosphate buffer at a pH 2.5 on a basic (propranolol), a neutral (praziquantel), and an acidic (warfarin) drug [85]. Eight factors were tested and studied on the effect of separation robustness: the highly sulfated cyclodextrin concentration; the pH of the buffer; the buffer concentration; the separation temperature; the applied voltage; the injection time; and the rinse volume of the separation electrolyte. The result showed that the separation was influenced more by the separation temperature, than by the highly sulfated cyclodextin concentration. Also, the pH should be strictly controlled, especially for the separation of acidic compounds. Very good precision was found using 4 different batches of highly sulfated cyclodextrins. Precision values for propanlol, praziquantel and warfarin were 1.3% RSD, 4.9%

RSD and 1.2% RSD, respectively. Precision values in resolution for these drugs ranged from 1.5% to 3.8% RSD. For batch-to-batch variations, less than 5% variations were obtained for migration times, selectivity, resolutions and peak areas.

In another study, the enantiomers of a novel antidepressant, E-6606, were successfully separated using sulfated β-cyclodextrins [86]. Precision values of the intraday operation for migration times were less than 0.6% RSD while the peak area was less than 3.3% RSD. A comparison of migration times and selectivities using different batches of sulfated β-cyclodextrins indicated no significant effect in method applicability with changes in the selector batch.

Due to their minimal lot-to-lot variations and heterogeneity in interactions, initial chemical intuition led to the conclusion that single isomer sulfated-cyclodextrins provided more rugged separation selectivity than randomly-sulfated cyclodextrins; however, more recent studies suggest that substitutional heterogeneity of randomly sulfated cyclodextrins do not significantly affect separation robustness due to the minimal substitutional variations. Under identical operational conditions, randomly-sulfated cyclodextrins were shown to be superior to the single isomer sulfated cyclodextrin system [61,67,87]. Indeed, direct lot-to-lot variations can be assessed by comparisons of migration times. Remarkably good enantiomeric reproducibilities of 0.3% and 1.3% RSD using sulfated β-cyclodextrin to separate several basic pharmaceutical compounds was reported by Zhou and co-workers [67], and 0.6% and 1.2% RSD using sulfated α-cyclodetrin to separate M3 antagonists was reported by Song *et. al*. [88]. These results are consistent with the minimal variations for direct measurement of batch-to-batch variation in substitutional heterogeneity [56].

2.8 Separation Strategy

Several publications have addressed the development of strategies for enantiomeric separations using charged cyclodextrins, in particular, sulfated β-cyclodextrins [67,89-90]. Below is a summary of separation strategies and the limitations [46] for enantiomeric analysis in CE using highly sulfated cyclodextrins as indicated in the literature.

- Randomly sulfated cyclodextrins with high degree of sulfation (11-13) is recommended.
- The reversed polarity mode with the anode at the detector end should be used. However, high cyclodextrin concentrations may be needed to generate a negative mobility for charged analyte-cyclodextrin complexes.
- Low voltage should be applied when using higher sulfated cyclodextrin concentrations to reduce the generation of high current and Joule heating in the capillary. Also, higher cyclodextrin concentrations may lead to electrodispersion, resulting in band broadening.
- Using a low pH background electrolyte buffer to minimize EOF results in analytes migrating towards the anode, primarily due to interactions with the sulfated cyclodextrins.

In addition, we have two additional recommendations.

- If high concentrations of sulfated cyclodextrins are detrimental to a separation or produce undesirable results, use lower concentrations and the normal polarity mode.
- If neither separation mode produces a sufficient separation, add neutral cyclodextrins or micelles to the run buffer as well (Figure 4).

2.9 Conclusions

Sulfated cyclodextrins demonstrate successful enantioseparation of compounds with a wide application range. Good precision, high selectivity for different compounds, and good reproducibility of the separations using different batches makes this type of cyclodextrin

derivative a good choice for screening new chiral compounds. The combination of sulfated cyclodextrins with high efficiency CE provides a versatile and alternative technique for enantiomeric separations. With this review, we hope to provide the background information needed to assist the analyst in the development of this powerful analytical approach and to provide a more universal, comprehensive separation strategy for future enantiomeric separations.

CHAPTER 3

STUDY OF A NEW CHIRAL SELECTOR FOR CAPILLARY ELECTROPHORESIS: SODIUM ARSENYL-(L)-(+)-TARTRATE

3.1 Abstract

Sodium arsenyl (L)-(+) tartrate ($Na_2[As_2(+)\text{-}tart_2]\cdot 3H_2O$) was examined and evaluated as a chiral selector using capillary electrophoresis. This chiral selector showed enantioselective associations with many cationic analytes, including primary, secondary, and tertiary amines. Also, baseline separations of ruthenium (II) polypyridyl complexes were achieved within 10 minutes. The effect of buffer type, chiral selector concentration, voltage applied, buffer concentration, buffer pH and organic modifier concentration were examined and optimized.

3.2 Introduction

Capillary electrophoresis (CE) has been found to be an effective and efficient alternative to chromatography for analytical enantiomeric separations over the last few decades due to its several known advantages such as short analysis time, high efficiency, low sample consumption, and simple instrumentation [89,91-96]. Unlike HPLC, the most common approach for enantiomeric separation in CE involves the addition of one or more chiral selectors into the run buffer. In spite of a vast number of chiral selectors reported in the literature for enantiomeric separations, only a few classes have been successfully used in CE because of some inherent requirements for CE chiral selectors: high water solubility; high stability in aqueous medium; and low UV absorptivity. Indeed, the dominant chiral selectors in CE are cyclodextrins and their derivatives, many of which were originally developed for HPLC and thin layer chromatography

[10-12,17]. The demand for and continuous exploration of new chiral selectors, however, is necessary due to the increase of structural complexity of new synthetic chiral molecules.

Several tartrate-based compounds have been employed as chiral selectors for enantiomeric separations with marginal success. For example, L-n-octyl tartrate was reported to separate propranolol enantiomers using an indirect chiral separation [97]. Enantiomeric separations of aminoalcohols, amines and alkyl tropate were reported using (2R,3R)-di-n-butyl tartrate and (2R,3R)-di-n-propyl tartrate in HPLC [98-99]. Sodium-(S)-(+)-tartrate was used as a run buffer additive in CE to separate several cobalt (III) ethylenediamine complexes [100]. Molecular recognition of metal tartrates in solution phase and gas phase using mass spectrometry were also reported [101]. Recently, potassium antimony-d-tartrate and dibenzoyl-L-tartrate have been reported to separate enantiomers of several metal complexes including Ru, Cr, Ni, Co and Fe [102]. However, the resolution and efficiency of these metal complex separations were not reported. In this work, we introduce sodium arsenyl (L)-(+)-tartrate as a new chiral selector for CE.

A member of tartrate-based transition metal complexes, sodium arsenyl (L)-(+) tartrate ($Na_2[As_2(+)\text{-}tart_2]\cdot 3H_2O$), (subsequently refered to as arsenyl tartrate) is a tartrato (4-)-bridged binuclear, metal tartrate-based compound (Figure 5). It was previously reported for diastereoselective precipitations of chiral ruthenium racemic complexes [103]. This metal tartrate-based compound has 4 stereogenic centers located at the only carbon atom that also have a hydrogen attached. In agreement with X-ray crystallographic analysis of the molecular structure of arsenyl tartrate [104-105], the modeling study shows that the tartrate compound is highly hydrophilic with all oxygen atoms located around the surface of the molecule to form a rugby-ball-shaped complex with no readily accessible cavity. (Figure 6a) The mean distance between the arsenic atom and the oxygen (CO-) is typically about 1.8Å, while the mean

distance between the arsenic atom and the oxygen (COO-) is about 2.04Å. The distance between the two arsenic atoms is about 4.6Å. (Figure 6b). The arsenyl tartrate molecule is negatively- charged and only stable at pHs 5 or above since this molecule slowly decomposes in an acidic environment. [106] A UV absorption spectrum of arsenyl tartrate in aqueous solution at pH 8.0 was obtained. (Figure 7) It shows that no substantial UV absorption at wavelengths of 230nm or above. To our knowledge, there has been no reported use of arsenyl tartrate as a chiral selector in capillary electrophoresis.

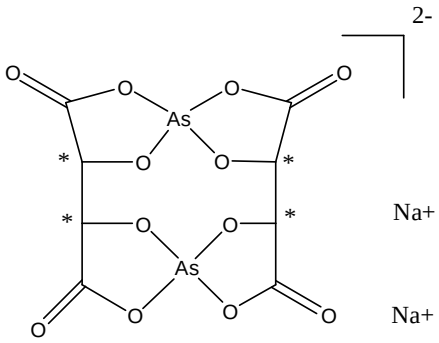


Figure 5. Sodium arsenyl (L)-(+)-tartrate. Stereogenic centeres are marked with an asterisk.

(a) (b)

Figure 6. Structure of sodium arsenyl (L)-(+) tartrate. (a) Space-filling molecular model, (b) stick capped model. Color denotation: red-oxygen; pink-arsenic; black-carbon; white-hydrogen.

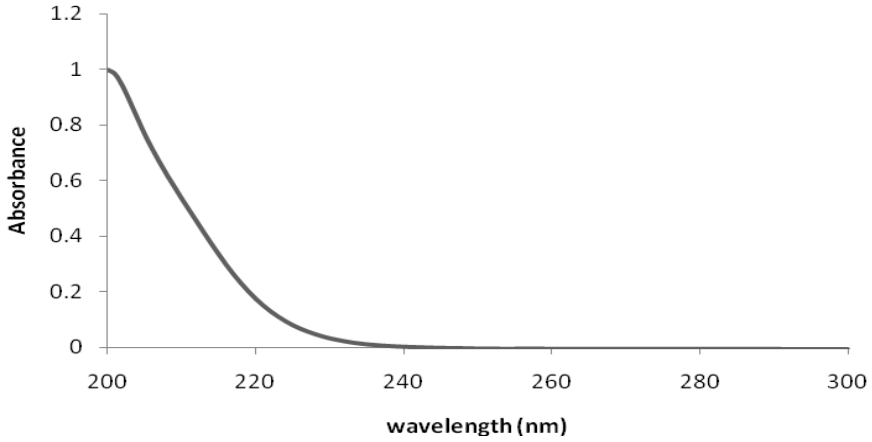


Figure 7. UV spectrum of arsenyl tartrate at Tris buffer pH 8.0

3.3 Materials and Methods

3.3.1 Materials

Sodium arsenyl (L)-(+) tartrate was synthesized as previously reported [107]. All ruthenium (II) polypyridyl complexes were synthesized as previously reported and their structures were confirmed by H-NMR [108-112]. Phosphoric acid, sodium hydroxide, HPLC grade methanol, sodium phosphate and sodium carbonate were all purchased from Fisher Scientific (St. Louis, MO, USA). Tris(hydroxylmethyl)aminomethane was acquired from Aldrich (Milwaukee, WI, USA). The fused-silica capillaries were obtained from Polymicro technologies (Phoenix, AZ, USA).

3.3.2 Methods

All separations were performed on a Beckman Coulter P/ACE MDQ system capillary electrophoresis equipped with a photodiode array detector. The capillary used for all separations was 50 µm i.d. x 358 o.d. with a total length of 30 cm (20 cm from inlet to detection

window). The capillary was maintained at a temperature of 25˚C. Tris(hydroxylmethyl)-aminomethane was dissolved in denionized water and adjusted to the desired pH with hydrochloric acid or sodium hydroxide as the background buffer. An organic modifier was added, based on volume percentage, prior to the addition of chiral selectors. Chiral selectors were then added into the buffer solution as running buffer. Racemic samples or artificial mixtures of enantiomers were dissolved in the background buffer to make sample solutions. All the electropherograms were obtained with detection at 214 nm in a normal polarity mode. All data were analyzed with Beckman System Gold Software.

New capillaries were initially conditioned with the following rinses: 1M sodium hydroxide and deionized water each for 5 min. Before each run, the capillaries were washed with 1M sodium hydroxide, deionized water and running buffer each for 1 min. The sample solution was then injected hydrodynamically at 0.5 psi for 5 seconds.

The parameters were calculated as follows: Resolution (Rs) =2(tm2-tm1)/(w1+w2), the number of theoretic plates (N) = $16*(tm1/w1)^2$, selectivity (α)= tm2/tm1 where tm1 and tm2 are the migration times of the first and second peaks, and w1 and w2 are the extrapolated peak widths at the baseline.

3.3.3 Synthesis of Sodium Arsenyl-(L)-(+)-Tartrate

The procedure for the synthesis of arsenyl tartrate was previously reported [107]. In brief, L(+)-tartaric acid (20 g, 0.133 mol) and NaOH (5.33 g, 0.133mol) were dissolved in water (150mL), and the solution was heated to reflux. As_2O_3 (13.1 g, 0.066 mol) was added and the resulting slurry refluxed for 45 min until the solution became clear. The solution then was filtered and 300 mL ethanol was added to the filtrate, which resulted in some precipitation. The resulting mixture was then cooled to 4^0C for 12 h, upon which a large mass of white crystals formed. The crystals were isolated by filtration and washed with cold ethanol and air-dried.

3.4 Results and Discussion

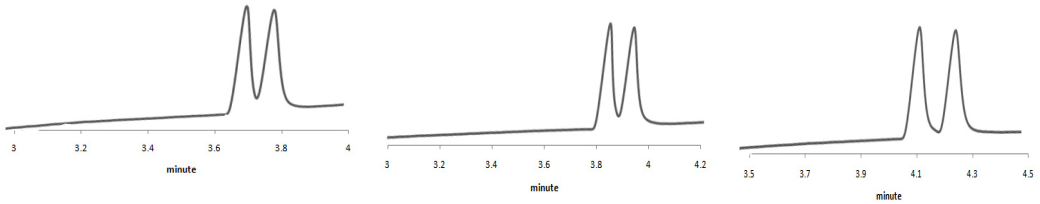
3.4.1 Factors Affecting Enantioseparations

Buffer type, buffer pHs and concentrations, chiral selector concentration addition of organic modifiers, variations of applied voltages are common factors that are varied to optimize the enantiomeric separations in capillary electrophoresis [13,53,113-115].

The buffer controls the ionic strength of the solution, stabilizes the current, controls pH, maintains the EOF and also modifies the interaction between chiral selectors and analytes. [13,116]. Three different types of buffers were studied using racemic mianserin as a test analyte and the electropherograms are shown in Figure 8. Tris(hydroxylmethyl)aminomethane buffer, overall, provides the best enantiomeric separation with baseline resolution in a reasonable time. Therefore it was used for the rest of this study. High buffer concentrations might inhibit the electrostatic interactions that contribute to the association between the analytes and the chiral selectors. Table 2 summarizes the effect of different concentrations of Tris buffer. The results show that high buffer concentrations hurt the enantioresolution and produce longer migration times. This finding indicates the importance of electrostatic interactions for enantioseparation with arsenyl tartrate. Figure 5 shows the electropherograms of the enantiomeric separations of mianserin at different pHs. Baseline separations were obtained at pHs 8.02 or below due to the fact that the lower the pHs slow the EOFs, which in turn improves selectivity and enhance enantioresolution.

Varying chiral selector concentrations have been reported to be an effective way to improve enantioresolution [117-118]. The separations of brompheniramine at four different arsenyl tartrate concentrations (while the other conditions were kept the same) are summarized in Table 3. Baseline separation was obtained at 70mg/mL of arsenyl tartrate concentration. The enantioresolutions were improved and the migration times increased with an increase in arsenyl

tartrate concentration. This is due to the fact that when the chiral selector is increased; the interaction between the analyte and the chiral selectors was increased, thus improving



(a) (b) (c)

Figure 8. The effect of different buffer types on enantioseparation of mianserin. Condition: Arsenyl tartrate Conc.: 30mg/mL; 30cm capillary (20cm to the detector) with 50 µm I.D capillary; +15 kV; Detection at 214nm; a) 30mM sodium phosphate buffer at pH 8.02; b) 30mM sodium carbonate buffer at pH 8.02; c) 30mM Tris buffer at pH 8.02.

Table 2. The Effect of Different Buffer Concentration on Enantioseparation of Pheniramine.

Tris buffer conc. (mM) at pH 8.02	Tm_1	Tm_2	W_1	W_2	Rs	N	α
5	12.53	12.95	0.32	0.3	1.4	24500	1.03
35	12.92	13.33	0.35	0.4	1.1	21800	1.03
55	13.09	13.42	0.35	0.35	0.9	22400	1.03
75	12.82	13.13	0.33	0.4	0.8	24100	1.02

Condition: Arsenyl tartrate Conc.: 40mg/mL; 30cm capillary (20cm to the detector) with 50 µm I.D capillary; +6 kV; Detection at 214nm; Tm_1 and Tm_2: migration time of peak 1 and peak 2 respectively, W_1 and W_2: peak width of peak 1 and peak 2 respectively; Rs: separation resolution; N: number of theoretical plates; α: selectivity.

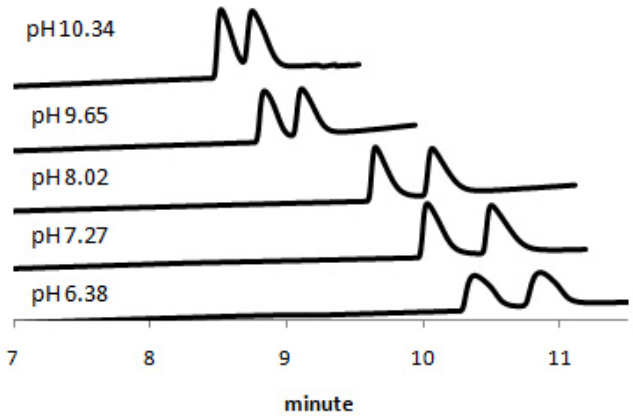


Figure 9. The effect of buffer pHs on enantioseparation of mianserin. Condition: Arsenyl tartrate Conc.: 30mg/mL; 30cm capillary (20cm to the detector) with 50 µm I.D capillary; +8 kV; buffer : 50mM Tris; Detection at 214nm.

Table 3. The Effect of Arsenyl Tartrate Concentration on Enantioseparation of Brompheniramine.

Arsenyl tartrate conc. (mg/mL)	T_1	T_2	W_1	W_2	Rs	N	α
10	(NO SEPARATION)						
40	8.95	9.25	0.28	0.35	1.0	16300	1.034
70	13.50	14.30	0.47	0.50	1.6	13200	1.059
110	21.75	24.23	0.95	0.90	2.7	8400	1.114

Condition: Buffer: 30mM Tris at pH 8.02; 30cm capillary (20cm to the detector) with 50 µm I.D capillary; +8 kV; Detection at 214nm; Tm_1 and Tm_2: migration time of peak 1 and peak 2 respectively, W_1 and W_2: peak width of peak 1 and peak 2 respectively; Rs: separation resolution; N: number of theoretical plates; α: selectivity.

enantioresolution. Furthermore, increasing the chiral selector concentration also increase the ionic strength and the viscosity of the run buffer, which, in turn, contributes to longer migration times.

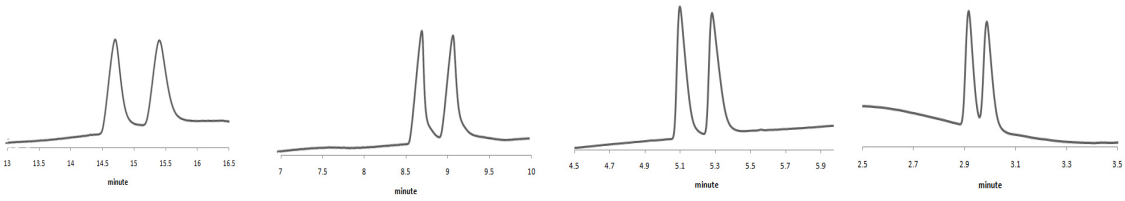
Addition of organic modifiers to the running buffer not only increases the solubility of hydrophobic analytes but also slows the EOF and suppresses the joule heating by lowering the current. [51,119]. These factors can improve enantiomeric separation. Upon the addition of methanol, the EOF decreased, causing the resolution to increase with longer migration times (see Table 4). In this study, 10% (v/v) of methanol was added into the run buffer in order to increase the solubility of the analytes.

Table 4. The Effect of Addition of Methanol on Enantioseparation of Trimipramine

Methanol % (v/v)	T_1	T_2	W_1	W_2	Rs	N	α
0	5.75	6.04	0.15	0.21	1.6	23500	1.05
10	7.15	7.55	0.2	0.2	2.0	20500	1.06
20	8.92	9.54	0.26	0.24	2.5	18800	1.07
30	10.72	11.74	0.32	0.3	3.3	18000	1.10
40	12.45	13.73	0.38	0.33	3.6	17200	1.10

Condition: Buffer: 50mM Tris at pH 8.02; 30cm capillary (20cm to the detector) with 50 µm I.D capillary; +15 kV; Detection at 214nm; Tm_1 and Tm_2: migration time of peak 1 and peak 2 respectively, W_1 and W_2: peak width of peak 1 and peak 2 respectively; Rs: separation resolution; N: number of theoretical plates; α: selectivity.

The effect of applied voltage also was studied using racemic tetrahydrozoline as an example. Figure 10 shows the electropherograms of the enantioseparations with varying applied voltages. As the voltage increased, the migration times and resolutions both decreased. Increases the applied voltages greatly increase the Joule heating in the capillary, which, in turn, causes faster EOF, hurting the enantioseparations.



(a) (b) (c) (d)

Figure 10. The effect of applied voltages on enantioseparation of tetrahydrozoline. Condition: Arsenyl tartrate Conc.: 50mg/mL; 30cm capillary (20cm to the detector) with 50 µm I.D capillary; Buffer: 50mM Tris at pH 8.02; Detection at 214nm; a) +5kV; b) +8kV; c) +12kV; d) +17kV.

3.4.2 Overview of Enantioseparation Results

Twenty-six amine-containing compounds showed enantioselectivity within reasonable time. Thirteen of them were baseline separated. All results are summarized in Table 5. In this study, all separations were run at pH 8.02 where the arsenyl tartrate (the chiral selector) was negatively-charged and where all amine-containing analytes were positively-charged. This is based on the fact that charged chiral selectors often produce the best resolving power with analytes of the opposite charge due to strong electrostatic interactions between analyte-selector complexes, as well as their countercurrent migrations, thus enhancing the selectivity factor [1,49]. Compared to separations that use anionic cyclodextrin chiral selectors, the migration times were similar, while the charged cyclodextrins, overall, showed better enantioseparations

Table 5. Experimental Data for Enantiomeric Separations of Amine-containing Compounds Using Sodium Arsenyl-(L)-(+)-tartrate.

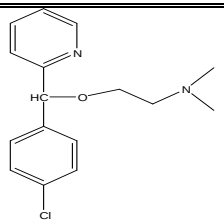
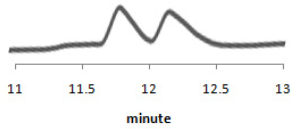
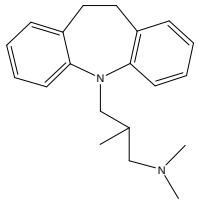
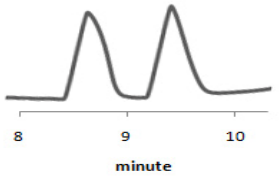
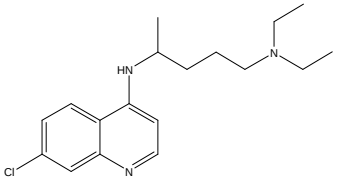
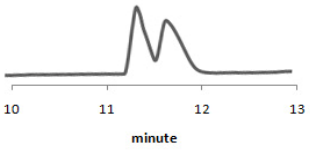
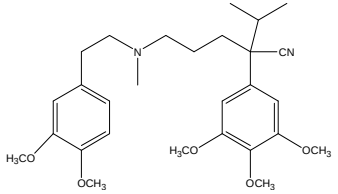
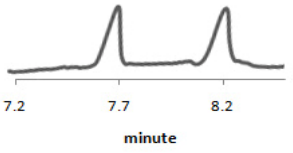
#	Structure and Name of analyte	Arsensyl tartrate Concentration (mg/mL)	Tm1 (min)	Tm2 (min)	Rs	N	α	Electropherogram
1	 Carbinoxamine	100	11.82	12.28	1.5	24800	1.04	
2	 Trimipramine	60	8.73	9.54	2.0	7600	1.10	
3	 Chloroquine	100	11.33	11.64	0.8	12800	1.03	
4	 Methoxyverapamil	60	7.64	8.23	4.0	41500	1.08	

Table 5 – *Continued.*

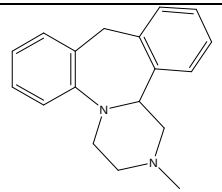
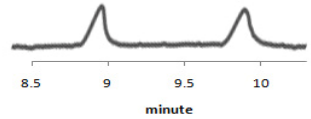
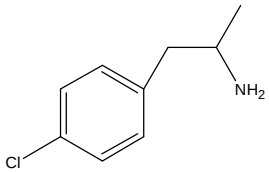
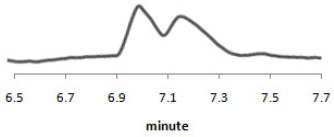
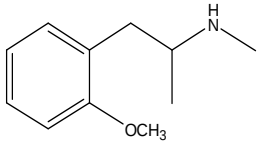
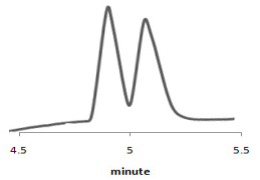
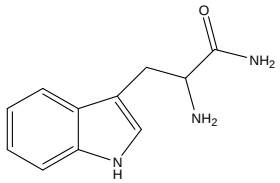
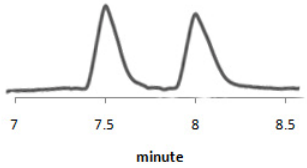
#	Structure and Name of analyte	Arsensyl tartrate Concentration (mg/mL)	Tm1 (min)	Tm2 (min)	Rs	N	α	Electropherogram
5	 Mianserin	60	8.94	9.92	4.8	20500	1.11	
6	 Chloroamphetamine	120	6.97	7.16	0.6	12000	1.03	
7	 Methoxyphenamine	60	4.92	5.17	1.1	6200	1.05	
8	 Tryptophanamide	60	7.48	8.03	1.8	22400	1.07	

Table 5 – *Continued.*

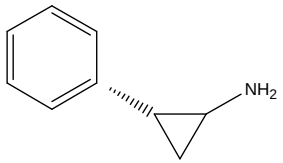
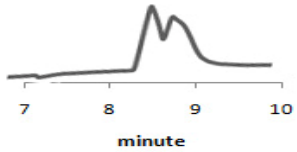
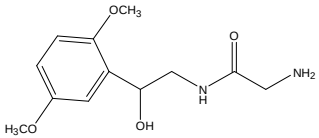
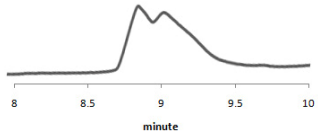
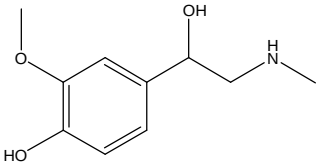
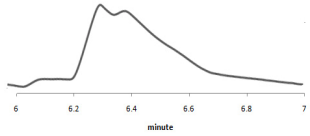
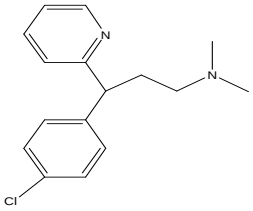
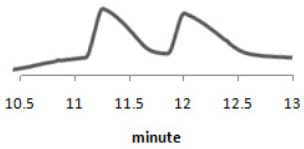
#	Structure and Name of analyte	Arsensyl tartrate Concentration (mg/mL)	Tm1 (min)	Tm2 (min)	Rs	N	α	Electropherogram
9	 Tranylcypromine	100	8.62	8.84	0.5	13000	1.03	
10	 Midodrine	100	8.87	9.18	0.3	8300	1.03	
11	 Metanephrine	60	6.33	6.43	0.3	6400	1.02	
12	 Chlorpheniramine	50	11.38	12.23	1.6	13000	1.07	

Table 5 – *Continued.*

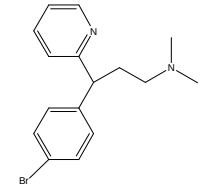
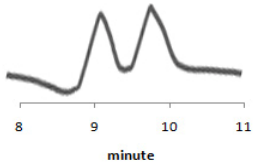
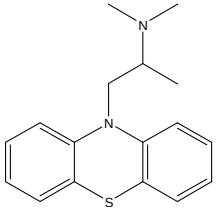
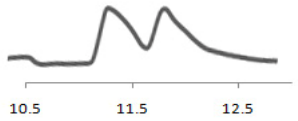
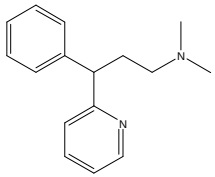
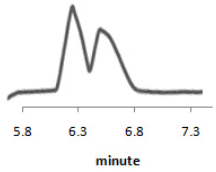
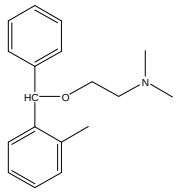
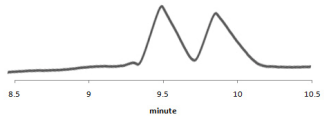
#	Structure and Name of analyte	Arsensyl tartrate Concentration (mg/mL)	Tm1 (min)	Tm2 (min)	Rs	N	α	Electropherogram
13	 Brompheniramine	100	9.08	9.89	1.6	13000	1.09	
14	 Promethazine	100	11.32	11.84	1.0	12000	1.05	
15	 Pheniramine	100	6.23	6.62	0.9	6900	1.06	
16	 Orphenadrine	100	9.44	9.95	1.5	15000	1.04	

Table 5 – *Continued.*

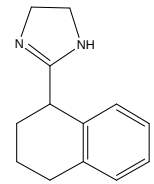
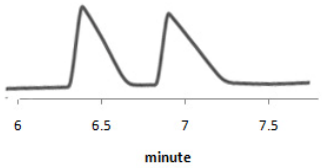
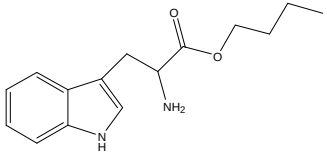
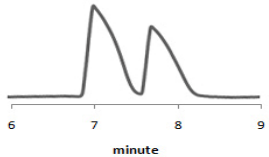
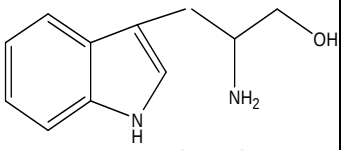
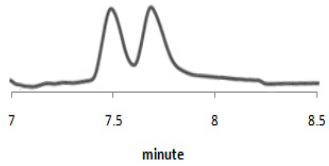
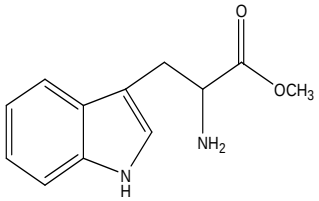
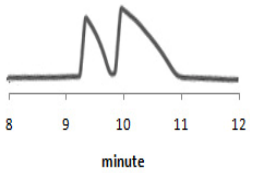
#	Structure and Name of analyte	Arsensyl tartrate Concentration (mg/mL)	Tm1 (min)	Tm2 (min)	Rs	N	α	Electropherogram
17	 Tetrahydrozoline	100	6.36	6.92	1.7	16200	1.09	
18	 Tryptophan butyl ester	60	7.05	7.76	1.6	3800	1.10	
19	 Tryptophanol	60	7.48	7.75	1.0	23300	1.04	
20	 Tryptophan methyl ester	60	9.47	10.08	1.5	9200	1.06	

Table 5 – *Continued.*

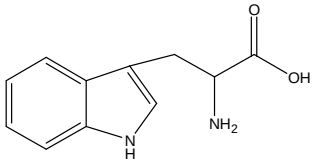
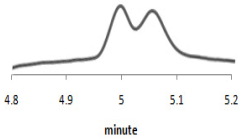
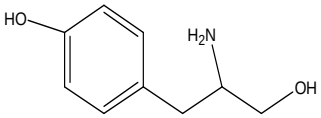
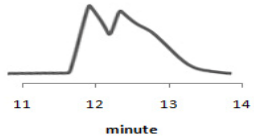
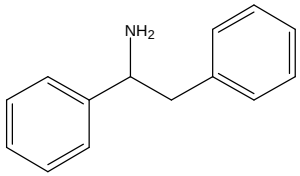
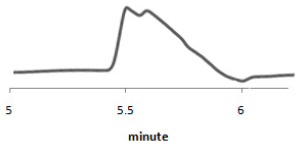
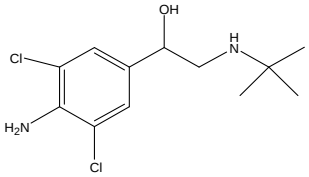
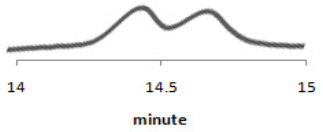
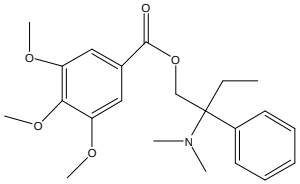
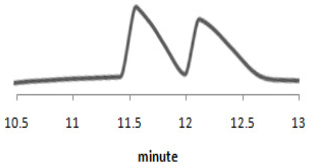
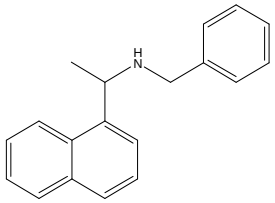
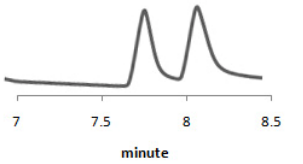
#	Structure and Name of analyte	Arsensyl tartrate Concentration (mg/mL)	Tm1 (min)	Tm2 (min)	Rs	N	α	Electropherogram
21	 Tryptophan	60	4.97	5.08	0.7	34000	1.02	
22	 Tyrosinol	100	11.96	12.48	0.6	8500	1.04	
23	 1,2-diphenylethylamine	60	5.52	5.63	0.3	6500	1.02	
24	 Clenbuterol	100	14.47	14.74	1.0	22000	1.02	

Table 5 – *Continued.*

#	Structure and Name of analyte	Arsensyl tartrate Concentration (mg/mL)	Tm1 (min)	Tm2 (min)	Rs	N	α	Electropherogram
25	 Trimebutine	50	11.54	12.26	1.5	9000	1.06	
26	 (*S*)-*N*-Benzyl-1-(1-naphthyl)ethylamine	60	7.76	8.18	1.8	24100	1.05	

a) Conditions: 30cm capillary (20cm to the detector) with 50 µm I.D capillary; +10-15 kV; buffer : 5mM Tris at pH 8.02 with 10% of methanol (v/v); detection at 214nm

b) Rs: separation resolution

c) N: number of theoretical plates calculated from the first detected peak

d) α: selectivity

for this specific group of analytes [59,62]. Also, the concentration of arsenyl tartrate required is higher than that of separations that use charged cyclodextrins. The possible reason is that enantioselective recognition mechanism of these two chiral selectors is different. Charged cyclodextrins involve electrostatic interactions between charged chiral selectors and oppositely charged analytes and inclusion or exclusion complexation [2]. However, electrostatic forces represent the only attractive interactions for arsenyl tartrate chiral selectors. Most of the separations were accomplished in 10 minutes. With a careful examination of data, we see that compound resolution increased as the size of the ester group increase. For example, compound #18 (Tryptophan butyl ester) was better separated than compound #20 (Tryptophan methyl ester). Another interesting phenomenon is that compounds with more benzyl or more fused rings gave better separations. (i.e. compounds # 2, #5, #8, #17 and #26).

Besides the amine-containing compounds, ten ruthenium(II) polypyridyl complexes were baseline separated within 10 minutes. All results are summarized in Table 6. Ruthenium (II) polypyridyl complexes have been reported to be useful in several applications such as catalysts for asymmetric synthesis, as DNA recognition probes or cleavage agents for DNA by generation of reactive oxygen species under a hypoxic environment [120-121]. The right- and left-handed configurations of these metal complexes are referred to as Δ and Λ enantiomers, respectively [103,121-122]. Several analytical techniques including chromatographic methods and diastereomeric formation using ion-pairing agent have been utilized to separate these racemates of metal complexes. [103,123-124]. Sodium L-(+)-arsenyl tartrate has been previously reported for diastereoselective precipitations of ruthenium complexes by our group [103]. In this study, 10 ruthenium (II) polypyridyl complexes (nine monomers and one dimer) were all baseline separated within 10 minutes. The structures of these complexes are shown in Figure 7. Compared to neutral or charged cyclodextrins, the arsenyl tartrate provided shorter

analysis times, better efficiency, and higher resolution, especially for the separation of the bridged dimer complex ($[Ru_2(phen)_4tatpp](Cl)_4$) [53].

Table 6. Experimental Data for Enantiomeric Separations of Ruthenium(II) Polypyridyl Complexes Using Sodium Arsenyl-(L)-(+)-tartrate

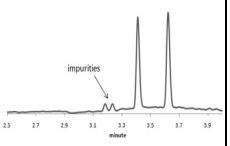
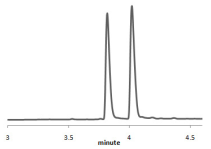
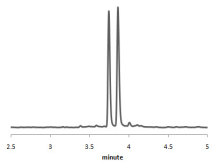
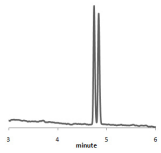
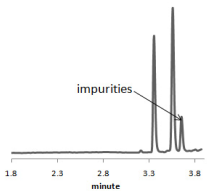
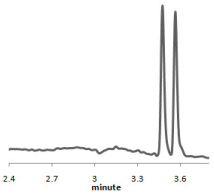
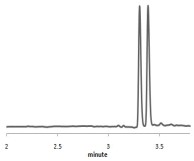
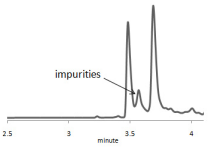
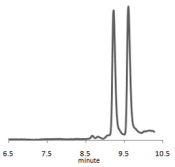
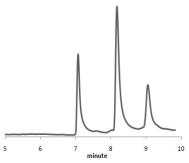
Name of structure	Arsenyl tartrate conc. (mg/mL)	Tm1 (min)	Tm2 (min)	Rs	N	α	Electropherogram
$[Ru(phen)_3](Cl)_2$	30	3.41	3.62	5.0	101000	1.06	
$[Ru(phen)_2nitrophen](Cl)_2$	30	3.82	4.02	3.3	69400	1.05	
$[Ru(phen)_2phendione](Cl)_2$	30	3.74	3.86	2.4	97100	1.03	
$[Ru(bpy)_3](Cl)_2$	60	4.74	4.83	1.4	90500	1.02	
$[Ru(phen)_2aminophen](Cl)_2$	30	3.35	3.55	4.5	88600	1.06	

Table 6 – *Continued.*

Name of structure	Arsenyl tartrate conc. (mg/mL)	Tm1 (min)	Tm2 (min)	Rs	N	α	Electropherogram
$[Ru(phen)_2tatpp](PF_6)_2$	30	3.47	3.56	2.0	95300	1.03	
$[Ru(phen)_2py_2](Cl)_2$	30	3.30	3.39	2.0	99000	1.03	
$[Ru(phen)_2phendiamine](PF_6)_2$	30	3.48	3.69	3.1	50400	1.06	
$[Ru(nitrophen)_2phendione](Cl)_2$	50	9.22	9.60	2.6	64600	1.04	
$[Ru_2(phen)_4tatpp](Cl)_4$[e]	60	7.07	8.18	6.7	38000	1.16	
		8.18	9.06	4.3	25200	1.11	

a)Conditions: 30cm capillary (20cm to the detector) with 50 µm I.D capillary; +6 kV; buffer : 5mM Tris at pH 8.02 with 10% of methanol (v/v); detection at 214nm
b)Rs: separation resolution
c)N: number of theoretical plates calculated from the first detected peak
d)α: selectivity
e)Top row for peak 1 and 2; bottom row for peak 2 and 3

Several EOF markers such as acetone, DMSO, benzyl alcohol and mesityl oxide were used in this study. None of them were detectable in any of the electropherograms. The selectivity (α), therefore, is only estimated by dividing of the two migration times of the two enantiomeric peaks, i.e. tm2/tm1 (see materials and methods 2.2).

3.5 Concluding Remarks

Sodium arsenyl (L)-(+)-tartrate showed enantioselectivity towards amine-containing compounds. Most separations were achieved in 10 minutes. Electrostatic interactions play an important role in the enantioseparations. Compounds with more benzyl or fused rings showed better separations. Compared to charged cyclodextrins, arsenyl tartrate showed shorter analysis times for amine-containing compounds. Resolutions were improved and migration times were increased with increasing arsenyl tartrate concentrations. Lower buffer pH increased the enantioresolution by slowing the EOF. Increasing pH buffer concentration increased migration times and decreased resolutions. Addition of methanol in run buffer increased migration times as well as solubility of hydrophobic analytes. Besides amine-containing compounds, racemates of ruthenium (II) polypyridyl complexes were also enantioseparated using arsenyl tartrate. Shorter analysis times and better resolutions were achieved using arsenyl tartrate than using neutral or charged cyclodextrins.

3.6 Acknowledgement

We gratefully acknowledge the support of this project by the National Institute of Health (NIH 5 ROICM053825-12).

CHAPTER 4

ENANTIOMERIC SEPARATION OF CHIRAL RUTHENIUM (II) COMPLEXES USING CAPILLARY ELECTROPHORSIS

4.1 Abstract

Capillary zone electrophoresis (CZE) and micellar capillary electrophoresis (MCE) were applied for the enantiomeric separation of nine mononuclear tris(diimine)ruthenium(II) complexes as well as the separation of all stereoisomers of a dinuclear tris(diimine)ruthenium(II) complex. Nine cyclodextrin (CD) based chiral selectors were examined as run buffer additives to evaluate their effectiveness in the enantiomeric separation of tris(diimine)ruthenium(II) complexes. Seven showed enantioselectivity. Sulfated γ-cyclodextrin (SGC), with four baseline and three partial separations, was found to be the most useful chiral selector. In CZE mode, the derivatized γ-CDs were more effective than β-CDs while sulfated CDs work better than carboxymethyl CDs. In MCE mode, hydroxypropyl β-CD separated the greatest number of tris(diimine)ruthenium(II) complexes. The effects of chiral selector concentration, run buffer pH and concentration, the concentration ratio between chiral selector and other factors were investigated.

4.2 Introduction

Ruthenium(II) tris(diimine) complexes are inherently chiral compounds (see Figure 11) [125]. They have been investigated extensively as chiral catalysts [126-129], chiral dopants [130], molecular recognition probes [131-135] and tumor-inhibiting prodrugs [136]. Recently, Ruthenium(II) tris(diimine) complexes have also been developed as chiral mobile phase

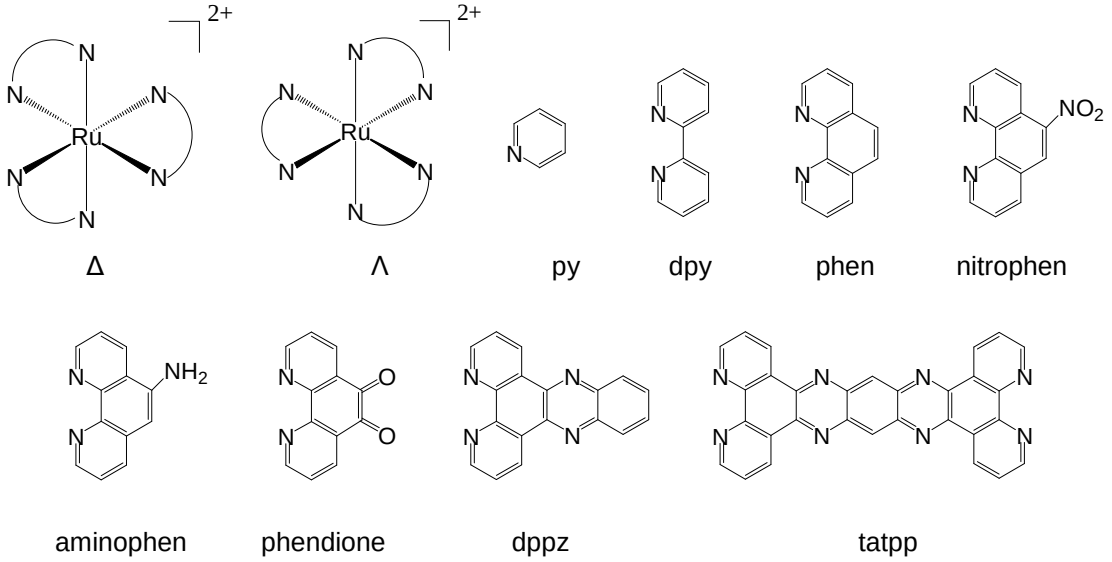


Figure 11. Structure of $Ru(diimine)_3^{2+}$ and diimmine ligands; N N=diimine ligands; py=pyridine; dpy=2,2'-dipyridine; phen=1,10 phenanthroline; nitrophen=5-nitro-1,10 phenanthroline; aminophen =5-amino-1,10 phenanthroline; phendione=1,10 phenanthroline-5,6-dione; dppz=dipyrido[a:3,2 -h:2'3'-c-]phenazine; tatpp=9,10,20,22-tetraaza[3,2-a:2'3'-c:3'',2''-h,2''',3'''-j]tetrapyrido-pentacene

additives [137] and clay-ruthenium complex adduct chiral stationary phases [138-141] for the enantiomeric separation of chiral compounds on HPLC. As the Δ and Λ enantiomers can have very different properties in these applications, enantiomerically pure compounds are usually preferred and a knowledge of enantiomeric purity is almost always necessary. Unfortunately, tris(diimine)ruthenium(II) complexes are usually synthesized as racemates or mixtures of moderate enantiomeric excess [108-109,111-112,143-144]. Therefore, effective and efficient methods to evaluate enantiomeric purity are needed.

Traditionally, the enantiomeric separation of tris(diimine)ruthenium(II) complexes has been achieved by diastereoisomeric salt formation and recrystallization[112,145-150] or chromatographic techniques. Spectroscopic methods, including NMR with chiral shift reagents

[149] and linear and circular dichroism [143,151] are used for enantiomeric recognition. Cation exchange chromatography with chiral anions as eluent additives was developed by Keene et al as a classical chromatograpic approach for the separation of some metal di(imine) complexes [152-159]. The Δ and Λ forms of tris(diimine)ruthenium(II) complexes are known to have different affinities to DNA. Therefore calf thymus DNA was immobilized on-column and used for the enantiomeric separation of tris(diimine)ruthenium(II) complexes and other transitional metal complexes[160-162]. A teicoplanin based chiral stationary phase was used for the separation of stereoisomers of dinuclear ruthenium(II) complexes and enantiomers of tris(diimine)ruthenium(II) complexes [163-164].

Many reviews [89,95-96,165,171-176] have been published on the extensive use of chiral capillary electrophoresis (CE) in recently years. CE has been used for the enantioseparation of pharmaceutical compounds [173-176], biological samples [165,168,114], and asymmetric synthesis products and intermediates [151,177-183]. There have been relatively few reports on the enantiomeric separation of transition metal poly(diimine) complexes[100,151,177-181,183-184]. Chiral additives used for the CE separation of transition metal complexes are usually chiral anions, including carboxymethyl-β-cyclodextrin [151,177-180], tartrate and its derivatives [151,177,178,180], isocitrate and amino acids derivatives [179] and bile salts [178-179]. In our study, nine cyclodextrin based chiral additives were evaluated by CE and micellar CE for the separation of nine tris(diimine)ruthenium(II) complexes and all stereoisomers of a dinuclear ruthenium(II) tris(diimine) complex (See Figure 1 for diimine ligand structures). Among these ten ruthenium complexes, only $Ru(phen)_3^{2+}$, $Ru(bpy)_3^{2+}$, and cis-$Ru[(bpy)_2py_2]^{2+}$ have been separated by CE using other chiral anions [151,177,180] or liquid chromatography [109,152,155-156,160]. There has been no report on the enantioseparation of the other six ruthenium complexes.

4.3 Materials and Methods

4.3.1 Materials

Sulfated γ-cyclodextrin (SGC), carboxymethyl γ-cyclodextrin (CMGC) and carboxyethyl γ-cyclodextrin (CEGC) were purchased from Cyclodextrin Technologies Development, Inc. (High Springs, FL, USA). Sulfated β-cyclodextrin (SBC), hydroxypropyl γ-cyclodextrin (HPGC) and hydroxypropyl β-cyclodextrin (HPBC) were purchased from Sigma-Aldrich Company (Saint Louis, MO, USA). Sulfobutyl ether β-cyclodextrin (SBE) was purchased from Advanced Separation Techniques, Inc. (Whippany, NJ, USA). Carboxymethyl β-cyclodextrin (CMBC) was obtained from American Maize Products (Hammond, IN, USA). The tris(diimine)ruthenium(II) complexes were synthesized as previously reported [108-109,111-112,143-144]. The EOF marker, dimethyl sulfoxide or mesityl oxide, and sodium dodecyl sulfate were purchased from Sigma-Aldrich Corporate (St. Louis, MO, USA). HPLC-grade ethanol and acetonitrile, phosphoric acid and sodium hydroxide were all purchased from Fisher Scientific (St. Louis, MO, USA). The fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA).

4.3.2 Methods

Separations were performed on a Beckman P/ACE 5000 (Fullerton, CA, USA) capillary electrophoresis system equipped with a UV-visible detector or a Beckman Coulter P/ACE MDQ capillary electrophoresis system equipped with a photodiode array detector and a 50 μm i.d. x 358 μm o.d. capillary. The length of the capillary on P/ACE 5000 system was 37 cm (30 cm to detector) while on P/ACE MDQ the capillary length was 50 cm (40 cm to detector). All the samples were detected at either 214 nm or 254 nm. Sodium phosphate, monobasic or dibasic was dissolved in deionized water and adjusted with concentrated sodium hydroxide or phosphoric acid to the desired pH. Buffer additives (chiral selector and SDS) were added to this buffer solution to make run buffer. Racemic samples or artificial mixtures of enantiomers were dissolved in buffer solution (50 mM SDS in run buffer for MCE samples) to make the sample

solutions. Organic modifiers were added by volume percentage prior to the addition of chiral run buffer additives. The capillary was rinsed with water for one min, 1 M sodium hydroxide for 5 min and water again for 5 min for conditioning. Between each run, the capillary was rinsed with methanol for 1 min, water for 1 min, 1 M sodium hydroxide for 1 min, and again water for 1 min and followed by run buffer for 2 min. Subsequently the sample solution was injected hydrodynamically at 0.5 psi for 5 seconds. All CZE separations were done in the normal polarity mode with a pH 7.5 buffer P/ACE 5000 system and 6.9 for P/ACE MDQ system. Micellar CE (MCE) separations were completed in the reverse polarity mode with a buffer pH of 2.6. Sample identity was confirmed by UV spectrum obtained with PDA detector in P/ACE MDQ system. The electromigration order was determined by spiking with a pure enantiomer.

The resolution (Rs) was calculated as: $Rs=2(t_{m2}-t_{m1})/(w_1+w_2)$, the apparent mobility (μ_{app}) was calculated as: $\mu_{app}=L*L_{total}/(t_mV)$, the electroosmotic mobility (μ_{eof}) was calculated as $\mu_{eof}= L*L_{total}*/(t_{eof}V)$, the electrophoretic mobility (μ) was calculated as $\mu=L*L_{total}*(1/t_m-1/t_{eof})/V$, the mobility difference ($\Delta\mu$) was calculated as $\Delta\mu=\mu_1-\mu_2$, the selectivity (α) was calculated as: $\alpha=\mu_{app1}/\mu_{app2}$, and the number of theoretical plates (N) was calculated as $N=16*(t_m/w)^2$, where t_{m1} and t_{m2} are the migration times of the first and second peak, t_{eof} is the migration time of EOF marker, and w is the baseline peak width. L is the length of capillary from the injection end to the detection window, L_{total} is the total length of capillary. The resolution (Rs) can also be expressed as: $Rs=\Delta\mu*N^{1/2}/(4\mu_{app,avg})$[173]. As the selectivity term $\Delta\mu/\mu_{app,avg}=2(\mu_{app2}-\mu_{app1})/(\mu_{app1}+\mu_{app2})=(\alpha-1)/(\alpha+1)$, $Rs=(\alpha-1)/(\alpha+1)N^{1/2}/(2\mu_{app,avg})$.

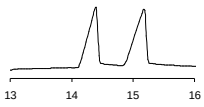
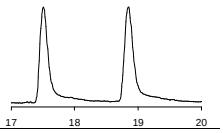
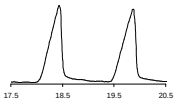
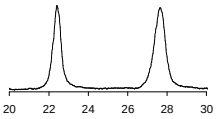
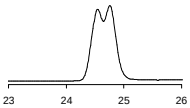
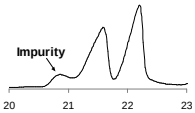
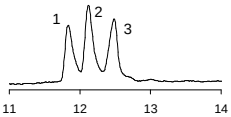
4.4 Results and Discussion

According to Kano's NMR study, only anionic γ -or β- cyclodextrins (per-CO_2-γ-CD and per-CO_2-β-CD) showed chiral recognition for $Ru(phen)_3(ClO_4)_2$ [149]. As the ten ruthenium trisdiimine complexes (Table 7 and Figure 11) are all positively charged, they will preferably

interact with negatively charged chiral selectors. Also it is well known that negatively charged selectors provide the largest separation window for positively charged analytes, which was first reported in 1994 [183]. Therefore, the five anionic chiral cyclodextrin derivatives, SGC, CMGC, SBC, CMBC and SBE were initially evaluated as chiral selectors. Tables 7-11 give the structure of ruthenium complexes, migration times for the first eluted peaks, cyclodextrin concentrations, resolutions, selectivities, efficiencies (the average of the two peaks), electromigration orders and electropherograms of the optimized separations. SGC appeared to be the most powerful chiral selector, giving the largest resolution for the greatest number of racemates, and it did so at lower concentrations. According to our results, sulfated cyclodextrins separated a greater number of ruthenium complexes. Derivatized γ- cyclodextrins also gave a larger number of separations with higher resolutions at lower chiral selector concentrations.

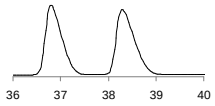
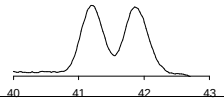
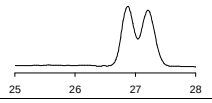
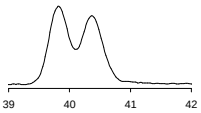
No peaks were observed for the following three ruthenium complexes in CZE mode: $[Ru(phen)_2dppz]^{2+}$, $[Ru(phendione)_3]^{2+}$ and $[Ru(dppz)_3]^{2+}$. Therefore, SDS was added to the running buffer to improve the solubility of these complexes. Neutral cyclodextrins, HPGC, HPBC, DMBC and γ-CD as well as CMGC, CMBC and SBE were tested as chiral selectors in micellar capillary electrophoresis (MCE). In the MCE mode, the pH was adjusted to 2.6 to reduce the EOF [185]. The results are listed in Tables 12-13. The peak for $Ru(phendione)_3^{+2}$ was never observed. Only the neutral chiral selectors HPBC and HPGC produced enantiomeric separations for any ruthenium complexes and then only at very high concentrations of both cyclodextrin and SDS. Also, this approach was characterized by longer analysis times and lower resolutions compared to conventional CZE (except for $Ru(phendione)_3^{+2}$). Other cyclodextrin derivatives showed limited solubility in the presence of high SDS concentrations.

Table 7. CZE separations of tris(diimine)ruthenium complexes with sulfated γ-cyclodextrin[a)]

Structure	t_{m1} (min)	[SGC] (mg/mL)	Rs	α	N_{avg}	EMO	Electropherogram
$[Ru(phen)_3]^{2+}$	14.39	10	1.9	1.06	20000	Λ,Δ	
$[Ru(phen)_2 nitrophen]^{2+}$	17.51	10	5.3	1.08	81000	Λ,Δ	
$[Ru(phen)_2 aminophen]^{2+}$	18.43	30	2.9	1.08	24000	Δ,Λ	
$[Ru(phen)_2 phendione]^{2+}$	22.35	10	5.4	1.24	11000	Δ,Λ	
$[Ru(bpy)_3]^{2+}$	24.54	60	0.6	1.01	31000	Δ,Λ	
Cis-$[Ru(phen)_2py_2]^{2+}$	21.58	110	1.1	1.03	24000	Λ,Δ	
$[Ru(phen)_2 dppz]^{2+}$	No analyte peak was observed						
$[Ru(phendione)_3]^{2+}$	No analyte peak was observed						
$[Ru(dppz)_3]^{2+}$	No analyte peak was observed						
[b)] $[Ru_2(phen)_4(tatpp)]^{4+}$	11.83 12.12	20	1.2 1.4	1.02 1.03	41000 41000	NA	

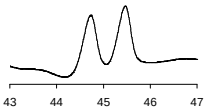
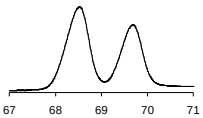
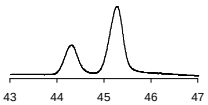
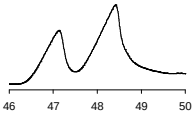
a) Data obtained on Beckman P/ACE 5000 CE system with 50 μm ID capillary with a total length of 37 cm (30 cm to detection window. Separation conditions: 60 mM phosphate, pH=7.5, +5 kV, detected at 214 nm.
b) Top row is for peak 1,2, bottom row for peak 2,3

Table 8. CZE Separations of Tris(diimine) Ruthenium Complexes with Carboxymethyl γ-Cyclodextrin[c)]

Structure	t_{m1} (min)	[CMGC] (mg/mL)	Rs	α	N_{avg}	EMO	Electropherogram
$[Ru(phen)_3]^{2+}$	36.80	260	2.1	1.04	46000	Λ,Δ	
$[Ru(phen)_2nitrophen]^{2+}$	41.17	260	1.0	1.02	57000	Λ,Δ	
$[Ru(bpy)_3]^{2+}$	26.86	260	0.8	1.01	58000	Δ,Λ	
Cis-$[Ru(phen)_2py_2]^{2+}$	39.80	260	0.7	1.01	47000	Δ,Λ	

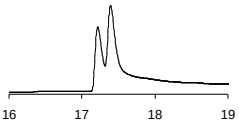
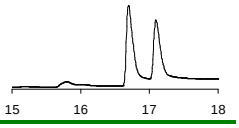
c) Data obtained on Beckman P/ACE 5000 CE system with 50 μm ID capillary with a total length of 37 cm (30 cm to detection window. Separation conditions: 60 mM phosphate, pH=7.5, +5 kV, detected at 214 nm. Analytes with no separation or no observation of peak are not listed.

Table 9. CZE Separations of Ruthenium Tris(diimine) Complexes with Sulfated β-Cyclodextrin[d)]

Structure	t_{m1} (min)	[SBC] (mg/mL)	Rs	α	N_{avg}	EMO	Electropherogram
$[Ru(phen)_3]^{2+}$	44.73	250	1.4	1.02	120000	Λ,Δ	
$[Ru(phen)_2nitrophen]^{2+}$	68.52	250	1.2	1.02	84000	Λ,Δ	
$[Ru(bpy)_3]^{2+}$	44.04	250	1.8	1.02	120000	Λ,Δ	
Cis-$[Ru(phen)_2py_2]^{2+}$	47.13	250	1.2	1.03	31000	Λ,Δ	

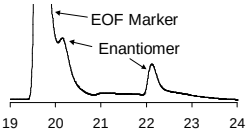
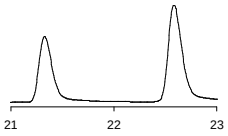
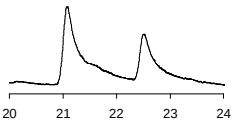
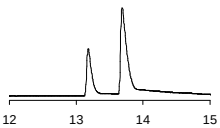
d) Sample solutions are made from artificial mixtures of two enriched enantiomers. Data obtained on Beckman P/ACE MDQ CE system with 50 μm ID capillary with a total length of 50 cm (40 cm to detection window). Separation conditions: 50 mM phosphate, pH=6.9, + 8kV, detected at 214 nm. Analyte with no separation or no observation of peak are not listed.

Table 10. CZE Separations of Ruthenium Tris(diimine) Complexes with Carboxymethyl β-Cyclodextrin[d)]

Structure	t_{m1} (min)	[CMBC] (mg/mL)	Rs	α	N_{avg}	EMO	Electropherogram
$[Ru(phen)_2nitrophen]^{2+}$	17.22	90	1.0	1.01	130000	Δ,Λ	
Cis-$[Ru(phen)_2py_2]^{2+}$	16.70	90	1.9	1.02	100000	Δ,Λ	

d) Sample solutions are made from artificial mixtures of two enriched enantiomers. Data obtained on Beckman P/ACE MDQ CE system with 50 µm ID capillary with a total length of 50 cm (40 cm to detection window). Separation conditions: 50 mM phosphate, pH=6.9, + 8kV, detected at 214 nm. Analytes with no separation or no observation of peak are not listed.

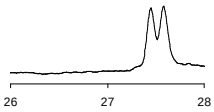
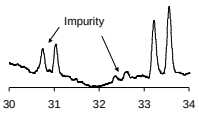
Table 11. CZE Separations of Tris(diimine) Ruthenium Complexes with Sulfobutyl Ether β-Cyclodextrin [d)]

Structure	t_{m1} (min)	[SBE] (mg/mL)	Rs	α	N_{avg}	EMO	Electropherogram
$[Ru(phen)_3]^{2+}$	20.18	80	4.7	1.10	42000	Δ,Λ	
$[Ru(phen)_2nitrophen]^{2+}$	21.33	80	5.2	1.06	130000	Δ,Λ	
$[Ru(phen)_2aminophen]^{2+}$	21.08	80	1.2	1.07	6200	Δ,Λ	
$[Ru(bpy)_3]^{2+}$	13.18	50	3.3	1.04	130000	Λ,Δ	

d) Sample solutions are made from artificial mixture of two enriched enantiomers. Data obtained on Beckman P/ACE MDQ CE system with 50 µm ID capillary with a total length of 50 cm (40 cm to detection window). Separation conditions: 50 mM phosphate, pH=6.9, +8 kV, detected at 214 nm. Analytes with no separation or no observation of peak are not listed.

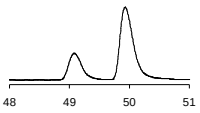
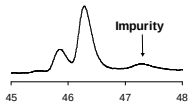
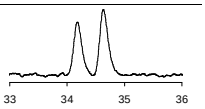
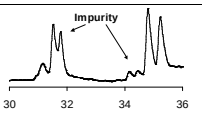
Increasing the concentration of chiral selector is known as an effective way to improve enantiomeric separations [13,113,165-166,168-170]. The CE separation of $[Ru(phen)_2nitrophen]^{2+}$ enantiomer with SGC is a typical example. All conditions including buffer concentration, pH, voltage and so forth were equivalent for all runs as the SGC concentration was altered (Figure 12). As the SGC concentration was increased from 3 mg/mL to 30 mg/mL, the enantioresolution improved from a partial separation (Rs=0.6) to more than a baseline separation (Rs=3.3) due to an improvement in selectivity. The analysis time increased from 5 min to 9 min. The effect of chiral selector concentration on the enantiomeric separation of $[Ru(bpy)_3]^{2+}$ using micellar CE also was studied and is shown in Figure 13. Similar trends were observed. With a fixed SDS concentration and all other conditions the same, a longer analysis time and higher resolution were observed at higher HPBC concentrations

Table 12. Micellar CE Separations of Tris(diimine) Ruthenium Complexes with Hydroxypropyl γ-Cyclodextrin[e)]

Structure	t_{m1} (min)	[SDS] (mM)	[HPGC] (mg/mL)	Rs	α	N_{avg}	EMO	Electropherogram
$[Ru(phen)_2 aminophen]^{2+}$	27.45	210	395	0.8	1.01	390000	Δ,Λ	
$[Ru(phendione)_3]^{2+}$	No analyte peak was observed							
$[Ru(dppz)_3]^{2+}$	33.22	200	395	1.8	1.01	510000	NA	

e) $[Ru(phen)_2aminophen]^{2+}$ is an artificial mixture. Data obtained on Beckman P/ACE MDQ CE system with 50 µm ID capillary with a total length of 50 cm (40 cm to detection window). Analyte with no separation was not listed. Separation conditions: 50 mM phosphate, pH=2.6, +30 kV; all samples are dissolved in 50 mM SDS, 50 mM phosphate with pH=2.6, detected at 214 nm

Table 13. Micellar CE Separations of Tris(diimine) Ruthenium Complexes with Hydroxypropyl β-Cyclodextrin[e)f)]

Structure	t_{m1} (min)	[SDS] (mM)	[HPBC] (mg/mL)	Rs	α	N_{avg}	EMO	Electropherogram
$[Ru(phen)_3]^{2+}$	49.08	210	365	2.0	1.02	220000	Λ,Δ	
$[Ru(phen)_2$ aminophen$]^{2+}$	45.85	210	365	0.9	1.01	150000	Λ,Δ	
$[Ru(bpy)_3]^{2+}$	34.18	200	365	1.6	1.01	240000	Λ,Δ	
$[Ru(dppz)_3]^{2+}$	34.80	200	365	1.1	1.01	130000	NA	

e) Data obtained on Beckman P/ACE MDQ CE system with 50 µm ID capillary with a total length of 50 cm (40 cm to detection window). Analyte with no separation was not listed. Separation conditions: 50 mM phosphate, pH=2.6, +30 kV; all samples are dissolved in 50 mM SDS, 50 mM phosphate with pH=2.6, detected at 214 nm

f) $[Ru(phen)_3]^{2+}$,$[Ru(phen)_2aminophen]^{2+}$, and $[Ru(bpy)_3]^{2+}$ are all artificial mixtures. No analyte peak was observed for $[Ru(phendione)_3]^{2+}$

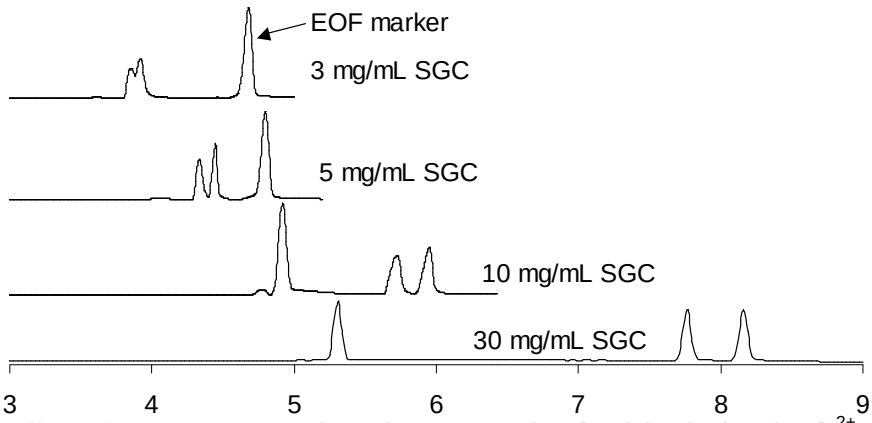


Figure 12. Effect of SGC concentration when separating $[Ru(phen)_2nitrophen]^{2+}$. Separation conditions: Data obtained on Beckman P/ACE 5000 CE system with 50 µm ID capillary with a total length of 37 cm (30 cm to detection window). Separation conditions: 60 mM phosphate, pH=8.5, +10 kV, detected at 214 nm.

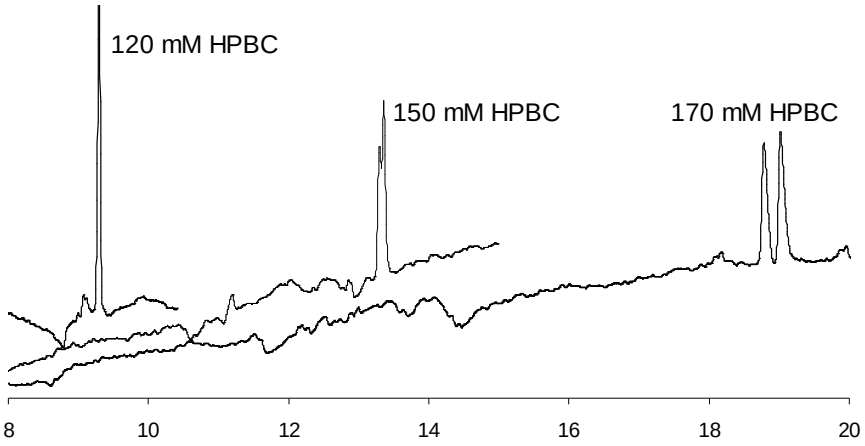


Figure 13. Effect of chiral selector (HPBC) concentration at Fixed SDS concentration (200 mM) when separating $[Ru(bpy)_3]^{2+}$. Data obtained on Beckman P/ACE MDQ CE system with 50 µm ID capillary with a total length of 50 cm (40 cm to detection window). Separation conditions: 50 mM phosphate, pH=2.6, -30 kV; all samples are dissolved in 50 mM SDS, 50 mM phosphate with pH=2.6, detected at 214 nm.

Run buffer pH can affect the enantioseparation by affecting EOF mobility [185], changing the charge state of the analyte and chiral selector, which in turn affects their association behavior[13.113]. The effect of pH on CE enantiomeric separations has been reported many times[114,167-168,170,186]. Four pH values were evaluated for the enantioseparation of $[Ru(phen)_2aminophen]^{2+}$. Results are given in Table 14. As the pH increased from 5.0 to 10.5, both the EOF marker and analyte migrated much faster, which reduced the analysis time by 50%. This is because of the deprotonation of a higher percentage of silanol groups on the capillary wall, which greatly increased the EOF mobility [185]. From pH 5.0 to pH 10.5, the average apparent mobility almost doubled, however, mobility differences between the two enantiomers remained essentially the same.

Table 14.The Effect of pH on the Enantiomeric Separation of $[Ru(phen)_2aminophen]^{2+}$ g)h)

pH	t_{m1} (min)	t_{m2} (min)	t_{eof} (min)	Rs	Δμ	$\mu_{app,avg}$	$\Delta\mu/\mu_{app,avg}$	α	N_{avg}
5.0	16.43	18.58	10.50	5.6	0.98	8.0	0.12	1.13	34000
6.8	10.41	11.23	7.26	4.6	0.98	12.8	0.08	1.08	58000
8.7	9.60	10.30	6.86	4.0	0.97	14.0	0.07	1.07	54000
10.5	8.53	9.10	6.03	3.9	1.04	15.7	0.07	1.07	56000

g) The unit for mobilities are all $cm^2kV^{-1}min^{-1}$

h) Data obtained with compound $[Ru(phen)_2aminophen]^{2+}$ on Beckman P/ACE 5000 CE system with 50 μm ID capillary with a total length of 37 cm (30 cm to detection window. Separation conditions: 40 mg/mL SGC, 60 mM phosphate, +8kv, detected at 214 nm.

In enantioselective CE separations, the buffer plays an important role [185]. In addition to its buffering capacity, it controls the ionic strength of the solution. It stabilizes the current, which minimizes baseline noise and also helps to maintain a constant EOF [179,185]. Also, the proper buffer suppresses electromigration dispersion [13,181] which leads to improved efficiency. However, excessive Joule heating due to high current would be expected at high concentrations of buffer. Buffer may also affect the association chemistry between analytes and the chiral selector. In our study, electrostatic interaction is significant in the complexation of the positively charged analytes and negatively charged chiral selectors. Higher buffer concentration provides higher ionic strength, which suppresses the electrostatic interaction between analytes and chiral selectors. Figure 14 shows the electropherograms of the CE separation of $[Ru(phen)_3]^{2+}$ with SGC at different phosphate buffer concentrations, while all other conditions remained the same. At 10 mM sodium phosphate, the analyte peak was very wide. As the phosphate concentration increased to 70 mM, the efficiency improved significantly. This is because at a higher ionic strength run buffer, there are more buffer ions that can disrupt the electrostatic

attraction between analytes and chiral selectors. The analytes migrated faster than the EOF marker at high buffer concentration (above 70 mM) but slower than the EOF marker at low buffer concentration (below 30 mM). This also indicates that higher ionic strength suppresses the binding of analytes and the chiral selector, since longer migration times indicate stronger binding of analyte to the negatively charged chiral selectors. When the buffer concentration was further increased to 110 mM, the enantioresolution was almost lost as the interaction between analyte and chiral selector was greatly suppressed. Optimum resolution was observed around 70 mM phosphate buffer.

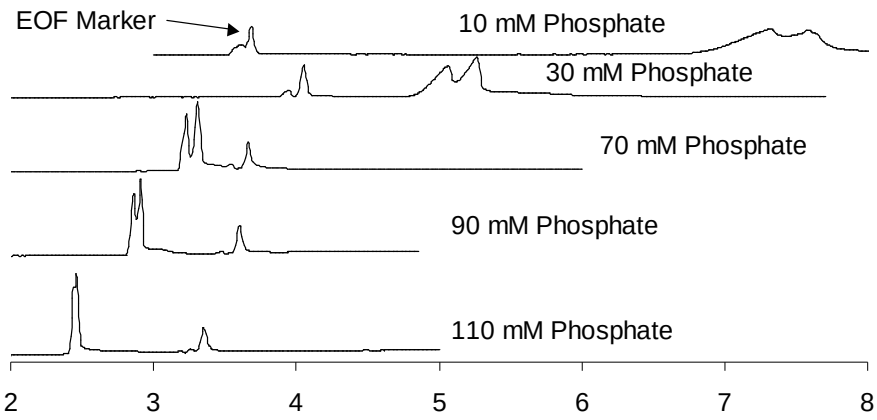


Figure 14. Effect of buffer (sodium phosphate) concentration when separating $[Ru(phen)_3]^{2+}$. Data obtained on Beckman P/ACE 5000 CE system with 50 µm ID capillary with a total length of 37 cm (30 cm to detection window). Separation conditions: 9 mg/mL SGC, pH=8.5, +10 kV, detected at 214 nm.

Increasing the applied voltage is an effective way to shorten analysis times [185]. Increased voltage greatly decreases analysis time, which in turn suppresses molecular diffusion leading to sharper peaks [185]. Increased voltage also produces higher current and more Joule

heating, which hurts the efficiency. The data in Table 15 shows the effect of voltage on the separation of [Ru(phen)phendione]$^{2+}$ enantiomers. As expected, analysis time was decreased from 17 min to about 2 min as the voltage was increased from 4 kV to 15 kV. The efficiency reached at a maximum at 12kV and then decreased at higher voltage. The enantioresolution and selectivity decreases as the voltage was increased.

The effect of organic modifier on enantiomeric separations can be very complicated [13, 165]. It can modify several parameters, including the association constants between analyte and the chiral selector, the EOF, the conductivity of run buffer, in turn the Joule heating [13, 165, 188]. The effect of methanol and acetonitrile on the separation of [Ru(phen)$_3$]$^{2+}$ enantiomers are given in Table 16. Both organic modifiers suppressed the EOF, with methanol showing a greater effect. The selectivity, however, was slightly increased by methanol but significantly decreased by higher concentrations of acetonitrile. Both organic modifiers decreased the efficiency, with acetonitrile producing a more pronounced effect. Overall, the addition of methanol increased analysis times and produced slightly decreased resolution. Acetonitrile produced slightly decreased analysis times, and severely decreased resolution. This behavior is in contrast with most chiral organic compounds, where there is often a beneficial effect with the addition of an optimum level of an organic modifier [113-114,167-170,188].

Table 15.The Effect of Voltage on the Enantiomeric Separation of $[Ru(phen)_2phendione]^{2+}$ g)i)

Voltage (kV)	t_{m1} (min)	t_{m2} (min)	t_{eof} (min)	Rs	μ_{eof}	μ_1	μ_2	$\Delta\mu$	$\mu_{app,avg}$	$\Delta\mu/\mu_{app,avg}$	N_{avg}
4	15.10	16.92	11.20	4.3	24.8	6.4	8.4	2.0	17.4	0.11	23000
8	6.21	6.77	4.89	3.5	28.4	6.0	7.9	1.8	21.4	0.086	26000
12	3.12	3.29	2.67	2.6	34.6	5.0	6.5	1.5	28.9	0.053	38000
15	1.85	1.90	1.74	1.1	42.5	2.5	3.6	1.1	39.5	0.027	27000

g) The unit for mobilities are all $cm^2kV^{-1}min^{-1}$
i) Data obtained with compound $[Ru(phen)_2phendione]^{2+}$ on Beckman P/ACE 5000 CE system with 50 µm ID capillary with a total length of 37 cm (30 cm to detection window. Separation conditions: 10 mg/mL SGC, 60 mM phosphate, pH=7.5, detected at 214 nm.

Table 16.The Effect of Organic Modifier on the Enantiomeric Separation of $[Ru(phen)_3]^{2+}$ g)j)

Organic modifier	t_{m1} (min)	t_{m2} (min)	t_{eof} (min)	Rs	$\Delta\mu$	$\mu_{app,avg}$	$\Delta\mu/\mu_{app,avg}$	α	N_{avg}
0%	4.92	5.07	5.22	1.4	0.83	27.9	0.030	1.03	84000
4% MeOH	5.19	5.37	5.68	1.2	0.90	26.3	0.034	1.04	21000
8% MeOH	6.11	6.32	7.15	1.0	0.76	22.3	0.034	1.03	15000
4% ACN	4.92	5.08	5.51	0.9	0.89	27.8	0.032	1.03	14000
8% ACN	4.68	4.78	5.70	0.2	0.61	29.3	0.021	1.02	3000

g) The unit for mobilities are all $cm^2kV^{-1}min^{-1}$
j) Data obtained with compound $[Ru(phen)_3]^{2+}$ on Beckman P/ACE 5000 CE system with 50 µm ID capillary with a total length of 37 cm (30 cm to detection window. Separation conditions: 8 mg/mL SGC, 60 mM phosphate, +8kV, pH=7.5, detected at 214 nm.

In micellar CE, both the concentration of the chiral selector and the surfactant have a significant effect on enantioseparation. Generally, higher chiral selector concentrations improve enantioresolution, but lead to longer analysis times. Higher surfactant concentrations, and therefore micellar concentrations, shorten analysis times and decrease enantioresolution. The effect of these two buffer additive concentrations at a fixed ratio (0.8) was studied for the separation of $[Ru(bpy)_3]^{2+}$. Electropherograms are shown in Figure 15. At 120 mM HPBC and 150 mM SDS, no enantioseparation was observed. As the concentrations of HPBC and SDS increased, the analysis time and enantioresolution increased. At 200 mM HPBC and 250 mM SDS, the enantioresolution was greatly improved to 1.4.

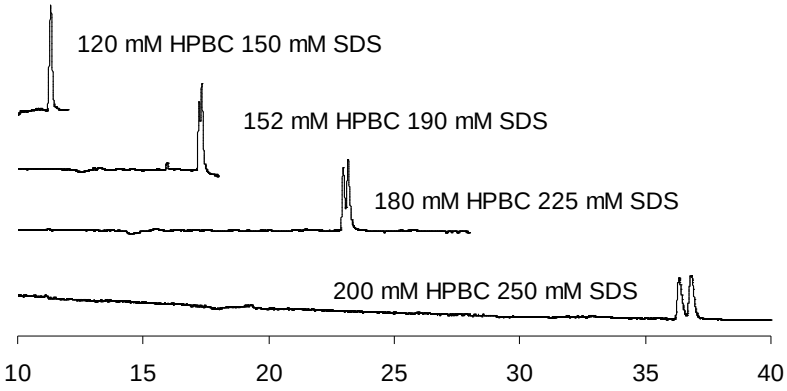


Figure 15. Effect of varying the concentration of a fixed ratio of HPBC and SDS (0.8) when separating $[Ru(bpy)_3]^{2+}$. Data obtained on Beckman P/ACE MDQ CE system with 50 μm ID capillary with a total length of 50 cm (40 cm to detection window). Separation conditions: 50 mM phosphate, pH=2.6, -30 kV; all samples are dissolved in 50 mM SDS, 50 mM phosphate with pH=2.6, detected at 214 nm.

The effect of surfactant concentration at fixed chiral selector concentration also was studied on the enantiomeric separation of $[Ru(bpy)_3]^{2+}$. The electropherogram is shown in Figure 16. When the surfactant concentrations were increased, the analysis times were shortened, and the enantioresolutions decreased, which is consistent with previous finding [167,169].

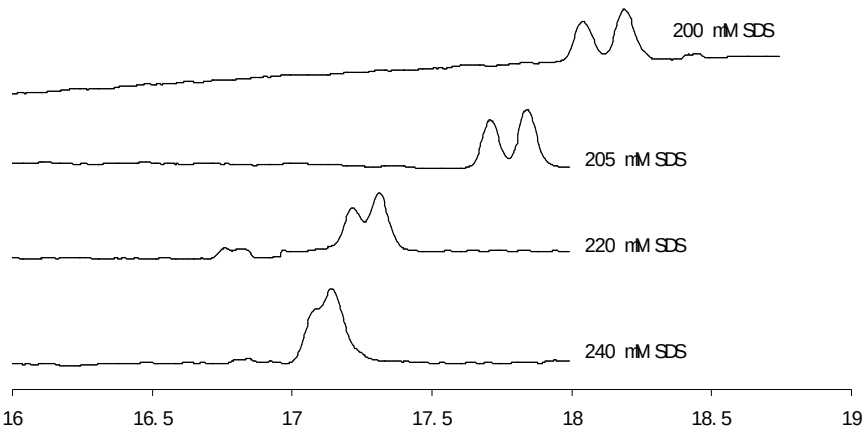


Figure 16. Effect of SDS concentration at a fixed HPBC concentration (160 mM) when separating $[Ru(bpy)_3]^{2+}$. Data obtained on Beckman P/ACE MDQ CE system with 50 µm ID capillary with a total length of 50 cm (40 cm to detection window). Separation conditions: 50 mM phosphate, pH=2.6, -30kv; all samples are dissolved in 50 mM SDS, 50 mM phosphate with pH=2.6, detected at 214 nm.

4.5 Concluding Remarks

Nine cyclodextrin based chiral selectors were examined for the CE enantioseparation of nine chiral ruthenium (II) tris(diimine) complexes and the separation of all stereoisomers of one dinuclear tris(diimine)ruthenium(II) complexes. Seven of the chiral organometallic compounds were separated by one or more chiral selectors. $[Ru(phendione)_3]^{2+}$ was not eluted in any mode. In both CZE and MCE modes, enantioresolution can be significantly improved by increasing the chiral selector concentration. In the CZE mode, better resolutions were obtained at lower pHs and lower applied voltages, but with longer analysis time. In MCE mode, higher surfactant concentrations speed up analysis, but with decreased enantioresolution. Increasing the concentration of a fixed ratio of surfactant and chiral selector helps to optimize an MCE mode enantioseparation but leads to longer analysis times.

4.6 Acknowledgement

We gratefully acknowledge the support of National Institute of Health (NIH 5 ROICM053825-11).

CHAPTER 5

SYNTHESIS AND EXAMINATION OF SULFATED CYCLOFRUCTANS AS A OVEL CLASS OF CHIRAL SELECTORS FOR CAPILLARY ELECTROPHORESIS

5.1 Abstract

Cyclofructans are a class of cyclic oligosaccharides with a crown ether skeleton. No enantioseparations have previously been reported using this class of chiral oligosaccharides in chromatography or electrophoresis. Cyclofructans and their sulfated derivatives were examined as chiral selectors using capillary electrophoresis. The native cyclofructans showed no enantioselectivity toward any tested compounds, while the sulfated cyclofructan showed exceptional selectivity toward many cationic analytes, including primary, secondary, and tertiary amines and amino acids. Enantiomeric resolution factors (as high as 15.4) were achieved within short analysis times (generally below 10 min). The effect of buffer type, buffer concentration, buffer pH, chiral selector concentration and organic modifier concentration were examined and optimized.

5.2 Introduction

The modern technique of capillary electrophoresis (CE) has several known advantages such as high efficiency, short analysis times, low sample consumption, simple instrumentation and a generally low operation cost [13,89,91-96,113,171]. In spite of the large number of chiral selectors used in modern enantiomeric separations, relatively few classes (all of which originated from LC) have been as successful in chiral CE [12,189-191]. This is due to the

inherent requirements of CE chiral selectors: low UV absorption, high solubility in water, minimum interaction with the fused silica wall, etc. To date, cyclodextrins (CDs) and especially their derivatives (charged and uncharged) have dominated chiral CE separations. In this work, we introduce cyclofructans (CFs) and their derivatives as a new class of chiral selectors. Cyclofructans may be the first class of chiral selectors that show comparable suitability to cyclodextrins for CE enantiomeric separations.

Cyclofructans (CFs), also known as cycloinulo-oligosaccharides, are enzymatic digestion products of inuline by the extracellular enzyme, cycloinulo-oligosaccharides transferase [192-197]. As shown in Figure 17, cyclofructans consist of a crown ether skeleton and fructofuranose residues that are linked to the crown ether ring in a spiral arrangement [198-200]. Each fructofuranose moiety has three hydroxyl groups, which not only make cyclofructans highly soluble in aqueous solution (>1.2g/ml), but also provide multiple H-bonding sites. In addition, CFs are UV transparent to nearly 200 nm. These unique characteristics make them ideal candidates for chiral selectors in CE. Unlike cyclodextrins, which possess a truncated cone shape [201-202]. Cyclofrutans are more disc-shaped with central indentation [200]. Since the first report in 1989 [192], cyclofructans have been used in many applications including: hardening accelerators in adhesives [203], silver halide photographic materials [204-206] gelling-prevention agents for frozen eggs [207], complexation agents [200,208-210], drug carriers and health food additives [211-214] as well as bad taste inhibitors [215-216]. However, few reports have been found concerning the application of cyclofructan as chiral selectors [217-219]. To our knowledge, there has been no reported use of any cyclofructan as a chiral selector in electrophoresis or chromatography.

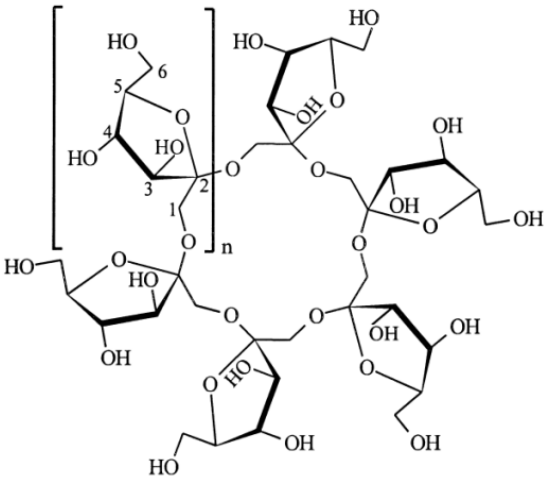


Figure 17. Structure of cyclofructan. n=1, CF6; n=2, CF7; n=3, CF8. Reprint from Immel *et. al.* [200]

5.3 Materials and Methods

5.3.1 Materials

Cyclofructan 6 (CF6) and Cyclofructan 7(CF7) were gifts from Dr. Mari Yasuda at the Mitsubishi Chemical Group (Tokyo, Japan). Dimethyl sulfoxide, pyridine, sulfur trioxide pyridine complex, sodium acetate, tetraethylammonium nitrate, tetrabutylammonium nitrate and all chiral analytes tested were purchased from Sigma-Aldrich (Milwaukee, WI, USA). HPLC-grade methanol, phosphoric acid, glacial acetic acid, and sodium hydroxide were purchased from VWR (Bridgeport, NJ, USA). Ammonium acetate was purchased from Fisher Scientific (St. Louis, MO, USA). The fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA) and Beckman Coulter (Fullerton, CA, USA).

5.3.2 Methods

All separations were performed on a Beckman Coulter P/ACE MDQ system capillary electrophoresis system equipped with a photodiode array detector. The capillary (50 µm i.d. ×358 µm o.d.) was used with a total length of 40 cm (30 cm from inlet to detection window). All

the electropherograms were obtained with detection at 214 nm and sample identity was confirmed by UV spectrum. Ammonium acetate was dissolved in deionized water and adjusted to desired pH with glacial acetic acid or phosphoric acid and used as the background buffer in normal polarity mode. Phosphoric acid was dissolved in deionized water and adjusted to desired pH with hydrochloric acid to be used as the background buffer in reverse polarity mode. Organic modifiers were added, based on volume percentage, prior to the addition of chiral selectors. Chiral selectors were then added to the background buffer solution to make run buffer. Due to the hydrolysis of the sulfate group on sulfated cyclofructans, fresh run buffer was made every 4 to 6 hours. Racemic samples or artificial mixtures of enantiomers were dissolved in the corresponding background buffer or water to make sample solutions.

When a new capillary was installed, it was rinsed with 1 M sodium hydroxide solution for 5 min, and then deionized water for 5 min for capillary conditioning. Between each run, the capillary was rinsed with 1 M hydrochloric acid solution for 1 min, deionized water for 1 min, 1 M sodium hydroxide solution for 1 min, deionized water for 1 min and then run buffer for 2 min. Subsequently, the sample solution was injected hydrodynamically at 0.5 psi for 3 seconds. All the compounds were first tested and the separation conditions were optimized in the normal polarity mode. Subsequently reverse polarity with low pH buffers was used to minimize the wall interaction of cationic analytes. The electromigration order was determined by spiking with a pure enantiomer.

The parameters were calculated as follows: Resolution (Rs): $Rs=2(t_{m2}-t_{m1})/(w_1+w_2)$, the apparent mobility (μ_{app}): $\mu_{app}=L*L_{total}/(t_mV)$, mobility difference ($\Delta\mu$): $\Delta\mu=\mu_{app1}-\mu_{app2}$, electroosmotic mobility (μ_{eof}): $\mu_{eof}= L*L_{total}*/(t_{eof}V)$, electrophoretic mobility (μ): $\mu=L*L_{total}*(1/t_m-1/t_{eof})/V$, selectivity ($\alpha$): $\alpha=\mu_{app1}/\mu_{app2}$, and the number of theoretical plates (N): $N=16*(t_m/w)^2$, where t_{m1} and t_{m2} are the migration times of the first and second peak, t_{eof} is the migration time of the EOF marker, and w is the baseline peak width. L represents the length of the capillary from the injection end to the detection window and L_{total} is the total length of capillary. When the

separation showed severe tailing, it was difficult to measure w, therefore, Rs was estimated by comparing to computer generated chromatograms.[220] The resolution (Rs) can also be expressed as: $Rs=\Delta\mu*N^{1/2}/(4\mu_{app,avg})$.[221] As the selectivity term $\Delta\mu/\mu_{app,avg}=2(\mu_{app2}-\mu_{app1})/(\mu_{app1}+\mu_{app2})=(\alpha-1)/(\alpha+1)$, $Rs=(\alpha-1)/(\alpha+1)N^{1/2}*(2\mu_{app,avg})$).

5.3.3 Sulfation of Cyclofructans

The procedure for the sulfation of CF6 and CF7 was developed by following a previously reported procedure for the sulfation of cyclodextrins [222]. Sodium sulfated cycloinulohexaose (SCF6) and sodium sulfated cycloinuloheptaose (SCF7) were synthesized in an analogous manner. Specifically, sulfur trioxide pyridine complex (6.82 g, 0.043 mols) was dissolved in anhydrous pyridine (12-15 mL) and heated to 80-85℃ for 20 min. Next, the native cyclofructan (1.135 g) was added and the mixture was stirred and heated at 80-85℃ for 6 hrs. After this time, the reaction mixture was allowed to cool to room temperature. The mixture was then processed with methanol (10 x 100mL); decanting in between washings. One more extraction was performed by allowing the semi-solid product to be stirred in methanol (100 mL) overnight. After decanting the final washing, the brownish semi-solid product was dissolved in 30% sodium acetate (6.5 mL, 0.024 mols). Then, deionized water (7 mL) was added and the solution was stirred at room temperature for 2 hrs. Next, the solution was slowly added to methanol (100 mL) and stirred for 1 hr., resulting in the precipitation of the product. Suction filtration, washing with ethanol, and drying yielded the pure sulfated cyclofructans in the sodium salt form. The product composition was examined with ESI-MS. The mass spectrum showed that SCF6 is a mixture containing 11-15 sulfate groups and SCF7 is a mixture containing 16-20 sulfate groups.

5.4 Results and Dicussion

5.4.1 Binding Mechanism

Cyclofructans were reported to form complexes with certain metal ions [200,208,300], in an analogues manner to crown ethers. It is known that changes in the electrophoretic mobility of an analyte at various chiral selector concentrations can be used to estimate the binding constant between the analyte and chiral selector [223-226]. Since SCFs are highly negatively charged species, analytes will show a lower mobility toward the anode when they associate with SCFs. Among the tested neutral compounds containing no nitrogen, all except catechin showed no binding to SCFs, since all of them coelute with the EOF marker in the normal polarity mode. Other nitrogen containing compounds, including amino acid amides/esters, amino acids and other amine-containning compounds, showed lower mobilities toward the anode in the presence of SCFs. Clearly, electrostatic interactions play an important role in the binding of analytes to SCFs. Generally, cationic analytes, including amines, and amino acid esters, bind more strongly than neutral analytes, such as amides and amino acids (which are zwitterions at the buffer pH used). Analytes with multiple positive charges bind more strongly than singly charged analytes. Figure 18 shows the electropherograms obtained for selected compounds with similar structures. The electromigration order was obtained by spiking compounds with known structures. It was interesting that 3-amino-3-phenylpropionic acid, which is a β-amino acid, showed significantly stronger binding to SCF than its corresponding α-amino acid, phenylalanine.

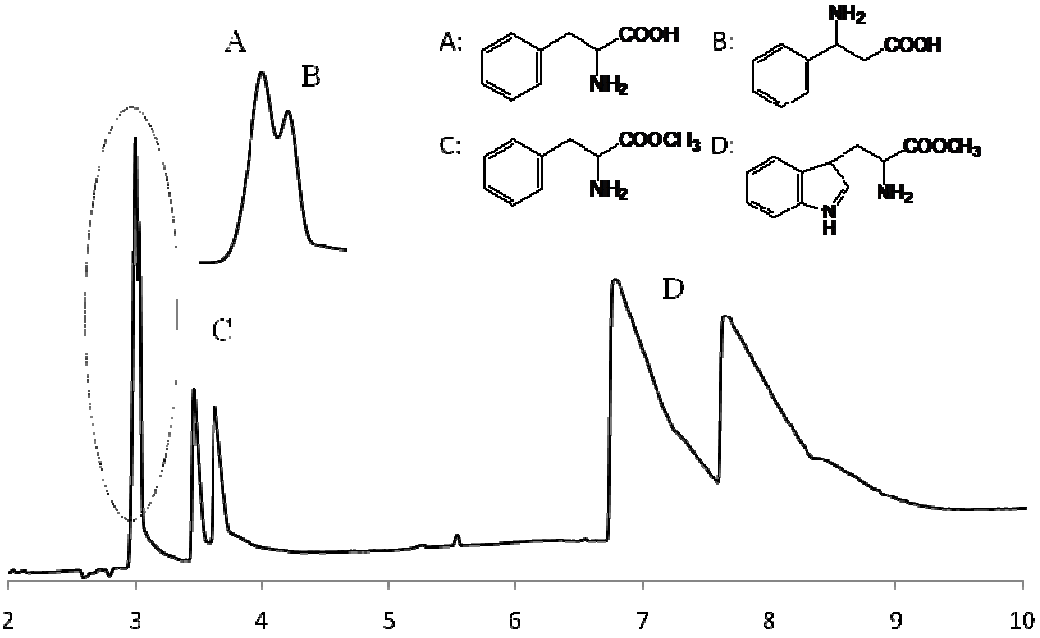


Figure 18. Electromigration order of 4 compounds with similar structures. Electropherogram was obtained at 214 nm with 5 mM SCF6. Conditions: +25 kV, 30/40 cm 50 µm I.D capillary, 4 mM ammonium acetate, 5%MeOH, pH=4.1.

5.4.2 Overview of Enantioseparation Results

In order to examine the enantioselective capabilities of sulfated cyclofructans, over 200 pairs of enantiomers were tested. A series of amines and amino acids were used to examine optimization parameters. In the normal polarity mode, only cationic amines showed enantioselectivity. However, some analytes showed severe tailing, probably due to adverse wall interactions. Therefore, reverse polarity using a background buffer with pH around 2 was examined. This approach greatly improved the efficiency for compounds with severe tailing (as shown in Figure 19). The low pH also allowed for the protonation of the carboxylic group of amino acids, which enabled their subsequent enantioseparation. All results are summarized in Tables 17-23 and Figure 20. Among the 110 amine-containing compounds tested (including two sets of diastereoisomers of which each contains two pairs of enantiomers), 90 of them showed

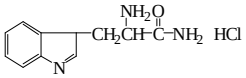
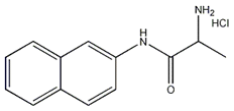
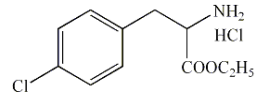
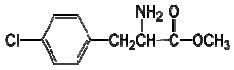
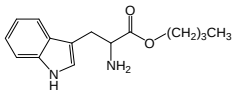
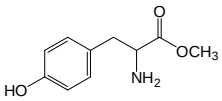
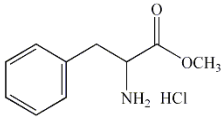
enantioselectivity, with 66 of them being baseline separated by one or both of the SCFs. This is a relatively high percentage. In fact, a resolution of 15.4 was easily achieved for compound #19 (2-amino-1,1,3-tripheyl-1-propanol), within 7 minutes (Table 18). All the analytes showed similar migration time under the same SCFs concentration, which indicates similar binding strength of analytes to both SCFs. In addition, it was observed that in all cases in which the electromigration order was determined, the electromigration order was the same for both SCF6 and SCF7. Among the 90 pairs of enantiomers that showed enantioselectivity, 84 of them were separated by both SCFs. These three facts indicate that similar molecular recognition mechanisms may be operative for both cyclofructans. However, for a few specific analytes, the two SCFs showed significant difference in enantioselectivity. For example, compound #38 (idazoxan) was only separated on SCF6 (Rs=4.3) while compound #37 (fenfluramine) was only separated on SCF7 (Rs=4.2).

With a careful examination of data, certain interesting facts were noticed. First, it was observed that the resolution (Rs) for the separation of amino acid esters increased as the size of the ester group increase. For example, compound #3 (4-chlorophenylalanine ethyl ester) was better separated than compound #4 (4-chlorophenylalanine methyl ester). Another interesting phenomenon is that a chloro- substituent in the *p*-position of phenyl groups (i.e. compounds #4, #14 and #29) can increase the selectivity compared to their non-halogenated counterparts (i.e. compounds #7, #13 and #27, respectively).

5.4.3 Factors Affecting Enantioseparations

Several factors, such as buffer type and concentration, pH, chiral selector concentration, and organic modifiers, are commonly used to optimize enantiomeric separations [13,91,93,113-115,170-171,227].

Table 17. Experimental Data for Enantiomeric Separations of Amino Acid Amides and Amino Acid Esters with SCF6 and SCF7.

#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
1	 Tryptophanamide	8.700	1.18	1.27	1.6	1100	-	SCF6 10 mM Buffer 2
		6.262	0.85	1.12	1.9	4200	-	SCF7 10 mM Buffer 2
2	 DL-alanine-β-naphthylamide hydrochloride	12.354	0.08	1.02	1.5	-	-	SCF6 15 mM Buffer 2
		6.688	0.33	1.05	2.6	42000	-	SCF7 10 mM Buffer 2
3	 DL-4-Chlorophenylalanine ethyl ester hydrochloride	3.933	0.92	1.08	4.3	52000	-	SCF6 15 mM Buffer 2
		6.742	1.21	1.20	8.1	33000	-	SCF7 15 mM Buffer 2
4	 DL-4-Chlorophenylalanine methyl ester hydrochloride	4.037	0.67	1.06	1.3	-	-	SCF6 15 mM Buffer 2
		7.537	0.66	1.12	4.6	30000	-	SCF7 15 mM Buffer 2
5	 DL-Tryptophan butyl ester hydrochloride	5.142	1.66	1.22	5.7	16000	-	SCF6 15 mM Buffer 2
		6.667	1.46	1.25	3.4	3800	-	SCF7 15 mM Buffer 2
6	 Tyrosine methyl ester	3.104	0.38	1.03	0.9	-	-	SCF6 15 mM Buffer 2
		4.829	0.17	1.02	0.7	-	-	SCF7 15 mM Buffer 2
7	 DL-Phenylalanine methyl ester hydrochloride	4.379	0.61	1.06	1.5	-	L>D	SCF6 15 mM Buffer 2
		5.217	0.53	1.06	1.5	-	L>D	SCF7 15 mM Buffer 2

Unit for time (tm_1) is min, mobility (Δμ) is $cm^2kV^{-1}min^{-1}$. Conditions: 30/40 cm 50 μm I.D capillary; +25 kV; buffer 1: 5 mM ammonium acetate, pH=4.1; buffer 2: 4 mM ammonium acetate, 5% MeOH, pH=4.1; buffer 3: 4 mM ammonium acetate, 5% MeOH, pH=3.7; buffer 4: mixture of 20 mM ammonium acetate and 10 mM phosphoric acid, pH=4.7. Buffer 1,2,3 were used with capillary batch 2, buffer 4 was used with capillary batch 1.

Table 17-C*ontinued.*

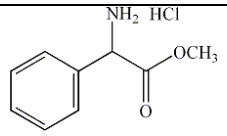
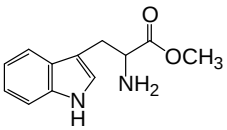
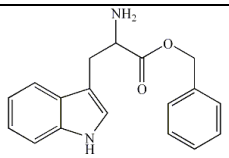
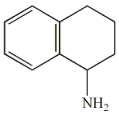
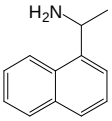
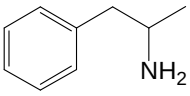
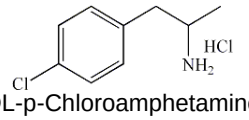
#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
8	 (±)-2-Phenylglycine methyl ester hydrochloride	3.604	0.49	1.04	2.2	59000	L>D	SCF6 15 mM Buffer 2
		4.404	2.29	1.27	7.9	48000	L>D	SCF7 15 mM Buffer 2
9	 DL-Tryptophan methyl ester	8.275	0.59	1.11	1.1	-	L>D	SCF6 15 mM Buffer 2
		10.392	0.32	1.07	1.1	-	L>D	SCF7 15 mM Buffer 2
10	 DL-Tryptophan benzyl ester	6.979	1.75	1.34	4.0	2200	-	SCF6 10 mM Buffer 2
		4.092	1.56	1.15	2.7	6500	-	SCF7 10 mM Buffer 2

Table 18. Experimental Data for Enantiomeric Separations of Primary Amines with SCF6 and SCF7.

#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
11	 1,2,3,4-Tetrahydro-1-naphthylamine	4.625	0.10	1.01	0.4	-	-	SCF6 15 mM Buffer 2
		5.329	0.40	1.05	1.4	-	-	SCF7 15 mM Buffer 2
12	 1-(1-Naphthyl)ethylamine	7.263	1.63	1.33	3.4	3400	-	SCF6 5 mM Buffer 4
		5.400	1.49	1.20	4.2	9700	-	SCF7 5 mM Buffer 4
13	 DL-Amphetamine sulfate salt	3.413	0.40	1.03	1.0	-	-	SCF6 15 mM Buffer 2
		5.217	0.25	1.03	0.8	-	-	SCF7 15 mM Buffer 1
14	 DL-p-Chloroamphetamine HCl	3.096	0.49	1.03	2.1	68000	-	SCF6 15 mM Buffer 2
		6.500	0.25	1.04	1.5	-	-	SCF7 15 mM Buffer 1

Unit for time (tm_1) is min, mobility (Δμ) is $cm^2kV^{-1}min^{-1}$. Other conditions are same as table 1.

Table 18 –*Continued.*

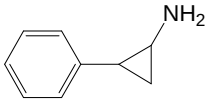
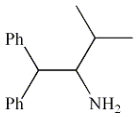
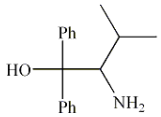
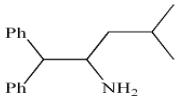
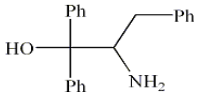
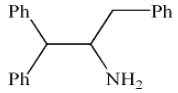
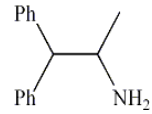
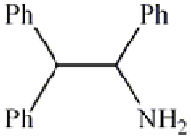
#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
15	 trans-2-Phenylcyclopropylamine	4.475	0.84	1.08	1.4	-	-	SCF6 15 mM Buffer 2
		10.254	1.20	1.26	1.5	-	-	SCF7 15 mM Buffer 2
16	 (±)-2-Amino-3-methyl-1,1-diphenylbutane	4.117	0.17	1.02	0.9	-	-	SCF6 15 mM Buffer 2
		3.604	-	-	-	-	-	SCF7 15 mM Buffer 2
17	 (±)-2-Amino-3-methyl-1,1-diphenyl-1-butanol	4.529	0.37	1.04	0.9	-	R>S	SCF6 15 mM Buffer 2
		3.254	0.19	1.01	0.9	-	R>S	SCF7 15 mM Buffer 1
18	 (±)-2-Amino-4-methyl-1,1-diphenylpentane	4.483	0.38	1.04	2.2	58000	R>S	SCF6 15 mM Buffer 2
		3.608	0.70	1.06	1.5	-	R>S	SCF7 15 mM Buffer 2
19	 (±)-2-Amino-1,1,3-triphenyl-1-propanol	4.904	1.37	1.16	9.4	62000	R>S	SCF6 15 mM Buffer 2
		3.879	2.38	1.24	15.4	85000	R>S	SCF7 15 mM Buffer 1
20	 (±)-1-Benzyl-2,2-diphenylethylamine	5.746	0.14	1.02	1.1	-	R>S	SCF6 15 mM Buffer 2
		7.558	0.88	1.16	3.4	12000	R>S	SCF7 15 mM Buffer 1
21	 (±)-1,1-Diphenyl-2-aminopropane	4.579	0.25	1.02	1.1	-	R>S	SCF6 15 mM Buffer 2
		3.850	0.24	1.02	1.3	-	R>S	SCF7 15 mM Buffer 2
22	 (±)-1,2,2-Triphenylethylamine	4.204	0.83	1.08	2.9	23000	R>S	SCF6 15 mM Buffer 2
		3.438	0.83	1.06	3.3	49000	R>S	SCF7 15 mM Buffer 2

Table 18 –*Continued.*

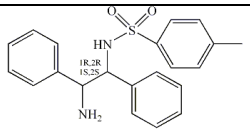
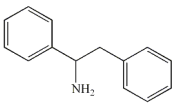
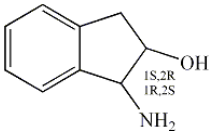
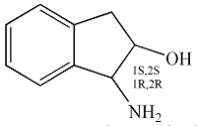
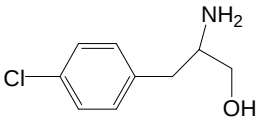
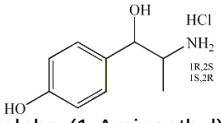
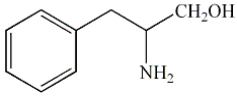
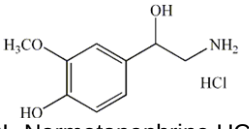
#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
23	 (±)-N-p-Tosyl-1,2-diphenylethylenediamine	3.038	-	-	-	-	-	SCF6 15 mM Buffer 2
		2.783	1.27	1.08	0.9	-	-	SCF7 15 mM Buffer 2
24	 1,2-Diphenylethylamine	3.450	1.25	1.10	6.0	79000	-	SCF6 15 mM Buffer 2
		3.850	1.10	1.10	3.0	27000	-	SCF7 15 mM Buffer 2
25	 (±) cis-1-Amino-2-indanol	4.546	-	-	-	-	-	SCF6 15 mM Buffer 2
		6.400	0.82	1.12	1.5	-	-	SCF7 15 mM Buffer 1
26	 (±) trans-1-Amino-2-indanol	3.330	0.03	1.00	0.6	-	-	SCF6 15 mM Buffer 2
		4.771	0.21	1.02	1.0	-	-	SCF7 15 mM Buffer 1
27	 DL-4-Chlorophenyl alaninol	6.554	0.58	1.09	4.9	57000	-	SCF6 15 mM Buffer 4
		8.525	0.47	1.09	5.2	56000	-	SCF7 15 mM Buffer 4
28	 (±)-alpha-(1-Aminoethyl)-4-hydroxybenzyl alcohol HCl	6.792	0.18	1.03	0.8	-	-	SCF6 18 mM Buffer 3
		4.258	-	-	-	-	-	SCF7 15 mM Buffer 1
29	 2-Amino-3-phenyl-1-propanol	5.092	0.52	1.06	2.4	29000	S>R	SCF6 15 mM Buffer 4
		6.558	0.49	1.07	1.8	10000	S>R	SCF7 15 mM Buffer 4
30	 DL-Normetanephrine HCl	5.883	0.19	1.02	0.6	-	-	SCF6 18 mM Buffer 3
		5.263	0.21	1.02	1.1	-	-	SCF7 15 mM Buffer 2

Table 18 –Continued.

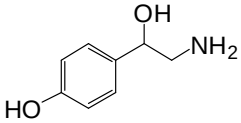
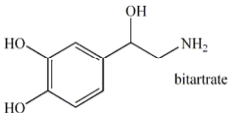
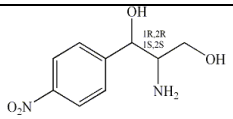
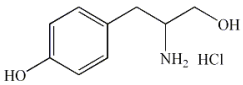
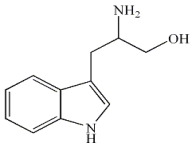
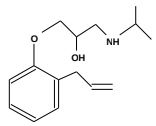
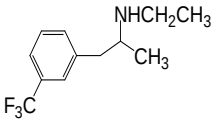
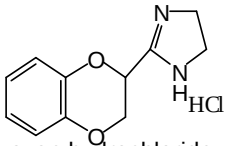
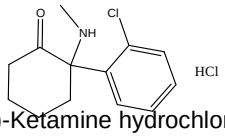
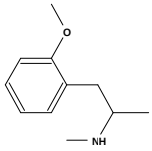
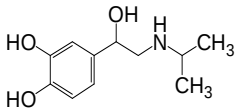
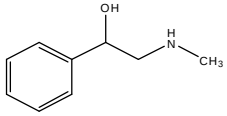
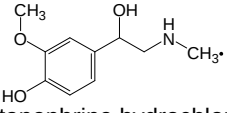
#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
31	 Norphenylephrine HCl	3.817	0.15	1.01	0.5	-	-	SCF6 15 mM Buffer 2
		4.892	0.17	1.02	0.7	-	-	SCF7 15 mM Buffer 2
32	 (+/-)-Norepinephrine L-bitartrate hydrate	7.213	0.13	1.02	0.6	-	-	SCF6 18 mM Buffer 3
		5.875	0.21	1.03	1.2	-	-	SCF7 15 mM Buffer 1
33	 (±)-2-Amino-1-(4-nitrophenyl)-1,3-propanediol	8.208	0.38	1.07	3.7	52000	S,S> R,R	SCF6 15 mM Buffer 4
		6.171	0.44	1.06	1.5	-	S,S> R,R	SCF7 15 mM Buffer 2
34	 DL-Tyrosinol hydrochloride	5.888	0.43	1.06	2.5	33000	L>D	SCF6 15 mM Buffer 4
		4.638	0.33	1.03	1.1	-	L>D	SCF7 15 mM Buffer 2
35	 DL-Tryptophanol	7.021	0.98	1.17	1.5	3400	L>D	SCF6 15 mM Buffer 2
		11.963	1.23	1.44	4.4	18000	L>D	SCF7 15 mM Buffer 2

Table 19 Experimental Data for Enantiomeric Separations of Secondary Amines with SCF6 and SCF7.

#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
36	 Alprenolol	9.625	0.16	1.03	0.8	-	-	SCF6 15 mM Buffer 4
		4.517	0.37	1.04	1.1	-	-	SCF7 15 mM Buffer 1
37	 (±)-Fenfluramine hydrochloride	6.333	0.13	1.02	0.6	-	-	SCF6 15 mM Buffer 2
		5.146	0.44	1.05	2.2	33000	-	SCF7 15 mM Buffer 2
38	 Idazoxan hydrochloride	6.104	0.86	1.12	3.0	16000	-	SCF6 15 mM Buffer 2
		7.025	-	-	-	-	-	SCF7 15 mM Buffer 2
39	 (±)-Ketamine hydrochloride	5.358	0.26	1.03	0.8	-	-	SCF6 15 mM Buffer 2
		4.317	0.51	1.05	1.4	-	-	SCF7 15 mM Buffer 1
40	 Methoxyphenamine	4.848	-	-	-	-	-	SCF6 15 mM Buffer 2
		5.296	0.28	1.03	1.1	-	-	SCF7 15 mM Buffer 2
41	 DL-Isoprenaline hydrochloride	4.750	0.12	1.01	0.7	-	-	SCF6 15 mM Buffer 2
		3.625	0.14	1.01	0.6	-	-	SCF7 15 mM Buffer 2
42	 alpha-(Methylaminomethyl) benzyl alcohol	4.338	-	-	-	-	-	SCF6 15 mM Buffer 2
		5.229	0.13	1.01	0.8	-	-	SCF7 15 mM Buffer 2
43	 Metanephrine hydrochloride	5.667	0.38	1.04	0.9	-		SCF6 15 mM Buffer 2
		3.604	0.23	1.02	0.9	-		SCF7 15 mM Buffer 2

Unit for time (tm_1) is min, mobility (Δμ) is $cm^2kV^{-1}min^{-1}$. Other conditions are same as Table 1.

Table 19 –*Continued.*

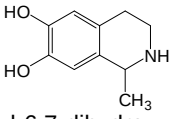
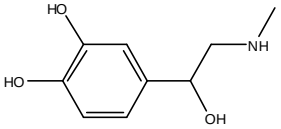
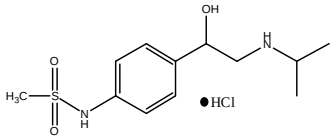
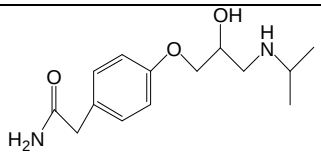
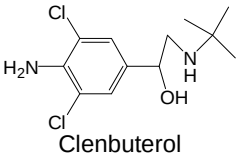
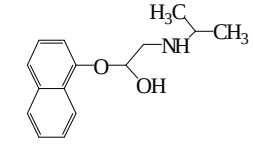
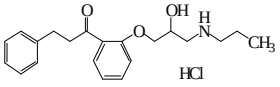
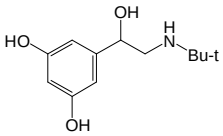
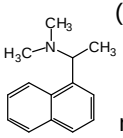
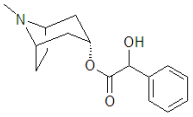
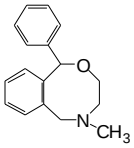
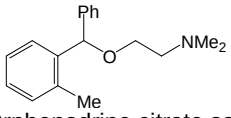
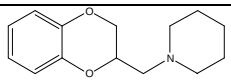
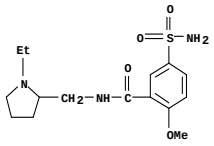
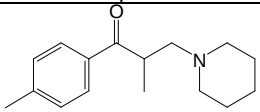
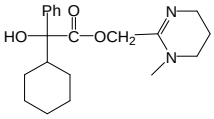
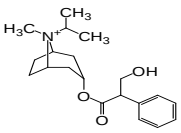
#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
44	 1-Methyl-6,7-dihydroxy-1,2,3,4 -tetrahydroisoquinoline HCl	4.783	0.95	1.10	1.4	-	-	SCF6 15 mM Buffer 2
		4.217	0.19	1.02	1.1	-	-	SCF7 15 mM Buffer 2
45	 Epinephrine	6.350	0.14	1.02	0.6	-	-	SCF6 15 mM Buffer 2
		6.550	0.12	1.02	1.0	-	-	SCF7 15 mM Buffer 2
46	 (±)-Sotalol hydrochloride	8.721	0.18	1.03	0.7	-	-	SCF6 15 mM Buffer 2
		9.271	0.06	1.01	0.4	-	-	SCF7 15 mM Buffer 2
47	 Atenolol	7.646	0.10	1.02	0.5	-	-	SCF6 18 mM Buffer 3
		6.792	0.23	1.03	0.9	-	-	SCF7 15 mM Buffer 1
48	 Clenbuterol	6.092	-	-	0.3	-	-	SCF6 15 mM Buffer 2
		4.787	0.41	1.04	1.8	-	-	SCF7 18 mM Buffer 3
49	 DL-Propranolol hydroxide	8.154	0.53	1.10	2.0	19000	-	SCF6 15 mM Buffer 2
		6.592	0.63	1.10	2.5	12000	-	SCF7 15 mM Buffer 2
50	 Propafenone HCl	3.163	0.29	1.02	0.6	-	-	SCF6 15 mM Buffer 2
		5.067	0.51	1.06	1.8	19000	-	SCF7 15 mM Buffer 1
51	 Terbutaline hemisulfate salt	2.592	-	-	-	-	-	SCF6 15 mM Buffer 2
		3.533	0.13	1.01	0.5	-	-	SCF7 15 mM Buffer 2

Table 20. Data for Enantiomeric Separations of Tertiary Amines with SCF6 and SCF7.

#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
52	 (R)-(+)-N,N-Dimethyl-1-(1-naphthyl) ethylamine (S)-(−)-N,N-Dimethyl-1-(1-naphthyl) ethylamine	5.633	1.65	1.24	1.7	1600	-	SCF6 10 mM Buffer 2
		3.496	1.71	1.14	1.6	2500	-	SCF7 10 mM Buffer 2
53	 DL-Homatropine hydrobromide	6.300	0.58	1.08	0.5	-	-	SCF6 15 mM Buffer 2
		7.233	0.28	1.04	0.8	-	-	SCF7 15 mM Buffer 2
54	 Nefopam hydrochloride	4.938	0.73	1.08	3.2	28000	-	SCF6 15 mM Buffer 2
		7.371	0.76	1.13	2.2	6600	-	SCF7 15 mM Buffer 2
55	 Orphenadrine citrate salt	3.837	0.24	1.02	1.2	-	-	SCF6 15 mM Buffer 2
		3.733	0.35	1.03	1.2	-	-	SCF7 15 mM Buffer 2
56	 dl-Piperoxan 2-(N-Piperidinomethyl)-1,4-benzodioxane	10.371	0.32	1.07	3.1	32000	-	SCF6 15 mM Buffer 4
		8.950	0.37	1.07	3.7	44000	-	SCF7 15 mM Buffer 4
57	 Sulpiride	7.375	0.14	1.02	1.1	-	-	SCF6 15 mM Buffer 2
		3.671	0.32	1.02	0.7	-	-	SCF7 15 mM Buffer 2
58	 Tolperisone hydrochloride	2.521	-	-	-	-	-	SCF6 15 mM Buffer 2
		6.263	-	-	0.4	-	-	SCF7 15 mM Buffer 2
59	 Oxyphencyclimine HCl	2.929	1.36	1.08	4.1	43000	-	SCF6 15 mM Buffer 2
		5.342	1.12	1.12	5.1	31000	-	SCF7 15 mM Buffer 1

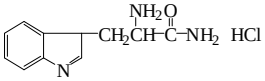
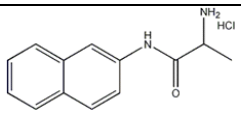
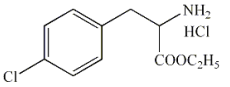
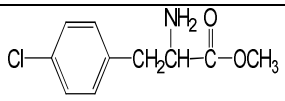
Unit for time (tm_1) is min, mobility (Δμ) is $cm^2kV^{-1}min^{-1}$. Other conditions are the same as Table 1.

Table 21. Data for Enantiomeric Separations of Quantiary Amine with SCF6 and SCF7.

#	Compound	tm_1	Δμ	α	Rs	N	EMO	Condition
60 & 61	 33%Ipratropium bromide monohydrate 67%8-S isomer	6.963	0.19	1.03	1.1	-	-	SCF6 15 mM Buffer 2
		7.713	0.15	1.02	1.1	-	-	
		5.388	0.32	1.04	2.3	68000	-	SCF7 15 mM Buffer 2
		5.779	0.24	1.03	2.2	94000	-	

Unit for time (tm_1) is min, mobility (Δμ) is $cm^2kV^{-1}min^{-1}$. Other conditions are the same as Table 1.

Table 22. Experimental Data for Enantiomeric Separations of Amines with SCF6 and SCF7 in the Reverse Polarity Mode.

#	Compound	V	L	t_{m1}	Δμ	α	Rs	N	Conditions
1	 Tryptophanamide	-18	20	2.150	1.06	1.07	1.6	8100	SCF6 10 mM Buffer 6
		-18	20	1.892	0.81	1.05	0.8	4200	SCF7 10 mM Buffer 6
2	 DL-alanine-β-naphthylamide HCl	-18	20	2.875	0.13	1.01	0.6	40000	SCF6 10 mM Buffer 6
		-18	20	2.467	0.99	1.08	4.3	50000	SCF7 10 mM Buffer 6
3	 DL-4-Chlorophenylalanine ethyl ester HCl	-25	20	3.904	0.96	1.19	7.4	31000	SCF6 10 mM Buffer 7
		-18	20	3.842	0.91	1.12	7.1	68000	SCF7 10 mM Buffer 6
4	 DL-4-Chlorophenylalanine methyl ester hydrochloride	-18	20	3.929	0.92	1.12	5.3	34000	SCF6 10 mM Buffer 6
		-18	20	3.625	0.49	1.06	3.0	47000	SCF7 10 mM Buffer 6

Any compound that didn't show peaks or enantioseparation is not listed. Unit for applied voltage (V) is kV, capillary length to detection window (L) is cm, total length Ltot=L+10 cm, time (t_{m1}) is min, mobility (Δμ) is $cm^2kV^{-1}min^{-1}$. Conditions: 50 μm I.D capillary; buffer 5, 4 mM phosphate with 5%MeOH, adjust with 1 M HCl, pH=1.96; buffer 6: 5 mM phosphoric acid, pH=2.45;buffer 7: 4 mM phosphoric acid, 5%MeOH, pH=2.45

Table 22 –*Continued.*

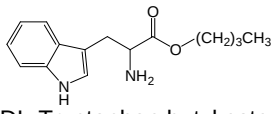
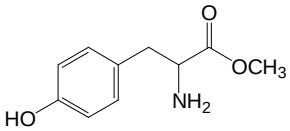
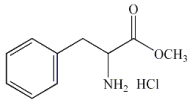
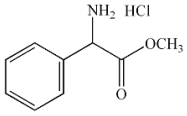
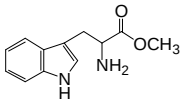
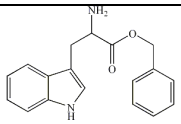
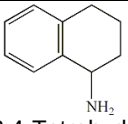
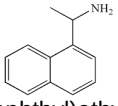
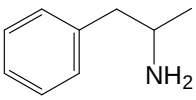
5	 DL-Tryptophan butyl ester HCl	-18	20	2.763	1.75	1.17	5.8	23000	SCF6 10 mM Buffer 6
		-18	20	2.467	1.33	1.11	2.5	9400	SCF7 10 mM Buffer 6
6	 Tyrosine methyl ester	-18	20	5.013	0.55	1.09	2.8	17000	SCF6 10 mM Buffer 6
		-18	20	7.608	0.08 1	1.02	1.1	53000	SCF7 10 mM Buffer 6
7	 DL-Phenylalanine methyl ester HCl	-18	20	5.075	0.65	1.10	2.3	8200	SCF6 10 mM Buffer 6
		-18	20	4.688	0.48	1.07	1.2	4400	SCF7 10 mM Buffer 6
8	 (±)-2-Phenylglycine methyl ester HCl	-18	20	6.583	0.67	1.15	4.2	15000	SCF6 10 mM Buffer 6
		-18	20	4.471	1.83	1.32	6.3	11000	SCF7 10 mM Buffer 6
9	 DL-Tryptophan methyl ester	-18	20	2.554	0.67	1.05	1.4	12000	SCF6 10 mM Buffer 6
		-18	20	2.996	0.29	1.03	0.5	6400	SCF7 10 mM Buffer 6
10	 DL-Tryptophan benzyl ester	-18	20	2.333	1.55	1.12	4.8	28000	SCF6 10 mM Buffer 6
		-18	20	2.271	1.67	1.13	6.6	48000	SCF7 10 mM Buffer 6
11	 1,2,3,4-Tetrahydro-1-naphthylamine	-18	20	5.050	-	-	-	3100	SCF6 10 mM Buffer 6
		-18	20	3.933	0.44	1.05	1.0	5600	SCF7 10 mM Buffer 6
12	 1-(1-Naphthyl)ethylamine	-18	20	2.987	1.55	1.16	3.5	9000	SCF6 10 mM Buffer 6
		-18	20	2.663	1.28	1.11	3.0	13000	SCF7 10 mM Buffer 6
13	 DL-Amphetamine	-18	20	7.463	0.37	1.10	2.6	15000	SCF6 10 mM Buffer 5
		-18	30	12.625	0.19	1.04	1.8	40000	SCF7 20 mM Buffer 5

Table 22 –*Continued.*

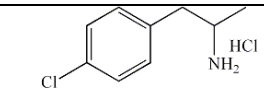
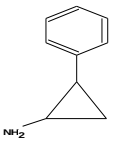
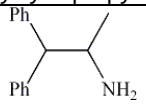
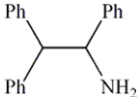
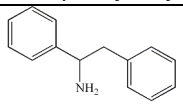
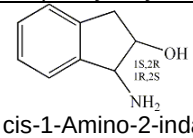
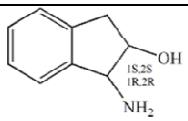
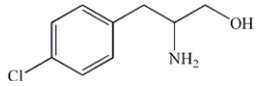
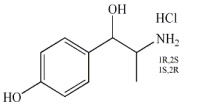
14	 DL-p-Chloroamphetamine HCl	-20	20	3.129	1.29	1.16	3.8	12000	SCF6 10 mM Buffer 6
		-18	20	3.658	0.20	1.02	1.4	65000	SCF7 10 mM Buffer 6
15	 trans-2-Phenylcyclopropylamine	-20	20	4.65	0.34	1.06	2.6	38000	SCF6 10 mM Buffer 6
		-18	20	3.754	1.15	1.15	3.5	10000	SCF7 10 mM Buffer 6
21	 (±)-1,1-Diphenyl-2-aminopropane	-18	20	6.283	0.26	1.05	1.3	10000	SCF6 10 mM Buffer 6
		-18	20	5.596	0.25	1.04	2.3	45000	SCF7 10 mM Buffer 6
22	 (±)-1,2,2-Triphenylethylamine	-18	20	6.583	0.90	1.22	9.0	34000	SCF6 10 mM Buffer 6
		-18	20	5.958	0.91	1.19	5.3	22000	SCF7 10 mM Buffer 6
24	 1,2-Diphenylethylamine	-18	20	4.146	1.46	1.22	8.9	35000	SCF6 10 mM Buffer 6
		-18	20	3.846	1.21	1.16	7.3	38000	SCF7 10 mM Buffer 6
25	 (±) cis-1-Amino-2-indanol	-18	20	4.592	0.18	1.03	0.7	13000	SCF6 10 mM Buffer 6
		-12	20	9.033	0.44	1.09	1.6	8100	SCF7 20 mM Buffer 5
26	 (±) trans-1-Amino-2-indanol	-18	20	6.129	0.091	1.02	0.7	33000	SCF6 10 mM Buffer 6
		-12	20	14.329	0.16	1.05	1.8	24000	SCF7 20 mM Buffer 5
27	 DL-P-Chlorophenylalaninol	-25	20	3.954	0.47	1.08	2.5	16000	SCF6 10 mM Buffer 7
		-18	20	3.821	0.35	1.04	2.8	71000	SCF7 10 mM Buffer 6
28	 (±)-α-(1-Aminoethyl)-4-hydroxybenzyl alcohol	-25	20	9.554	0.07	1.03	0.9	21000	SCF6 10 mM Buffer 7
		-12	20	20.104	0.005	1.00	0.6	17000	SCF7 20 mM Buffer 5

Table 22 –*Continued.*

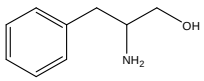
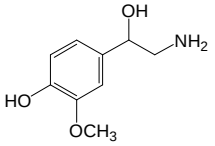
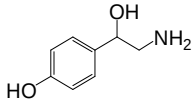
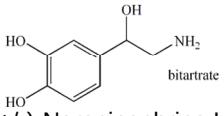
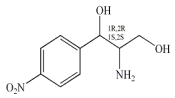
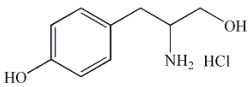
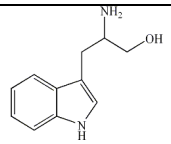
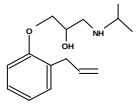
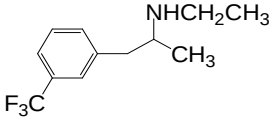
29	 2-Amino-3-phenyl-1-propanol	-18	20	4.888	0.68	1.12	2.1	6600	SCF6 10 mM Buffer 6
		-12	20	7.500	0.93	1.16	2.1	3700	SCF7 10 mM Buffer 6
30	 DL-Normetanephrine	-25	20	3.675	0.19	1.03	1.1	23000	SCF6 10 mM Buffer 7
		-12	20	9.146	0.16	1.03	1.7	45000	SCF7 20 mM Buffer 5
31	 Norphenylephrine HCl	-18	20	5.696	0.17	1.03	1.1	24000	SCF6 10 mM Buffer 6
		-18	20	6.354	0.19	1.04	1.2	19000	SCF7 10 mM Buffer 6
32	 (+/-)-Norepinephrine L-bitartrate hydrate	-18	20	5.737	0.16	1.03	0.9	15000	SCF6 10 mM Buffer 6
		-18	20	5.438	0.15	1.03	1.5	58000	SCF7 10 mM Buffer 6
33	 (±)-2-Amino-1-(4-nitrophenyl)-1,3-propanediol	-18	20	4.563	0.26	1.04	2.1	54000	SCF6 10 mM Buffer 6
		-18	20	4.208	0.41	1.05	3.2	60000	SCF7 10 mM Buffer 6
34	 DL-Tyrosinol HCl	-18	20	12.967	0.083	1.03	1.9	55000	SCF6 10 mM Buffer 6
		-18	20	5.162	0.28	1.05	1.8	25000	SCF7 10 mM Buffer 6
35	 DL-Tryptophanol	-18	20	3.563	1.28	1.16	1.7	2200	SCF6 10 mM Buffer 6
		-18	20	2.275	0.88	1.06	0.9	3400	SCF7 10 mM Buffer 6
36	 Alprenolol	-18	20	7.329	0.22	1.05	0.8	4700	SCF6 10 mM Buffer 6
		-12	20	20.721	0.24	1.11	1.7	4200	SCF7 20 mM Buffer 5
37	 (±)-Fenfluramine HCl	-18	20	4.892	-	-	-	17000	SCF6 10 mM Buffer 6
		-18	20	4.371	0.49	1.07	4.2	81000	SCF7 10 mM Buffer 6

Table 22 –*Continued.*

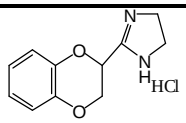
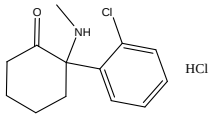
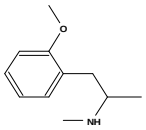
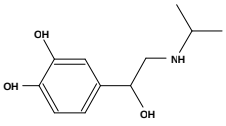
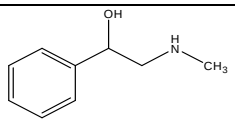
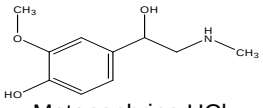
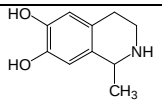
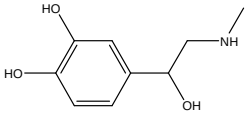
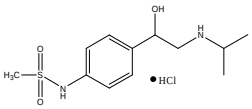
38	 Idazoxan HCl	-18	20	3.529	0.83	1.10	4.3	39000	SCF6 10 mM Buffer 6
		-17	20	3.358	-	-	-	38000	SCF7 10 mM Buffer 6
39	 (±)-Ketamine HCl	-18	20	6.671	0.92	1.23	7.8	27000	SCF6 10 mM Buffer 6
		-18	20	5.987	0.26	1.05	1.3	13000	SCF7 10 mM Buffer 6
40	 Methoxyphenamine	-18	20	5.146	0.18	1.03	1.1	25000	SCF6 10 mM Buffer 6
		-12	20	11.979	0.25	1.06	2.2	20000	SCF7 20 mM Buffer 5
41	 (±)-Isoproterenol	-18	20	8.283	0.18	1.05	1.3	19000	SCF6 10 mM Buffer 6
		-18	20	9.838	0.16	1.05	1.4	13000	SCF7 10 mM Buffer 6
42	 α-(Methylaminomethyl) benzyl alcohol	-18	20	5.638	0.20	1.04	1.5	30000	SCF6 10 mM Buffer 6
		-18	20	4.904	0.15	1.02	1.0	32000	SCF7 10 mM Buffer 6
43	 Metanephrine HCl	-25	20	4.404	0.26	1.05	1.6	17000	SCF6 10 mM Buffer 7
		-18	20	4.279	0.28	1.04	1.4	26000	SCF6 10 mM Buffer 6
44	 1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline	-18	20	4.213	0.89	1.13	4.1	20000	SCF6 10 mM Buffer 6
		-18	20	4.779	0.69	1.11	3.7	22000	SCF7 10 mM Buffer 6
45	 Epinephrine	-18	20	7.879	0.14	1.04	1.3	22000	SCF6 10 mM Buffer 5
		-18	20	7.912	0.14	1.04	0.8	8500	SCF7 10 mM Buffer 6
46	 (±)-Sotalol HCl	-25	20	5.246	0.071	1.02	0.5	18000	SCF6 10 mM Buffer 7
		-12	20	10.517	-	-	-	22000	SCF7 20 mM Buffer 5

Table 22 –*Continued.*

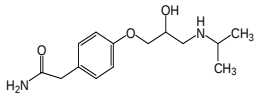
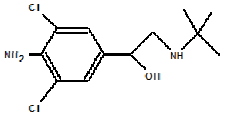
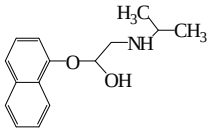
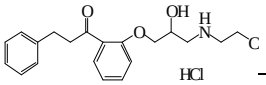
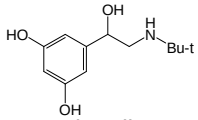
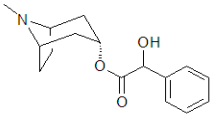
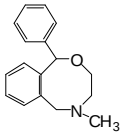
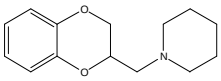
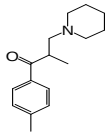
47	 Atenolol	-18	20	5.200	0.096	1.02	0.6	32000	SCF6 10 mM Buffer 6
		-18	20	4.825	0.11	1.02	0.9	52000	SCF7 10 mM Buffer 6
48	 Clenbuterol	-18	20	6.287	0.17	1.03	1.4	28000	SCF6 10 mM Buffer 6
		-18	20	5.796	0.36	1.07	3.2	39000	SCF7 10 mM Buffer 6
49	 DL-Propranolol HCl	-20	20	2.95	1.27	1.14	2.9	8100	SCF6 10 mM Buffer 6
		-18	20	3.029	0.66	1.06	2.4	26000	SCF7 10 mM Buffer 6
50	 Propafenone HCl	-18	20	5.129	0.36	1.06	3.0	44000	SCF6 10 mM Buffer 6
		-18	20	3.604	0.35	1.04	2.5	66000	SCF7 10 mM Buffer 6
51	 Terbutaline hemisulfate salt	-18	20	14.788	0.24	1.12	1.2	2000	SCF6 10 mM Buffer 6
		-18	20	13.017	0.18	1.08	1.2	4200	SCF7 10 mM Buffer 6
53	 DL-Homatropine HBr	-18	20	3.579	0.20	1.02	1.1	43000	SCF6 10 mM Buffer 6
		-18	20	3.567	0.17	1.02	0.9	47000	SCF7 10 mM Buffer 6
54	 Nefopam HCl	-18	20	4.075	0.60	1.08	1.8	9200	SCF6 10 mM Buffer 6
		-18	20	3.267	0.48	1.05	1.1	9000	SCF7 10 mM Buffer 6
56	 dl-Piperoxan	-25	20	3.925	0.32	1.05	1.0	7700	SCF6 10 mM Buffer 7
		-18	20	3.542	0.34	1.04	2.2	60000	SCF7 10 mM Buffer 6
58	 Tolperisone HCl	-18	20	4.888	0.22	1.03	1.0	15000	SCF6 10 mM Buffer 6
		-12	20	11.004	-	-	-	11000	SCF7 20 mM Buffer 5

Table 22 –*Continued.*

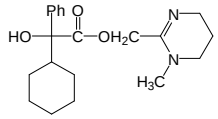
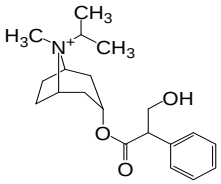
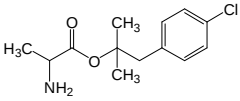
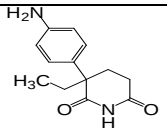
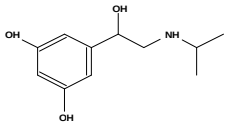
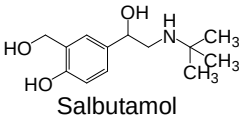
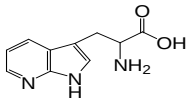
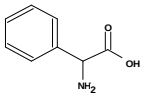
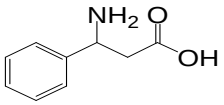
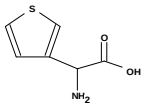
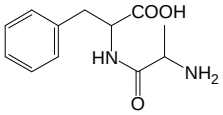
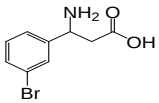
59	 Oxyphencyclimine HCl	-20	20	3.304	2.91	1.47	10.0	13000	SCF6 10 mM Buffer 6
		-18	20	4.4	1.34	1.21	7.9	30000	SCF7 10 mM Buffer 6
60 & 61	 Ipratropium bromide monohydrate	-18	20	5.342	0.11	1.02	0.8	33000	SCF6 10 mM Buffer 6
				5.833	0.15	1.03	1.2	34000	SCF6 10 mM Buffer 6
		-18	20	4.704	0.20	1.03	1.6	51000	SCF7 10 mM Buffer 6
				5.079	0.28	1.04	2.4	53000	SCF7 10 mM Buffer 6
62	 Alaproclate HCl	-25	20	9.375	0.11	1.05	0.5	3000	SCF6 10 mM Buffer 7
		-12	20	13.333	0.12	1.03	1.0	17000	SCF7 20 mM Buffer 5
63	 DL-Aminoglutethimide	-18	20	4.033	1.01	1.14	7.7	56000	SCF6 10 mM Buffer 6
		-17	30	10.996	0.23	1.04	2.6	79000	SCF7 20 mM Buffer 5
64	 Metaproterenol	-18	20	11.654	0.20	1.08	1.1	3500	SCF6 10 mM Buffer 6
		-18	20	9.408	0.26	1.08	1.9	10000	SCF7 10 mM Buffer 6
65	 Salbutamol hemisulfate salt	-18	20	11.917	-	-	-	8300	SCF6 10 mM Buffer 6
		-18	20	9.346	0.044	1.01	0.5	2100	SCF7 10 mM Buffer 6

Table 23. Experimental Data for Enantiomeric Separations of Amino Acids with SCF6 and SCF7 in the Reverse Polarity Mode.

#	Compound	V	L	t_{m1}	Δμ	α	Rs	N	Condition
66	 DL-7-Azatryptophan hydrate	-16	20	1.825	-	-	-	16000	SCF6 15 mM Buffer 5
		-17	30	3.308	0.29	1.01	0.8	56000	SCF7 20 mM Buffer5
67	 DL-alpha-Aminophenyl-acetic acid	-16	20	20.488	0.10	1.06	1.3	8500	SCF6 15 mM Buffer5
		-12	20	22.813	0.39	1.21	5.7	16000	SCF7 20 mM Buffer 5
68	 DL-β-Phenylalanine	-16	20	9.692	0.22	1.06	2.5	29000	SCF6 15 mM Buffer 5
		-18	30	17.079	0.16	1.04	2.1	40000	SCF7 20 mM Buffer 5
69	 DL-alpha-Amino-3-thiopheneacetic acid	-16	20	14.088	0.096	1.04	1.4	24000	SCF6 15 mM Buffer 5
		-20	30	17.575	0.43	1.15	6.1	32000	SCF7 15 mM Buffer 5
70 & 71	 DL-Ala-DL-Phe	-16	20	12.183	0.072	1.02	1.0	28000	SCF6 15 mM Buffer 5
				15.054	0.17	1.07	3.0	29000	
		-18	20	7.029	0.050	1.01	0.4	24000	SCF7 10 mM Buffer 6
				8.325	0.082	1.02	1.0	36000	
72	 3-Amino-3-(3-bromophenyl)propionic acid	-16	20	7.654	0.16	1.03	1.5	35000	SCF6 15 mM Buffer 5
		-17	30	12.596	0.23	1.04	2.6	63000	SCF7 20 mM Buffer 5

Any amino acid that didn't show peaks or enantioseparation is not listed. Unit for applied voltage kV) is kV, capillary length to detection window (L) is cm, total length Ltot=L+10 cm, time (tm_1) is min, mobility (Δμ) is $cm^2kV^{-1}min^{-1}$. Conditions: 50 μm I.D capillary; buffer 5, 4 mM phosphate with 5%MeOH, adjust with 1 M HCl, pH=1.96; buffer 6: 5 mM phosphoric acid, pH=2.45;buffer 7: 4 mM phosphoric acid, 5%MeOH, pH=2.45.

Table 23 –*Continued.*

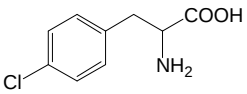
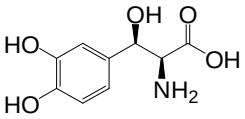
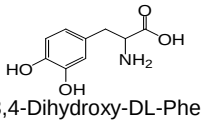
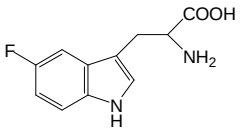
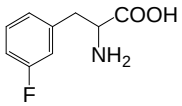
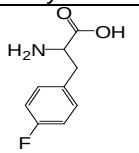
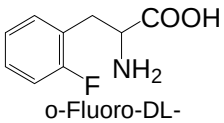
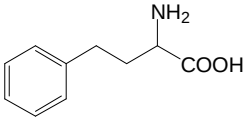
73	 **4-Chloro-DL-phenylalanine**	-16	20	9.779	0.33	1.09	3.4	22000	SCF6 15 mM Buffer 5
		-17	30	17.413	0.35	1.09	4.9	48000	SCF7 20 mM Buffer 5
74	 DL-threo-β-(3,4-Dihydroxyphenyl)serine	-18	20	16.171	0.077	1.04	1.2	17000	SCF6 10 mM Buffer 6
		-18	20	15.729	0.066	1.03	0.7	7400	SCF7 10 mM Buffer 6
75	 3,4-Dihydroxy-DL-Phe	-18	20	13.008	0.075	1.03	1.0	18000	SCF6 10 mM Buffer 6
		-17	30	21.379	0.12	1.04	1.8	40000	SCF7 20 mM Buffer 5
76	 5-Fluoro-DL-tryptophan	-16	20	5.042	0.68	1.10	3.4	21000	SCF6 15 mM Buffer 5
		-17	30	7.933	0.78	1.10	3.4	22000	SCF7 20 mM Buffer 5
77	 m-Fluoro-DL-phenylalanine	-16	20	11.133	0.25	1.08	3.3	29000	SCF6 15 mM Buffer 5
		-17	30	19.104	0.43	1.13	6.4	43000	SCF7 20 mM Buffer 5
78	 p-Fluoro-DL-phenylalanine	-16	20	14.667	0.24	1.10	3.6	22000	SCF6 1 mM Buffer 5
		-17	30	19.654	0.23	1.07	3.4	42000	SCF7 20 mM Buffer 5
79	 o-Fluoro-DL-phenylalanine	-18	20	16.375	0.070	1.04	1.4	24000	SCF6 10 mM Buffer 6
		-18	30	No peak was observed for 42 min					SCF7 10 mM Buffer 6
80	 DL-Homophenylalanine	-16	20	11.271	0.13	1.04	1.8	32000	SCF6 15 mM Buffer 5
		-17	30	22.150	0.065	1.02	0.9	32000	SCF7 20 mM Buffer 5

Table 23 –*Continued.*

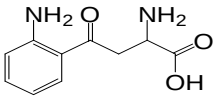
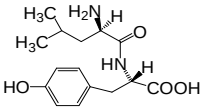
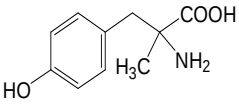
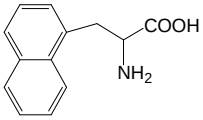
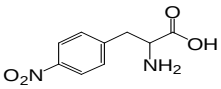
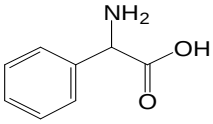
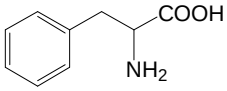
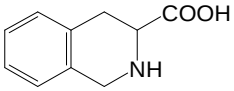
81	 DL-Kynurenine	-16	20	2.862	0.64	1.05	2.7	48000	SCF6 15 mM Buffer 5
		-20	30	3.621	0.69	1.04	1.4	17000	SCF7 15 mM Buffer 5
82	 DL-Leucyl-DL-Tyrosine	-18	20	12.863	0.071	1.03	0.8	14000	SCF6 10 mM Buffer 6
		-12	20	21.404	0.051	1.02	0.8	24000	SCF7 20 mM Buffer 5
83	 α-Methyl-DL-tyrosine	-18	20	13.825	0.043	1.02	0.5	14000	SCF6 10 mM Buffer 6
		-18	20	12.304	0.062	1.02	0.8	18000	SCF7 10 mM Buffer 6
84	 3-(1-Naphthyl)-DL-alanine	-16	20	6.563	0.804	1.16	5.7	24000	SCF6 15 mM Buffer 5
		-12	20	7.175	1.16	1.20	7.7	29000	SCF7 20 mM Buffer 5
85	 4-Nitro-DL-phenylalanine	-16	20	6.429	0.25	1.05	2.0	33000	SCF6 15 mM Buffer 5
		-17	30	11.979	0.22	1.04	2.7	79000	SCF7 20 mM Buffer 5
86	 2-Phenylglycine	16	20	6.100	0.39	1.07	2.8	31000	SCF6 15 mM Buffer 5
		17	30	26.692	0.47	1.22	7.8	29000	SCF7 20 mM Buffer 5
87	 Phenylalanine	16	20	7.575	1.24	1.34	9.8	19000	SCF6 15 mM Buffer 5
		20	30	17.658	0.37	1.12	5.6	37000	SCF7 15 mM Buffer 5
88	 1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid	16	20	11.650	0.28	1.10	3.5	25000	SCF6 15 mM Buffer 5
		12	20	11.313	0.87	1.24	6.9	16000	SCF7 20 mM Buffer 5

Table 23 –*Continued.*

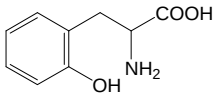
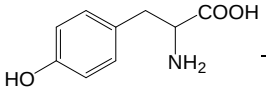
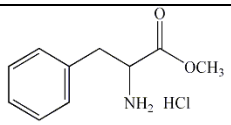
89	 DL-o-Tyrosine	18	20	18.229	0.20	1.12	2.9	10000	SCF6 10 mM Buffer 6
		18	20	22.754	0.16	1.12	4.0	22000	SCF7 10 mM Buffer 6
90	 Tyrosine	18	20	12.483	0.11	1.04	1.4	20000	SCF6 10 mM Buffer 6
		20	30	18.329	0.15	1.05	2.7	56000	SCF7 15 mM Buffer 5

Table 24. Effect of Batches of Capillary

Compound	Capillary batch	tm_1	t_{eof}	Δμ	$\mu_{app,avg}$	α	Rs	N
 DL-Phenylalanine methyl ester hydrochloride	#1	7.029	3.317	0.63	6.52	1.05	2.2	24000
	#2	3.658	6.096	0.61	12.8	1.10	3.6	33000

Unit for time (tm_1) is min, mobility (Δμ) is $cm^2kV^{-1}min^{-1}$. Conditions: SCF6 15 mM, 30/40 cm 50 μm I.D capillary; +25 kV; buffer: mixture of 20 mM ammonium acetate and 10 mM phosphoric acid, pH=4.7.

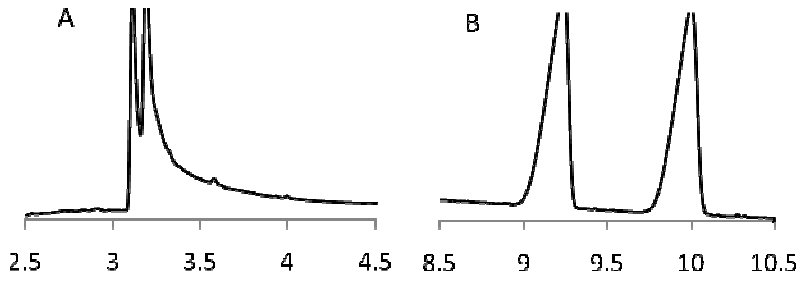


Figure 19. Comparison of normal polarity and reverse polarity. Conditions: tyrosine methyl ester, SCF6 15 mM. A: 4 mM ammonium acetate, adjust with 1 M HCl to pH 4.1, +25 kV, 30 cm/40 cm capillary with 50 μm i.d.; B: 4 mM phosphate, 5%MeOH, pH=2.0, -16 kV, 20 cm/30 cm capillary with 50 μm i.d.

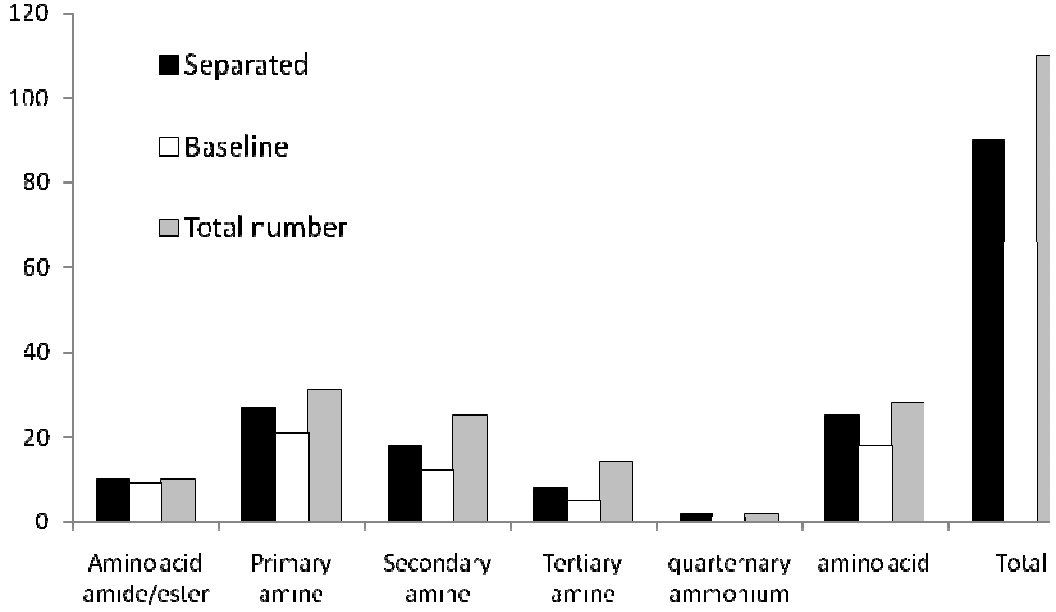


Figure 20. Summary of separation of amines and amino acids with SCF6 and SCF7 in both normal and reverse polarity mode.

Buffer plays an important role in enantioselective separations. It controls the pH, stabilizes the current, and maintains the EOF [13,221]. It can also modify the interaction between an analyte and chiral selector. Four types of buffers were tested in this study, and the results are shown in Figure 21. Overall, ammonium acetate produced the best enantiomeric resolutions within reasonable analysis times. The buffer concentration effect also was studied and the results are shown in Figure 22. The optimum buffer concentration was in the range of 4 mM to 7 mM, which is significantly lower than typical optimum buffer concentration used for sulfated cyclodextrins [13,89,91,114]. The finding that high buffer concentration suppresses the association of analyte and SCFs indicates the importance of electrostatic interactions for enantioselectivity by SCFs. The buffer pH can affect the charge state of analyte and chiral selector as well as the EOF. Lower pH slows the EOF, which in turn greatly improves selectivity and thus the resolution by decreasing the apparent mobility of the analytes (see Figure 23) [79,221].

Altering the chiral selector concentration has been shown to be an effective way to improve enantioresolution [13,91]. Electropherograms obtained for the separation of alprenolol using with different SCF7 concentrations (while other conditions remain the same) are shown in Figure 24. The optimum concentration was determined to be 15 mM, which is close to the optimum concentration when chiral crown ethers are used as chiral selectors [228], but significantly lower than a typical optimum sulfated cyclodextrin concentration [13].

Another important experimental factor that affects enantioseparations is the organic modifier [13]. Organic modifiers not only increase the solubility of hydrophobic analytes, but also suppress the joule heating by lowering the current and slowing the EOF. These effects can improve selectivity. However, the organic modifier may also compete for the chiral selector, thus disrupting the association between analyte and selector. Figure 25 shows the effect of methanol percentage on the separation of p-chloroamphetamine. The observed effects are significantly different than what is observed with sulfated cyclodextrins [13,53,114], in that the resolution was

not affected to a great degree. This allows for the determination that the chiral recognition mechanism of sulfated cyclofructans is significantly different than sulfated cyclodextrins, in that hydrophobic inclusion complexation is not as important with SCFs.

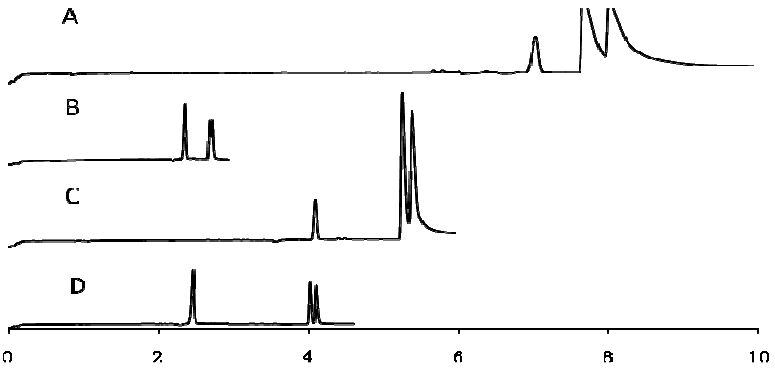


Figure 21. Effect of buffer type when using 5 mM SCF7 separating *p*-chloroamphetamine. Buffer: 20 mM, pH=4.7, +25 kV, 30/40 cm 50 µm I.D capillary. A: tetrabutylammonium nitrate and sodium acetate; B: phosphate; C: sodium acetate; D: ammonium acetate. First peak is EOF marker.

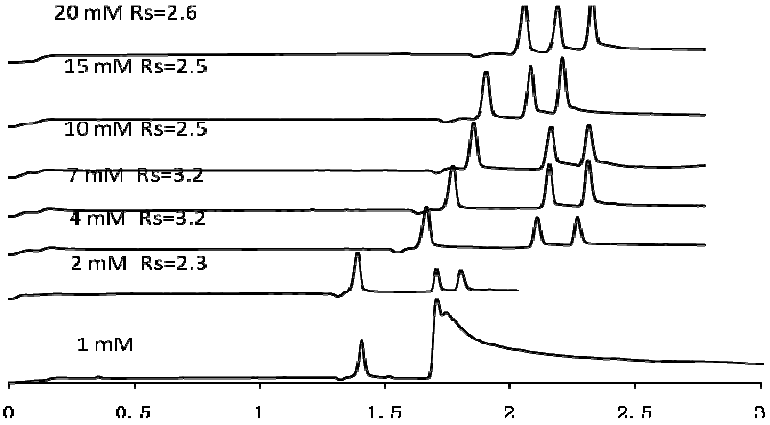


Figure 22. Effect of Buffer (ammonium acetate) concentration when using 5 mM SCF7 to separate 1,2-diphenylethylamine. pH=4.7, +25 kV, 30/40 cm 50 µm I.D capillary. First peak is EOF marker.

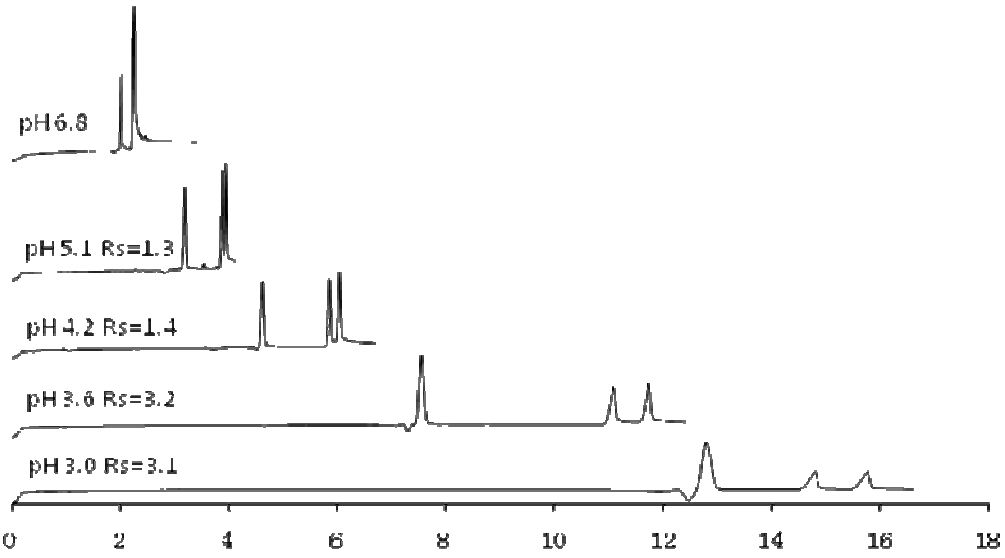


Figure 23. Effect of pH when using 5 mM SCF7 separating *p*-chloroamphetamine. Buffer: 20 mM ammonium acetate, +25 kV, 30/40 cm 50 µm I.D capillary. First peak is EOF marker.

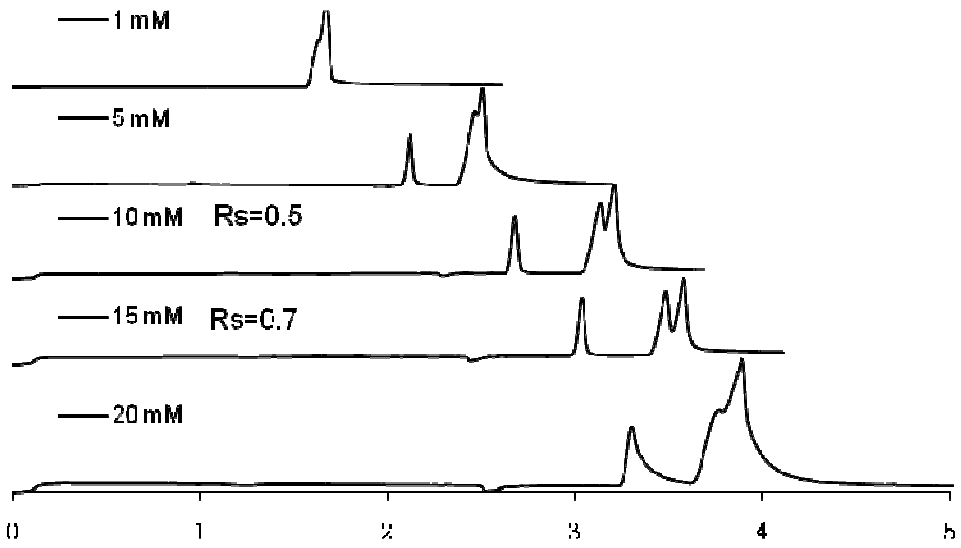


Figure 24. Effect of SCF7 concentration when separating alprenolol. Buffer: 5 mM ammonium acetate, pH=4.7, 30 cm (from inlet to detection window)/37 cm capillary, 50 µm i.d., +25 kV. First peak is EOF marker.

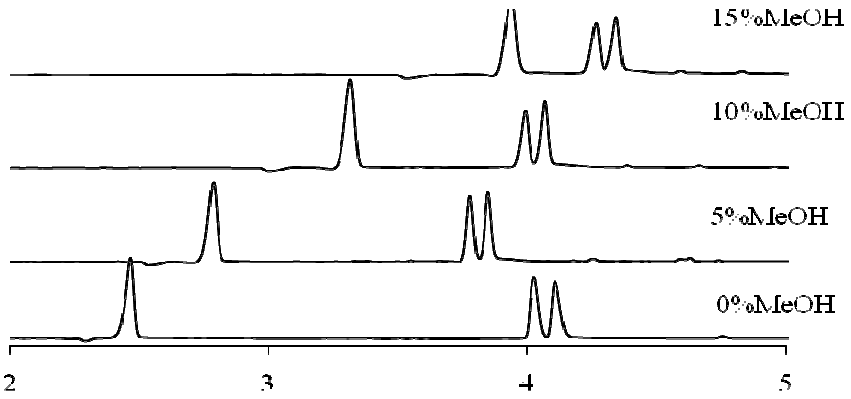


Figure 25. Effect of MeOH percentage (v/v) when using 5 mM SCF7 separating *p*-chloroamphetamine. Buffer: 20 mM ammonium acetate, pH=4.7, +25 kV, 30/40 cm 50 µm I.D capillary. First peak is EOF marker.

Different capillary batches can have different surface properties. Therefore, different wall interactions and EOFs can be observed under the same conditions, which may result in different enantioresolutions. Table 24 and Figure 26 show the electropherograms of same compound under identical separation conditions in the normal polarity mode on capillaries of different batches (see experimental). Employment of reverse polarity with low pH (around 2) buffer can minimize this difference (as shown in Figure 3).

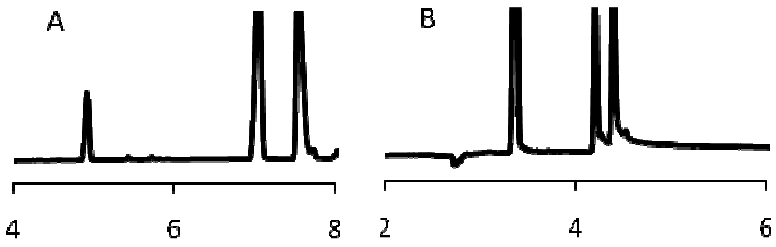


Figure 26. Comparison of capillary batches. Conditions: *p*-chloroamphetamine, SCF6 15 mM, 10 mM ammonium acetate,10 mM phosphoric acid, pH=4.7,+25 kV, 30 cm/40 cm capillary with 50 µm i.d. A: capillary batch #1; B: capillary batch #2. First peak is EOF marker.

5.5 Concluding Remarks

Sulfated cyclofructan 6 and 7 showed high enantioselectivity towards all types of amine containing compounds. Fast separations (<10 min) were achieved for most of the analytes separated. Electrostatic interaction plays an important role in both association and molecular recognition. SCFs showed similarities and differences to both crown ether and sulfated cyclodextrin chiral selectors. While chiral crown ethers showed enantioselectivity to mainly primary amines, SCFs showed good enantioselectivity to all amines. Compared to sulfated cyclodextrins, SCFs showed better selectivities for amine-containing compounds, allowing faster baseline separations. Their high solubility, UV transparency and minimum wall interaction enable them to be useful and competitive chiral selectors for capillary electrophoresis.

5.6 Acknowledgement

We gratefully acknowledge the support of the National Institute of Health (NIH 5 ROICMO53825-11) and Dr. Mari Yasuda at Mitsubishi Chemical Group (Tokyo, Japan) the kind donation of cyclofructan 6 and 7.

CHAPTER 6

GENERAL CONCLUSIONS

Chapter 2 provides a review of enantiomeric separations using sulfated cyclodextrins. Successful enantioseparations were demonstrated using the combination of sulfated cyclodextrins with high efficiency CE. Also, good precision and high selectivity for different compounds, and as well as good reproducibility of the separations using different batches makes this type of cyclodextrin derivative a good choice for screening new chiral compounds.

Chapter 3 presents sodium arsenyl (L)-(+)-tartrate, a new chiral selector for CE. It shows enantioselectivity towards amine-containing compounds and ruthenium (II) polypyridyl complexes. Most separations were achieved in 10 minutes. Electrostatic interactions play an important role in the enantioseparations when using this chiral selector. Compounds with more benzyl or fused rings showed better separations.

Chapter 4 examines the CE enantiomeric separation of nine ruthenium (II) polypyridyl complexes using different types of cyclodextrin selectors. Separations using cyclodextrin-modified capillary electrophoresis (CD-MCE) mode was compared to basic capillary zone electrophoresis (CZE). In the CZE mode, higher resolutions were obtained at lower pHs and lower applied voltages, but with longer analysis times. In the MCE mode, higher surfactant concentrations shorten analysis, but with decreased enantioresolution.

Chapter 5 demonstrates a new chiral selector, sulfated cyclofrutans. Cyclofructans consist of a crown ether skeleton and fructofuranose residues that are linked to the crown ether ring in a spiral arrangement. However, unlike chiral crown ethers that only show enantioselectivity for primary amine containing compounds, sulfated cyclofrutans showed high enantioselectivity towards all types of amine containing compounds. Fast separations, less than

10 minutes were achieved for most of the analytes separated. Electrostatic interaction plays an important role in both association and molecular recognition.

APPENDIX A

CREDITS TO AUTHORS ON THESIS CHAPTERS

Chapter 1. Introduction
Author: Man-Yung Benjamin Tong

Chapter 2. Review: Enantiomeric Separations Using Sulfated Cyclodextrins in Capillary Electrophoresis
Authors: Man-Yung Benjamin Tong, Daniel W. Armstrong
Publication: A manuscript published in Beckman Coulter P/ACE Setter 2008, (12), 5-10.

Chapter 3. Study of a New Chiral Selector for Capillary Electrophoresis: Sodium Arsenyl-(L)-(+)-Tartrate
Authors: Man-Yung Benjamin Tong, Tharanga Payagala, Sirantha Perera, Frederick M. Macdonnell, Daniel W. Armstrong
Publication: A manuscript has been submitted to the Journal of Chromatographic A.

Chapter 4. Enantiomeric Separation of Chiral Ruthenium (II) Complexes Using Capillary Electrophoresis
Authors: Chunxia Jiang, Man-Yung Benjamin Tong, Daniel W. Armstrong, T. Sampatha S. Perera, Ye Bao, Frederick M. MacDonnell
Publication: A manuscript published in Chirality 2008, (21), 208-217.

Chapter 5. Synthesis and Examination of Sulfated Cyclofructans as a Novel Class of Chiral Selectors for Capillary Electrophoresis
Authors: Chunxia Jiang, Man-Yung Benjamin Tong, Zachary S. Breitbach, Daniel W. Armstrong
Publication: A manuscript has been submitted to Electrophoresis

Chapter 6. General Conclusions
Author: Man-Yung Benjamin Tong

REFERENCES

1. De Boer, T., De Zeeuw, R. A., De Jong, G. J., Ensing K., *Electrophoresis* **2000**, *21*, 3220.
2. Bressolle, F., Audran, M., Pham T-N., Vallon, J-J., *J. Chromatogr. B* **1996**, *687*, 303.
3. Rocheleau, M-J., *Electrophoresis* **2005**, *26*, 2320.
4. Crosby, J., Collins, A. N. Chirality in Industry, John Wiley & Sons, Chichester, UK, 1992, p.2.
5. Skoog, D. A., Holler, F. J., Principles of instrumental analysis, 6th ed. Chapter 30. Belmont, CA 2007.
6. Gassmann, E., Kuo, J. E., Zare, R. N. *Science* **1995**, *230*, 813.
7. Beale, S. C. *Anal. Chem.* **1998**, *70*, 279R.
8. St. Clarire, R. L*. Anal. Chem*. **1996**, *68*, 569R.
9. Antczak, A., Ramstad, T., Johnson, R. *Chromatographia* **2006**, *64*, 57.
10. Armstrong, D. W., Demond, W. *J., Chromatogr. Sci.* **1984**, *22*, 411.
11. Armstrong, D. W., Ward, T. J., Armstrong, R. D., Beesley, T. E. *Science* **1986**, *232*, 1132.
12. Armstrong, D. W., Faulkner, J. R.J., Han, S. M. *J. Chromatogr.* **1988**, *452*, 323.
13. Fanali, S. *J. Chromatogr. A* **2000**, *875*, 89.
14. Mitchell, C. R., Armstrong, D. W., In Chiral Separations: Methods and Protocols, Humana Press: Totowa, NJ, 2003; pp 61.
15. Wren, S. A. C. *J. Chromatogr*. **1995**, *636*, 57.
16. FDA Policy Statement on Stereoisomeric Drug Products, FDA 92D-0211, *Fed. Reg.* **1992**, 57, 2249.
17. Armstrong, D. W.; He, F. Y.; Han, S. M. *J. Chromatogr.* **1988**, *488,* 345.
18. Smolkova-Keulemansova, E. *J. Chromatogr.* **1982**, *251,* 17.
19. Armstrong, D. W.; Li, W.; Pitha, J. *Anal. Chem.* **1990,** *62,* 914.
20. Li, W. Y.; Jin, H. L.; Armstrong, D. W.; *J. Chromatogr.* **1990**, *509,* 303.
21. KÖnig, W. A.; Lutz, S.; Wnaz, G. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 979.
22. Schurig, V.; Nowotny, H. P.; Schmalzing, D. *Angew. Chem.* **1989**, 101, 785.
23. Berthod, A.; Li, W.; Armstrong, D. W. *Anal. Chem.* **1992,** *64,* 873.
24. Armstrong, D. W.; Tang, Y.; Ward, T.; Nichols, M. *Anal. Chem.* **1993,** *65,* 1114.
25. Armstrong, D. W.; Chang, L.W.; Chang, S. C.; Wang, X.; Ibrahim, X.; Reid, G. R. III; Beesley, T. E. *J. Liq. Chromatogr. Relat. Technol.* **1997**, *20,* 3279.
26. Issaq, H. J. *J. Liq. Chromatogr. Relat. Technol.* **2002**, *25,* 1153.
27. Fillet, M.; Hubert, Ph.; Crommen, J. *J. Chromatogr. A* **2000**, *875,* 123.
28. Lurie, I. S. *J. Chromatogr. A* **1997**, *792,* 297.
29. Surapaneni, S.; Ruterbories, K.; Lindstrom, T. *J. Chromatogr. A* **1997**, *761,* 249.
30. Gahm, K-H.; Lee, J-T,; Chang, L. W.; Armstrong, D. W. *J. Chromatogr. A* **1998**, *793,* 135.
31. Gahm, K-H.; Chang, L. W.; Armstrong, D. W. *J. Chromatogr. A* **1998**, *793,* 135.
32. Grard, S.; Morin, P.; Dreux, M.; Ribet, J-P. *Electrophoresis* **2000**, *21*, 3028.
33. Lin, C-E.; Lin, S-L; Liao, W-S; Liu, Y-C *J. Chromatogr. A* **2004,** *1032,* 227.
34. Abushoffa, A.M.; Burjanadze, N.; Blaschke, G.; Crommen, J.; Chankvetadze, B. *J Sep. Sci.* **2002,** *25*, 10.
35. Matthijs, N.; Hemelryck, S. V.; Maftouh, M.; Massart, D. L.; Vander Heyden, Y. *Anal. Chim. Acta* **2004,** *525,* 247.
36. Nishi, H.; Izumoto, S-I. *Electrophoresis* **1999**, *20*, 189.

37. Fanali, S. *J. Chromatogr. A* **1996**, *735,* 77.
38. Vespalec, R.; Boček, P. *Chem. Rev.* **2000**, *100,* 3715.
39. Vigh, G.; Sokolowski, A. D. *Electrophoresis* **1997**, *18*, 2305.
40. Schmitt, U.; Branch, S. K.; Holzgrabe, U. *J. Sep. Sci.* **2002**, *25*, 959.
41. Gűbitz, G.; Schmid, M. G. *J. Chromatogr.* **1997**, *792*, 179.
42. Szejtli J. *Chem. Rev.* **1998,** *98*, 1743.
43. Michotte, Y.; Eeckhaut, A.V. *Electrophoresis* **2006**, *27,* 2880.
44. Sandra, P.; Verleysen K. *Electrophoresis* **1998**, *19,* 2798.
45. Shpigun, O. A.; Ananieva, I. A.; Budanova, N. Y.; Shapovalova, E. N. *Russ. Chem. Rev.* **2003**, *72* 1035.
46. Wang, F.; Khaldei, M. G. *Electrophoresis* **1998**, *19,* 2095.
47. Zhu, W.; Vigh, G. *Electrophoresis* **2003,** *987,* 459.
48. Gratz, S.R.; Stalcup, A.M. *Anal. Chem.* **1998,** *70,* 5166.
49. Williams, B.; Vigh, G. *J. Chromatogr. A* **1997**, *777,* 295.
50. Wu, W.; Stalcup, A. M. *J. Liq. Chromatrogr.* **1995**, *18,* 1289.
51. Stalcup, A. M.; Gahm, K-H. *Anal. Chem.* **1996**, *68,* 1360.
52. Chankvetadze, B.; Endresz, G.; Blaschke, G. *Chem. Soc. Rev.* **1996**, *25*, 141.
53. Jiang, C.; Tong, M-Y.; Armstrong, D. W.; Perera, S.; Bao, Y.; Macdonnell, F. M. *Chirality* **2008**, *21*, 208.
54. Verleysen, K.; Sandra, P.; Vander Bosch, T. *Electrophoresis* **1999,** *20,* 2650.
55. Chen, F.T.A.; Shen, G, Evangelista, R. A. *J. Chromatogr. A* **2001**, *924,* 523.
56. Chen, F.T.A.; Evangelista, R. A *J. Chin. Chem. Soc.* **1999**, *46*, 847.
57. Breslow, R.; Ueno, A. Tetrahedron Letter **1982**, *23*, 3451.
58. Robb, C. S. *J. Liq. Chromatogr. Relat. Technol.* **2007**, *30,* 729.
59. Rudaz S.; Calleri, E.; Geiser, L.; Cherkaoui S.; Prat, J.; Veuthey, J-L. *Electrophoresis* **2003**, *24,* 2633.
60. Iwata, Y.T.; Garcia, A.; Kanamori, T.; Inoue, H.; Kishi, T.; Lurie, I.S. *Electrophoresis* **2002,** *23,* 1328.
61. Foulon, G.; Goossens, J.F.; Fourmaintraux, E.; Bonte, J.P.; Vaccher, C. *Electrophoresis* **2002,** *23,* 1121.
62. Yang, G.S.; Chen, D.M.; Yang, Y.; Tang, B.; Gao, J.J.; Aboul-Enein, H.Y.; Koppenhoefer, B. *Chromatographia* **2005**, *62*, 441.
63. Phinney, K.W.; Jackson, J.W.; Sander, L.C. *Electrophoresis* **2002,** *23,* 1380.
64. Jin, L.J.; Li, S.F.Y. *J. Chromatogr. B* **1998**, *708,* 257.
65. Xu, X.; Stewart, J.T. *J. Pharm. Biomed. Anal.* **2000,** *23,* 735.
66. Zakaria, P.; Macka, M.; Haddad, P.R. *J. Chromatogr. A* **2003**, *985*, 493.
67. Zhou, L.; Thompson, R.; Song, S.; Ellison, D.; Wyvratt, J.M. *J. Pharm. Biomed. Anal.* **2002**, *27,* 541.
68. Massart D. L.; Perrin, C.; Vander Heyden, Y.; Maftouh, M. *Electrophoresis* **2001**, *22*, 3203.
69. De Boer, T.; Mol, R.; Zeeuw, R. A.; Jong, G. J.; Ensing, K. *Electrophoresis* **2001**, *22*, 1413.
70. Mateus, L.; Cherkaoui, S.; Christen, P.; Veuthey, J-L. *J. Chromatogr. A* **2000**, *868,* 285.
71. Bitar, Y.; Holzgrabe, U. *Electrophoresis* **2007**, *28*, 2693.
72. Wedig, M.; Holzgrabe, U. *Electrophoresis* **1999**, *20*, 1555.
73. Li, S.F.Y.; Lee, H.K.; Wu, Y.S. *Electrophoresis* **2000,** *21,* 1611.
74. Li, S.F.Y.; Lee, H.K.; Wu, Y.S. *J. Chromatogr. A* **2001**, *912,* 171.
75. Vaccher, M.P.; Bonet, J.P.; Vaccher, C. *Chromatographia* **2006**, *64*, 51.
76. Kirschner, D.L.; Jaramillo, M.; Green, T.K. *Anal. Chem.* **2007**, *79,* 736.
77. Muzikfár, M.; Havel, J.; Macka, M. *Electrophoresis* **1992**, *23*, 1796.
78. Armstrong, D. W.; Lee, J. T.; Chang, L. W. *Tetrahedron:Asymmetry* **1998**, *9*, 2043.
79. Wang, F.; Khaledi, M.G. *J. Chromatogr. A* **2000**, *875,* 277.

80. Danel, C.; Foulon , C.; Goossens, J. F.; Bonte, J. P.; Vaccher, C. *Chromatographia* **2006**, *63*, 353.
81. Chiral Analysis for Capillary Electrophoresis. 2008 Beckman Coulter, Inc. Accessed date on 10 Nov 2008. <http://www.beckmancoulter.com/products/ splashpage/ chiral38/ default.asp.>
82. Zhou, L.; Johnson, B.D.; Miller, C.; Wyvratt, J.M. *J. Chromatogr. A* **2000**, *875,* 389.
83. Kvasnička, F.; Bíba, B.; Cvak, L.; Krátká, J.; Voldřich, M. *J. Chromatogr. A* **2005**, *1081,* 87.
84. Vander Heyden, Y.; Matthijs, N. *Biomed. Chromatogr.* **2006**, *20,* 696.
85. Perrin, C.; Fabre, H.; Maftouh, M.; Massart, D. L.; Vander Heyden, Y. *J. Chromatogr. A* **2003**, *1007,* 165.
86. Gómez-Gomar, A.; Ortega, E.; Calvet, C. Andaluz, B.; Mercé, R.; Frigola,J. *J. Chromatogr. A* **2003**, *990,* 91.
87. Gotti, R.; Cavrini, V.; Pomponio, R.; Andrisano, V. *J. Pharm. Biomed. Anal.* **2001**, *24,* 863.
88. Song, S.; Zhou, L.; Thompson, R.; Yang, M.; Ellison, D.; Wyvratt, J.M. *J. Chromatogr. A* **2002,** *959,* 299.
89. Evans, C.E.; Stalcup, A.M. *Chirality* **2003**, *15*, 709.
90. Matthijs, N.; Massart D. L.; Perrin, C.; Vander Heyden, Y. Maftouh, M. *J. Pharm. Biomed. Anal.* **2002**, *27,* 515.
91. Wan, H., Blomberg, L. G, *J. Chromatogr., A* **2000**, *875*, 43.
92. Nishi, H., Kuwahara, Y. *Journal of Biochemical and Biophysical Methods* **2001**, *48*, 89.
93. Blaschke, G., Chankvetadze, B. *J. Chromatogr A* **2000**, *875,* 3.
94. Stalcup, A. M. *Kirk-Othmer Encycl. Chem. Technol. (5th Ed.)* **2004**, *6*, 72.
95. Chankvetadze, B. *J. Chromatogr., A* **2007**, *1168*, 45.
96. Ali, I., Kumerer, K., Aboul-Enein, H. Y. *Chromatographia* **2006**, *63*, 295.
97. Peng, X-H., Huang, K-L., Jiao, F-P., Zhao, X-H., Yu, J-G *Zhongnan Daxue Xuebao, Ziran Kexueban* **2005**, *36*, 983.
98. Pettersson, C., Heldin, E., Stuurman, H. W. *J. Chromatogr. Sci*. **1990**, *28*, 413.
99. He, H., Xu, X., Zhang, D., Chen, *J. Anal. Chim. Acta* **2005**, *536*, 15.
100. Fanali, S., Ossicini, L., Foret, F., Bocek, P. *J. Microcol. Sep*. 1989, *1*, 190.
101. Wijeratue, A. B., Schug, K. A. J. Sep. Sci. 2009, 32, 1537.
102. Martin, S. E., Maggie, C. R., Kane-Maguire, N. A. P. Wheeler, J. F. *Anal. Chim. Acta* **2001**, *445*, 21.
103. Sun, P., Krishnan, A., Yadav, A., Singh, S., MacDonnell, F. M., Armstrong, D. W. *Inorg. Chem.* **2007***, 46*, 10312.
104. Bott, R. C., Smith,G., Sagatys, D. S., Lynch, D. E. Kennard, C. H. L. *Aust. J. Chem.* **2000**, *53*, 917.
105. Matsumoto, K., Kawaguchi, H., Kuroya, H., Kawaguchi, S. *Bull. Chem. Soc. Jap*. **1973**, *48*, 2424.
106. Tapscott, R. E., Robert, E., Belford, R. L., Paul, I. C. *Coord. Chem. Rev*. **1969**, *4*, 323.
107. Schlessinger, G. *Inorg. Synth*. **1970**, *12*, 267.
108. Burstall, F. H. *J. Chem. Soc*. **1936**, *173*.
109. Goss, C. A., Abruna, H. D. *Inorg. Chem*. **1985**, *24*, 4263.
110. Sullivan, F. G., Cagle, F. W. J. *J. Org. Chem.* **1947**, *12*, 781.
111. Konduri, R. Ph.D. Thesis, University of Texas at Arlington, Arlington, TX, 2003.
112. Broomhead, J. A., Young, C. G., Hood, P. *Inorg. Synth*. **1982**, *21*, 127.
113. Rizzi, A. *Electrophoresis* **2001**, *22*, 3079.
114. Jiang, C., Armstrong, D. W., Lantz, A. W., Peter, A., Toth, G. *J. Liq. Chromatogr. Related Technol.* **2007**, *30*, 1421.
115. Armtrong, D. W., Rundlett, K. L., Chen, J. R. *Chirality* **1994**, *6*, 496.
116. Altria, K. D., Goodall, D. M., Rogan, M. M. *Chromatographia* **1994**, *38*, 723.

117. Wren, S. A. C., Rowe, R. C. *J. Chromatogr*. **1992**, *603*, 235.
118. Wren, S. A. C., Rowe, R. C. *J. Chromatogr.* **1992**, *609*, 363.
119. Ward, T. J. Nichols, M., Sturdivant, L., King, C. C. *Amino Acids*, **1995**, *8*, 337.
120. Torres, A. S., Maloney, D. J., Tate, D., Saad, Y., MacDonnell, F. M. *Inorg. Chim. Acta* **1999***, 293*, 37.
121. MacDonnell, F. M., Kim, M-J., Wouters, K. L., Konduri, R. *Coord. Chem. Rev.* **2003**, *242*, 47.
122. Janaratne, T. K., Yadav, A., Ongeri, F., MacDonnell, F. M. *Inorg. Chem.* **2007**, *46,* 3420.
123. Smith, J. A., Keene, F. R., *Chem. Commun*. **2006***, 24*, 2583.
124. Monchaud, D., Lacour, J., Coudret, C., Fraysse, S., *J. Organomet. Chem*. **2001**, *624*, 388.
125. Patterson, B.T., Foley, F.M., Richards, D., Keene, F.R. *Dalton Trans* **2003,** 709.
126. Eustache, F., Dalko, P.I., Cossy, J. *Org. Lett.* **2002**, *4*, 1263.
127. Yamagishi A. *Chem. Aust.* **1987**, *54*, 278.
128. Fujita, S., Sato, H., Kakegawa, N., Yamagishi, A. *J Phys Chem B* **2006**,*110*, 2533.
129. Hartshorn, R.M., Barton, J.K. *J Am. Chem. Soc.* **1992**,*114*, 5919.
130. Hoshino, N., Matsuoka, Y., Okamoto, K., Yamagishi, A. *J. Am. Chem. Soc.* **2003**,*125*, 1718.
131. Szmacinski, H., Terpetschnig, E., Lakowicz, J.R. *Biophys. Chem.* **1996**,109.
132. Guo, X., Castellano, F.N., Li, L., Lakowicz, J.R. *Biophys. Chem.* **1998**, *71*, 51.
133. Szmacinski, H., Castellano, F.N., Terpetschnig, E., Dattelbaum, J.D., Lakowicz, J.R., Meyer, G.J. *Biochimica et Biophysica Acta* **1998**, *1383*, 151.
134. Li, L., Szmacinski, H., Lakowicz, J.R. *Biospectroscopy* **1997**, *3*, 155.
135. Barton, J.K. *Science* **1986,** 233, 727.
136. Pieper, T., Keppler, B.K. *Analysis* **1998**, 26.
137. Yuan, D., Pietrzyk, D.J. *J Chromatogra.* **1990**, *509*, 357.
138. Yamagishi, A., Ohnishi, R. *J Chromatogra.* **1982**, *245*, 213.
139. Yamagishi, A., Taniguchi, M., Imamura, Y., Sato, H. *Applied Clay Science* **1996**, *11*, 1.
140. Yamagishi A. *Seramikkusu* **1989**, *24,*1048.
141. Yamagishi A., Makino, H., Nakamura, Y., Sato, H. *Clays and Clay Minerals* **1992**, *40*, 359.
142. Sauaia, M.G., Tfouni, E., De Almeida Santos, R. E., Do Prado Gambardella, M. T., Del Lama, M. P. F. M.,Guimarães, L. F., Da Silva, R. F. *Inorg. Chem Comm*. **2003**, *6*, 864.
143. Bosnich, B., Dwyer, F.F. *Aust. J. Chem*. **1966**, *19*, 2229.
144. Kim, M-J., Konduri, R., Ye, H., MacDonnell, F.M., Puntoriero, F., Serroni, S., Campagna, S., Holder, T., Kinsel, G., Rajeshwar, R. *Inorg. Chem.* **2002**, *41*, 2471.
145. Rutherford, T.J., Quagliotto, M.G., Keene, F.R. *Inorg. Chem*. **1995**, *34,* 3857.
146. Hua, X., von Zelewsky, A. *Inorg. Chem.* **1995**, *34*, 5791.
147. Watson, R.T., Jackson, J.L., Harper, J.D., Kane-Maguire, K.A., Kane-Maguire, L.A.P., Kane-Maguire, N.A.P. *Inorg. Chim. Acta* **1996**, *249*, 5.
148. Hiort, C., Lincoln, P., Norden, B. *J. Am. Chem. Soc*. **1993**, *115*, 3448.
149. Kano, K., Hasegawa, H. *Chem. Lett.* **2000**, 698.
150. Hiort, C., Nordén, B., Rodger, A. *J. Am. Chem. Soc*. **1990**,*112*, 1971.
151. Herbert, B.J., Carpenter, H.E., Kane-Maguire, N.A.P., Wheeler, J.F. *Anal. Chim. Acta* **2004**, *514*, 27.
152. Rutherford, T.J., Pellegrini, P.A., Aldrich-Wright, J., Junk, P.C., Keene, F.R. *Eur. J. Inorg. Chem.* **1998**,1167.
153. Keene, F.R. *Chem. Soc. Rev*. **1998**, 7, 185.
154. Fletcher, N.C., Junk, P.C, Reitsma, D.A., Keene, F.R. *J. Chem. Soc. Dalton Trans.* **1998**,133.
155. Yoshikawa, Y., Yamasaki, K. *Coord. Chem. Rev.* **1979**, *28*, 205.
156. Aldrich-Wright, J., Vagg, R.S., Williams, P.A. *Aust. J. Chem*. **2003**, *56*, 1193.
157. Fletcher, N.C., Keene, F.R. *J. Chem. Soc. Dalton*. **1999**, 683.

158. Dmochowski, I.J., Winkler, J.R., Gray, H.B. *J. Inorg. Biochem*. 2000,*81*,221.
159. Aoki, F., Hesek, D., Ishida, H., Inoue, Y. *Chromatography* **2000**, *21*, 133.
160. Aldrich-Wright, J., Greguric, I., Vagg, R.S., Vickery, K., Williams, P.A. *J. Chromatogra. A* **1995**, *718*, 436.
161. Baker, A.D., Morgan, R.J., Strekas, T.C. *J. Am. Chem. Soc*. **1991**, *113*, 1141.
162. Smith, J.A., Keene, F.R. *Chem. Commun*. **2006**, 2583.
163. Browne, W.R., O'Connor, C.M., Villani, C., Vos, J.G. *Inorg. Chem*. **2001**, *40*, 5461.
164. Gasparrini, F., D'Acquarica, I., Vos, J.G., O'Connor, C.M., Villani, C. *Tetrahedron: Asymmetry* **2000**, *11*, 3535.
165. Wan, H., Blomberg, L.G. *J. Chromatogra. A* **2000**, *875*, 43.
166. Fanali, S. *J. Chroatogra. A* **1989**, *474*, 441.
167. Egger, M.D., Liu, Y., Sevčík, J., Etsařová, E., Rozhkov, R., Larock, R.C., Armstrong, D.W. *Electrophoresis* **2003**, *24*, 2650.
168. Rodriguez, M.A., Liu, Y., McCulla, R., Jenks, W.S., Armstrong, D.W. *Electrophoresis* **2003**, *23*, 1561.
169. Lantz, A.W., Rozhkov, R.V., Larock, R.C., Armstrong, D.W. *Electrophoresis* **2004**, *25*, 2727.
170. Jiang, C., Armstrong, D.W., Péter, A., Fülöp, F. *J. Liq. Chromatogra. Rel. Tech.* **2007**, *30*, 1709.
171. Sánchez-Hernández, L., Crego, A.L., Marina, M.L., García-Ruiz, C*. Electrophoresis* **2008**, *29*, 237.
172. Lin, C., Li, Y., Chen, S. *Electrophoresis* **2003**, *24*, 4106.
173. Zhang, J., Konecny, J., Glatz, Z., Hoogmartens, J., Van Schepdael, A. *Curr. Anal. Chem.* **2007**, *3*, 197.
174. Altria, K.D., Chen, A.B., Clohs, L. *LC-GC Europe* **2001**, *14*, 740.
175. Ahmad, A. *Electrophoresis* **2001**, *22*, 3107.
176. Haginaka, J. *J Chromatogr. A* **2000**, *87*, 235.
177. Holder, E., Schoetz, G., Schurig, V., Lindner, E. *Tetrahedron: Asymmetry* **2001**, *12*, 2289.
178. Holder, E., Trapp, O., Trapp, G., Marin, V., Hoogenboom, R., Schubert, U.S. *Chirality* **2004**, 363.
179. Holder, E., Trapp, G., Grimm, J.C., Schurig, V., Lindner, E.. *Tetrahedron: Asymmetry* **2002**, *13*, 2673.
180. Shelton, C.M., Seaver, K.E., Wheeler, J.F., Kane-Maguire, N.A.P. *Inorg. Chem.* **199**7, *36*, 1532.
181. Elshihabi, S., Black, K.D., Sutton, J.K., Woody, K.A., Burke, Jr. J.A., Bushey, M.M. *Electrophoresis* **2001**, *22*, 3771.
182. Chankvetadze, B., Endresz, G., Blaschke, K. Electrophoresis **1994**, *15*, 804.
183. See, M.M., Elshihabi, S., Burke, Jr. J.A., Bushey, M.M. *J. Microcol. Sep*. **1995**, *7*, 199.
184. Harris, J.E., Desai, N., Seaver, K.E., Watson, R.T. *J. Chromatogra. A* **2001**, *919*, 427.
185. Grossman, P.D., Colburn, J.C. Capillary Electrophoresis, Theory and Practice Academic Press, 1992
186. Pak, C., Marriott, P.J., Carpenter, P.D., Amiet, R.G. *J. Chromatogra. A* **1998**, *793*, 357.
187. Altria, K.D., Goodall, D.M., Rogan, M.M. *Chromatographia* **1992**, *34*, 19.
188. Guttman, A., Paulus, A., Cohen, A.S., Geinberg, N., Karger, B.L. *J. Chromatogra.* **1998**, 448, 41.
189. Stalcup, A. M., Chang, S. C., Armstrong, D. W., *J. Chromatogr.* **199**1, *540*, 113.
190. Armstrong, D. W., Nair, U. B., *Electrophoresis* **1997**, *18*, 2331.
191. Hilton, M., Armstrong, D. W., *J. Liq. Chromatogr.* **1991**, *14*, 9.
192. Kawamura, M., Uchiyama, T., Kuramoto, T., Tamura, Y., Mizutani, K., *Carbohydr. Res.* **1989**, *192*, 83.
193. Kamata, A., *Bio Ind.* **1992**, *9*, 654.

194. Kawamura, M., Uchiyama, T., *Biosci., Biotechnol., Biochem.* **1993**, *57*, 343.
195. Kushibe, S., Sashida, R., Morimoto, Y., *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1136.
196. Kushibe, S., Yamagishi, M., Sashida, R., *Res. Dev. Rev. - Mitsubishi Kasei Corp.* **1994**, *8*, 44.
197. Lim, C.-K., Kim, H.-C., Kim, K.-H., Kim, B.-W., Nam, S.-W., *Han'guk Misaengmul-Saengmyongkong Hakhoechi* **2004**, *32*, 60.
198. Sawada, M., Tanaka, T., Takai, Y., Hanafusa, T.*, et al.*, *Chem. Lett.* **1990**, 2011.
199. Sawada, M., Tanaka, T., Takai, Y., Hanafusa, T.*, et al.*, *Carbohydr. Res.* **1991**, *217*, 7.
200. Immel, S., Schmitt, G. E., Lichtenthaler, F. W., *Carbohydr. Res.* **1998**, *313*, 91.
201. Armstrong, D. W., *J. Liq. Chromatogr.* **1984**, *7*, 353.
202. Armstrong, D. W., Ward, T. J., Armstrong, R. D., Beesley, T. E., *Science* **1986**, *232*, 1132.
203. Kase, M., Edasawa, K., Sugimae, T., Iida, K., (Koatsu Gas Kogyo, Japan; Mitsubishi Chemical Industries Co., Ltd.). Application: JPJP 1994, p. 6 pp.
204. Nishio, S., Komatsu, H., Shimizu, H., (Konishiroku Photo Ind, Japan). Application: JP JP 1996, p. 14 pp.
205. Meji, I., (Konishiroku Photo Ind, Japan). Application: JP JP 1994, p. 18 pp.
206. Nishio, S., (Konishiroku Photo Ind, Japan). Application: JP JP 1994, p. 61 pp.
207. Miura, Y., Juki, A., (Mitsubishi Kagaku KK, Japan). Application: JP JP 1995, p. 4 pp.
208. Shizuma, M., Takai, Y., Kawamura, M., Takeda, T., Sawada, M., *J. Chem. Soc., Perkin Trans.* **2001**, 1306.
209. Uchiyama, T., Kawamura, M., Uragami, T., Okuno, H., *Carbohydr. Res.* **1993**, *241*, 245.
210. Reijenga, J. C., Verheggen, T. P. E. M., Chiari, M., *J. Chromatogr., A* **1999**, *838*, 111.
211. Ishikawa, K., Nanjo, F., (Mitsui Norin Co., Ltd., Japan). Application: JP JP 2008, p. 9pp.
212. Ishikawa, K., Nanjo, F., (Mitsui Norin Co., Ltd., Japan). Application: JP JP 2005, p. 19 pp.
213. Kis, G. L., Schoch, C., Lohmann, D., (Novartis AG, Switz.; Novartis Pharma G.m.b.H.). Application: WO WO 2003, p. 24 pp.
214. Ishikawa, K., Nanjo, F., (Mitsui Norin Co., Ltd., Japan). Application: JP JP 2008, p. 8pp.
215. Nishioka, M., Mori, H., Nanjo, F., (Mitsui Norin Co., Ltd., Japan). Application: JP JP 2004, p. 15 pp.
216. Nishioka, M., Mori, H., Nanjo, F., (Mitsui Norin Co., Ltd., Japan). Application: JP JP 2004, p. 17 pp.
217. Sawada, M., Takai, Y., Shizuma, M., Takeda, T.*, et al.*, *Chem. Commun. (Cambridge)* **1998**, 1453.
218. Shizuma, M., *Kagaku to Kogyo (Osaka)* **2000**, *74*, 495.
219. Sawada, M., Takai, Y., Yamada, H., Hirayama, S.*, et al.*, *J. Am. Chem. Soc.* **1995**, *117*, 7726-7736.
220. Snyder, L. R., *J. Chromatogr. Sci.* **1972**, *10*, 200.
221. Grossman, P. D. a. C., J.C., 1992.
222. Sun, Q., Liu, C., Li, G., Yu, S.*, et al.*, *Dier Junyi Daxue Xuebao* **2004**, *25*, 1259.
223. Kim, J.-k., Song, S., Kim, J., Kim, T. H.*, et al.*, *Bulletin of the Korean Chemical Society* **2006**, *27*, 1577.
224. Rundlett, K. L., Armstrong, D. W., *Electrophoresis* **2001**, *22*, 1419.
225. Rundlett, K. L., Armstrong, D. W., *Electrophoresis* **1997**, *18*, 2194.
226. Rundlett, K. L., Armstrong, D. W., *J. Chromatogr., A* **1996**, *721*, 173.
227. Rundlett, K. L., Gasper, M. P., Zhou, E. Y., Armstrong, D. W., *Chirality* **1996**, *8*, 88.
228. Kuhn, R., *Electrophoresis* **1999**, *20*, 2605.

.

BIOGRAPHICAL INFORMATION

Man-Yung Benjamin Tong was originally from Hong Kong Special Administrative Region, China. In 2000, he went to the United States of America and obtained his Bachelor of Science degree from Winona State University majoring in Chemistry and Biochemistry with minoring in Biology. He then went on to study analytical chemistry under Dr. Daniel Armstrong at Iowa State University and the University of Texas at Arlington. He finished his Master of Science in August 2009 working on research in areas such as enantiomeric separations and determination of microbes in biological samples using different analytical techniques including capillary electrophoresis and high pressure liquid chromatography. After that, he is planning to obtain a degree in Master of Business Administration then keep going on to finish his doctor of philosophy in analytical chemistry.