CAPILLARY ELECTROPHORESIS SEPARATION OF NEUTRAL ORGANIC COMPOUNDS, PHARMACEUTICAL DRUGS, ENANTIOMERS AND ANIONS

by

QING FENG

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

December 2011

ACKNOWLEDGEMENTS

I would like to give thanks to my Heavenly Father, who guides me through my whole life.

I would like to express my sincere appreciation to my research supervisor, Prof. Daniel W. Armstrong for his guidance and support during my graduate study. He is such a wonderful mentor that I consider myself extremely fortunate to have this great opportunity to work in his laboratory. His creative thinking, scientific attitude and broad knowledge have impressed and inspired me not only in the area of chemistry, but also being a more confident person effusing his excellence in every aspect of life.

I would also like to thank Dr. Subhrangsu S. Mandal and Dr. Zoltan A. Schelly as my committee members, for their time and very inspiring suggestions. It has been really a great honor to have you serve on my committee. Also, I would like to express my gratitude to all the professors who have taught me during the last three years and shaped me into a scientist.

I greatly appreciate the assistance from my research group, especially Barbara Smith for taking care of my orders and paperwork, Violet Jiang and Ben Tong for their professional advice, and all other group members (Ping, Chunlei, Koko, Xiaotong, Zach, Eranda, Sophie, Yasith, Sarantha, Jonathon, Edra, Eva, Nilusha, Tharanga, Dilani, Aruna,Jason, Ross, Choyce, Tran, Pavlina, Yuncheol, Sarah, Victor and Beatrie) for their help and friendship.

I would like to dedicate this thesis to my parents for their unconditionally support, love and encouragement. At last but not least, I am also grateful to my colleagues from the University of Texas at Arlington and my friends for collaboration, sharing of knowledge and suffering, and everything you have done to me.

November 8 2011

ABSTRACT

CAPILLARY ELECTROPHORESIS SEPARATION OF NEUTRAL ORGANIC COMPOUNDS, PHARMACEUTICAL DRUGS, ENANTIOMERS AND ANIONS

Qing Feng, M.S.

The University of Texas at Arlington, 2011

Supervising Professor: Daniel W. Armstrong

The focus of this thesis is on using capillary electrophoresis (CE) with on-column UV detection in achiral and chiral separations. Chapter 1 provides a brief introduction to the principle, mechanism, meaning and application of the CE method. Chapter 2 presents the CE separation of multivalent anions by using di- and tetracationic ion pairing reagents. Two newly developed UV transparent phosphonium-based cationic reagents were evaluated as additives to the background electrolyte of CE for the separation of multiply charged anions, including several complex anions. These cationic reagents showed moderate suppression of the electroosmotic flow (EOF), interacted with the analytes to improve their separation and often improved the peak shape. The effects of the additives and their concentration on the separation were studied, as well as the buffer type, pH and voltage. Chapter 3 examines the CE enantiomeric separation of sixteen 4,5-disubstituted imidazoles. Different cyclodextrin-based chiral selectors were used and the separation conditions were optimized. Meanwhile, comparisons of these results to HPLC separations were made.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Figure Page

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 General Introduction

The term “electrophoresis” was first coined in 1909 by Michaelis[1]. It was developed as a separation techniques and first used to separate serum proteins by Tiselius in 1937[2]. It was the pioneering experiment of Hjerten in 1950s that provided ground work for the capillary electrophoresis (CE) and its use for the analysis of various analytes, ranging from small molecules to proteins and virus[3]. Virtanen followed this work using smaller internal diameter (0.2mm) tubes, which greatly simplified the CE instrumentation[4]. In 1981, Jorgenson and Lukacs[5] described electrophoresis in micron-scale (20-100) capillaries and introduced CE to the analytical scene to fulfill the increasing demands for high resolution, high efficiency and fast analysis. Over the last thirty years, CE has been demonstrated as an efficient and versatile analytical tool that combines simplicity with highly reproducibility [6, 7]. Especially since the 1990s, the growth of CE literature was exponentially and right now, CE has been one of the most widely used analytical techniques for the separation and analysis of ionic substances, based on the analytes’ charge and frictional forces in the interior solution of the capillary. In this paper, achiral separations and chiral separations in CE system will be discussed; comparison of CE and HPLC in enantiomeric separations will be included as well.

1.2 Separation on anionic species by using cationic reagent in CE

The analysis of anionic species, including inorganic and small organic anions is of a great importantance. Established techniques that are used to analyze anions include flow injection analysis[8, 9],ion chromatography[10-14], ion selective electrode[15, 16] and also spectroscopic techniques, such as UV-vis[17, 18] and fluorescence [19, 20], among others. Newer methods involving electromigration techniques have become complementary tools for the analysis of small ions[21] .

Thus there is a growing body of work involving capillary electrophoresis (CE) and related microfluidic techniques for anion analysis [22-26].

Effective CE separations of some anions have been reported [27-32]. It has been shown that a novel class of ion interaction reagents can be added to the separation buffers in order to enhance the separations and detection of anions[28, 29]. Our group has demonstrated that some mono- and dicationic reagents have the ability to suppress EOF and enable highly efficient and reliable baseline separation of six inorganic and seven organic anions which were singly charged [33]. Another nitrogen-based dicationic complexing reagent was found to be very useful in a CE-ESI-MS method for separation and identification of four anions. Also it was applied to quantitative water analysis [29].

In a further CE study (Chapter 2), the CE separation of anions and their identification were extended to anions of greater charge and complexity. This was in contrast to previous work which focused on using phosphonium-based dicationic agent to characterize singly charged inorganic and small organic anions in CE and CE-ESI-MS. Tetracationic phosphonium-based pairing reagents, which had not been utilized or reported in CE, were shown to the particularly useful for CE separations of trivalent anions and mixtures of divalent and trivalent anions. The dicationic ion pairing reagent best separated eight divalent anions. The separation performance is affected by the cationic additive's concentration, the BGE composition, pH and voltage. Under optimized conditions, satisfactory separations with relatively short analysis time, high efficiencies and good resolutions were achieved. In this case the pairing agent may serve a dual role, i.e., as CE separation agent in solution, and as a positive mode ESI-MS detection agent in the gas phase.

1.3 Enantiomeric separations by capillary electrophoresis and HPLC

Chirality is an important structural property of some molecules. A chiral molecule cannot be superimposable with its mirror image. The two "mirror images" isomers are called "enantiomers". They can exhibit different pharmacological and toxicological effects when

interacting with chiral biological and biochemical entities [27]. Thus separation of racemic drugs has become a great concern in modern research.

Capillary electrophoresis (CE) is a separation technique based on the velocity difference of analytes in the fluid-filled interior of a capillary, in the presence of an electric field. It is an effective alternative enantiomeric separation technique besides HPLC and GC (the most commonly used technique for enantiomeric separations is high performance liquid chromatography (HPLC)[28]. It has several advantages including extremely high efficiencies, fast analysis times, and low sample requirements[29]. Cyclodextrins (CDs) and their derivatives have been used as CE chiral selectors for the enantioseparation of large numbers of analytes, mainly of pharmaceutical interest [30]. Cyclodextrins have a shape of a truncated cone with an open hydrophobic cavity and a hydrophilic outer rim [30-32]. Charged CD derivatives have greater chiral resolving capacity for opposite charged analytes, since electrostatic forces play an important role in selector-selectant interactions [33]. Several reviews have been published on applications of sulfated β-CDs (SBCDs) as chiral selectors in CE [34, 35].

In Chapter 3, the enantiomeric separation of 15 newly synthesized 4,5-disubstituted imidazoles was explored. Both HPLC and CE were tested and compared for their abilities to separate the enantiomers of these chiral analytes. Using HPLC, 14 of the 15 compounds were separated, whereas using CE, only 8 were separated. Though HPLC appears to be more broadly applicable for separating these compounds, it should be noted that when a separation was obtained by both separation techniques, the CE method usually produced greater resolution. This is a common observation in the general comparison between HPLC and CE analyses, and this study was no exception. Also, it is important to note that the one compound that was not separated by HPLC was separated by CE, and by using both techniques, the entire set of analytes was separated. The optimization of these separations was discussed and a comparison between the chiral selectors used was made.

CHAPTER 2

SEPARATION OF MULTIPLE CHARGED ANIONS BY CAPILLARY ELECTROPHORESIS USING ALKYL PHOSPHONIUM PAIRING AGENTS

2.1 Abstract

Two newly developed UV transparent phosphonium-based cationic reagents were evaluated as additives to the background electrolyte for the CE separation of multiply charged anions, including several complex anions. These cationic reagents showed moderate suppression of the EOF, interacted with the analytes to improve their separation and often improved the peak shape. The effects of the additives and their concentration on the separation were studied, as well as the buffer type, pH and voltage. The dicationic reagent effectively separated eight divalent anions within 17 min and the tetracationic reagent best separated nine trivalent anions, as well as a mixture of all the anions.

2.2 Introduction

The analytical identification and quantification of anions remains an important task in many scientific disciplines, including pharmaceutical, biomedical, environmental and the food/beverage industry. Established techniques that are used to analyze anions include flow injection analysis[8, 9],ion chromatography[10-14], ion selective electrode[15, 16] and also spectroscopic techniques, such as UV-vis[17, 18] and fluorescence [19, 20], among others. Each approach has its own virtues and shortcoming in terms of universality, specificity, sensitivity and sample treatment requirements [36]. Newer methods involving electromigration techniques have become complementary tools for the analysis of small ions [21] . Thus there is a growing body of work involving capillary electrophoresis (CE) and related microfluidic techniques for anion analysis [22-26].

CE is a powerful technique for the recognition and separation of ionic species. Its often reported advantages include very high efficiencies, small sample consumption and quick analysis times compared to HPLC. Successful CE separations of some anions have been reported [37-42]. Many small anions have a high charge to size ratio giving them high electrophoretic mobilities, which can be higher than the EOF mobility. Thus, the reverse polarity mode often is used for the separation and detection of anions [43]. However, this so-called counter-electroosmotic CE approach often gives poor shaped peaks. Furthermore, in systems with very fast EOFs, migration times of some inorganic anions become unacceptable long [43]. In order to detect and separate anions, several approaches have been developed [23, 26, 44]. One is to suppress the EOF, which can be achieved by employing a buffer with low pH (typically 2.5-4.5 leads to a reduction of the EOF, and then the separation of anions can be performed in the reversed polarity mode. However, the most common CE method for anion separation involves the so called co-electroosmotic migration of the anions with an anodic EOF. The reversal of EOF is achieved by dynamically coating the inner capillary wall via addition of cationic surfactants to the background electrolytes (BGE) [44-46]. Positively charged additives are adsorbed onto the negative charged silanol groups of the capillary wall and change the wall surface charge to positive, which creates the reversal of EOF. Typical additives used in this method are positively charged surfactants such as cetyltrimethylammonium bromide (CTAB) [47], dodecytrimethylammonium bromide (DTAB) [48], and tetradecyltrimethylammonium bromide (TTAB) [22, 49]. However, TTAB strongly coats the surface of a fused silica capillary and results in a rapid change in the magnitude and direction of the EOF at very low concentrations, while at slightly higher concentrations, cationic micelles are formed and this reverses the direction of the mobility of the anions [50]. A combination of DTAB and TTAB additives was reported and its advantages were demonstrated [51]. Surfactants with two hydrocarbon chains such as didodecyldimethylammonium bromide, were shown to be able to form a more uniform planar dynamic coating of the capillary wall [52, 53]. Also, the use of soluble polymers as cationic

additives to the BGE was reviewed [54]. In particular, poly (diallyldimethylammonium chloride) (PDDAC) was shown to be effective in modifying the relative migration of anions [55, 56]. Noteworthy are the zwitterionic surfactant additives. They possess interesting properties as they adsorb on the inner surface of capillary wall and suppress EOF without inducing its reversal [57, 58].

Recently, a novel class of ion interaction reagents has been added to separation buffers. The utilization of ionic liquids (ILs) has attracted a great deal of interest. The term “Ionic Liquid” can be applied to any salt with a melting point below 100℃ and “Room-temperature Ionic Liquids” (RTILs) are liquid salts at ambient temperature [59]. ILs have negligible vapor pressures at room temperature; possess high thermal stability and wide range of viscosities. And due to those unique properties, ILs are widely used as biosensors [60], for support of catalysts [61], especially in separation science (e.g. gas chromatography and extractions) [62, 63, 63, 64]. However, it must be noted that once dissolved in solution, they are no longer ILs, but just another charged, dissolved additive. In CE, polymeric ionic liquids have been used as BGE modifiers for the separation of aromatic acids and basic proteins with high efficiency, high speeds and good reproducibility [65]. The most widely used ILs are imidazolium-based, which have been used in CE as additives for the separation of basic and acidic proteins [66-68]. In these cases, the ILs dynamically or covalently coat the capillary wall and the coating reverses the EOF and significantly improves the separation efficiency and peak shapes.

Most recently, several phosphonium-based cationic reagents were used as additives to characterize and separate anions. Phosphonium-based monocationic, dicationic, tricationic and tetracationic reagents were previously shown to be very useful in gas chromatography [59, 69, 70] and ESI-MS in the fluoride ion form [59, 71-73] for characterization of various anions. Among these, some mono- and dicationic reagents also showed the ability to suppress EOF and enable highly efficient and reliable baseline separation of six inorganic and seven organic anions which were singly charged [43]. Another nitrogen-based dicationic complexing reagent was found to be very

useful in a CE-ESI-MS method for separation and identification of four anions. Also it was applied to quantitative water analysis [39]. In this work, we expand the category of both the phosphonium-based ion pairing reagents and the types of anions separated. Especially the first tetracationic interaction reagent is used to separate multivalent anions. These results were compared to those obtained with the best dicationic reagent found in a previous study [43].

2.3 Materials and methods

2.3.1. Chemicals

All chemicals and solvents used were of HPLC grade. 2-amino-2hydroxymethylpropane-1,2-diol (Tris), hydrochloric acid and sodium hydroxide were purchased from EMD chemicals (Darmstadt, Germany). Phosphoric acid and sodium dihydrogen phosphate were obtained from EM Science (Gibbstown, NJ, USA). Sodium phosphate and boric acid were purchased from Fisher Scientific (St. Louis, MO, USA). Other chemicals including all the anions and Amberlite IRA-400 (Cl) ion exchange resin were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Tetracationic ion pairing reagent (Figure 2.1a) was also synthesized in bromide [72]. Dicationic phosphonium salt, propane-1,3-bis(tripropylphosphonium) (Figure 2.1b) was synthesized in the bromide form following procedures described by Remsburg et al [74]. Deionized water for preparation of all solutions was produced by Milli-Q system (Millopore, Billerica, MA, USA).

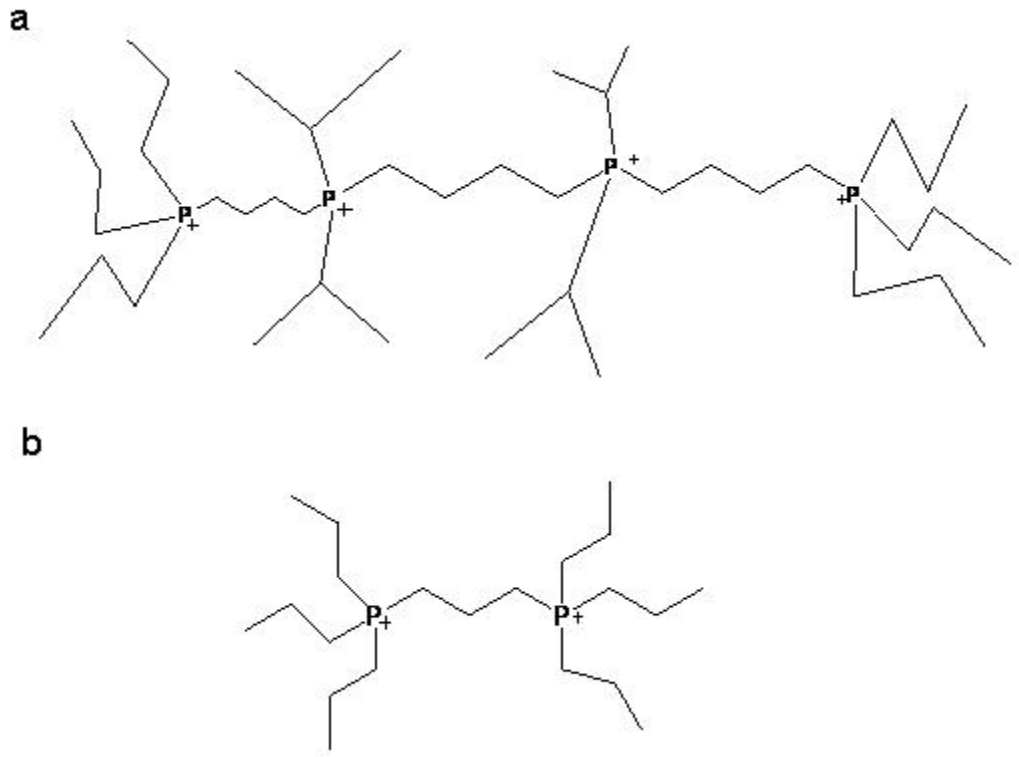


Figure 2.1 Structure of (a) tetra- and (b) dicationic phosphonium-based reagents tested in this study.

2.3.2. BGEs and Samples

The cationic reagents were all synthesized in the bromide-ion form and converted to the fluoride form in order to get best separation effect during electrophoresis. Those cations were exchanged as follows. An aliquot of strongly basic anion-exchange resin (Amberlite IRA-400) in the chloride form was packed in a 10-mL disposable syringe and washed by 10 column volumes of 1M NaOH to be converted to the hydroxide form. Next the resin was become fluoride form by passing 10 column volumes of 0.5 M NaF, and then followed by washing the same amount water. The cationic salts dissolved in 0.1 M solution in 5mL was passed through the resin bed by water and collected in a 10 mL volumetric flask at a concentration of 50mM stock solution. The stock solution was then diluted to different desired concentration, base (such as Tris) was added in and the pH of it was adjusted by acid.

Samples were prepared in 1mM by dissolving in deionized water.

2.3.3. Instrumental

CE experiments were carried out using a Beckman Coulter P/ACE MDQ system CE system equipped with a photodiode array detector (Beckman Instruments, Fullerton, CA, USA). Fused-silica capillaries (50 μm id) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Capillaries were cut to 30cm length (20cm to detector). Prior to use, a new capillary was first flushed of 140 kPa with 1M NaOH for 10 min and water for 10 min. The capillary cartridge was thermostated to 25 ℃. The UV detector was operated at 214 nm. The samples were injected hydrodynamically for 4s at the pressure of 3.5 kPa. Separations were performed under reversed polarity mode. Between each run, the capillary was flushed for 3 min with 1M NaOH, 3 min with water, and 2 min with running buffer using a pressure of 140 kPa.

2.3.4 Theoretical

When using hydrodynamic injection, the migration time t_i is defined as[75]

$$t_i = \frac{l \times l_{eff}}{U \times \mu_i} \qquad (1)$$

$$\mu_i = \mu_{ep,i} - \left(\mu_{eof} - \vartheta_p \times \frac{1}{U}\right) \qquad (2)$$

Where L_{eff} is the effective length (distance from the inlet end of the capillary to the detector), μ_i is the total mobility of the ion, U is the voltage, ϑ_p is the velocity of the hydrodynamic flow. The difference $\left(\mu_{eof} - \vartheta_p\left(\frac{1}{U}\right)\right)$ can be considered as the elecroosmotic mobility of EOF reduced by applying the hydrodynamic pressure. During the electrophoresis, some additional system peaks do not hold for Eq. (1) due to the applied hydrodynamic pressure. These changes are less than the migration time of anions and result in changing the order of the ion peaks [76] in different running which could be observed in the following.

2.4 Results and Discussion

2.4.1 Tested anions

The structure of the eight divalent anions and nine trivalent anions studied in this work are listed in Tables 2.1 and 2.2 respectively. They include both inorganic and organic species. Among them, tartrazine is a food colorant that has been found to be associated with a variety of children's behavioral changes when ingested [77]. Sodium fumarate can be used as an acidity regulator in processed foods and also work as a terminal electron acceptor in the cultivation of some anaerobic microorganisms. Sodium selenite is used in food supplements to provide the selenium nutritive element. Many of the inorganic anions are complex ions, such as sodium hexachloroplatinate hexahydrate, sodium tetrabromopalladate, and sodium hexanitrocobaltate, etc.

Table 2.1. Structures of the divalent anions

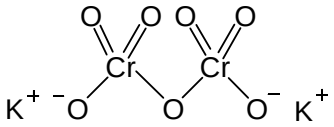
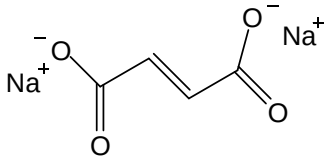
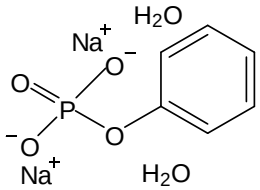
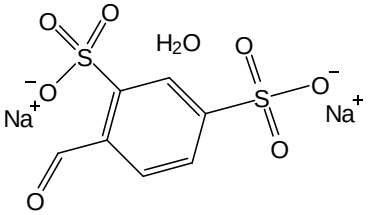
#	Name	Formula/Structure
d1	Potassium Dichromate	
d2	Sodium fumarate dibasic	
d3	Sodium phenyl phosphate dibasic dehydrate	
d4	4-Formyl-1,3-benzenedisulfonic acid, disodium salt hydrate	

Table 2.1 - *Continued*

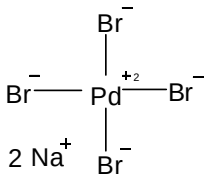
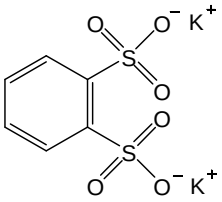
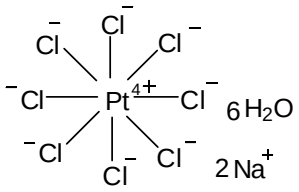
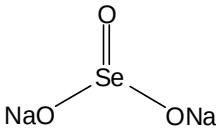
d5	Sodium tetrabromopalladate	
d6	1,2-Benzenedisulfonic acid, dipotassium salt	
d7	Sodium hexachloroplastinate hexahydrate	
d8	Sodium selenite	

Table 2.2 Structures of the trivalent anions

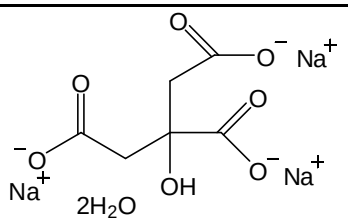
#	Name	Structure/Formula
t1	Sodium citrate tribasic dihydrate	

Table 2.2 - *Continued*

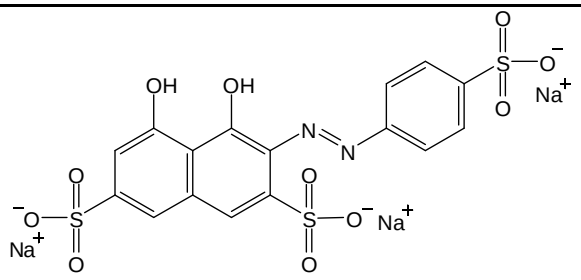
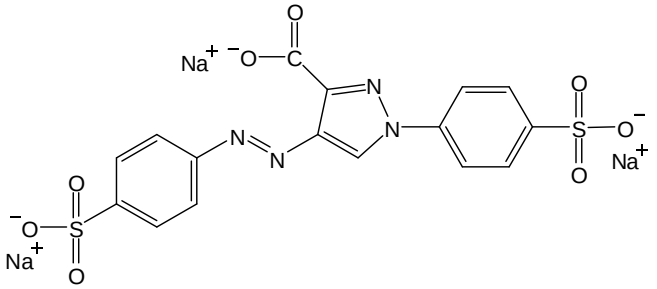
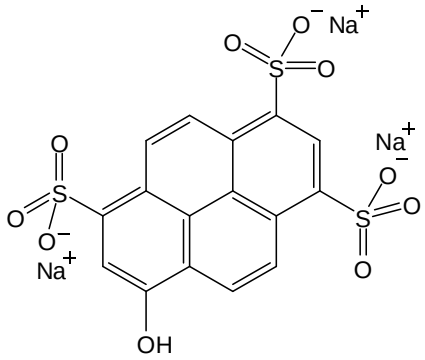
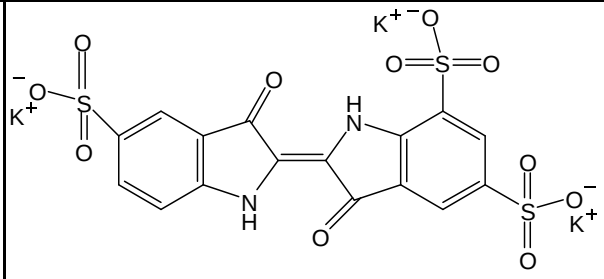
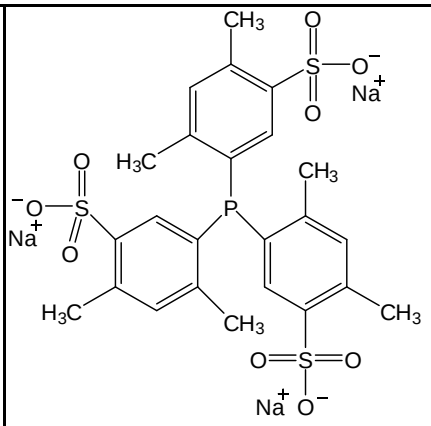
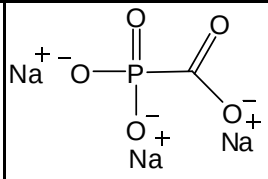
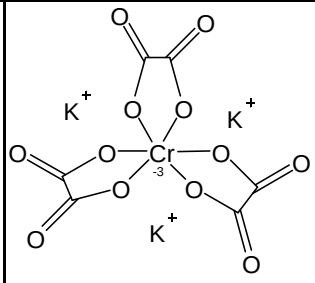
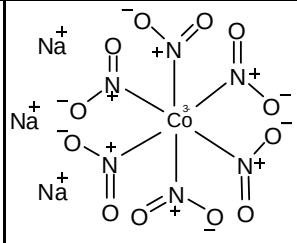
t2	Sulfanilic acid azochromotrop	
t3	Tartrazine	
t4	Pyranine	
t5	Potassium indigotrisulfonate	

Table 2.2 – *Continued*

t6	Tris(2,4-dimethyl-5-sulfophenyl) phosphine trisodium salt	
t7	Sodium phosphonoformate tribasic hexahydrate	
t8	Potassium chromium(III) oxalate trihydrate	
t9	Sodium hexanitrocobaltate(III)	

2.4.2 Optimization of CE separation on divalent anions

In a previous study, a background electrolyte containing 50mM concentration of tris buffer and 20mM dicationic reagent was found to reverse the usual direction of electroosmotic migration in a fused-silica capillary [43]. This study has now been extended to those multivalent anions. Figure 2 shows the effect of the concentrations of the cationic run buffer additive on the

separation of the multivalent anions. The optimum concentration of dicationic additives in tris buffer was 30mM. At 10mM cationic additive, several anions were co-eluted and the separations were less efficient compared to those in higher concentration. However, as the concentration of cationic reagent increases, some analytes, such as sodium phenyl phosphate dibasic dehydrate (d3), begin to show long migration times (Figure 2c).

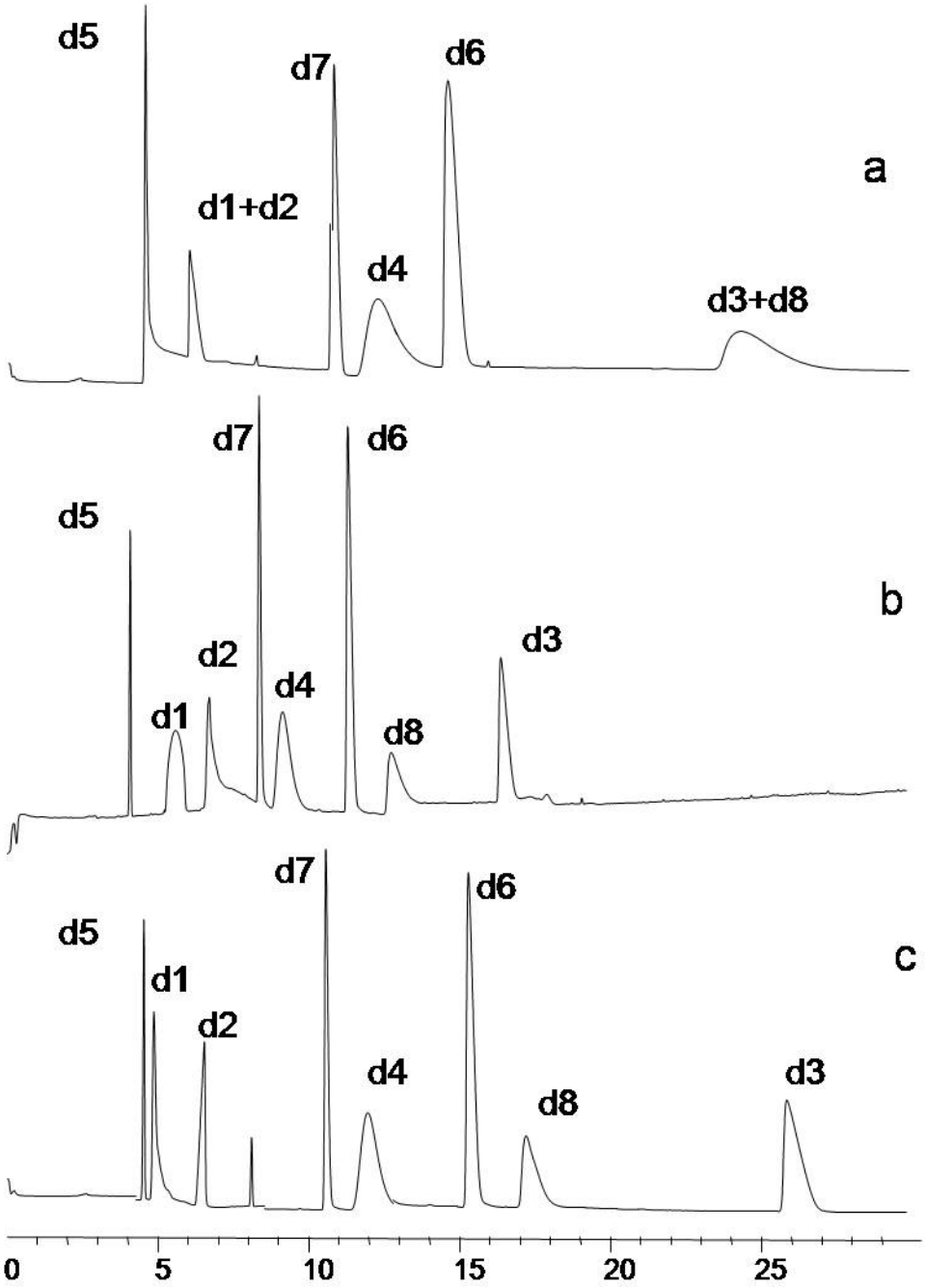


Figure 2.2 Effect of concentrations of dicationic ion pairing reagent when using 50mM tris solution, pH=7, -6kV, 20/30cm capillary with 50 m id. Conditions: a): 10mM; b): 20mM ; c): 30mM.

Meanwhile, buffer types play an important role for it controls pH, stabilizes current and maintains the EOF [78, 79]. Commonly used CE buffers are ammonium acetate, borate, tris, and

phosphate buffer [39, 43, 65, 76]. Four types of buffer (ammonium acetate buffer, borate buffer, tris buffer and phosphate buffer) were evaluated in this study, and only tris and phosphate buffer produced reasonable separations of the multivalent anions herein. Figure 2.3 compares the optimized separation of the eight divalent anions in tris and phosphate buffer with the dicationic complexing reagent, as well as the best separation using neat buffer. Though the neat phosphate buffer enabled the baseline separation of five divalent anions, sodium phenyl phosphate dibasic dehydrate, 4-formyl-1,3-benzenedisulfonic acid, disodium salt hydrate and sodium selenite co-eluted and had poor peak shapes. The importance of addition of a cationic ion pairing reagent to the BGE becomes obvious when comparing the Figure 2.3 a, b and c electropherograms. The separations with phosphate buffer and 20mM dicationic pairing agent (Figure 2.3c) produced the highest efficiencies and the shortest migration times.

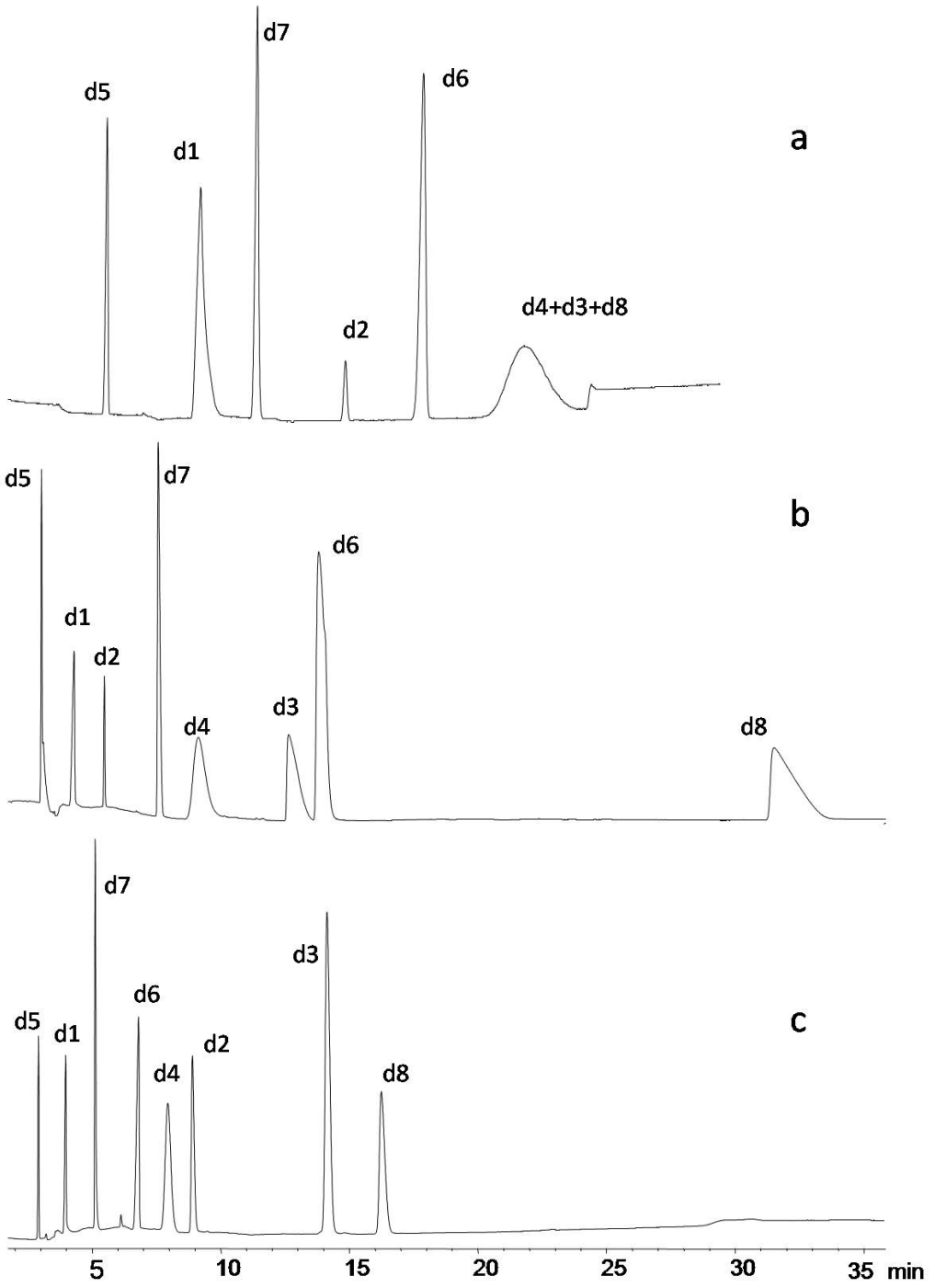


Figure 2.3 Comparison of best separation on divalent anions by using phosphate buffer (with or without cationic reagent) and tris buffer. Conditions: a): 50mM phosphate, pH=7, -9kV, 20/30cm capillary with 50 m id; b): 50mM tris, pH=8, -9kV, 30mM dicationic ion pairing reagent, 20/30cm capillary with 50 m id; c): 50mM phosphate, pH=8, -9kV, 20mM dicationic ion pairing reagent, 20/30cm capillary with 50 m id.

The pH of the BGE is one of the most significant parameter for the separations in CE. It affects the zeta potential of the capillary as well as the charge state of analytes and in the same cases the cationic additives. Tris-HCl buffer was tested in the pH range from 5 to 9. Baseline separation of the eight anions was achieved from pH 6 to 8. Figure 2.4 illustrates the changes in the effective mobilities of the anions in that pH range. Lower pH does not favor the ionization of silanol groups and thus slows the EOF and also affects the separation. A steep increase in the effective mobilities were observed from pH 6 to pH 7 and reasonable resolutions were obtains at pH 7 (Figure 2.2 c). When pH is higher than 8, no improvements in terms of resolution or selectivity were observed (data not shown).

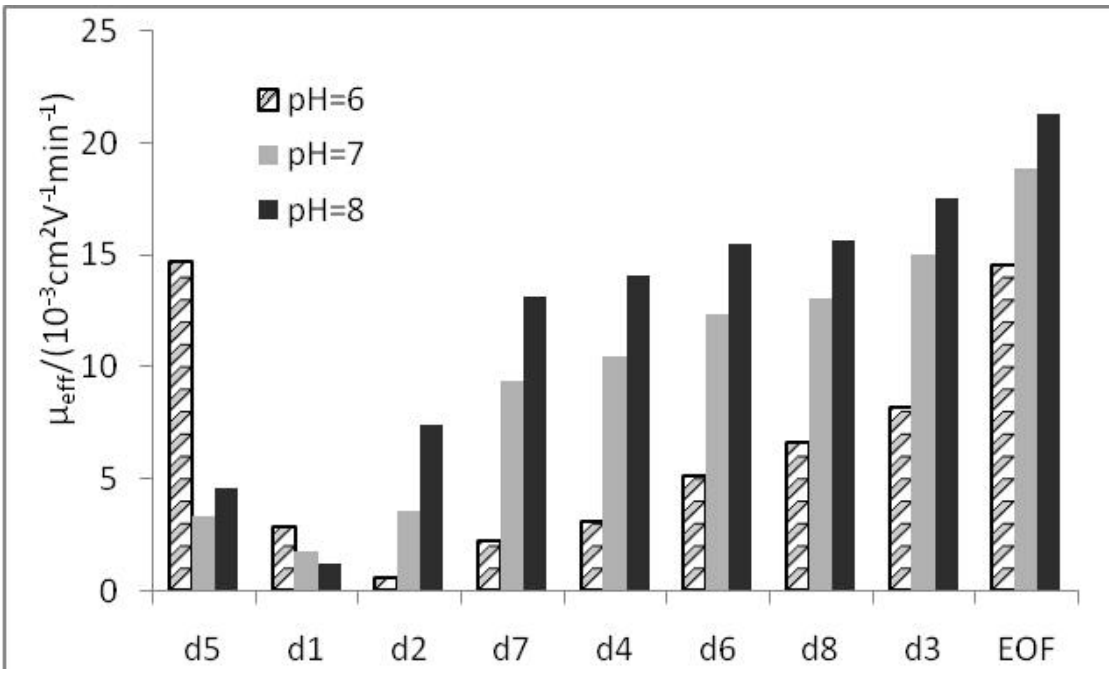


Figure 2.4 Dependence of effective mobilities on pH; 50mM Tris-phosphate buffer, 30mM dicationic ion pairing reagent, -6kV. Other experimental conditions were the same as in Figure 2.2.

The applied voltage affects the separation results as well. Figure 2.5 compares the voltage effect on the separation of anions at two different voltages. Figure 2.5a illustrates that higher voltage dramatically increases the apparently mobility of all analytes .Figure 2.5b indicates that higher voltages benefited the separation efficiencies as well. Although all the analytes can be

well separated at 6 kilovolts when using 20mM dicationic additives in phosphate buffer at pH 8, the analysis time is over 30 min (results not shown). However, when the voltage was increased to 9 kilovolts, all anions were baseline separated within 17 minutes (Figure 2.3c) and the peak shapes were improved as well. It should be noted that even greater voltage (higher than 9kV) further decreased the distances between the analytes peaks and some peaks were even overlapped. Thus this higher voltage failed to benefit the separation.

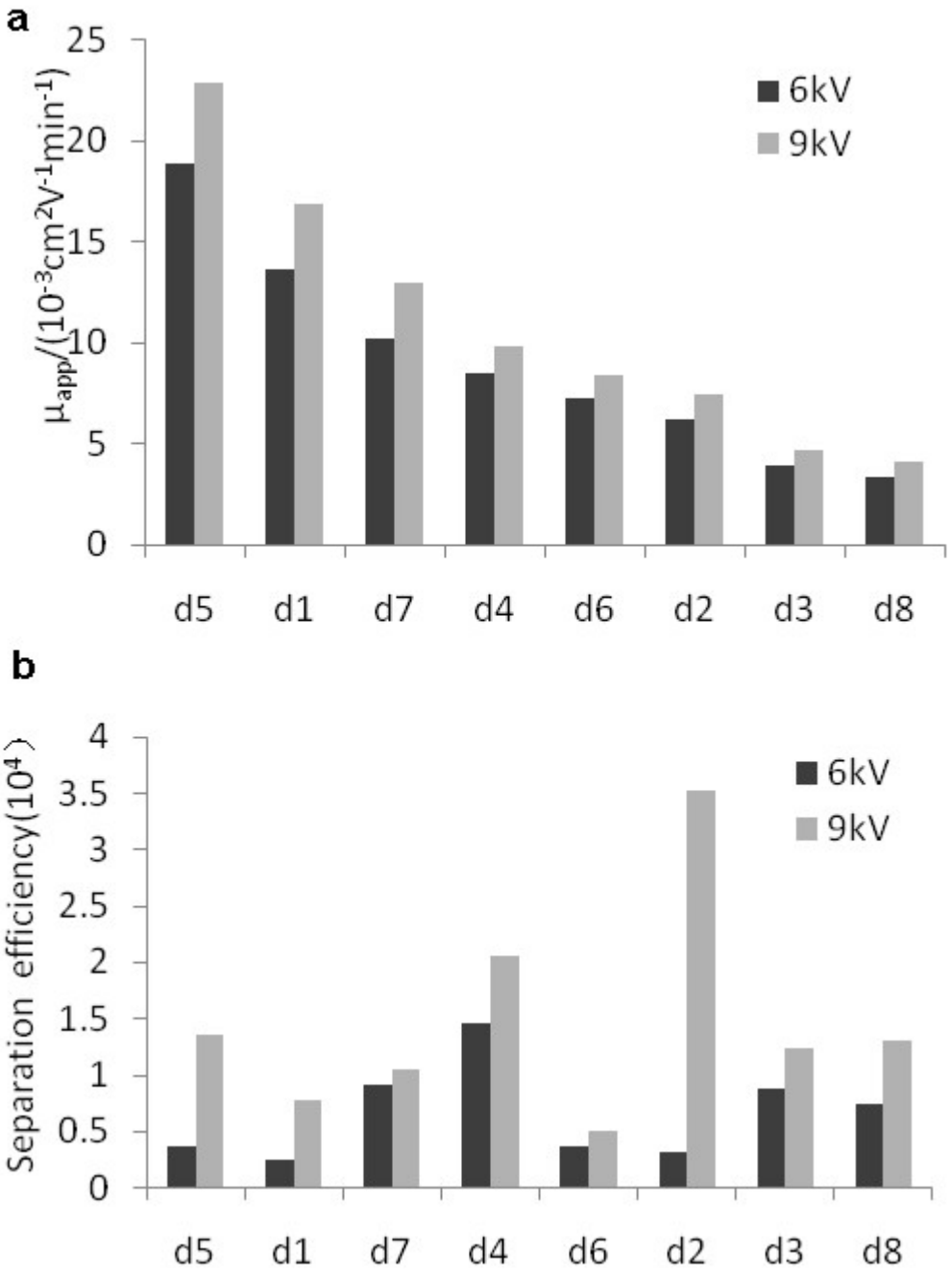


Figure 2.5 Comparison of different voltages on the separation of anions. a: effect of voltage on effective mobilities; b: effect of voltage on efficiencies. Electrophoresis condition: 50mM phosphate buffer of pH 8 containing 20mM dicationic ion pairing reagent, reversed polaity mode in different voltage. Other experimental conditions were the same as in Figure 2.2.

The effect of tetracationic ion pairing reagent (Figure 2.1a) was compared to that of the dicationic ion pairing reagent, Figure 2.6 illustrates the best separation of the eight divalent

anions when using the two different cationic additives. It can be seen that the tetracationic reagent gave shorter analysis time than did the dicationic reagent. However, it failed to provide enough analysis time for a fully effective separation and resulted in somewhat broaden peaks for dibasic phenyl phosphate (d3) and selenite (d8).

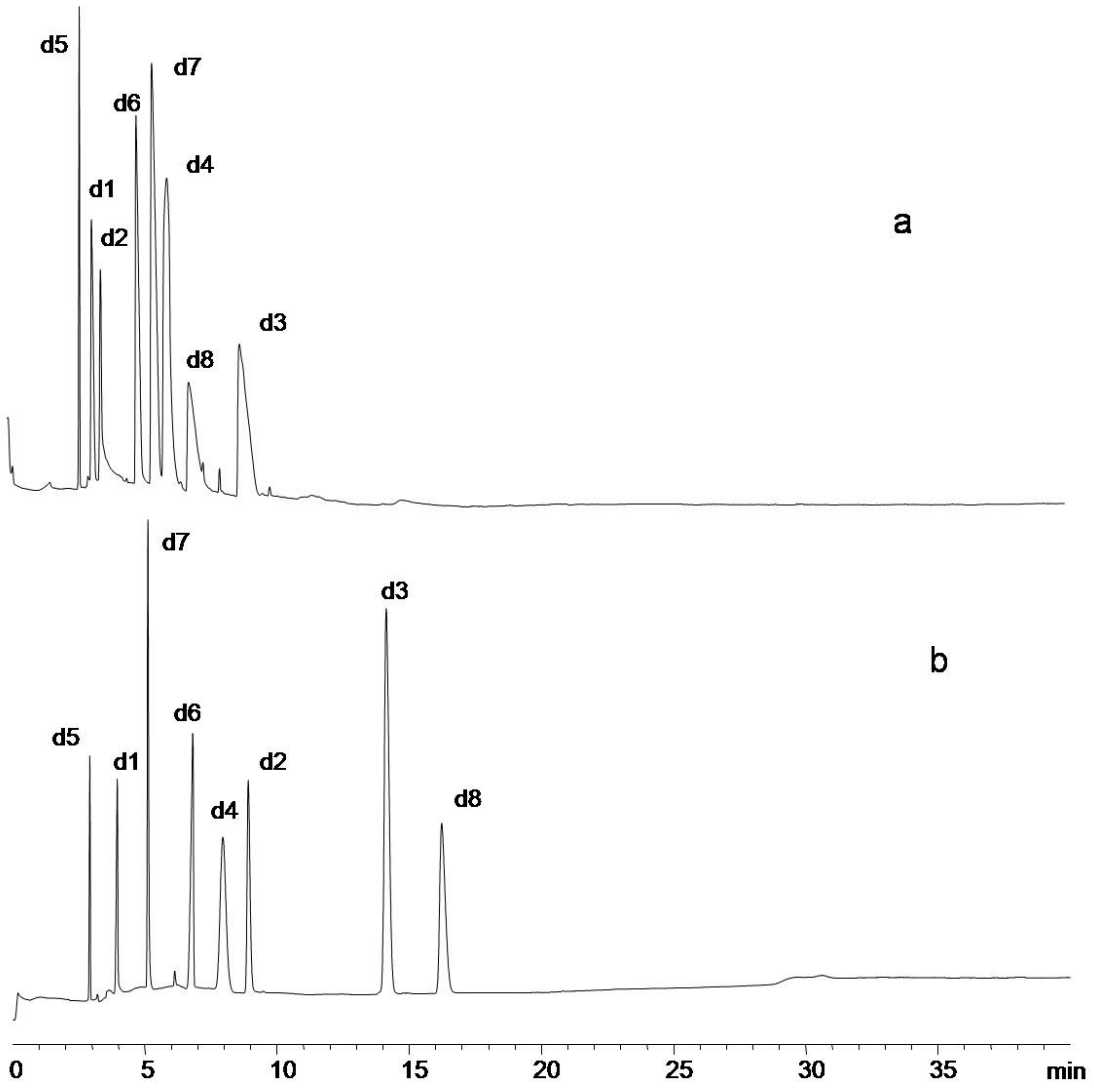


Figure 2.6 Comparison of the best separation done by different cations. Condition: a): 50mM tris, pH=6, -6kV, 30mM tetracationic ion pairing reagent; b): 50mM phosphate, pH=8, -9kV, 20mM dicationic ion pairing reagent. Other experimental conditions were the same as in Figure 2.2.

2.4.3 CE separation of trivalent anions and mixtures

The separations of the trivalent anions were somewhat more difficult to achieve. Also it is interesting to note that the separations produced with the dicationic ion pairing reagent were significantly worse than those with the utilized tetracationic reagent (data not shown). Figure 2.7 shows the best separations obtained using the tetracationic ion pairing reagent in tris and phosphate buffer, as well as the results in neat phosphate buffer. The separations of the trivalent anions obtained using tris buffer were better that the same separations done with (Figure 2.7b, c), when using tetracationic ion pairing reagent for trivalent anions. The analytes migrated faster and produced sharper peak shapes when using tris buffer. All the anions were baseline separated except sulfanilic acid azochromotrop (t2) and potassium indigotrisulfonate (t5) in tris buffer, while in phosphate buffer, pyranine (t4) co-eluted with sulfanilic acid azochromotrop (t2) and potassium indigotrisulfonate (t5). When using the neat buffer without any additives, the analysis took longer and indigotrisulfonate (t5), chromium (III) oxalate (t8) were not well separated.

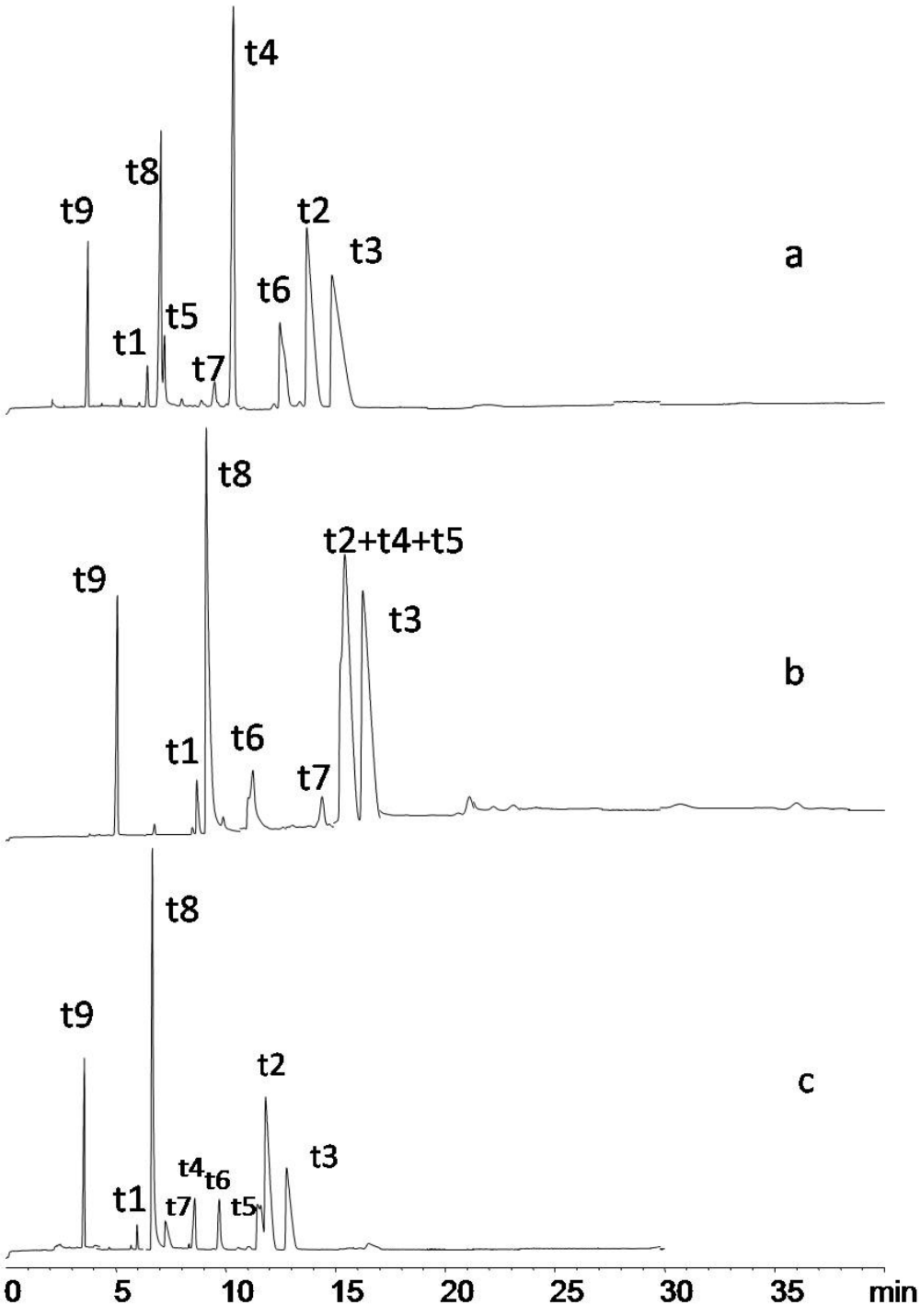


Figure 2.7 Comparison of optimized separation of trivalent anions in different BGE when suing 20/30cm capillary with 50 m id. Conditions: a): 50mM phosphate, pH=6, -9kV; b): 50mM phosphate, pH=6, -6kV, 5mM tetracationic ion pairing reagent; c): 50mM tris, pH=5, -9kV, 15mM tetracationic ion pairing reagent

When the divalent and trivalent anions were mixed together, the increased ionic strength of the sample solution helped to improve the peak shapes of all the analytes. However, the dicationic ion pairing reagent still produced poorer overall separations than tetracationic reagent for the mixture. Again, phosphate buffer produced a better overall separation as compared to tris buffer. Figure 2.8 illustrates the best separations made using tetracationic ion pairing reagent in tris and phosphate buffer, as well as the results in neat phosphate buffer. Thus for complex mixtures of multivalent ions the tetracationic ion pairing reagent appear to produce adequate selectivity plus the shortest analysis times and highest efficiencies. Furthermore, it has the dual potential of enhancing the sensitivity of multivalent anions in CE-ESI-MS [23, 43, 55-57].

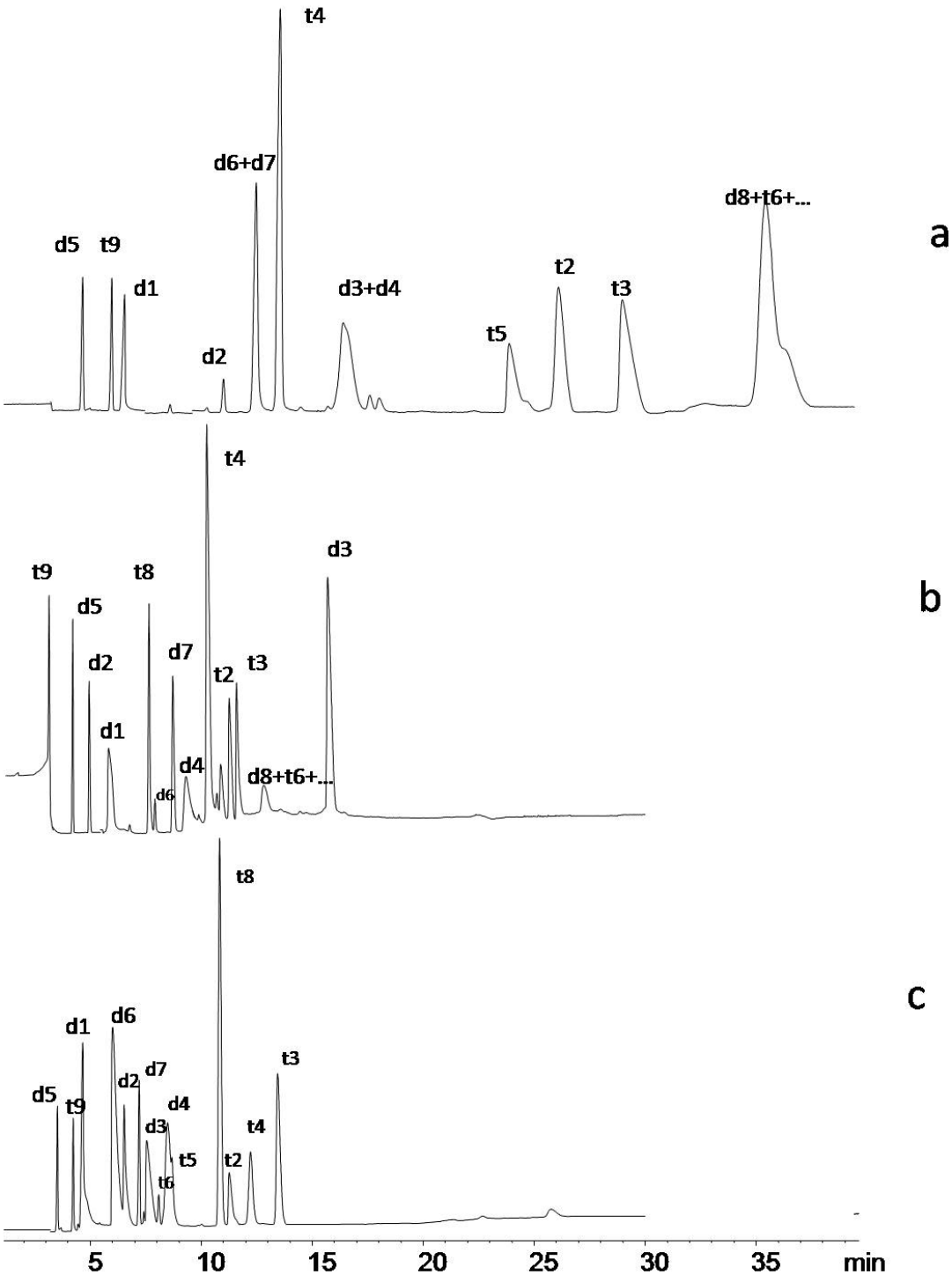


Figure 2.8 Comparison of optimized separation of divalent & trivalent anions in different BGE when using 20/30cm capillary with 50 m id. Conditions: a): 50mM phosphate, pH=8, -6kV; b): 50mM tris, pH=6, -6kV, 10mM dicationic ion pairing reagent; c): 50mM phosphate, pH=7, -6kV, 15mM tetracationic ion pairing reagent

2.5. Concluding remarks

In this study, the CE separation of anions and their identification were extended to anions of greater charge and completely. This was in contrast to previous work which focused on using phosphonium-based dicationic agent to characterize singly charged inorganic and small organic anions in CE and CE-ESI-MS. Tetracationic phosphonium-based pairing reagents, which have not been utilized or reported in CE, were shown to the particularly useful for CE separations of trivalent anions and mixtures of divalent and trivalent anions. The dicationic ion pairing reagent best separated eight divalent anions. The separation performance is affected by the cationic additive's concentration, the BGE composition, pH and voltage. Under optimized conditions, satisfactory separation with relatively short analysis time, high efficiencies and good resolutions were achieved. In this case the pairing agent may serve a dual role, i.e., as CE separation agent in solution, and as a positive mode ESI-MS detection agent in the gas phase.

2.6. Acknowledgement

We gratefully acknowledge the Robert A. Welch foundation, Y-0026 (D. W. Armstrong)

CHAPTER 3

THE ENANTIOMERIC SEPARATION OF 4, 5-DISUBSTITUTED IMIDAZOLES BY HPLC AND CE USING CYCLODEXTRIN-BASED CHIRAL SELECTORS

3.1 Abstract

The enantiomeric separation of a series of fifteen racemic 4,5-disubstituted imidazole compounds was examined by high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). These alkaloid analytes are important precursors for the total synthesis of the natural product calcaridine A and other *Leucetta*-derived alkaloids. Therefore, the enantiomeric analysis of these analytes is not only important in the production of enantiomerically pure calcaridine A, but also for a better understanding of the stereochemistry involved in related biosynthetic pathways. Several bonded cyclodextrin (both native and derivatized) stationary phases were evaluated for their ability to separate these racemates via HPLC. Likewise, several cyclodextrin derivatives were evaluated for their ability to separate this set of compounds via CE. Using HPLC, 14 of the 15 racemic compounds were separated. Eight of the analytes were separated using CE with resolutions up to 7.0. Using both HPLC and CE approaches, the entire set of analytes was separated. The optimization of these separations was discussed and a comparison between the chiral selectors used was made. Lastly, the similarity of the 15 analytes allowed for insight into the mechanism of chiral recognition.

3.2 Introduction

The synthesis of natural products is of great interest to organic and medicinal chemists. Bioactive molecules and their metabolites, as well as, any related chemical derivatives or analogues are an abundant resource for the development of new pharmaceutical compounds.[80-

[84]. The intricate structural constitution of many naturally occurring molecules can make synthesis a challenging undertaking. Furthermore, the stereochemical requirements often increase the burden of the task and may require the use of asymmetric synthetic pathways or stereoselective separation techniques. Considering the potential pharmacological importance of natural products, researchers are continually searching for new, undiscovered, molecules. Recognizing that about 70% of the earth's surface is water, many of these researchers have intuitively turned to the seas and marine organisms in the search for these compounds [85-94]. Currently, one group of organisms that has come under scrutiny is marine sponges.

The Fijian sponge, *Leucetta* sp., has been shown to be a good source of some very interesting alkaloid imidazole compounds, such as calcaridine A, spirocalcaridine A and B, and spiroleucettadine [86, 90-94]. The latter, spiroleucettadine, has been shown to posses antibacterial properties that are comparable to those of vancomycin and penicillin [93]. Calcaridine A is thought to be a precursor in the biosynthesis of spiroleucettadine. Recently, calcaridine A has been successfully synthesized though a multi-step process that concluded with an oxidative rearrangement of a 4,5-disubstituted imidazole [86, 94]. Through-out this synthetic pathway, several newly synthesized chiral alkaloids were produced. These chiral precursors were synthesized and used in their racemic form, such that, when the oxidative rearrangement step was performed, the synthetic material was formed as a racemic mixture of calcaridine A. In addition to calcaridine A, the C14-epimer was formed in the oxidative rearrangement. The enantiomeric separation and purification of any of these racemic precursors would allow for the production of enantiomerically pure calcaridine A. Furthermore, enantioselective separation techniques that are applicable to separate these types of compounds may be helpful in understanding when and how proper stereochemistry is introduced throughout the biosynthetic pathways involving these alkaloids. For these reasons, the stereoselective separation of these precursors is of relevance.

Currently, the most commonly used technique for enantiomeric separations is high performance liquid chromatography (HPLC) [28]. One popular set of chiral HPLC stationary phases are cyclodextrins and their derivatives. Cyclodextrins have been used to separate a vast number of chiral analytes and have shown to be extremely applicable towards the separation of racemic analytes containing aromatic functionality.[20-25] Under reverse phase solvent conditions, aromatic moieties often form inclusion complexes with the cyclodextrins. Separation by HPLC offers the advantage of preparative capabilities, which could aid in the production of the pure enantiomers of calcaridine A in the long term.

Additionally, capillary electrophoresis (CE) may offer another means by which these racemic precursors could be resolved. Cyclodextrins and their derivatives dominate the field of chiral selectors used in CE [95-97]. Though these separations offer limited potential in the preparative production of enantiomerically pure calcaridine A, they could be useful for determining enantiomeric excesses of asymmetric synthetic steps.

In this study, the enantiomeric separation of 15 newly synthesized 4,5-disubstituted imidazoles was explored. Both HPLC and CE were tested and compared for their abilities to separate the enantiomers of these chiral analytes. To our knowledge, none of these compounds have previously been subjected to any chiral chromatographic or electrophoretic technique. Also, the structural similarity of this series of analytes may allow some insight into their chiral recognition by cyclodextrins and provide a basis for exploration of related systems.

3.3 Materials and methods

3.3.1 Materials

HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from VWR (West Chester, PA, USA). Deionized water was produced by a Milli-Q system (Millipore, USA). Ammonium acetate (H_4NOAc), glacial acetic acid (HOAc), triethylamine (TEA), sodium phosphate monobasic and dibasic, phosphoric acid, and sodium hydroxide (NaOH) were

obtained from Sigma-Aldrich (Milwaukee, WI, USA). All the chiral analytes listed in Figure 3.1 were synthesized according to literature by Koswatta et al [86, 94]. Note compound 5 possesses an azide group and was stored away from light. All HPLC columns used were of analytical dimensions (25 cm x 4.6 mm, 5 µm) and were obtained from Advanced Separation Technologies (Whippany, NJ, USA). The columns used were as follows: β-CD (β-cyclodextrin), HP-RSP (high performance hydroxypropyl ether β-cyclodextrin), RSP (hydroxypropyl ether β-cyclodextrin), AC (acetylated β-cyclodextrin), RN and SN (R- and S-naphtylethyl carbamate β-cyclodextrin), DMP (dimethylphenyl carbamate β-cyclodextrin), DNP (2,6-dinitro-4-trifluoromethylphenyl ether β-cyclodextrin), and DM (dimethylated β-cyclodextrin). CE chiral selectors were obtained from Sigma-Aldrich (Milwaukee, WI, USA) and were as follows: SBCD (sulfated β-cyclodextrin), DMBCD (dimethylated β-cyclodextrin), and HPBCD (hydroxypropyl β-cyclodextrin). The untreated fused silica capillaries (50 µm i.d.) for CE were purchased from Polymicro Technologies (Pheonix, AX, USA).

3.3.2 Method

All HPLC analyses were performed on one of two chromatographic systems, both of which were produced by Shimadzu (Kyoto, Japan). The pumps used were models LC-6A and LC-10A, the detectors were SPD-6A and SPD-10A, and the data was recorded using a SPD-6A chromatographic integrator. In both cases, the samples were injected using a 6 port injector equipped with 10 µL injection loops.

All CE experiments were performed on Beckman Coulter P/ACE MDQ (Fullerton, CA, USA) capillary electrophoresis system equipped with an on column UV-visible detector.

3.3.3 HPLC Analyses

All samples were dissolved in MeOH for analyses done in reverse phase (RP) and in ACN when the tests were performed in the polar organic mode (PO). The wavelength of detection for all HPLC experiments was 254 nm. H_4NOAc buffers were adjusted to a desired pH using HOAc. The mobile phase compositions listed throughout this manuscript are in

volume to volume ratios. All mobile phases were degassed by ultrasonication under vacuum for 5 min before use. The mobile phase flow rate throughout the entire study was 1 mL/min.

3.3.4 CE Analyses

Unmodified fused-silica capillary was thermostated at 25 ℃, with the length being 30 cm to the end and 20 cm to the detection window. UV detection was accomplished at 214 nm. Sodium phosphate, monobasic and dibasic (1:1), were dissolved in deionized water and adjusted with concentrated phosphoric acid to the desired pH. Buffer additives (β-cyclodextrin derivatives) were added directly into the buffer. All samples were dissolved in a solution consisting of 20% methanol and 80% water. Normal polarity (NP) and reversed polarity (RP) analyses used voltages of +7 kV and -7 kV, respectively.

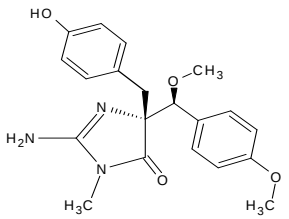
At the beginning of each series of experiments, the capillary was rinsed with 1 M sodium hydroxide for 5 min and water for 5 min for conditioning. Between each run, the capillary was flushed with 1 M sodium hydroxide for 2 min, water for 2 min, and buffer for 2 min. Then, the sample solution was injected hydrodynamically at 0.5 psi for 5 sec.

3.3.5 Calculations

The HPLC retention factors (k'_1), selectivities (α), and resolution (R_s) values were calculated as follows: $k'_1 = (t_{r1}-t_0)/ t_{r1}$; $\alpha = (t_{r2}-t_0)/ (t_{r1}- t_0)$; and $R_s = 2*(t_{r2}-t_{r1})/ W_1 + W_2)$; where t_{r1} and t_{r2} are the retention times of the first and second eluting peaks, t_0 is the dead time (found by calculating the void volume of the column), and W_1 and W_2 are the baseline widths of the first and second eluting peaks.

In CE, the migration time of the first peak is represented by t_{m1}. The efficiencies (N), selectivities (α), and resolution (R_s) values were calculated as follows: $N = 16(t_m/W)^2$; $\alpha=\mu_{app1}/\mu_{app2}$; $R_s = 2*(t_{r2}-t_{r1})/ (W_1 + W_2)$; where μ_{app1} and μ_{app2} represent the apparent electrophoretic mobilities of the first and second peaks. Dimethyl sulfoxide was used as the EOF marker. All other constants are the same as above.

A)



Calcaridine A

B)

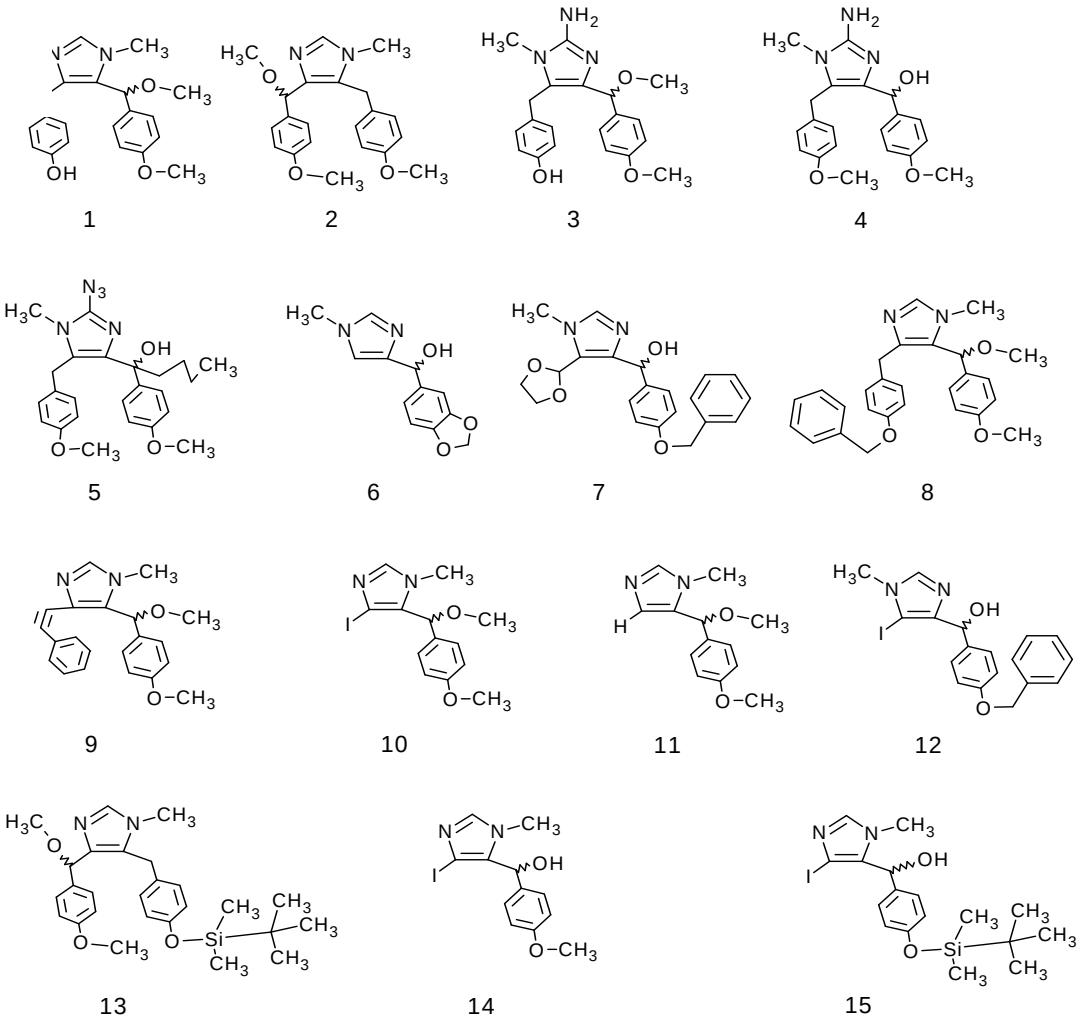


Figure 3.1 Structure of Calcaridine A (A) and the structures of the 15 racemates that were subjected to enantioselective HPLC and CE analyses in this study (B).

3.4 Results and Discussion

3.4.1 Analytes

Figure 3.1a shows the structure of calcaridine A. Also, in Figure 3.1b the chemical structures of the 15 racemic synthetic intermediates are shown. As can be seen, this set of intermediates offers systematic changes in functionalities, as well as, placement of the stereogenic center (being either alpha to the 4- or the 5-position of the imidazole core).

3.4.2 HPLC Analyses

A summary of the optimized HPLC separations data is in Table 3.1. The optimized conditions were considered to be when the resolution (R_s) values were greatest and the retention factors (k') were satisfactorily small. Enantioselectivity (α) was observed for 14 of the 15 compounds. These α values ranged from 1.08 (compounds 12 and 13) to 1.29 (compound 6). Compound 15 was not separable under the conditions used. The R_s values for these compounds ranged from 0.6 (compound 13) to 2.0 (compound 10). Seven of the compounds had R_s values greater than or equal to 1.5 and were considered to be baseline separated.

As can be seen in Table 3.1, the 3,5-dimethylphenyl derivatized β-cyclodextrin (DMP) CSP produced the most favorable HPLC separations for five of the 15 compounds. The hydroxypropyl-β-cyclodextrin derivatives (HP-RSP and RSP), as well as, the native β-cyclodextrin (β-CD) chiral stationary phase each accounted for three of the baseline separations. In fact, it was the native β-CD phase which produced in the greatest R_s value observed in this study (R_s = 2.0 for compound 10). It is important to note that just because a CSP doesn't appear in Table 1, does not necessarily mean it did not produce any separations, since Table 1 lists only the best results.

Table 3.1 Summary of the optimized HPLC enantiomeric separations.

#	CSP[a]	K'_1	α	R_s	Mobile Phase
1	HP-RSP	4.46	1.15	1.6	85/15 20mM H_4OAc / ACN pH=4.1
2	DMP	3.53	1.13	1.6	80/20 20mM H_4OAc / ACN pH=4.1
3	RN	11.81	1.06	1.3	75/25 20mM H_4OAc / ACN pH=4.1
4	SN	4.55	1.10	1.6	85/15 20mM H_4OAc / ACN pH=4.1
5	HP-RSP	3.45	1.09	1.1	85/15 20mM H_4OAc / ACN pH=4.1

Table 3.1 - *Continued*

6	DMP	2.04	1.29	1.8	95/5 20mM H_4OAc / ACN pH=4.1
7	DMP	0.78	1.17	1.3	99/1/.3/.2 ACN/MeOH/HOAc/TEA
8	β-CD	1.55	1.16	1.1	80/20 20mM H_4OAc / ACN pH=4.1
9	β-CD	7.97	1.19	1.6	85/15 20mM H_4OAc / ACN pH=4.1
10	β-CD	3.73	1.27	2.0	75/25 20mM H_4OAc / ACN pH=4.1
11	DMP	4.71	1.14	1.3	95/5 20mM H_4OAc / ACN pH=4.1
12	DMP	4.95	1.08	1.3	80/20 20mM H_4OAc / ACN pH=4.1
13	AC	1.10	1.08	0.6	80/20 20mM H_4OAc / MeOH pH=4.1
14	RSP	5.00	1.09	1.7	85/15 20mM H_4OAc / ACN pH=4.1
15	N/A	_	_	_	_

[a] For a list of the defined CSP abbreviations, see the Experimental section.
N/A means no enantioseparation was observed for this compound under any condition.
For the structures of the analytes, refer to Fig. 9.

A more complete comparison between the nine CSPs tested in this study can be seen in Figure 3.2 which depicts the total number of partial ($0.4 < Rs < 1.5$) and baseline ($Rs > 1.5$) separations obtained using each chiral selector. The chiral stationary phase that produced the greatest number of separated compounds was the HP-RSP/RSP media. This chiral selector offered enantioselectivity for 66% of the compounds tested. Additionally, this CSP produced four baseline separations; a number that was matched only by the S-naphthylethyl carbamate (SN) derivative of β-cyclodextrin. For this reason, it was determined that this stationary phase should be the recommended starting point when attempting to separate similar analytes. The chiral selectors AC, β-CD, SN, RN, and DMP (in Figure 3.2) separated only six to seven of the analytes. Keeping in mind that the DMP phase was previously acknowledged as the one that gave the greatest number of "best" separations, it is interesting to see that it only ranks modestly compared to other CSP in terms of total separations. This is a result of the DMP phase being complimentary to the other phases tested, which allowed for the separation of certain compounds that were not separable when using the other chiral selectors. The CSPs that performed poorly were the DNP and the DM, both of which only showed partial separations for four analytes and no baseline separations.

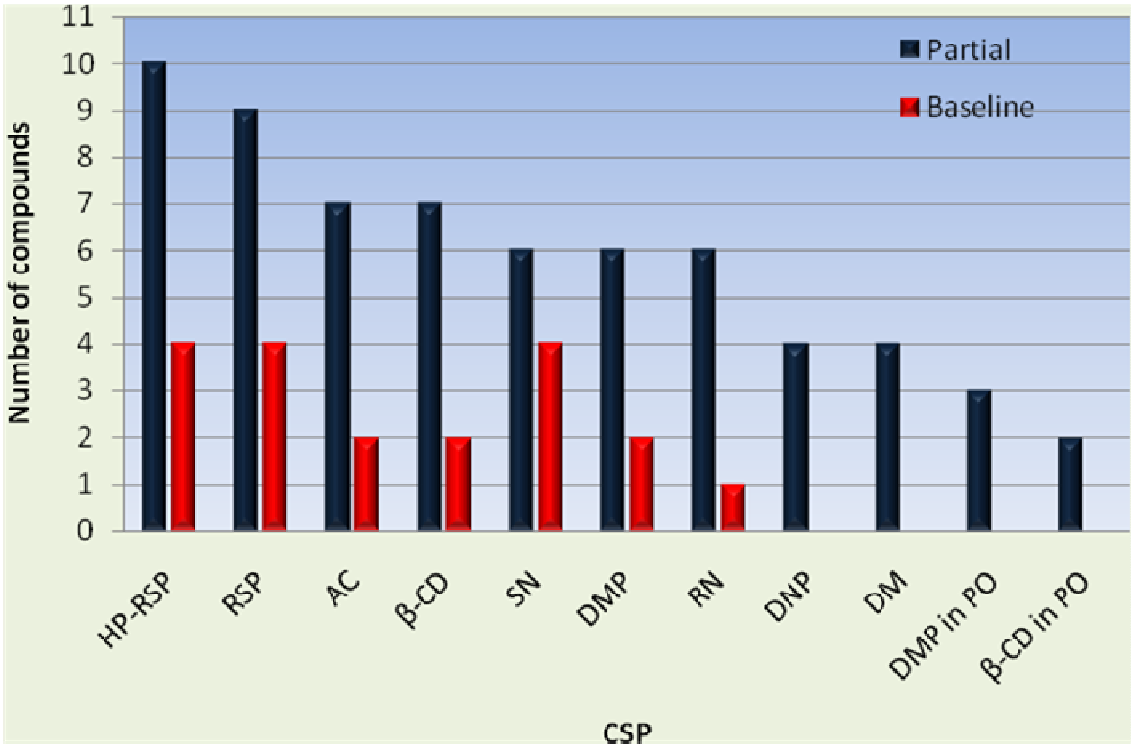


Figure 3.2 A representation of the total number of partial and baseline separations obtained using HPLC with different chiral stationary phases. Note, "in PO" means the separations were obtained in the polar organic mode, whereas, all other separations were obtained in the reverse phase mode. For a list of the defined CSP abbreviations, see the Experimental section.

In this study, both reverse phase and polar organic modes were tested. The normal phase also was attempted, but the initial results were very poor. In the reverse phase mode, both acetonitrile and methanol were tested as organic modifiers. In general, using acetonitrile as the organic modifier resulted in sharper peaks with smaller retention factors, whereas the use of methanol yielded the opposite, i.e., broader, longer retained peaks. For this reason, acetonitrile typically resulted in better (higher R_s) separations. As indicated in Table 3.1, only once did methanol produce a better separation than acetonitrile (compound 13). Considering all these compounds have an imidazole core, it was necessary to use a buffer (ammonium acetate in this study). Both the pH and the buffer concentration were examined in the optimization process. The optimum pH and buffer concentrations were determined to be 4.1 and 20 mM, respectively. Changing the ratio of the aqueous to organic cosolvents was the most important

tool in manipulating the separations. When using cyclodextrin based CSPs in the reverse phase, it is important to allow hydrophobic inclusion complexation to occur [98, 99]. The organic modifier competes with the analytes for the nonpolar cavity, thus increases in the acetonitrile (or methanol) percentage resulted in smaller retention factors. The occurrence of inclusion complexation with cyclodextrins has been extensively studied [100]. Throughout this work, optimum aqueous/organic ratios ranged from 75/25 to 95/5.

Conversely, the polar organic mode does not permit inclusion complexes to form as the high organic content in the mobile phase completely occupies the cyclodextrin cavity [101]. Rather, the dominant interaction in this operation is through hydrogen bonding. Thus, evaluation in the polar organic mode was also performed on all the CSPs which possessed free hydroxyl groups. Operating in the polar organic mode resulted in very few separations for this set of analytes. As listed in Table 3.1, only one of the optimized separations (compound 7) was obtained using polar organic mobile phases. Figure 3.2 shows all the separations obtained in the polar organic mode. The only CSPs that showed any enantioselectivity were the DMP and native β-CD stationary phases, which separated just three and two analytes, respectively. However, when compared to the separations obtained in the reverse phase mode, the separations found using the polar organic mode were of greater efficiency and of lower retention. This is a typical feature of the polar organic mode and arises from the faster adsorption-desorption kinetics involved in the hydrogen bonding interactions that occur at the mouth of the cyclodextrin cavity versus the slower kinetics involved with inclusion complexation. A prime example of this can be seen in Figure 3.3, were the separation of compound 7 on the DMP CSP is shown. Figure 3.3A is the separation in polar organic mode and 11B is the separation in the reverse phase. Notice the highly efficient separation and short analysis time in the upper chromatogram, while the lower chromatogram is of poorer efficiency and longer retention. Keep in mind that for this set of analytes, this is only one of a few examples for which the polar organic mode gave the superior separation.

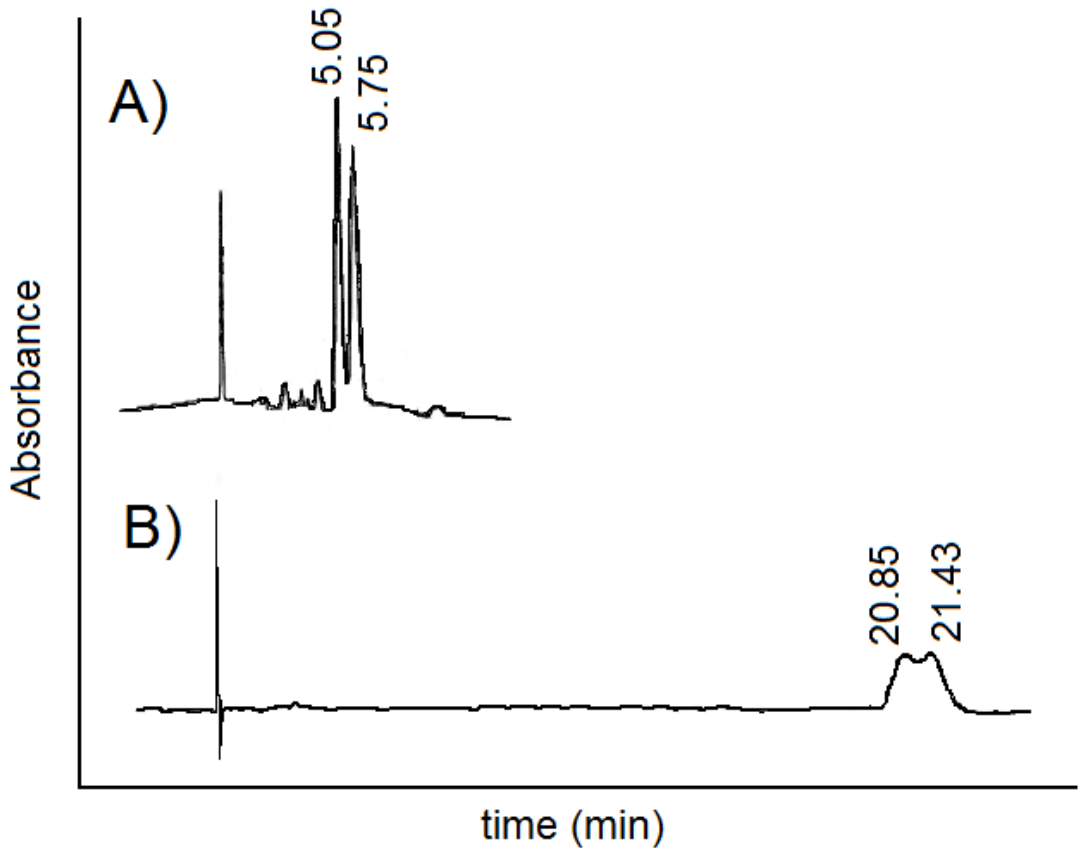


Figure 3.3 HPLC enantiomeric separations of compound #7 in the polar organic mode (A) and in reverse phase (B). The CSP used was DMP. The mobile phase conditions were: A) 99/1/.3/.2; ACN/MeOH/HOAc/TEA and B) 75/25; 20mM H4NOAc (pH 4.1)/ACN. For other chromatographic conditions see the Experimental section. See Figure 1 for the analyte structure.

Generally, the main cause for such poor results in the polar organic mode was lack of sufficient retention. Usually, the compounds that respond well to being chromatographed in the polar organic mode were those that had smaller retention factors in reversed phase conditions. Conversely, compounds that retained well in the reverse phase mode were not well retained in the polar organic mode. An example of this phenomenon is shown in Figure 3.4, were the retention curves for two different analytes (compounds 1 and 14) are shown. The left hand region of the plot shows the retention in reverse phase and the right hand side shows the retention in the polar organic mode. Note that the curves cross each other when the separation mode is changed. This indicates that compound 14 relies heavily on the formation of an

inclusion complex for retention, whereas, compound 1 does not. In the polar organic mode, when inclusion complexation is not available, compound 1 gives rise to longer retention. Apparently, compound 1 can interact with the CSP through hydrogen bonding more than compound 14 can. Comparing the structure of these two analytes reveals that compound 1 has more possible hydrogen bonding sites than compound 14.

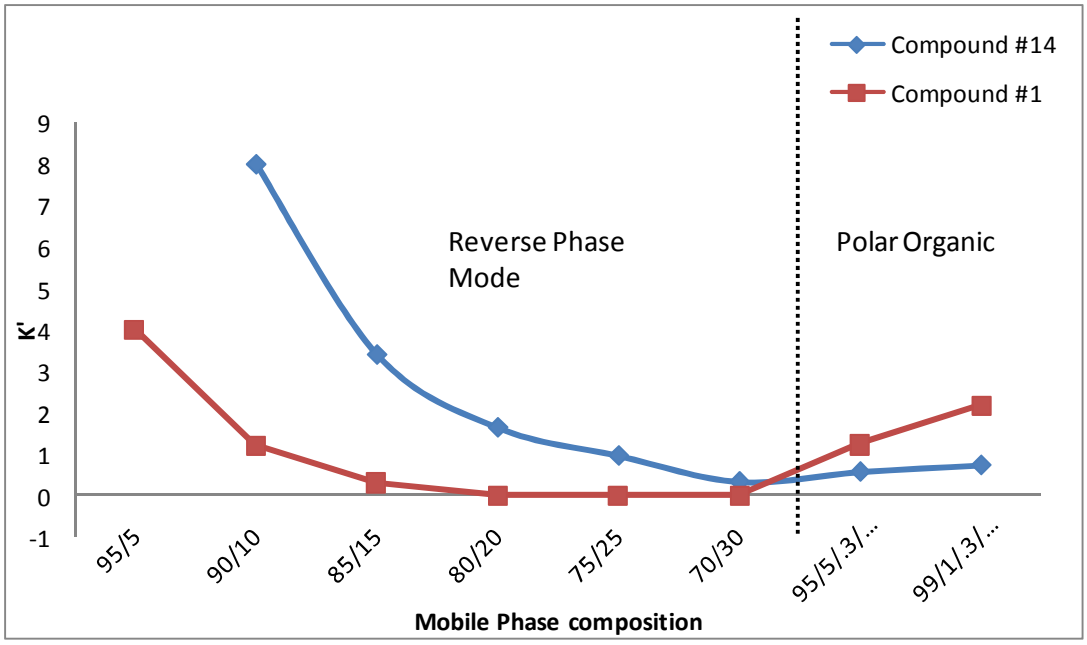


Figure 3.4 Retention curves for two of the analytes chromatographed using HPLC. The mobile phase composition in the reverse phase region is 20mM H4OAc (pH 4.1) / ACN. The mobile phase composition in the polar organic region is ACN/MeOH/HOAc/TEA. The stationary phase was native β-CD. See Figure 3.1 for analyte structures.

Perhaps the most interesting trends, which can lead to insights to the chiral recognition mechanism, were noted in the reverse phase mode. First, it was observed that the analytes which posses aromatic substituents in either the 4- or 5-position, but not both, were most easily separated. Examples of such analytes include compounds 6, 10, 11, and 14. When these analytes interact with the cyclodextrins via inclusion complexation, there is no competition as to which hydrophobic portion of the analyte will occupy the cyclodextrin's cavity. Thus, the stereogenic center is always near to the included moiety (i.e., the aromatic ring). Compounds that have hydrophobic aromatic moieties in both the 4- and 5- positions will dynamically

compete for inclusion in the cyclodextrin cavity. This being the case, the stereogenic center may not always be near to the chiral selector. Examples of analytes that posed such difficulties were compounds 1, 2, 3, 4, and 9. Yet, there were compounds (e.g. compounds 7 and 12) which possessed only one hydrophobic substituent, but were still difficult to separate. Since these two analytes share an additional terminal benzyl moiety, it is reasonable that this is the cause of these molecules being more challenging to separate. It is evident that the benzyl groups will most easily occupy the stationary phase cavity, which will in turn place the stereogenic center further from the chiral selector.

Compound 15 was the only analyte that was not separated by any CSP in either mode of operation. In fact, it was difficult in most cases to even get this molecule to elute from the stationary phase. This tenacious retention is surely an effect of the presence of the *tert*-butyldimethylsilane (TBDMS) functionality. However, compound 13 also possesses the TBDMS group and enantioselectivity was observed for it. This finding also supports the previous conclusion that analytes with two hydrophobic portions may compete with each other for the cavity of the cyclodextrin. Hence with compound 13, the addition of the second substituent allowed for shorter retention and the allowed for enantioselectivity to be observed.

3.4.3 CE Analyses

In addition to the evaluation by HPLC, all the analytes were also subjected to enantioselective CE using derivatized cyclodextrin additives as chiral selectors. A summary of the optimized CE separations is found in Table 3.2. As can be seen, eight out of the 15 compounds were separated by CE. Enantiomeric selectivity values (α) ranged from 1.02 (compound 7) to 1.34 (compound 11). Separation efficiency (N) values were observed from 2,000 (compound 11) to 27,000 (compound 7). Lastly, resolution values (R_s) ranged from 0.8 (compound 7) to 7.0 (compound 4). Of the eight compounds that were separated using CE, five of them were baseline separated, with the worst baseline separation still exhibiting a resolution greater than 2.0.

For the optimized separations shown in Table 3.2, the background electrolyte composition and pH, as well, as the chiral selector used are also listed. The concentration of the background electrolyte was evaluated at 10 mM intervals from 10 to 100 mM in order to determine the proper concentration. As can be seen, typical optimum concentrations fell between 30 and 70 mM. The pH of the electrolyte was also adjusted for optimum separations. As listed in Table 3.2, optimized pH values were found to be approximately 3 and 8, depending on which (reversed or normal polarity) mode was being employed. This will be discussed in detail below.

Table 3.2 Summary of the optimized CE enantiomeric separations.

Analyte no.	t_{m1}[a]	N	α	R_s	Condition[b]
1	9.20	19000	1.15	4.8	30mM SBCD 50mM phosphate pH=2.6
2	12.97	13000	1.21	5.5	50mM SBCD 50mM phosphate pH=8.0
3	N/A	-	-	-	
4	19.33	21000	1.22	7.0	70mM SBCD 50mM phosphate pH=8.0
5	N/A	-	-	-	
6	12.85	5000	1.08	1.3	60mM SBCD 50mM phosphate pH=8.0
7	10.14	27000	1.02	0.8	30mM SBCD 50mM phosphate pH=3.0[c]
8	N/A	-	-	-	
9	10.78	4000	1.19	2.1	50mM SBCD 50mM phosphate pH=2.6
10	N/A	-	-	-	
11	10.39	2000	1.34	2.7	45mM SBCD 50mM phosphate pH=8.0
12	N/A	-	-	-	
13	N/A	-	-		
14	N/A	-	-	-	
15	30.30	14000	1.05	1.3	30mM DMBCD 50 mM phosphate pH=3.6

[a] t_{m1} is listed in minutes.

[b] SBCD and DMBCD stand for sulfated β-cyclodextrin and dimethylated β-cyclodextrin, respectively.

[c] At sample was made and injected quickly to prevent hydrolysis of acetal and ketone formation.

N/A means no enantioseparation was observed for this compound under any condition.

For the structures of the analytes, refer to Fig. 9.

Almost certainly, the most effective means by which an enantiomeric separation can be modified in CE is by changing the chiral selector itself. Seven out of the eight best separations (Table 3.2) were obtained using sulfated β-cyclodextrin (SBCD) as the chiral selector. Throughout this work, several cyclodextrin based chiral selectors were evaluated. Figure 5 compares the efficacy of all the selectors tested. SBCD again shows its superiority by

occupying two-thirds of the entire chart, as well as, being a “co-selector” for three other separations. In fact, of the 12 total separations, only four of them were obtained using a chiral selector other than pure SBCD and only one was found without use of any SBCD. As can be seen, hydroxypropyl β-cyclodextrin (HPBCD) was unable to separate any analytes on its own; rather it needed to be used in combination with SBCD to obtain three separations. The only other cyclodextrin derivative determined to be useful for these enantiomeric separations was dimethylated β-cyclodextrin (DMBCD), which separated just one analyte.

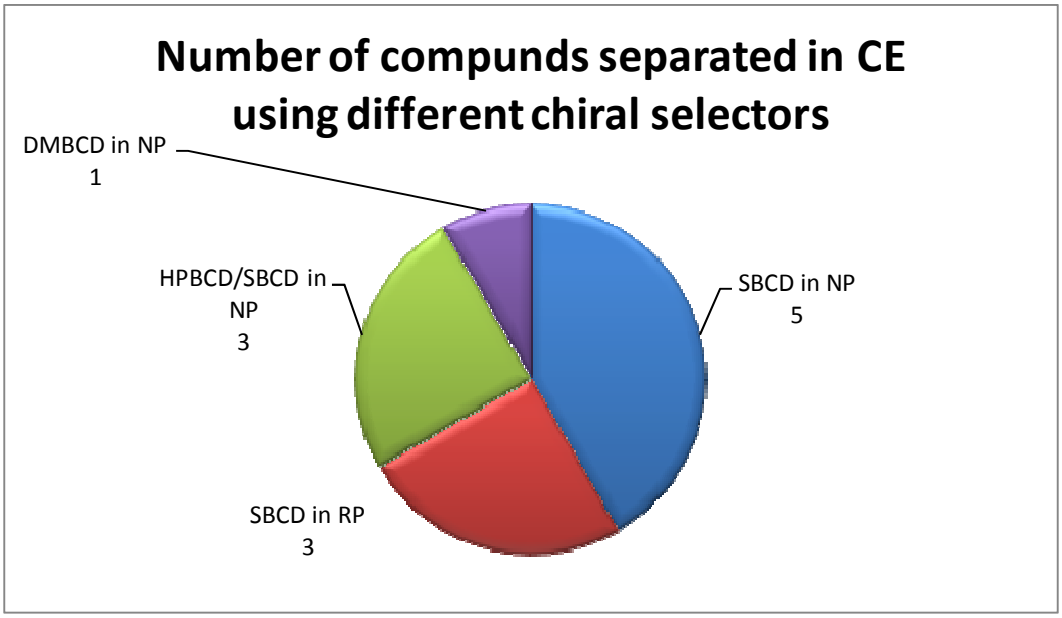


Figure 3.5 Pie chart representing the distribution of separation amongst different chiral selectors. Note, the 12 separations represented here are not necessarily unique. DMBCD, SBCD, and HPBCD/SBCD stand for dimethyl-β-CD, sulfated-β-CD, and a mixture of hydroxypropyl-β-CD and sulfated-β-CD, respectively. NP and RP denote normal polarity and reversed polarity modes.

Another facet to Figure 3.5 is the distinction of the separations found in the reverse polarity mode versus those found in the normal polarity mode. Totally, nine out of the 12 separations were found in the normal polarity mode, which therefore appears to be the mode of choice. Also, when comparing those separations obtained using just SBCD, the normal polarity conditions still give rise to a greater number of separations, but by a narrower margin. In the normal polarity mode, the electroosmotic flow (EOF) causes the bulk solution to migrate in the

direction of the cathode while the anionic chiral selectors (SBCD) migrate towards the anode (away from the detection window), giving rise to countercurrent interactions between the chiral selector and the analytes. As noted earlier, the optimum pH found in the normal polarity mode was 8.0. This is because higher pH conditions are required for a strong EOF. At this pH, the analytes will migrate and elute as neutral molecules. Conversely, the optimum pH found when using the reverse polarity mode was 2.6. In this mode, the EOF must be strongly suppressed, and the electrophoretic movement of anionic chiral selectors will be towards the anode (towards the detection window). For these reasons, separations done in the normal polarity mode were generally faster, but the resolution was usually lower. This effect can be seen in Figure 3.6, were the separation of compound 1 was performed with both reverse polarity (Fig 14A) and normal polarity (Fig 14B). Compound 1 was baseline separated under both conditions, but the analysis time is shorter for the separation done in the normal polarity mode. However, it should be noted that for this analyte, the efficiency and resolution was far greater for the separation performed in the reverse polarity mode.

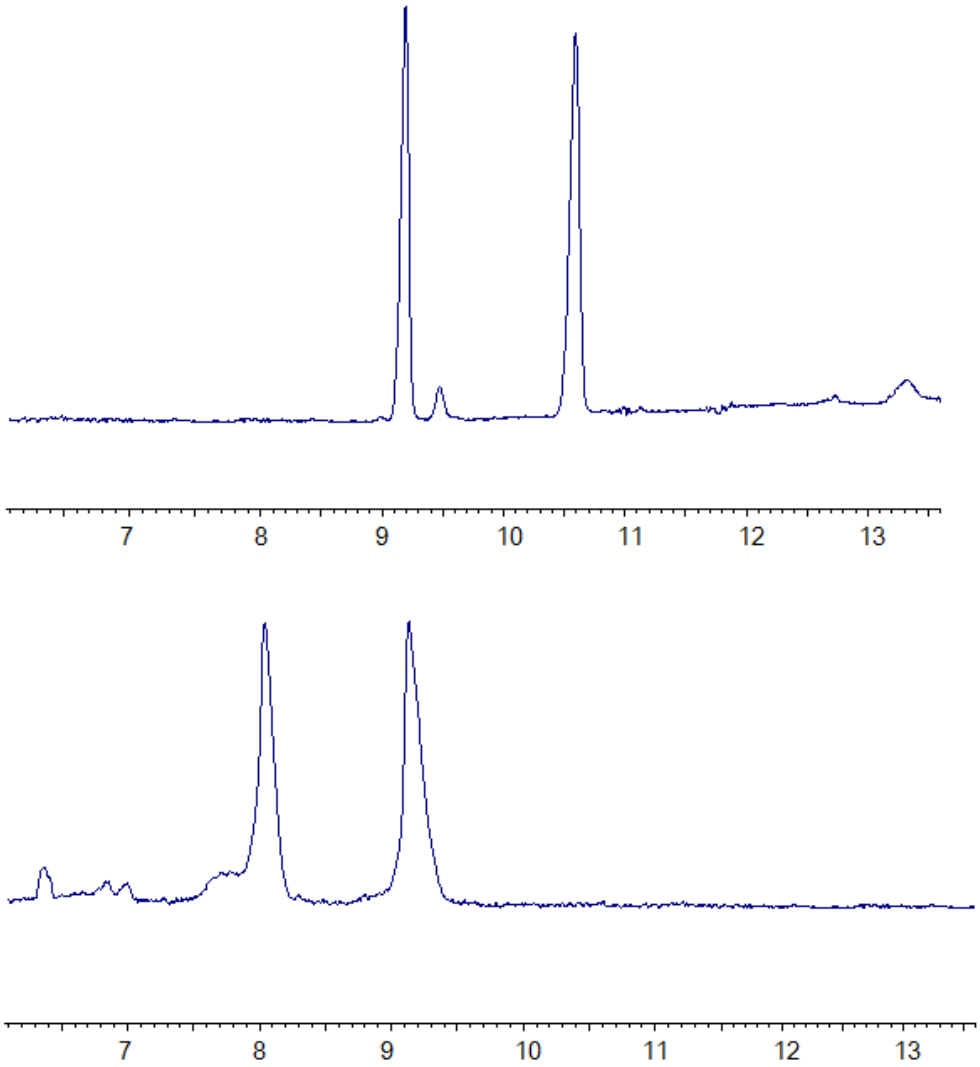


Figure 3.6 A comparison of the separation of compound #1 by CE in the reverse polarity mode (A) and in the normal polarity mode (B). Conditions were 50mM phosphate buffer (pH=2.6(A) and 8.0(B)) and 30mM SBCD. For other conditions, see Experimental section. See Figure 3.1 for analyte structure.

There was one exception to the pH rules outlined above. Compound 15 was separated in the normal polarity mode, yet the optimum pH of the buffer was 3.6. This is the only analyte listed in Table 1 that was separated with a neutral chiral selector (DMBCD). When compound 15 was run using SBCD and a basic buffer, the analyte was not eluted. It can be concluded that the attraction between SBCD and compound 15 was too strong, thus it was not a suitable chiral selector. It is probably not coincidental that compound 15 would not elute during HPLC

analyses in reverse phase conditions. Fortunately, the use of DMBCD and acidic pH values allowed for the enantiomeric separation of this analyte. When the pH was low enough to ionize the analyte, the electrophoretic mobility of the positively charged compound 15 was able to overcome the strength of the binding to the slowly moving chiral selector and reach the detector in a reasonable time.

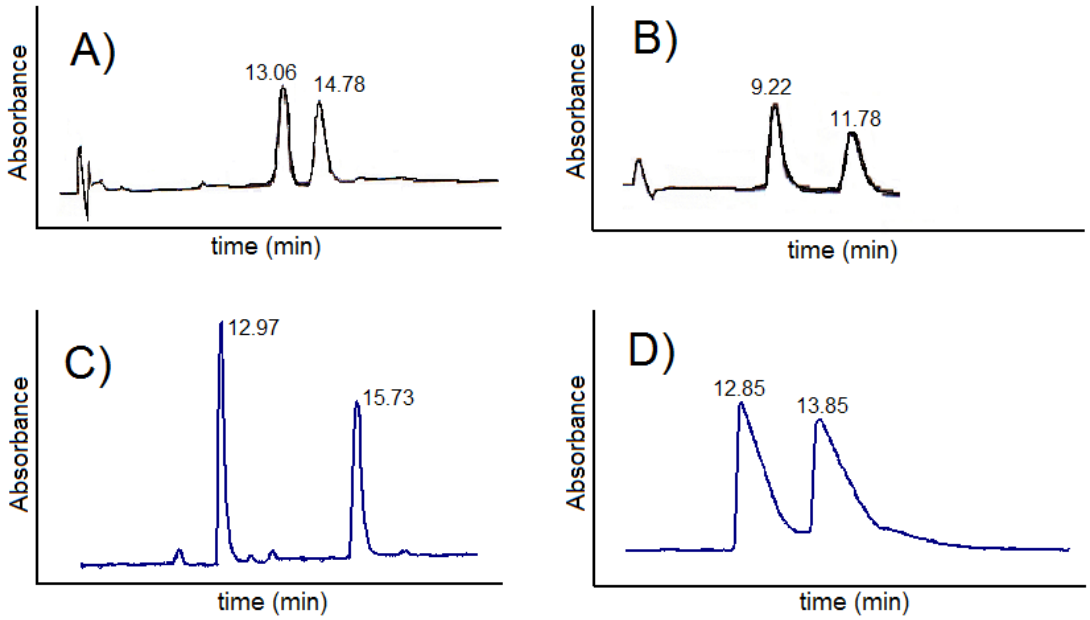


Figure 3.7 Enantioselective HPLC (A and B) and CE (C and D) analyses of compounds 2 (A and C) and 6 (B and D). The chiral selectors were DMP and SBCD for HPLC and CE, respectively. The HPLC mobile phases were 80/20 (A) and 95/5 (B); 20mMH4NOAc (pH=4.1)/ACN. The CE experiments were done in normal polarity using 50mM phosphate buffer (pH=8.0) with 50mM and 60mM SBCD (C and D, respectively). See Experimental section for other details. See Figure 3.1 for analyte structures.

3.4.4 Comparison between HPLC and CE Enantiomeric Separations:

It is interesting to make a brief comparison of the results obtained using HPLC to those using CE for the enantiomeric separation of these compounds. First, it should be understood that the main importance of this work is the ability to possibly purify one of these precursors for use in the production of enantiomerically pure calcaridine A. From this viewpoint, the HPLC results are more valuable. However, the separations made by CE are not without merit. For example, rapid separations of these compounds may be useful in following the stereochemistry

as it is introduced and/or controlled throughout biosynthetic pathways. For such a study, the potentiality for preparative separations is not necessary. Also, rapid enantiomeric excess measurements may be desirable if these analytes are the result of asymmetric syntheses.

Using HPLC, 14 of the 15 compounds were separated, whereas using CE, only 8 were separated. Though HPLC appears to be more broadly applicable to separating these compounds, it should be noted that when a separation was obtained by both separation techniques, the CE method was usually produced greater resolution. This is a common observation in the general comparison between HPLC and CE analyses, and this study was no exception. For example, the greatest R_s found using HPLC was 2.0, while the best CE separation had a R_s of 7.0. Figure 7 compares some separations obtained using HPLC (Fig 7A and 7B) and CE (Fig7C and 7D). The comparison made in the separation of compound 2 by HPLC (Fig 7A) and by CE (Fig 7C) shows the typical advantage of using CE. However, there were some cases were the HPLC separation was superior. One such example is shown in the separation of compound 6 by HPLC (Fig 7B) and by CE (Fig 7D). These observations indicate that both techniques have distinct advantages for this set of analytes. Also, it is important to note that the one compound that was not separated by HPLC was separated by CE (compound 15). Furthermore, by using both techniques, the entire set of analytes was separated.

3.5 Conclusions

In this study, fifteen chiral heterocycles were subjected to enantioselective HPLC and CE analyses using β-cyclodextrin and its derivatives. These compounds are important precursors in the synthesis of calcaridine A. The combination of HPLC and CE allowed for the separation of all 15 analytes. HPLC seems to be more broadly applicable for their separation, while the CE separations obtained were generally of high resolution. It was determined that in HPLC, reverse phase separations using the Cyclobond RSP and DMP phases resulted in the greatest number of separations. Throughout the CE analyses, SBCD proved to be the dominant chiral selector and the normal polarity mode produced more separations. In

summary, using cyclodextrin based chiral selectors via HPLC and CE is a viable means for separating this important set of analytes. Furthermore, this work will likely prove useful in future studies in the production of calcaridine A, as well as, in the study other related natural products.

3.6 Acknowledgements

We gratefully acknowledge the National Institute of Health (NIH 5 R01-GM53825-13) and NIH GM065503) and the Welch foundation (Welch Y-1362) for their financial support of this work.

CHAPTER 4

GENERAL CONCLUSIONS

In chapter 2, two newly developed UV transparent phosphonium-based cationic reagents were evaluated as additives to the background electrolyte of CE for the separation of multiply charged anions, including several complex anions. The dicationic reagent effectively separated eight divalent anions within 17 min and the tetracationic reagent best separated nine trivalent anions, as well as a mixture of all the anions. It is found that phosphate buffer with addition of dicationic reagent favors the separation of divalent anions, while tris buffer with addition of tetracatonic reagent worked better for the trivalent anions the and the mixture.

Chapter 3 examined the enantiomeric separation of a series of fifteen racemic 4,5-disubstituted imidazole compounds. Separations using high performance liquid chromatography (HPLC) were compared to capillary electrophoresis (CE). In HPLC system, 14 of the 15 racemic compounds were separated, while eight of the analytes were separated using CE with resolutions up to 7.0. Using both HPLC and CE approaches, the entire set of analytes was separated.

APPENDIX A

CREDITS TO AUTHORS ON THESIS CHAPTERS

Chapter 1. Introduction

Author: Qing Feng

Chapter 2. Separation of multiple charged anions by Capillary Electrophoresis using alkyl phosphonium pairing agents

Authors: Qing Feng, Eranda Wanigasekara, Zachary S. Breitbach, Daniel W. Armstrong

Publication: A manuscript has been submitted to the Electrophoresis

Chapter 3. The Enantiomeric Separation of 4, 5-disubstituted Imidazoles by HPLC and CE Using Cyclodextrin-Based Chiral Selectors

Authors: Zachary S. Breitbach, Qing Feng, Panduka B. Koswatta, Edra Dodbiba, Carl J. Lovely, and Daniel W. Armstrong

Publication: A manuscript published in *Supramolecular Chemistry*, 2010, (22), 1029-0478

Chapter 4. General Conclusions

Author: Qing Feng

REFERENCES

1 L. Michaelis, *Biochem. Z.*, 1909, 17, 231-234.

2 A. Tiselius, *Trans. Faraday Soc.,* 1937, **33**, 524-531.

3 S. Hjerten, *Chromatogr Rev,* 1967, **9**, 122-219.

4 R. Virtanen, *Acta Polytech. Scand. , Chem. Incl. Metall. Ser.,* 1974, **123**, 67.

5 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.,* 1981, **53**, 1298-1302.

6 W. G. Kuhr and C. A. Monnig, *Anal. Chem.,* 1992, **64**, 389R-407R.

7 C. A. Monnig and R. T. Kennedy, *Anal Chem,* 1994, **66**, 280R-314R.

8 J. F. van Staden and S. I. Tlowana, *Fresenius' J. Anal. Chem.,* 2001, **371**, 396-399.

9 K. Grudpan, J. Jakmunee and P. Sooksamiti, *Talanta,* 1999, **49**, 215-223.

10 W. Ahrer and W. Buchberger, *J. Chromatogr. , A,* 1999, **854**, 275-287.

11 V. V. Salov, J. Yoshinaga, Y. Shibata and M. Morita, *Anal. Chem.,* 1992, **64**, 2425-2428.

12 A. Dudoit and S. A. Pergantis, *J. Anal. At. Spectrom.,* 2001, **16**, 575-580.

13 Z. Guo, Q. Cai, C. Yu and Z. Yang, *J. Anal. At. Spectrom.,* 2003, **18**, 1396-1399.

14 H. El Aribi, Y. J. C. Le Blanc, S. Antonsen and T. Sakuma, *Anal. Chim. Acta,* 2006, **567**, 39-47.

15 E. M. Rachlin, 1983.

16 R. Virtanen, *Ion-Sel. Electrodes, Conf.,* 1978, , 589-595.

17 Z. Yang, K. Zhang, F. Gong, S. Li, J. Chen, J. S. Ma, L. N. Sobenina, A. I. Mikhaleva, B. A. Trofimov and G. Yang, *J. Photochem. Photobiol. , A,* 2011, **217**, 29-34.

18 M. Fotsing, B. Barbeau and M. Prevost, *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.,* 2011, **46**, 420-425.

19 *Pat.,* WO; 2011081939, 0707; Patent Application Date: 20101214.; Priority Application Date: 20091214.

20 X. Lv, J. Liu, Y. Liu, Y. Zhao, M. Chen, P. Wang and W. Guo, *Sens. Actuators, B,* 2011, **158**, 405-410.

21 P. R. Haddad, *J. Chromatogr. , A,* 1997, **770**, 281-290.

22 X. Huang, J. A. Luckey, M. J. Gordon and R. N. Zare, *Anal. Chem.,* 1989, **61**, 766-770.

23 D. Kaniansky, M. Masar, J. Marak and R. Bodor, *J. Chromatogr. , A,* 1999, **834**, 133-178.

24 A. R. Timerbaev, *Electrophoresis,* 2002, **23**, 3884-3906.

25 A. R. Timerbaev, *Electrophoresis,* 2004, **25**, 4008-4031.

26 A. Padarauskas, *Anal. Bioanal. Chem.,* 2006, **384**, 132-144.

27 J. Tomaszewski and M. M. Rumore, *Drug Dev. Ind. Pharm.,* 1994, **20**, 119-139.

28 G. Alves, A. Fortuna and A. Falcao, *Trends Chromatogr.,* 2008, **4**, 1-10.

29 P. D. Grossman, J. C. Colburn and Editors, 1992, , 352.

30 S. Fanali, *J. Chromatogr. , A,* 2000, **875**, 89-122.

31 R. Vespalec and P. Bocek, *Electrophoresis,* 1999, **20**, 2579-2591.

32 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.,* 1984, **22**, 411-415.

33 B. Chu, B. Guo, H. Zuo, Z. Wang and J. Lin, *J. Pharm. Biomed. Anal.,* 2008, **46**, 854-859.

34 T. J. Ward, *Anal. Chem.,* 2006, **78**, 3947-3956.

35 C. E. Evans and A. M. Stalcup, *Chirality,* 2003, **15**, 709-723.

36 W. W. Buchberger, *J. Chromatogr. , A,* 2000, **884**, 3-22.

37 T. Kishi, J. Nakamura and H. Arai, *Electrophoresis,* 1998, **19**, 3-5.

38 T. Krizek, Z. S. Breitbach, D. W. Armstrong, E. Tesarova and P. Coufal, *Electrophoresis,* 2009, **30**, 3955-3963.

39 X. Lin, A. R. Gerardi, Z. S. Breitbach, D. W. Armstrong and C. L. Colyer, *Electrophoresis,* 2009, **30**, 3918-3925.

40 J. Hernandez-Borges, T. Borges-Miquel, G. Gonzalez-Hernandez and M. A. Rodriguez-Delgado, *Chromatographia,* 2005, **62**, 271-276.

41 S. A. Steiner, D. M. Watson and J. S. Fritz, *J. Chromatogr. , A,* 2005, **1085**, 170-175.

42 H. Mo, L. Zhu and W. Xu, *J. Sep. Sci.,* 2008, **31**, 2470-2475.

43 T. Krizek, Z. S. Breitbach, D. W. Armstrong, E. Tesarova and P. Coufal, *Electrophoresis,* 2009, **30**, 3955-3963.

44 W. R. Jones, *J. Chromatogr.,* 1993, **640**, 387-395.

45 P. E. Jackson and P. R. Haddad, *Trends Anal. Chem.,* 1993, **12**, 231-238.

46 J. E. Melanson, N. E. Baryla and C. A. Lucy, *TrAC, Trends Anal. Chem.,* 2001, **20**, 365-374.

47 T. Kaneta, S. Tanaka, M. Taga and H. Yoshida, *Anal. Chem.,* 1992, **64**, 798-801.

48 C. Bjergegaard, P. Moller and H. Sorensen, *J. Chromatogr. , A,* 1995, **717**, 409-414.

49 A. H. Harakuwe and P. R. Haddad, *J. Chromatogr. , A,* 1996, **734**, 416-421.

50 X. Huang, J. A. Luckey, M. J. Gordon and R. N. Zare, *Anal. Chem.,* 1989, **61**, 766-770.

51 P. R. Haddad, A. H. Harakuwe and W. Buchberger, *J. Chromatogr. , A,* 1995, **706**, 571-578.

52 N. E. Baryla and C. A. Lucy, *J. Chromatogr. , A,* 2002, **956**, 271-277.

53 C. Johns, W. Yang, M. Macka and P. R. Haddad, *J. Chromatogr. , A,* 2004, **1050**, 217-222.

54 J. S. Fritz, M. C. Breadmore, E. F. Hilder and P. R. Haddad, *J. Chromatogr. , A,* 2002, **942**, 11-32.

55 J. Li, W. Ding and J. S. Fritz, *J. Chromatogr. , A,* 2000, **879**, 245-257.

56 J. S. Fritz and S. A. Steiner, *J Chromatogr A,* 2001, **934**, 87-93.

57 T. Yokoyama, M. Macka and P. R. Haddad, *Anal. Chim. Acta,* 2001, **442**, 221-230.

58 T. Yokoyama, M. Macka and P. R. Haddad, *Fresenius J Anal Chem,* 2001, **371**, 502-506.

59 Z. S. Breitbach, M. M. Warnke, E. Wanigasekara, X. Zhang and D. W. Armstrong, *Anal. Chem. (Washington, DC, U. S.),* 2008, **80**, 8828-8834.

60 R. Marcilla, M. Sanchez-Paniagua, B. Lopez-Ruiz, E. Lopez-Cabarcos, E. Ochoteco, H. Grande and D. Mecerreyes, *J. Polym. Sci. , Part A: Polym. Chem.,* 2006, **44**, 3958-3965.

61 M. J. Muldoon and C. M. Gordon, *J. Polym. Sci. , Part A: Polym. Chem.,* 2004, **42**, 3865-3869.

62 S. A. Shamsi and N. D. Danielson, *J. Sep. Sci.,* 2007, **30**, 1729-1750.

63 M. Lopez-Pastor, B. M. Simonet, B. Lendl and M. Valcarcel, *Electrophoresis,* 2008, **29**, 94-107.

64 A. Berthod, M. J. Ruiz-Angel and S. Carda-Broch, *J Chromatogr A,* 2008, **1184**, 6-18.

65 J. Li, H. Han, Q. Wang, X. Liu and S. Jiang, *Anal. Chim. Acta,* 2010, **674**, 243-248.

66 T. Jiang, Y. Gu, B. Liang, J. Li, Y. Shi and Q. Ou, *Anal. Chim. Acta,* 2003, **479**, 249-254.

67 X. Wu, W. Wei, Q. Su, L. Xu and G. Chen, *Electrophoresis,* 2008, **29**, 2356-2362.

68 A. M. Stalcup and B. Cabovska, *J. Liq. Chromatogr. Relat. Technol.,* 2004, **27**, 1443-1459.

69 J. V. Seeley, S. K. Seeley, E. K. Libby, Z. S. Breitbach and D. W. Armstrong, *Anal. Bioanal. Chem.,* 2008, **390**, 323-332.

70 Z. S. Breitbach and D. W. Armstrong, *Anal. Bioanal. Chem.,* 2008, **390**, 1605-1617.

71 R. J. Soukup-Hein, J. W. Remsburg, P. K. Dasgupta and D. W. Armstrong, *Anal. Chem. (Washington, DC, U. S.),* 2007, **79**, 7346-7352.

72 X. Zhang, E. Wanigasekara, Z. S. Breitbach, E. Dodbiba and D. W. Armstrong, *Rapid Commun. Mass Spectrom.,* 2010, **24**, 1113-1123.

73 Z. S. Breitbach, M. M. Warnke, E. Wanigasekara, X. Zhang and D. W. Armstrong, *Anal. Chem. (Washington, DC, U. S.),* 2008, **80**, 8828-8834.

74 J. W. Remsburg, R. J. Soukup-Hein, J. A. Crank, Z. S. Breitbach, T. Payagala and D. W. Armstrong, *J. Am. Soc. Mass Spectrom.,* 2008, **19**, 261-269.

75 S. N. Kalyakin, V. V. Sursyakova, G. V. Burmakina and A. I. Rubailo, *J. Anal. Chem.,* 2009, **64**, 398-403.

76 V. V. Sursyakova, S. N. Kalyakin, G. V. Burmakina and A. I. Rubaylo, *Electrophoresis,* 2011, **32**, 210-217.

77 K. S. Rowe and K. J. Rowe, *J Pediatr,* 1994, **125**, 691-698.

78 P. D. Grossman, T. Hino and D. S. Soane, *J. Chromatogr.,* 1992, **608**, 79-83.

79 S. Fanali, *J Chromatogr A,* 2000, **875**, 89-122.

80 K. Kumar and H. Waldmann, *Angew. Chem. , Int. Ed.,* 2009, **48**, 3224-3242.

81 D. J. Newman and G. M. Cragg, *J. Nat. Prod.,* 2007, **70**, 461-477.

82 M. A. Koch, A. Schuffenhauer, M. Scheck, S. Wetzel, M. Casaulta, A. Odermatt, P. Ertl and H. Waldmann, *Proc. Natl. Acad. Sci. U. S. A.,* 2005, **102**, 17272-17277.

83 S. Wetzel, A. Schuffenhauer, S. Roggo, P. Ertl and H. Waldmann, *Chimia,* 2007, **61**, 355-360.

84 B. B. Toure and D. G. Hall, *Chem. Rev. (Washington, DC, U. S.),* 2009, **109**, 4439-4486.

85 J. C. Morris and A. J. Phillips, *Nat. Prod. Rep.,* 2009, **26**, 245-265.

86 P. B. Koswatta and C. J. Lovely, *Tetrahedron Lett.,* 2009, **50**, 4998-5000.

87 I. Mancini, G. Guella and A. Defant, *Mini-Rev. Med. Chem.,* 2008, **8**, 1265-1284.

88 J. W. Blunt, B. R. Copp, M. H. G. Munro, P. T. Northcote and M. R. Prinsep, *Nat. Prod. Rep.,* 2004, **21**, 1-49.

89 R. K. Akee, T. R. Carroll, W. Y. Yoshida, P. J. Scheuer, T. J. Stout and J. Clardy, *J. Org. Chem.,* 1990, **55**, 1944-1946.

90 H. Y. He, D. J. Faulkner, A. Y. Lee and J. Clardy, *J. Org. Chem.,* 1992, **57**, 2176-2178.

91 P. Crews, D. P. Clark and K. Tenney, *J. Nat. Prod.,* 2003, **66**, 177-182.

92 I. Mancini, G. Guella, C. Debitus and F. Pietra, *Helv. Chim. Acta,* 1995, **78**, 1178-1184.

93 P. Ralifo and P. Crews, *J. Org. Chem.,* 2004, **69**, 9025-9029.

94 P. B. Koswatta, R. Sivappa, H. V. R. Dias and C. J. Lovely, *Org. Lett.,* 2008, **10**, 5055-5058.

95 M. D. Egger, Y. Liu, J. Sevcik, E. Tesarova, R. Rozhkov, R. C. Larock and D. W. Armstrong, *Electrophoresis,* 2003, **24**, 2650-2656.

96 A. W. Lantz, R. V. Rozhkov, R. C. Larock and D. W. Armstrong, *Electrophoresis,* 2004, **25**, 2727-2734.

97 C. Jiang, D. W. Armstrong, A. W. Lantz, A. Peter and G. Toth, *J. Liq. Chromatogr. Relat. Technol.,* 2007, **30**, 1421-1436.

98 D. W. Armstrong, T. J. Ward, R. D. Armstrong and T. E. Beesley, *Science,* 1986, **232**, 1132-1135.

99 P. Sun, c. Wang, D. W. Armstrong, A. Peter and E. Forro, *J. Liq. Chromatogr. Relat. Technol.,* 2006, **29**, 1847-1860.

100 G. L. Bertrand, J. R. Faulkner Jr., S. M. Han and D. W. Armstrong, *J. Phys. Chem.,* 1989, **93**, 6863-6867.

101 C. Wang, C. Jiang and D. W. Armstrong, *J. Sep. Sci.,* 2008, **31**, 1980-1990.

BIOGRAPHICAL INFORMATION

Qing Feng obtained her Bachelor of Science degree from Wuhan Institute of Technology majoring in Applied Chemistry. She then went to the United States of America and studied analytical chemistry under Dr. Daniel W. Armstrong. She finished her Master of Science in December 2011 working on the research in areas such as enantiomeric separation and achiral separation via Capillary Electrophoresis and High Performance Liquid Chromatography..