2012

Development of innovative stationary phases for chiral and protein separations in open tubular capillary electrochromatography

Leonard Moore, Jr.
Louisiana State University and Agricultural and Mechanical College

DEVELOPMENT OF INNOVATIVE STATIONARY PHASES FOR CHIRAL AND PROTEIN SEPARATIONS IN OPEN TUBULAR CAPILLARY ELECTROCHROMATOGRAPHY

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
Requirements for the degree of
Doctor of Philosophy

In
The Department of Chemistry

By
Leonard Moore Jr.
B.S., Grambling State University, 2004
B.S., Grambling State University, 2004
May, 2012

To my parents, Betty and Leonard Moore Sr., and siblings, Kevin White and Tanya White, for your continuous encouragement over the years.

ACKNOWLEDGEMENTS

The fruition of this work would not have been possible without the assistance and gracious support from the following people.

Dr. Isiah M. Warner, for the guidance, wealth of knowledge, patience, and support you have bestowed upon me. You have helped me to realize my true potential as a scientific researcher. Words cannot express the amount of gratitude that I have for your assistance in the development of my critical thinking.

Dr. Robert L. Cook, Dr. Jayne Garno, Dr. Kermit Murray, and Dr. Shabab Mehraeen, for time and expertise towards the completion of my research work.

Dr. Min Li, and Dr. Bilal El-Zahab, for helpful suggestions and proofing of manuscripts during my graduate school tenure.

Dr. Vivian Fernand, for your helpful suggestions as well as proofing of manuscripts and of this dissertation.

Dr. Lancia Darville and Atiya Jordan, for proofing of this dissertation and your friendship.

Dr. Danny Hubbard, for your support through my transition of becoming an independent scientist.

Dr. Arther Gates, for your support and friendship over the years.

The Warner Research Group, for providing challenging questions to continuously sharpen my scientific mind. I couldn't be more grateful for your support and friendships formed.

Funding Agencies: National Institutes of Health, National Science Foundation, Philip W. West Endowment, Graduate Alliance for Education in Louisiana, and Louis Stokes-Alliances for Minority Participation Bridge to Doctorate Fellowship

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Name
AFM	atomic force microscopy
amu	atomic mass unit
BGE	background electrolyte
[BMIM][OAc]	1-butyl-3-methylimidazolium acetate
Boc	butoxycarbonyl
BSA	bovine serum albumin
CA	cellulose acetate
CAB	cellulose acetate butyrate
CAP	cellulose acetate phthalate
CE	capillary electrophoresis
CEC	capillary electrochromatography
CGE	capillary gel electrophoresis
CIEF	capillary isoelectric focusing
CITP	capillary isotachophoresis
CMC	critical micelle concentration
CTAB	hexadecyltrimethylammonium bromide
CZE	capillary zone electrophoresis
DCC	N, N'-dicyclohexylcarbodiimde
DCM	dichloromethane
EOF	electroosmotic flow
ε-Boc-SUK	epsilon- butoxycarbonyl-sodium-undecenoyl lysinate

EtOAc	ethyl acetate
GC	gas chromatography
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
IgG	Immunoglobulin G
IL	ionic liquid
m-CEC	monolithic capillary electrochromatography
MEKC	micellar electrokinetic chromatography
NaCl	sodium chloride
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance
OT-CEC	open tubular capillary electrochromatography
OTS	octadecyltrichlorosilane
PC-CEC	packed column capillary electrochromatography
PEM	polyelectrolyte multilayer
pI	isoelectric point
poly- ε-SUK	poly-ε-sodium-undecanoyl lysinate
RSD	relative standard deviation
R6G	rhodamine 6G
RTIL	room temperature ionic liquid
SCF	supercritical fluid chromatography
SUK	sodium undecanoyl lysinate
Surfactant	surface active agent
TEM	transmission electron microscope

TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

ABSTRACT

Protein biomarkers in bodily fluids can lead to an indication of the onset of a disease. Due to low abundance of the biomarker at early stages of the disease and other protein interferences, detection may be challenging. Therefore, in order to achieve more selective protein separations, the development of a novel stationary phase has been investigated. Moreover, novel methods to apply polysaccharides as stationary phases were developed for protein separations as well as chiral separations. The initial discussions in this dissertation commences with the use of novel zwitterionic lysine-based molecular micelles as a coating for open tubular capillary electrochromatography (OT-CEC) for protein separations. Poly-ε-sodium-undecanoyl lysinate (poly-ε-SUK) was evaluated as a stationary phase by varying polymer concentration, voltage, and temperature in the separation of six acidic (myoglobulin, albumin, β-lactoglobulin A, β-lactoglobulin B, α-lactalbumin, deoxyribonuclease I) and four basic (α-chymotrypsinogen A, lysozyme, cytochrome *c*, and ribonuclease A) proteins. Additionally, NaCl concentration was assessed for enhancement of the separation due to increased interactions from polymer swelling. Nearly all protein peaks were baseline resolved. In addition, a separation of proteins in a human serum sample was evaluated under optimized acidic and basic conditions. Furthermore, a separation of proteins in a human serum sample was also evaluated under optimized acidic and basic conditions.

This dissertation also addresses chiral separations using polysaccharides derivatives (cellulose acetate (CA), cellulose acetate phthalate (CAP), and cellulose acetate butyrate (CAB)) for OT-CEC. These hydrophobic polymers were each dissolved in a room temperature ionic liquid (RTIL), 1-butyl-3-methylimidazolium acetate and deposited into capillaries. Four chiral analytes (thiopental, sotalol, labetalol, and ephedrine) were evaluated using the coated

capillaries. The system was optimized using pH, polymer concentration, voltage, and temperature studies. Examination of results indicated baseline resolution for thiopental using each polysaccharide, while only partial separation was achieved for sotalol, labetalol, and ephedrine. Lastly, protein separations were evaluated using polysaccharides as coatings for OT-CEC. Basic proteins, mentioned earlier, were investigated using CA, CAP, and CAB. CA had the fastest analysis time, and it was further investigated in this study. Polymer concentration, temperature, and voltage studies were conducted to determine optimized conditions. In addition, proteins were evaluated using CA nanofibers. Nanofibers were coated at various times onto the capillary wall for the separation of basic proteins.

CHAPTER 1
INTRODUCTION

1.1 Protein Biomarkers

Identification of proteins, such as lysozyme C and immunoglobulin, has garnered interest due to their correlation to diseases, e.g. for determining benign prostate disease versus malignant prostate disease.[1-3] Proteins are large macromolecules that consist of a tertiary, secondary, and primary structures. In addition, proteins are composed of amino acids which are covalently bonded by amide bonds $–CONH_2–$ formed by the $–COO^-$ carboxyl group of one molecule and the $–NH_2$ amine group of the other amino acid. Proteins are considered to be acidic or basic proteins depending on the isoelectric point (pI). The pI is the pH value at which a molecule is neutral, i.e. containing equal positive and negative charges, in aqueous solution. This value is an average of the pKa values of acidic and basic groups within a molecule. Thus, if the pI value is below pH 7 the protein is considered to be acidic while above pH 7 it is considered basic. Moreover, if the pH value of a solution containing a protein is above the pI the protein has an overall negative charge. If the pH value of the solution is lower than the pI, the protein has an overall positive charge. A number of protein biomarkers have been sought for analysis from biological fluids such as human serum, urine, and saliva.[4] Low detection limits could aid in early detection of diseases and therefore prevent disease progression.

1.2 Chirality

Separation of chiral molecules has become more valued due to events such as the infamous thalidomide tragedy. This tragedy occurred because the racemic drug, thalidomide, was given to expecting mothers to treat morning sickness. The unforeseen side effect of one enantiomer caused severe birth defects for fetuses or even death. As a result, the Food and Drug Administration set guidelines for chiral drugs which has led to drugs being sold in single

enantiomer purity yielding more of the therapeutic effects.[5] Chirality refers to a molecule comprised of enantiomers which are non-superimposable mirror images of each other as shown in Figure 1.1. A chiral molecule can contain one or more chiral tetrahedral carbon atoms with four different groups bonded to a carbon for induction of chirality. A molecule can also be chiral by having an asymmetric plane or axis of symmetry. Enantiomers can be L- or D- stereoisomers. D- stands for dextrorotary while L- for levorotary. This notation is due to the order of priority of the groups bonded to the chiral carbon. The order of importance for the groups bonded is –H being the lowest, with -CR following next, and –X being the highest priority.

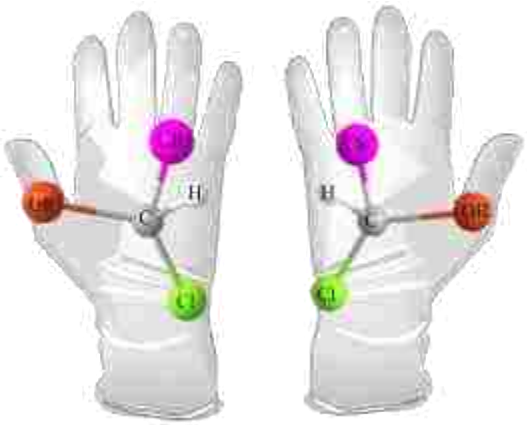


Figure 1.1 Example of chirality in two enantiomers

1.3 Chiral Discriminators

Chiral separations occur by chiral analyte interaction with a chiral selector. Numerous molecules have been used as chiral selectors including crown ethers,[6, 7] polysaccharides,[8, 9] cyclodextrins,[10, 11] and polymers.[12, 13] The mechanism of chiral separation by Easson and Stedman suggests one enantiomer has three interactions or more with a chiral selector while the other enantiomer has only one or two interactions with the molecules as demonstrated in Figure 1.2.[14] These interactions can be dipole-dipole, steric, or hydrogen bonding.[15] Factors such as buffer concentration, pH, voltage and temperature play a key role in the discrimination of chiral

molecules. For chromatographic separations, it is well established that the enantiomer with the greatest interactions will elute after the enantiomer with fewer interactions.

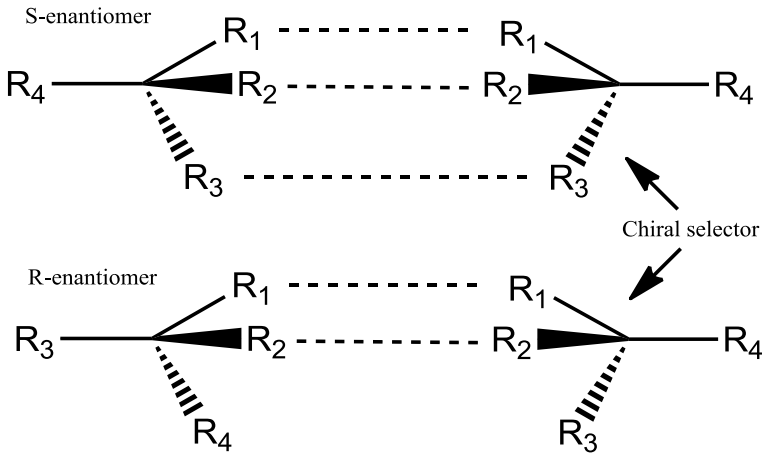


Figure 1.2 Demonstration of the three-point rule for chiral discrimination

1.4 Room Temperature Ionic Liquids

1.4.1 Structure and Properties

Ionic liquids (ILs) are molten salts with melting points below 100°C.[16] These molecules have lower melting points, compared to other salts, due to asymmetric packing of their anions and cations. These anions and cations can have varying degrees of hydrophobicity. Figure 1.3 shows typical decreasing relative hydrophobicity with common IL anions and cations. Room temperature ionic liquids (RTILs) are molten salts with melting points below 25°C. The first RTIL reported was ethylammonium nitrate, which has a melting point of 12°C discovered by Paul Walden in 1914.[17] Ionic liquids as well as room temperature ionic liquids also have interesting solubilizing properties.[18]

1.4.2 Applications

These low melting salts have use for many applications such as renewable solvents, stationary phases, or as transport media for electrons.[16, 19, 20] These materials have a wide range

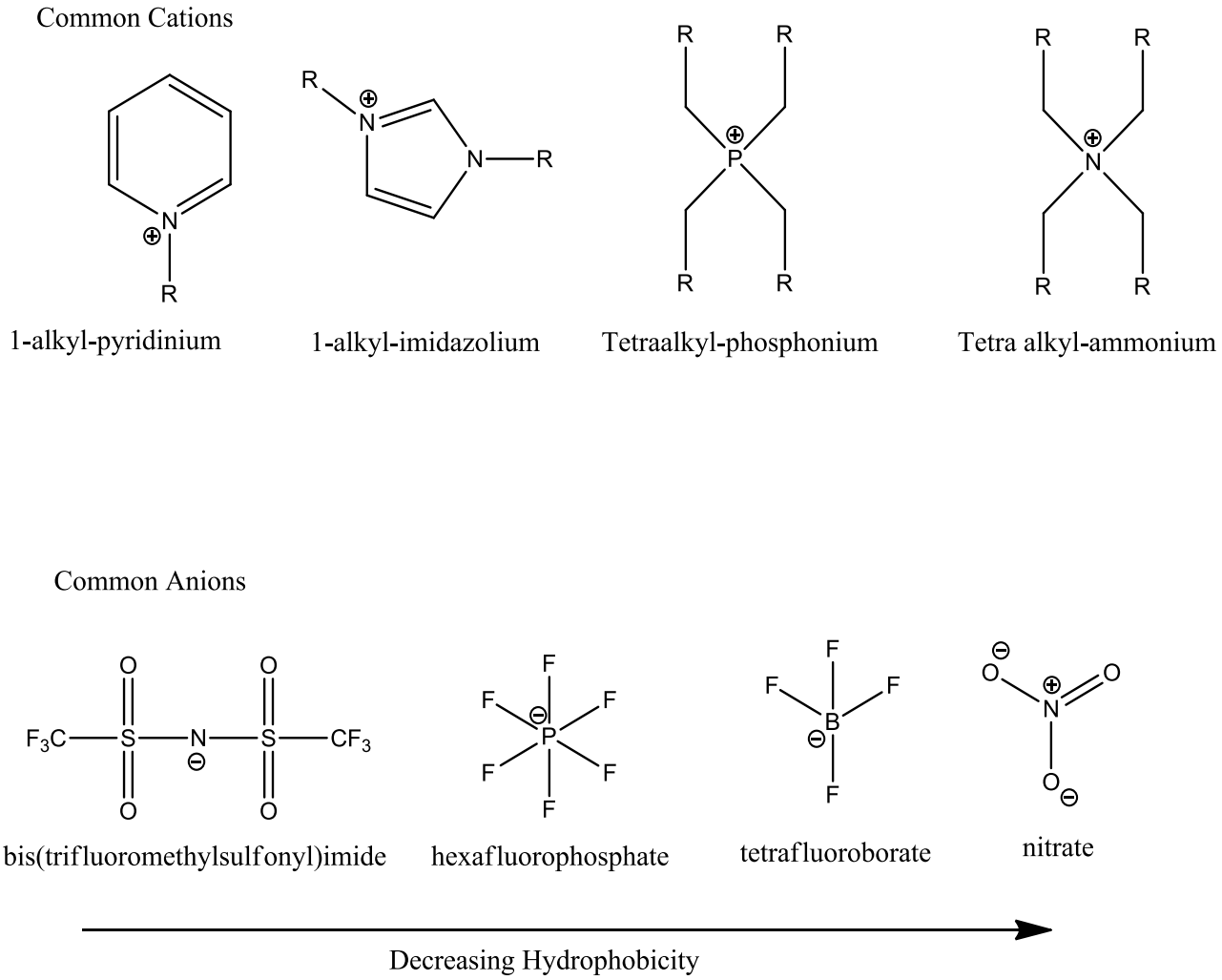


Figure 1.3 Molecular structures of common cations or anions used to form ILs

of applications due to the numerous compounds that can be formed by changing the cation or the anion to yield a new compound. It is estimated that RTILs can have up to 10^9 different combinations.[21] Thus, with the enormous library of ionic liquids to choose from, finding a RTIL for dissolution is as simple as a cation or anion exchange. Most chiral stationary phases have limited solubility in most organic and aqueous solvents. However, unique dissolution properties, as mentioned earlier, with RTILs could prove very useful. This dissertation reports the use of RTIL for the fabrication of a chiral stationary phase used for chiral separations.

1.5 Surfactants and Micelles

Surfactants are molecules which typically contain a polar head group (e.g. amine, sulfate, amino acid) and a nonpolar hydrocarbon tail (e.g. octyl, undecyl) as observed in Figure 1.4. In Figure 1.4 the red circle represents the polar head of the surfactant and the green line is the nonpolar tail. The term surfactant is an acronym for **surf**ace **act**ive **a**ge**nt**. This name is given due to the ability of the molecules to change the surface tension of a solution with increasing concentration of surfactant. Surfactants are known as organized media due to their ability to form higher ordered structures at a given concentration (i.e. critical micelle concentration, CMC). When adding these surfactants to a polar solvent before the CMC is reached, these molecules aggregate on the surface with the polar head group being solvated and the nonpolar tail extending outward and positioning itself away from the solvent. Once the CMC is reached, micellar formation is more thermodynamically favored versus having the surface of the solvent overcrowded with surfactant molecules. One type of structure these molecules can form is a micelle. These amphiphiles have been employed for applications such as detergents for cleaning,[22] fabric softeners,[23] or templates for nanoparticle synthesis.[24] Micelles have been explored for drug delivery systems due to their ability to solubilize hydrophobic drug molecules within its core and remain water soluble as a whole.[25]

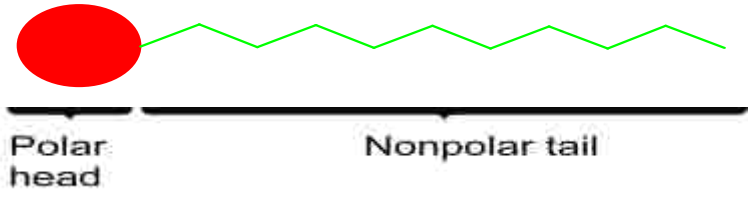


Figure 1.4 Typical surfactant notation

Molecular micelles are surfactant molecules that have been polymerized together via reacting terminal double bonds on the hydrophobic tails as a linkage. A conventional micelle compared to a molecular micelle in Figure 1.5. These molecules have an advantage over conventional micelles by eliminating the dynamic equilibrium between surfactant and micelle.

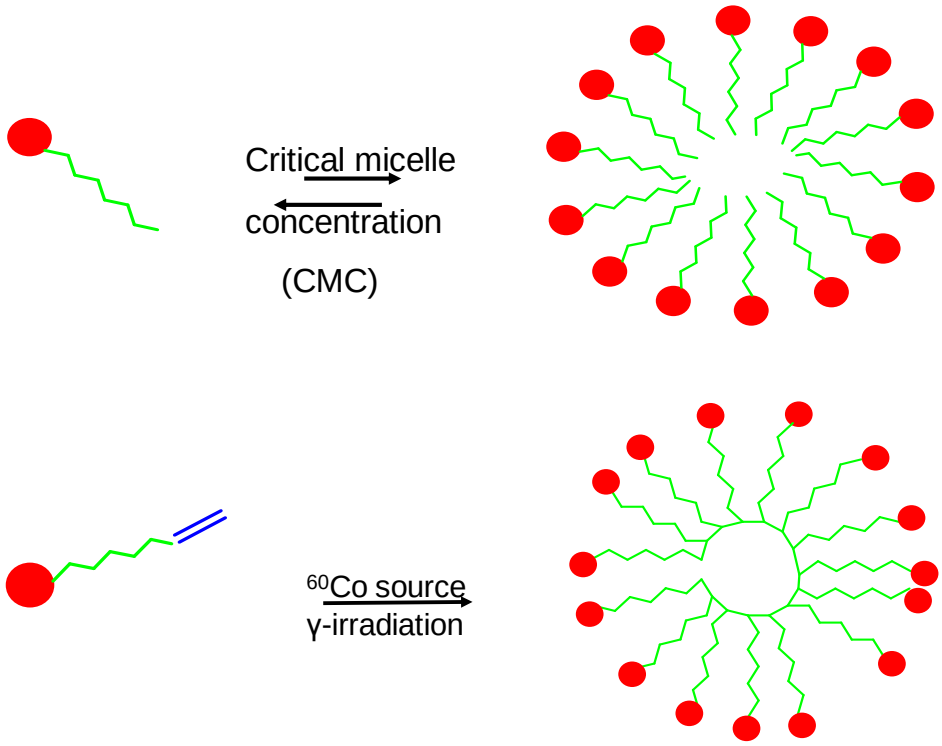


Figure 1.5 Conventional micelles versus molecular micelles

1.6 Capillary Electrophoresis

Capillary electrophoresis (CE) is an important analytical separations technique used to separate a wide range of analytes including pharmacedical drugs,[26, 27] amino acids,[28, 29] proteins,[30, 31] and DNA.[32, 33] CE has advantages of smaller sample consumption, rapid analysis times, better analyte peak shape, as well as higher efficiencies.[34] A diagram of a typical CE instrument is displayed in Figure 1.6. The instrumentation for CE is composed of a fused-silica capillary in which the ends are placed into buffer vials to aid in the generation of an electric

field. A high voltage power supply along with the cathode and anode are used to apply a voltage across the capillary. A sample vial is used for the storage and injection of the sample onto the capillary. The light source and detector are used for the recognition of analytes as they migrate through the capillary. Lastly, the instrument is interfaced with a computer for recording and processing of data. There are six distinctive modes of CE which include capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), capillary isotachophoresis (CITP), micellar electrokinetic chromatography (MEKC), and capillary electrochromatography (CEC). The most basic and most widely used mode of CE is CZE which will be explained later in this dissertation. CGE involves the use of a gel to separate molecules based on size. CIEF is used to separate proteins based on their pI using a pH gradient. CITP is a technique that separates compounds using fast leading and slow terminating electrolytes with known mobility. MEKC implements micelles as a pseudostationary phase in the background electrolyte (BGE) for analytes to partition with as they all migrate across the capillary. CEC requires a stationary phase to exhibit high selectivity and will be discussed in greater detail in this dissertation.

1.6.1 Capillary Zone Electrophoresis

In CZE, a bare capillary is typically conditioned with a strong base to expose negatively charged silanol groups and then rinsed with water. Next, the capillary is filled with background electrolyte solution. The sample is then injected onto the electrolyte filled capillary by using pressure or voltage. CZE is performed by applying a voltage to that fused-silica capillary, simultaneously inducing electrophoretic migration of ionic species and electroosmotic flow (EOF).[35]

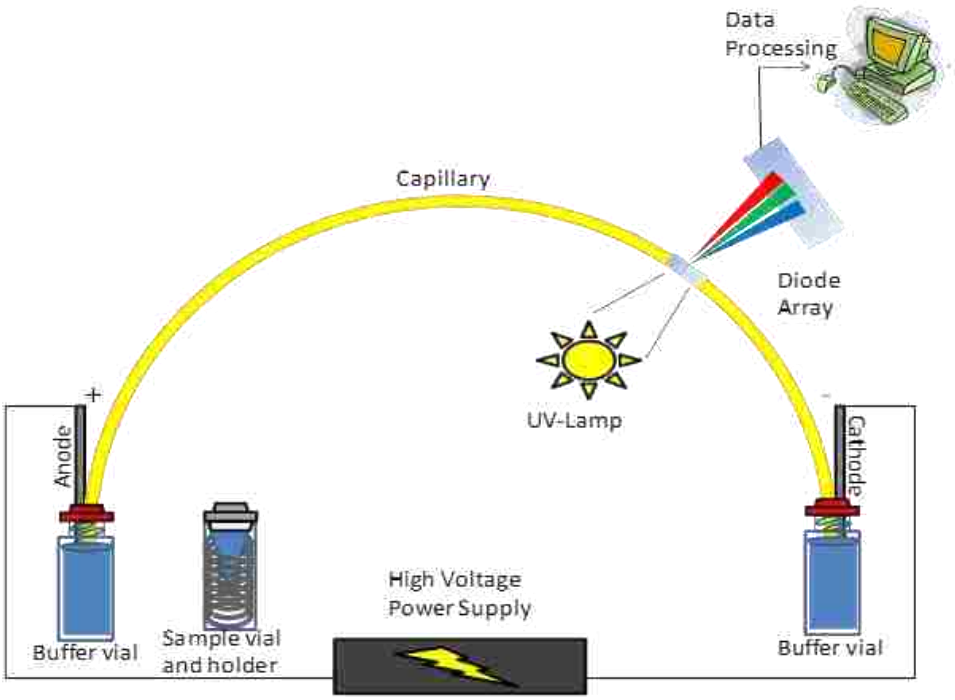


Figure 1.6 Schematic representation of a CE experiment.

The electric force (F_E) of a charged species in a given medium can be calculated by

$$F_E = qE \tag{1.1}$$

where q is the charge of the ion and E is the electric field of the medium. While the frictional force (F_F) for a spherical ion is given by the equation

$$F_F = -6\pi\eta r v \tag{1.2}$$

where η is the viscosity of the medium, r is the radius of the ion, and v is the velocity of the charged species. Replacing v from equation (1.2) with the relationship from the following equation

$$v = \mu_{ep} E \tag{1.3}$$

yields the new equation

$$F_F = -6\pi\eta r \mu_{ep} E \tag{1.4}$$

where μ_{ep} is the electrophoretic mobility of an ionic species. When the magnitude of equations (1.1) and (1.4) are equal but in opposite directions, the electrophoretic mobility of an ionic species can be approximated by combining these equations and solving for μ_{ep} gives the following relationship

$$\mu_{ep} = \frac{q}{6\pi\eta r} \tag{1.5}$$

where the relationship between charge-to-size and electrophoretic mobility is more apparent.

The EOF is the driving force of CZE. This force can allow cationic, neutral, and anionic species to be detected in a given separation. If the magnitude of the EOF, $\mu_{eo,}$ is greater than the electrophoretic mobility of a certain molecule then that molecule will migrate in the direction of the EOF regardless of which electrode molecules are attracted to. EOF is generated by applying a voltage to the buffer solution within a capillary that contains a charged surface. This charged surface is either the silanol groups that have been deprotonated yielding a negatively charged surface or an adsorbed positively charge molecule. In the case of a negatively charge surface, a positive voltage is applied and cations from the BGE solution are attracted to the negatively charged silanol groups to form the stern layer. This layer is followed by both anions and cations from the BGE to form the diffuse layer. The cations within the diffuse layer are then attracted to the cathode. Hence, bulk flow occurs due to the solvation of the cations with water molecules[36] as in Figure 1.7. This mode is called normal mode. The direction of the EOF can be reversed by coating the capillary wall with positively charged species such as amines and applying a negative voltage, i.e. reverse mode. Similarly anions from the BGE solution are attracted to the cationic surface to form the stern layer. The diffuse layer follows after the stern layer with anions being

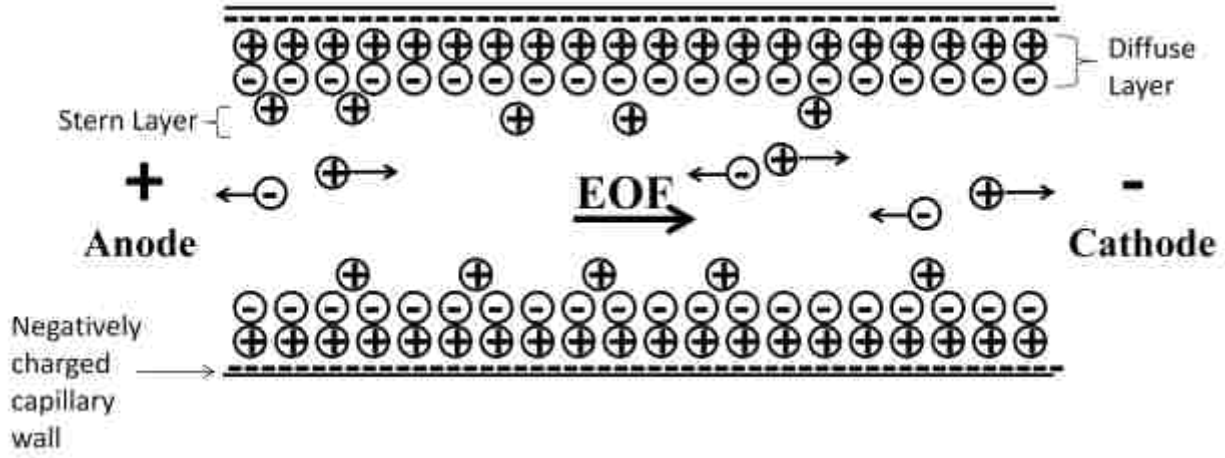


Figure 1.7 EOF generation in normal mode (cathodic EOF).

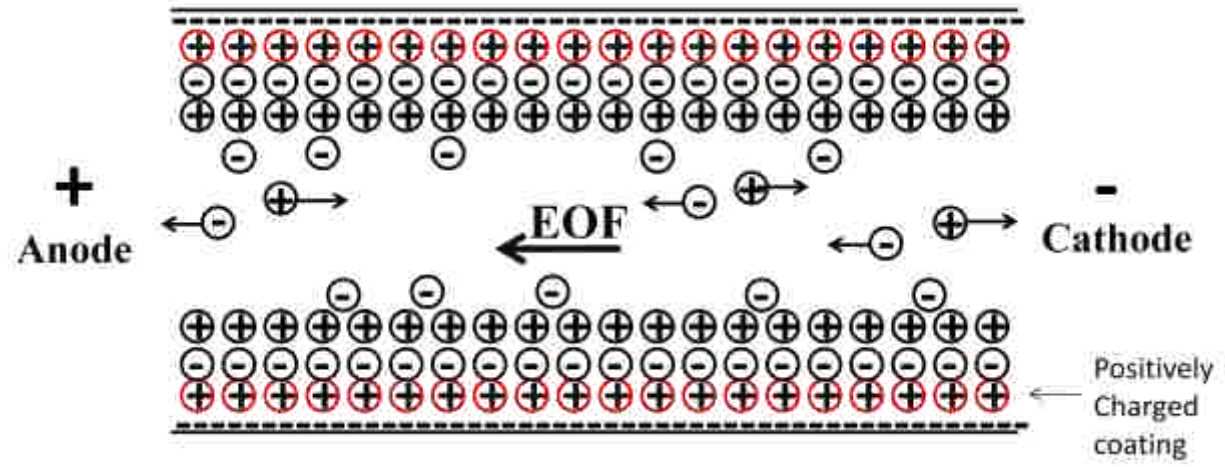


Figure 1.8 EOF generation in reverse mode (anodic EOF).

attracted to the anode. Therefore, bulk flow occurs in the opposite direction as observed in Figure 1.8.[37]

CE separations can be highly efficient due to the flat flow profile of the EOF which contrasts with the parabolic flow profile in high performance liquid chromatography (HPLC) as depicted in Figure 1.9. HPLC uses pressure to generate flow of a mobile phase where the center

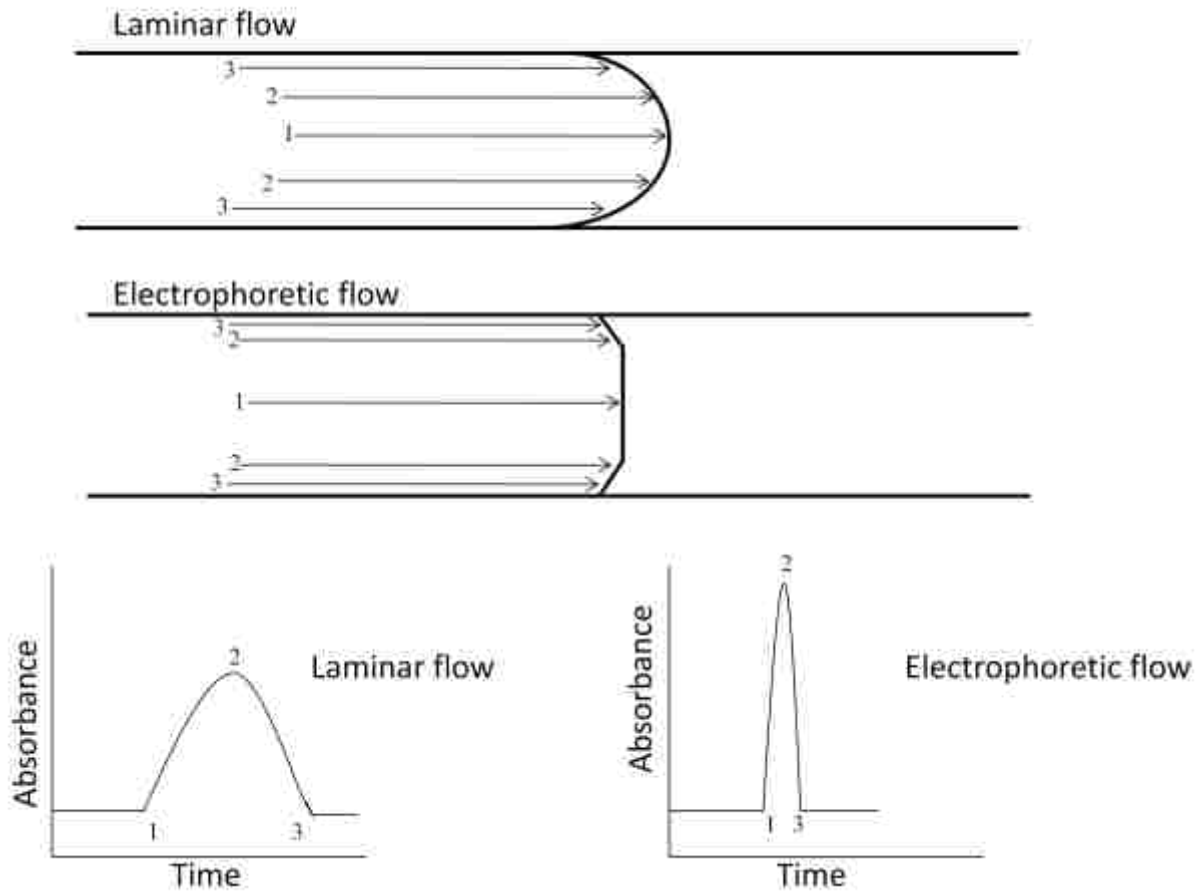


Figure 1.9 Laminar and electrophoretic flow profiles as well as their respective chromatograms

of the parabolic flow would be the fastest movement of the solvent and the solvent against the column would be slower due to friction. This results in broader analyte peaks. In contrast CE generates a flat flow profile based on the electric field being generated throughout the capillary with minor contribution from the capillary wall. Therefore, CE separations are typically highly efficient with very narrow analyte peaks. The magnitude of the EOF (μ_{eo}) is given by

$$\mu_{eo} = \frac{L_d L_t}{V t_m} \tag{1.6}$$

where L_t is the length of the capillary, L_d is the length to the detector, V is the applied electric field, and t_m is the migration time of the neutral species. The velocity (v_i) of a species is a combination of contributions from the EOF and electrophoretic mobility as is given by

$$\nu_i = \mu_{app} E = (\mu_{ep} + \mu_{eo}) \quad (1.7)$$

where μ_{app} is the apparent mobility of species. Analytes migrate to the electrode of opposing charge (i.e. positively charged molecules are attracted to the cathode while negatively charged molecules are attracted to the anode). The size of these molecules has an important role in combination with the amount of charge each molecule has for separations. The ratio of charge-to-size governs the separation mechanism of analytes. Therefore, smaller more charged particles will migrate faster than more bulky less charged molecules as can be observed in Figure 1.10. Neutral molecules have no net charge, and therefore are not attracted to the cathode nor anode; instead these molecules migrate with the EOF.

Protein separation can be accomplished using CZE; however, there are problems associated with using the bare capillary. These large macromolecules have hydrophobic as well as hydrophilic domains along with charged groups which can irreversibly adsorb to the hydrophobic capillary wall. Such problems associated with protein adsorption may include poor reproducibility, broad peaks, peak tailing, and also result in unstable baselines. McCormick separated various proteins using CZE; however, high concentration buffers with low pH were used.[38] Increasing to higher buffer concentration leads to joule heating which could denature proteins and affect the viscosity of the solution.

Chromatography is a widely used separations technique based on resolving analytes using interactions from their partitioning between the mobile and stationary phases. Techniques such as HPLC, Gas chromatography (GC), thin layer chromatography (TLC), and supercritical

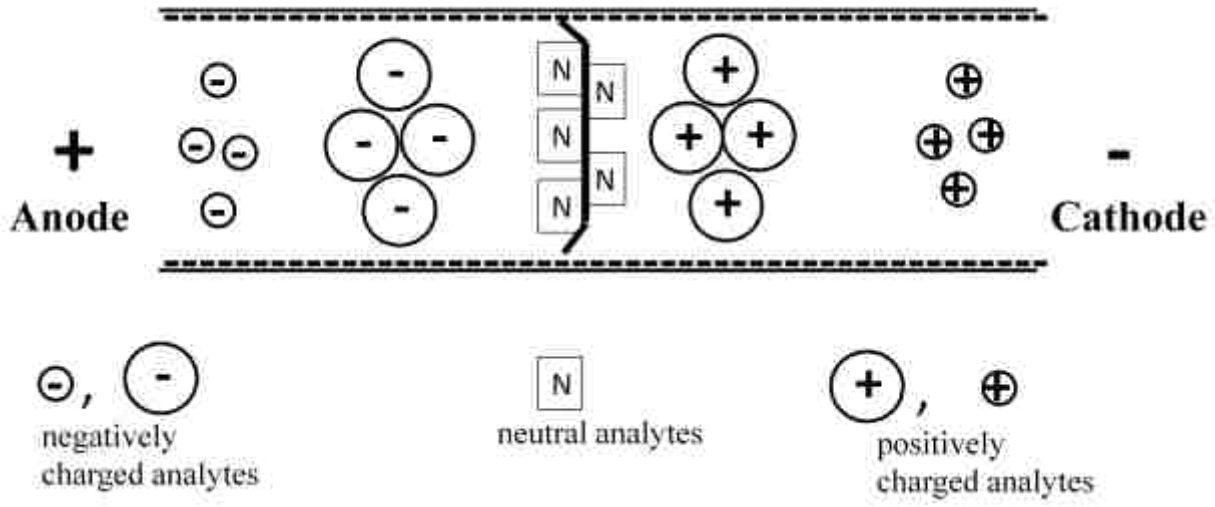


Figure 1.10 Migration order of analytes in CE

fluid chromatography (SCF) have been assessed for chiral separations.[39-41] SCF suffers from high energy usage from heating and cooling of carbon dioxide gas.[42] Gas chromatography (GC) is limited to chiral analytes that are volatile or thermally stable.[43] HPLC is the mostly used separations technique. In HPLC, mobile phases can be as polar as water or as nonpolar as hexane. Stationary phases can be as polar as propylamine or as nonpolar as an octyldecyl solid support.[44, 45] However, based on separation factors such as longer analysis times, large amounts of organic solvent consumption, and large analyte concentration requirements may make this technique less preferred.[46, 47] Chromatography can be quantified by how well one analyte is separated from the other using a calculated value called resolution (R_s). The calculation for resolution can be acquired using the following equation

$$R_s = 2\left[\frac{(t_r)_B - (t_r)_A}{w_B + w_A}\right] \tag{1.8}$$

where $(t_r)_A$ is the retention time for the first peak of interest, $(t_r)_B$ is the retention time for the second peak of interest, w_A is the width of the first peak, and w_B is the width of the second peak.

The peak efficiency (N) or the number of theoretical plates can be calculated from the following relationship below where t_r is the retention time of the peak of interest and $w_{1/2}$ is the half of the width of the peak of interest.

$$N = 5.54\left(\frac{t_r}{w_{1/2}}\right)^2 \tag{1.9}$$

1.6.2 Capillary Electrochromatography

CEC is a hybrid separation technique that employs the separation efficiency of CZE with the selectivity of HPLC. Using CEC allows one to exploit the advantage of high plate numbers without the high back pressures observed in HPLC. Moreover, with the large variety of stationary phases that can be implemented onto capillaries, selectivity can be tuned for a particular analyte. This technique was first established by Pretorius *et al*. in 1974.[48] Later, CEC became more popular with its application demonstrated by Jorgenson and Lukacs.[49] CEC uses weak interactions such as hydrophobic, electrostatic, hydrogen bonding, dipole-dipole, and π-π interactions to separate various compounds. There are three main types of CEC: monolith (m-CEC), packed column capillary electrochromatography (PC-CEC), and open tubular capillary electrochromatography (OT-CEC). These three techniques have their own advantages, as well as disadvantages.

1.6.2.1 Monoliths

Monoliths or continuous beds, are stationary phases generated by chemically bonding molecules to the capillary wall.[50] This type of stationary phase affords the use of frits which are applied on the ends of the capillary to enclose packing material within. These monoliths can be divided into two categories: rigid organic polymer-based or silica-based columns. Skerikova *et al*. synthesized hydrophilic monolithic columns to separate phenolic acids.[51]

Huang *et al*. prepared zwitterionic moieties to a silica monolith in the separation of nucleotides.[52] Monolithic columns contain chemical moieties which have specific interaction with analytes as they migrate across the capillary. Some monoliths developed without charged groups such as butyl methacrylate and ethylene dimethacrylate have led to weak EOF generation in the separation of charged samples as observed by Zhang *et al*.[53] Due to this depletion of the magnitude of the EOF, some monoliths have charged groups incorporated within them to reduce analysis times and aid EOF generation as studied by Karenga *et al*.[54] These columns are very durable; however, can be very rigorous to prepare due to lengthy modification procedures.[55]

1.6.2.2 Packed Capillary Electrochromatography

Silica particles modified with long hydrocarbon chains have typically been explored for PC-CEC.[56] Using 2-5 μm particles packed into a capillary provides larger surface area for analyte interactions.[55] Therefore, separations using PC-CEC can display high theoretical plate numbers. Smith *et al*. prepared 3 μm octyldecylsilane packed columns to separate diastereomers as well as other compounds from related impurities.[57] Wiedmer *et al*. used novel polyethyleneimine-functionalized metal oxide 5 μm particles as packing material to separate benzoic acid derivates.[58] Although PC-CEC have notable advantages, it also has drawbacks that include difficulties with frit preparation, packing particles within small diameter capillaries, short term stability of capillaries, and bubble formation during acquisition periods.[55, 59, 60]

1.6.2.3 Open Tubular Capillary Electrochromatography

In OT-CEC, the stationary phase is coated along the capillary wall for interaction with analytes. Chemical moieties are physically adsorbed onto the capillary wall via weak interactions, e.g. electrostatic and hydrophobic. Columns are prepared by flushing solutions with the coating material through the capillary. OT-CEC technique eliminates the use of frits unlike PC-CEC.[61] Moreover, bubble formation is minimized with OT-CEC. Simple column

preparation and robustness have made OT-CEC a very attractive technique. Coatings may consist of surfactants,[62] polymers,[13, 63] or nanoparticles.[64, 65] Pesek *et al*. prepared anionic as well as zwitterionic surfactant coated capillaries for the analysis of small basic compounds.[66] Yang *et al*. prepared 2-3 nm gold nanoparticle modified columns for the separation of a mixture of polycyclic aromatic hydrocarbons.[67] These coating materials can be composed of positive or negative charges or both as a zwitterionic species. Negatively charged species have been used in conjunction with positively charged species to form polyelectrolyte multilayered coating. Luces *et al*. separated basic proteins using polyelectrolyte multilayers consisting of cationic linear polymers and anionic molecular micelles.[68] Molecules are attached by way of electrostatic, hydrophobic, and other weak interactions. Surfactants can be categorized by the type of charge it possesses.

Non-charged surfactants have no charge (e.g. polyoxyethylene(23)dodecanol, Brij-35, [$C_{12}H_{25}(OC_2O_4)_{23}OH$]), whereas cationic surfactants have a positive charge (e.g. hexadecyltrimethylammonium bromide, CTAB, [$C_{16}H_{33}N^+(CH_3)_3Br^-$]). Furthermore, anionic surfactants have a negative charge (e.g. Sodium tetradecylsulfate, NaTDS, [$C_{14}H_{29}SO_4^-Na^+$]), while zwitterionic surfactants can have both positive and negative charges (e.g. N-dodecyl-N, N-dimethylammonium-3-propane-1-sulfonic acid, sulfobetaine, [$C_{12}H_{25}N^+(CH_3)_2(CH_2)_3SO_3^-$]). Negatively charged groups can be due to sulfates, sulfonates, and carboxylic acids. The selected molecules must be in a solution with a pH higher than its pKa to deprotonate the molecule and expose the negative charge. However, at lower pH values the group becomes neutral.

Positively charged groups are typically due to amines that have a quaternary orientation due to having a pH lower than the pKa of the amine which in turn protonates the amine. However, tetra alkyl amines retain positive charge for all pH values. Typically, zwitterionic molecules contain a quaternary amine with an anionic group that can be protonated. At low pH

the molecule has a positive charge. However, as the pH is increased, the molecule becomes more zwitterionic as the negative charges begin to equal the positive charges. It is worthy to note that at high pH these molecules stay zwitterionic. Other zwitterionic molecules contain two groups which can both become ionized with changes in pH e.g. amino acids. Similar to amino acids, these molecules can be more overall negatively charged under acidic conditions, positively charged under basic conditions, or equal positive and negative charge for the pH value at the pI.

Molecular micelles have proved useful with the separation of chiral analytes,[69-71] and proteins.[68, 72] Molecular micelles have advantages over regular micelles such as they can be used at concentrations lower than the CMC and maintain their shape. Moreover, if a surfactant has a high CMC value heat generation can become problematic due to high concentration of monomers being used during CE separations.[73] Furthermore, molecular micelles have ridged structures which can be used for interactions in a stationary phase for OT-CEC.

1.6.2.3.1 Protein Separations Using Capillary Electrochromatography

To minimize protein interactions with the capillary wall, it may be necessary to coat the capillary wall with a stationary phase. Moreover, one could obtain good selectivity with the use of various stationary phases. For instance, Zhou *et al*. modified capillary walls with carboxymethylchitan for the separation of trypsin ribonuclease A, lysozyme, and cytochrome *c*.[74] Carboxymethylchitan was covalently bonded to the capillary wall via a silane based molecule intermediate. Protein adsorption to the capillary wall was avoided and good RSD values for migration times of the peaks were reported. Hsieh *et al*. coated capillaries with titanium dioxide nanoparticles for the separation of ovalbumin, conalbumin, apo-transferrin, and bovine serum albumin.[75] The coating also resolved five glycoisoforms of ovalbumin under optimized conditions. Luces *et al*. developed a polyelectrolyte multilayer coating for the separation of basic proteins (lysozyme, ribonuclease A, cytochrome *c*, and α-chymotrypsinogen A).[68] The

multilayer coating consisted of bilayers formed by flushing a cationic polymer through the capillary followed by an anionic molecular micelle. It was observed that increasing the bilayer number to a certain extent resulted in higher efficiencies, increased resolution, and better reproducibility could be achieved. Qu *et al*. incorporated gold nanoparticles into their polyelectrolyte multilayer coating for the separation of acidic and basic proteins (ovomucoid, lysozyme, avidin, ovotransferrin, and ovalbumin).[76] The coating exhibited excellent RSDs of less than 1% for run-to-run, day-to-day, and capillary-to capillary reproducibilities. OT-CEC has proven to be a very versatile technique. Table 1.1 below shows the proteins investigated for Chapters 2 and 4 of this dissertation.

Table 1.1 Protein analytes

Protein	Molecular Weight (kDa)	pI
Albumin	69.0	4.7
α-lactalbumin	14.2	4.8
β-lactoglobulin A	36.7	5.1
β-lactoglobulin B	36.6	5.3
Myoglobin	17.0	6.8
α-chymotrypsinogen A	25.0	9.2
ribonuclease A	13.7	9.3
cytochrome *c*	12.4	10.2
Lysozyme	14.3	11.1

1.6.2.3.2 Chiral Separations Using Capillary Electrochromatography

There are some basic guidelines for the selection of a chiral selector which include firstly; the chiral selector of interest must have a wide range of uses. In addition, the chiral selector

must be a stable compound, insoluble and have the ability to separate compounds in various buffers used for CEC. Furthermore, the chiral selector should aid the generation of EOF. Using a charged selector could support faster chiral separations with improvement of the EOF. For immobilization on the capillary wall, the chiral selector must also possess functional groups which are compatible for attachment. Moreover, for a chiral selector to be effective it is important that the diffusion through the stationary phase, interactions with the analyte of interest, and mass transfer kinetics are favorable. Finally, a mixture of chemoselectivity along with enantioselectivity would improve the separation ability of the chiral selector. These factors would aid in the removal of impurities from the desired product.[77]

Cationic, anionic, zwitterionic, and nonionic surfactants have been explored for chiral discrimination for resolving chiral molecules. These separations have been based on using the surfactants as coating or as a pseudostationary phase in MEKC. Chiral cationic surfactants have been investigated in the enantioselective separation of chiral acid analytes by Shamsi and Rizvi.[78] They synthesized surfactants undecenoxycarbonyl-L-leucinol bromide and undecenoxycarbonyl-L-pyrrolidinol bromide using L-leucinol and N-methylpryrrolidinol, respectively, as precursors. To induce the positive charge from the tetraalkylated amine, the head group precursor was attached to the hydrophobic chain undec-10-enyl-2-bromoethylcarbamate. The monomer and polymerized monomer forms were compared in the separation of (±)-2-(2-chlorophenoxy)propanoic acid and (±)-α-bromophenylacetic acid. Resolutions values greater than 1.5 were obtained for monomer and polymer forms of both chiral selectors. However, the use monomer undecenoxycarbonyl-L-leucinol bromide and the polymer form of undecenoxycarbonyl-L-pyrrolidinol bromide resulted in faster analysis times than for their respective counterpart. In the separation of (±)-2-(2-chlorophenoxy)propanoic acid the use of both the monomer and polymer forms of the respective chiral selectors resulted in

resolutions being the same value. However, both polymers resulted in faster analysis times than their respective monomer counterpart.

Anionic chiral surfactants have been reported by Wang and Warner using an amino acid coupled to a hydrophobic tail in the enantioseparation of (±)-1,1'-bi-2-naphthol.[73] Studies involved the use of the synthesized monomer sodium undecylenyl-L-valine and its polymer form. The monomer form of sodium undecylenyl-L-valine using concentrations lower than its CMC could not resolve individual enantiomers of (±)-1,1'-bi-2-naphthol. However, the polymer counterpart poly(sodium *N*-undecylenyl-L-valinate) could resolve enantiomers at lower concentrations, than the CMC of the monomer, due to the polymerized micelle maintaining its shape. Moreover, there is an optimal value of concentration obtained when using these polymerized micelles while at concentrations higher than this value results in a decrease in resolution. This phenomenon can be explained by Wren and Rowe.[79] Chiral enantiomers of the same molecule within achiral buffer migrate through the capillary at equal electrophoretic mobilities. If a chiral selector is added to the buffer, enantiomers could interact with the selector to form a complex. The assumption is that the complexes formed with the individual enantiomers will have the equal electrophoretic mobilities. However, if the binding constant of the individual enantiomers with the chiral selector is different than the electrophoretic mobility of the complexed and non-complexed enantiomer will be different, thus resulting in a separation.

Zwitterionic chiral surfactants, 3-[(3-cholamidoproply)dimethylammonio]-2-hydroxy-1-propanesulphonate and 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate, were evaluated in the separation of chiral enantiomers of (±)-1,1'-binaphthyl-2,2'-diamine and Tröger's base.[80] When using concentrations higher than the CMC of 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate for MEKC, enantiometric resolution was achieved for the binaphtyl derivative. They proposed using higher concentrations than the CMC to yield an

enantioseparation was due to interactions of the micelle with the analyte than the monomer with the analyte. The hydroxyl modified form of the bile salt was evaluated at the same concentration yielding similar results. The authors observed an increase in selectivity and longer analysis times were observed. The separation of Tröger base resulted in similar results obtained as the separation of the binaphtyl derivative. At a concentration higher than the CMC for 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate, the individual enantiomers were baseline resolved. The hydroxyl derivative of the bile salt also resulted in baseline resolved enantiomers with an increase in retention time for the separation as well as increased selectivity.

Nonionic Triton X-100 has been investigated for the separation of amino acids (D,L-Leucine, D,L-Valine, D,L-Alanine, D,L-Proline, D,L-Glutamic acid, and D,L-Asparagine) derivatized with a fluorophore, 4-(3isothiocyanatopyrrolidin-1-yl)-77-nitro-2,1,3-benzoxadiazole.[65] This separation mechanism involves an indirect method of chiral separation by forming a diastereomertic compound and separations being based on a conventional achiral system. Triton X-100 shown increases in the sensitivity and enhancements in the fluorescence of the fluorophore due to interactions of the nonionic micelle interacting with the fluorophore to reduce the potential of nonradiative decay of the exited state of the molecule.

Previous research reports have observed that higher density of sterogenic centers on chiral selectors can result in more interactions with certain enantiomers of a chiral species.[81] Thus, providing higher selectivity for chiral separations. Natural biopolymers have saccharide based monomer units. Saccharides contain multiple chiral centers making the molecule highly selective for chiral species. Cellulose is a biopolymer which contains D-glucose units linked together by β-1, 4 glycosidic bonds as shown in Figure 1.11. Each monomer unit contains five chiral centers making this polymer valuable for chiral separations. Modified cellulose has been applied as a stationary phase in CEC in the separation of chiral drug enantiomers.[82, 83] These

previous methods either modify the capillary wall with the cellulose or pack the column with a slurry. However, coating the capillary wall with cellulose would provide a quick, facile preparation method for chiral separations. Typically, coatings in OT-CEC are applied by dissolving chiral selectors in solvent then flushing the capillary thereby allowing the chiral selector to adsorb to the capillary wall. Cellulose derivatives can be water insoluble and may require harsh solvents to dissolve them. There are several reports of RTILs used for the dissolution of polysaccharides.[84-86] A novel approach to applying these polysaccharides to the capillary wall can be via dissolution with ionic liquids which will be discussed later in this dissertation.

1.7 Other Analytical Techniques Used

1.7.1 Tensiometry

Tensiometry is commonly used to measure the CMC of surfactants. This technique employs a platinum-iridium ring that is dipped into a solution to determine the amount of force needed to break the interface when withdrawing the ring. A plot is generated of force versus increasing concentration of surfactant to determine the CMC as indicated by Figure 1.12. The plot illustrates a relationship that as the concentration increases force should decrease, until the

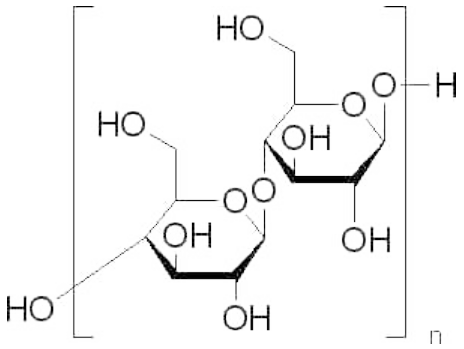


Figure 1.11 Polymeric notation of cellulose

CMC is reached. Then force will remain constant as surfactant concentration is further increased, which indicates higher order structures are being formed rather than aggregation on the solution surface.

1.7.2 Atomic Force Microscopy

Atomic Force Microscopy (AFM) is a technique which uses a nanosized probe to analyze materials and provide high resolution views of a sample.[87] AFM can be used independently or as a complimentary technique to gain information such as surface topography, mechanical and physical properties. Advantages of AFM compared to other microscopy and spectroscopy is the ability to image samples in air, vacuum, or liquid, and no special pretreatment of the sample such as staining or labeling is necessary to make measurements. Most impressive is that AFM instrumentation has the capability of investigating properties of materials from the micron level down to atomic level. The surface structure of materials can be observed in real time on a scale from microns down to nanometers.

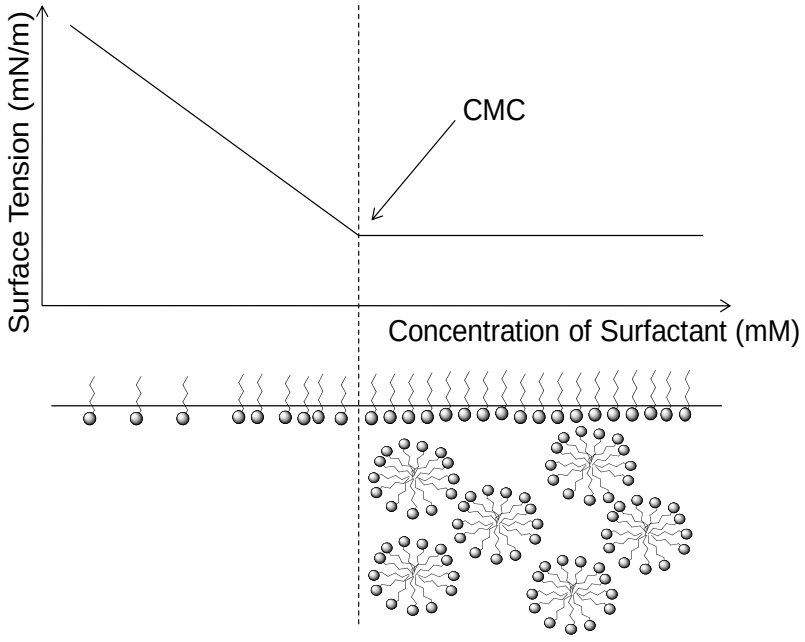


Figure 1.12 Determination of CMC

Components of an AFM include a cantilever, sample specific tip, detector, and a laser. A diode laser is focus on the backside of the cantilever while changes in the deflection are monitored in a quadrant photodetector. A schematic is displayed in Figure 1.13. In principle, an image is generated when the AFM tip is scanned across the material. During the scan, either attractive or repulsive force between the tip and the sample will cause the cantilever to bending. As the tip scans the surface it will experience difference in composition of the sample, resulting in the tip moving in the up and down or left to right direction. The computer used generates a 3-D digital image of the surface topography by mapping X-Y position of the tip.

There are over 30 different imaging modes of AFM all with the sample basic principle of operation. The three main modes of AFM are non-contact, intermittent, and contact. The images that are presented in this chapter of this dissertation were acquired using contact mode- AFM.

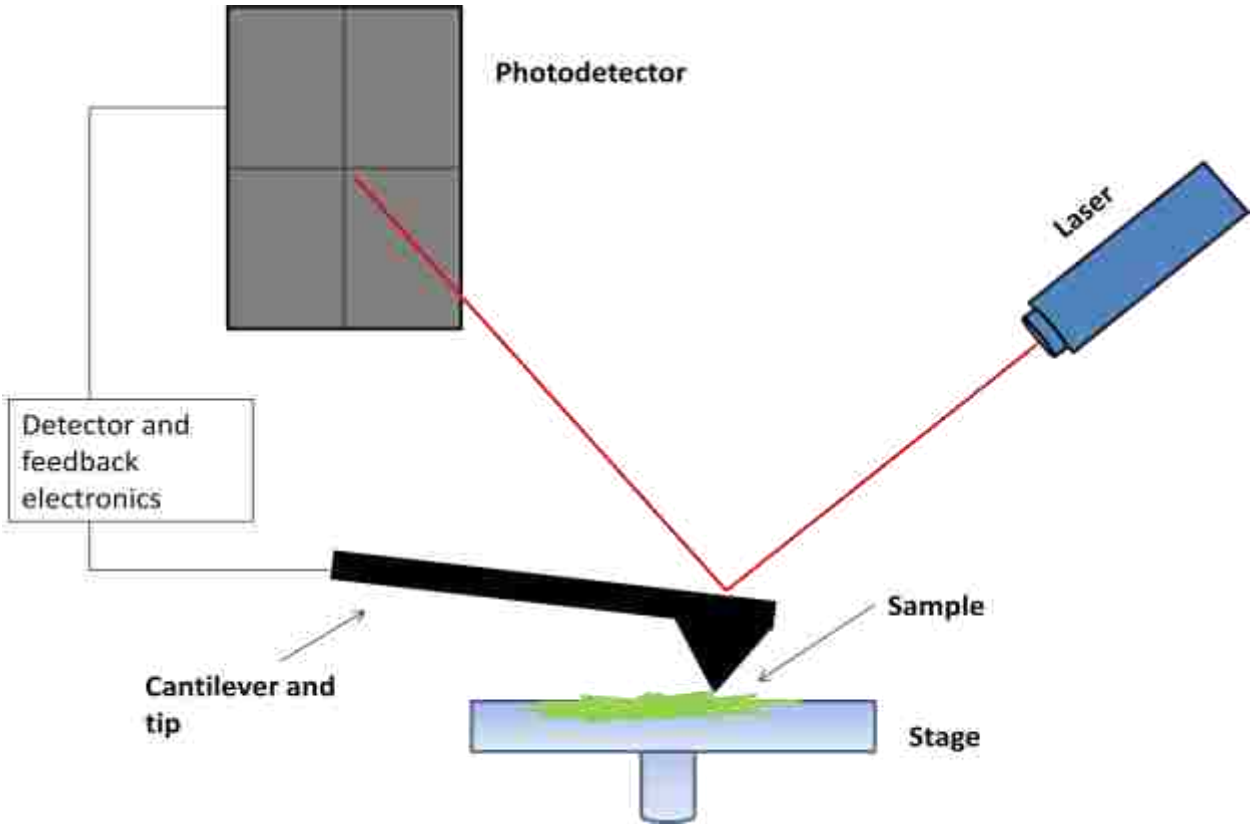


Figure 1.13 Schematic of AFM instrumentation

For contact mode, a constant force applied between the tip and sample is maintained by the feedback loop. The purpose of the feedback loop is to in contact mode, is to adjust the voltages applied to the piezoscanner to maintain the desired tip deflection as it scans the surface. In contact mode the lateral force image can be simultaneously acquired to give information regarding homogeneity of the sample and also differences in surface chemistry. Because the tip is in continuous contact with the surface high resolution images are obtainable with this mode.

1.8 Scope of Dissertation

The goal of this research is to develop stationary phases or alternate approaches to applying stationary phases on a capillary column for the separation of protein and chiral analytes in OT-CEC. The material, poly-ε-SUK, used in this study provide dual benefits for the separation of a wide range of protein biomarkers at acidic and basic pH. Preparing polysaccharides by dissolution with ionic liquids have advantages such as use of a non-harsh, environmentally green solvent. This method reduces column preparation time needed compared to lengthy wall modification synthesis methods which in turn makes the process more time efficient.

In Chapter 2, the synthesis and fabrication of novel poly-*ε*-sodium-undecanoyl lysinate (poly-*ε*-SUK) molecular micelle coated capillaries are discussed. This coating is used for the analysis of acidic and basic proteins biomarkers at both acidic and basic pH for OT-CEC. The lysine based coating exhibits an overall positive charge at acidic pH; however, at basic pH the overall charge is negative. The effect of polymer as well as sodium chloride concentration, voltage, and temperature were each individually investigated. A sample of human serum was also investigated using optimized acidic and basic conditions with protein standards. The

stability of the column was investigated with run-to-run, day-to-day, week-to-week, and capillary-to-capillary reproducibility using relative standard deviation of the EOF.

In Chapter 3, the use of cellulose derivatives i.e. cellulose acetate (CA), cellulose acetate phthalate (CAP), and cellulose acetate butyrate (CAB), as stationary phases for chiral separation is reported. Each cellulose derivative was dissolved in 1-butyl-3-methylimidizolium acetate to fill capillaries. Due to the ability of some RTIL to dissolve highly hydrophobic cellulose but remain water soluble, a simple water rinse is used to remove the RTIL leaving biopolymer on the capillary wall. The chiral selectors were used to analyze four chiral analytes thiopental, sotalol, labetalol, and ephedrine. During this investigation, a polymer concentration, temperature, and voltage study was performed to determine the optimum separation conditions. The column stability was investigated using RSD of the EOF with run-to-run and capillary-to-capillary studies.

In Chapter 4, the use of polysaccharide-based stationary phases for the separation of proteins biomarkers are discussed. Cellulose derivatives (CA, CAP, CAB) were used as stationary phases for the separation of four basic proteins lysozyme, ribonuclease A, cytochrome *c*, and α-chymotrypsinogen A. Polysaccharide CA revealed the best separation of the four basic proteins and was further optimized using voltage, temperature, and organic solvent type as well as percent concentration. In addition, CA nanofibers were investigated using optimized conditions with varying coating time to examine the effect on the separation of the four basic proteins.

1.9 References

(1) Ryu, O.; Atkinson, J.; Hoehn, G.; Illei, G.; Hart, T. Identification of Parotid Salivary Biomarkers in Sjogren's Syndrome by Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and Two-Dimensional Difference Gel Electrophoresis. *Rheumatology* **2006**, *45*, 1077-1086.

(2) Ye, B.; Skates, S.; Mok, S. C.; Horick, N. K.; Rosenberg, H. F.; Vitonis, A.; Edwards, D.; Sluss, P.; Han, W. K.; Berkowitz, R. S.; Cramer, D. W. Proteomic-Based Discovery and Characterization of Glycosylated Eosinophil-Derived Neurotoxin and COOH-Terminal Osteopontin Fragments for Ovarian Cancer in Urine. *Clin. Cancer Res.* **2006**, *12*, 432-441.

(3) Finnskog, D.; Jaras, K.; Ressine, A.; Marko-Varga, G.; Lilja, H.; Laurell, T. High-Speed Biomarker Identification Utilizing Porous Silicon Nanovial Arrays and MALDI-TOF Mass Spectrometry. *Electrophoresis* **2006**, *27*, 1093-1103.

(4) Phillips, T. M.; Dickens, B. F. Analysis of Recombinant Cytokines in Human Body Fluids by Immunoaffinity Capillary Electrophoresis. *Electrophoresis* **1998**, *19*, 2991-2996.

(5) Caner, H.; Groner, E.; Levy, L.; Agranat, I. Trends in the Development of Chiral Drugs. *Drug Discov. Today* **2004**, *9*, 105-110.

(6) Zhou, L. L.; Lin, Z. H.; Reamer, R. A.; Mao, B.; Ge, Z. H. Stereoisomeric Separation of Pharmaceutical Compounds Using CE with a Chiral Crown Ether. *Electrophoresis* **2007**, *28*, 2658-2666.

(7) Cho, S. I.; Lee, K.-N.; Kim, Y.-K.; Jang, J.; Chung, D. S. Chiral Separation of Gemifloxacin in Sodium-Containing Media Using Chiral Crown Ether as a Chiral Selector by Capillary and Microchip Electrophoresis. *Electrophoresis* **2002**, *23*, 972-977.

(8) Nishi, H. Enantioselectivity in Chiral Capillary Electrophoresis with Polysaccharides. *J. Chromatogr. A* **1997**, *792*, 327-347.

(9) Lv, C. G.; Liu, Y. Q.; Mangelings, D.; Vander Heyden, Y. Enantioselectivity of Monolithic Silica Stationary Phases Immobilized with Different Concentrations Cellulose Tris (3,5-dimethylphenylcarbamate), Analyzed with Different Mobile Phases in Capillary Electrochromatography. *Electrophoresis* **2011**, *32*, 2708-2717.

(10) Wang, H.; Gu, J. L.; Hu, H. F.; Dai, R. J.; Ding, T. H.; Fu, R. N. Study on the Chiral Separation of Basic Drugs by Capillary Zone Electrophoresis Using Beta-CD and Derivatized Beta-CDs as Chiral Selectors. *Anal. Chim. Acta* **1998**, *359*, 39-46.

(11) Belder, D.; Schomburg, G. Chiral Separations of Basic and Acidic Compounds in Modified Capillaries Using Cyclodextrin-Modified Capillary Zone Electrophoresis. *J. Chromatogr. A* **1994**, *666*, 351-365.

(12) Luces, C. A.; Warner, I. M. Achiral and Chiral Separations Using MEKC, Polyelectrolyte Multilayer Coatings, and Mixed Mode Separation Techniques with Molecular Micelles. *Electrophoresis* **2010**, *31*, 1036-1043.

(13) Zaidi, S. A.; Han, K. M.; Hwang, D. G.; Cheong, W. J. Preparation of Open Tubular Molecule Imprinted Polymer Capillary Columns with Various Templates by a Generalized Procedure and Their Chiral and Non-chiral Separation Performance in CEC. *Electrophoresis* **2010**, *31*, 1019-1028.

(14) Easson, L. H.; Stedman, E. Studies on the Relationship Between Chemical Constitution and Physiological Action. V. Molecular Dissymmetry and Physiological Activity. *Biochem. J.* **1933**, *27*, 1257-1266.

(15) Pirkle, W. H.; Pochapsky, T. C. Considerations of Chiral Recognition Relevant to the Liquid-Chromatographic Separation of Enantiomers. *Chem. Rev.* **1989**, *89*, 347-362.

(16) Seddon, K. R. Ionic Liquids for Clean Technology. *J. Chem. Technol. Biot.* **1997**, *68*, 351-356.

(17) Walden, P. *Bulletin de l'Academie Imperiale des Sciences de St. Petersburg* **1914**, 405-422.

(18) Zhao, H.; Jones, C. I. L.; Baker, G. A.; Xia, S.; Olubajo, O.; Person, V. N. Regenerating Cellulose from Ionic Liquids for an Accelerated Enzymatic Hydrolysis. *J. Biotechnol.* **2009**, *139*, 47-54.

(19) Gratzel, M. Conversion of Sunlight to Electric Power by Nanocrystalline Dye-Sensitized Solar Cells. *J. Photoch. Photobio. A* **2004**, *164*, 3-14.

(20) Anderson, J. L.; Armstrong, D. W. High-Stability Ionic Liquids. A New Class of Stationary Phases for Gas Chromatography. *Anal. Chem.* **2003**, *75*, 4851-4858.

(21) Mora-Pale, M.; Meli, L.; Doherty, T. V.; Linhardt, R. J.; Dordick, J. S. Room Temperature Ionic Liquids as Emerging Solvents for the Pretreatment of Lignocellulosic Biomass *Biotechnol. and Bioeng.* **2011**, *108*, 1229-1245.

(22) Miller, C. A.; Raney, K. H. Solubilization Emulsification Mechanisms of Detergency. *Colloids and Surface. A* **1993**, *74*, 169-215.

(23) Giolando, S. T.; Rapaport, R. A.; Larson, R. J.; Federle, T. W.; Stalmans, M.; Masscheleyn, P. Environmental Fate and Effects of Deedmac - A New Rapidly Biodegradable Cationic Surfactant for Use In Fabric Softeners. *Chemosphere* **1995**, *30*, 1067-1083.

(24) Spirin, M. G.; Brichkin, S. B.; Razumov, V. F. Synthesis and Stabilization of Gold Nanoparticles in Reverse Micelles of Aerosol OT and Triton X-100. *Colloid J+* **2005**, *67*, 485-490.

(25) Liu, J.; Lee, H.; Allen, C. Formulation of Drugs in Block Copolymer Micelles: Drug Loading and Release. *Curr. Pharm. Design* **2006**, *12*, 4685-4701.

(26) Haynes, J. L.; Billiot, E. J.; Yarabe, H. H.; Warner, I. M.; Shamsi, S. A. Chiral Separation with Dipeptide-Terminated Polymeric Surfactants: The Effect of an Extra Heteroatom on the Polar Head Group. *Electrophoresis* **2000**, *21*, 1597-1605.

(27) Williams, A. A.; Fakayode, S. O.; Huang, X. D.; Warner, I. M. Use of Multivariate Analysis for Optimization of Separation Parameters and Prediction of Migration Time, Resolution, and Resolution Per Unit Time in Micellar Electrokinetic Chromatography. *Electrophoresis* **2006**, *27*, 4127-4140.

(28) Cheng, Y.-F.; Dovichi, N. J. Subattomole Amino-Acid Analysis by Capillary Zone Electrophoresis and Laser-Induced Fluorescence *Science* **1988**, *242*, 562-564.

(29) Veledo, M. T.; Frutos, M. d.; Diez-Masa, J. C. Amino Acids Determination Using Capillary Electrophoresis with On-Capillary Derivatization and Laser-Induced Fluorescence Detection *J. Chromatogr. A* **2005**, *1079*, 335-343.

(30) Lauer, H. H.; McManigill, D. Capillary Zone Electrophoresis of Proteins in Untreated Fused Silica Tubing. *Anal. Chem.* **1986**, *58*, 166-169.

(31) McCormick, R. M. Capillary Zone Electrophoretic Separation of Peptides and Proteins Using Low pH Buffers in Modified Silica Capillaries. *Anal. Chem.* **1988**, *60*, 2322-2328.

(32) Strege, M.; Lagu, A. Separation of DNA Restriction Fragments by Capillary Electrophoresis Using Coated Fused-Silica Capillaries. *Anal. Chem.* **1991**, *63*, 1233-1236.

(33) Pariat, Y. F.; Berka, J.; Heiger, D. N.; Schmitt, T.; Vilenchik, M.; Cohen, A. S.; Foret, F.; Karger, B. L. Separation of DNA Fragments by Capillary Electrophoresis Using Replaceable Linear Polyacrylamide Matrices *J. Chromatogr. A* **1993**, *652*, 57-66.

(34) Jorgenson, J. W.; Lukacs, K. D. Zone Electrophoresis in Open-Tubular Glass-Capillaries. *Anal. Chem.* **1981**, *53*, 1298-1302.

(35) Jorgenson, J. W.; Lukacs, K. D. Capillary Zone Electrophoresis. *Science* **1983**, *222*, 266-272.

(36) Schwer, C.; Kenndler, E. Electrophoresis in Fused-Silica Capillaries: The Influence of Organic Solvents on the Electroosmotic Velocity and the .Zeta. Potential. *Anal. Chem.* **1991**, *63*, 1801-1807.

(37) Lucy, C. A.; Underhill, R. S. Characterization of the Cationic Surfactant Induced Reversal of Electroosmotic Flow in Capillary Electrophoresis. *Anal. Chem.* **1996**, *68*, 300-305.

(38) McCormick, R. M. Capillary Zone Electrophoretic Separation of Peptides and Proteins Using Low ph Buffers in Modified Silica Capillaries. *Anal. Chem.* **1988**, *60*, 2322-2328.

(39) Kim, K. H.; Lee, J. H.; Ko, M. Y.; Hong, S. P.; Youm, J. R. Chiral Separation of Beta-Blockers After Derivatization with (-)-(alpha)-methoxy-alpha-(trifluoromethyl)phenylacetyl Chloride by Gas Chromatography. *Arch. Pharm. Res.* **2001**, *24*, 402-406.

(40) Wang, R. Q.; Ong, T. T.; Ng, S. C. Synthesis of Cationic Beta-Cyclodextrin Derivatives and Their Applications as Chiral Stationary Phases for High-Performance Liquid Chromatography and Supercritical Fluid Chromatography. *J. Chromatogr. A* **2008**, *1203*, 185-192.

(41) Bhushan, R.; Tanwar, S. Different Approaches of Impregnation for Resolution of Enantiomers of Atenolol, Propranolol and Salbutamol using Cu(II)-L-Amino Acid Complexes for Ligand Exchange on Commercial Thin Layer Chromatographic Plates. *J. Chromatogr. A* **2010**, *1217*, 1395-1398.

(42) Van der Vorst, G.; Van Langenhove, H.; De Paep, F.; Aelterman, W.; Dingenen, J.; Dewulf, J. Exergetic Life Cycle Analysis for the Selection of Chromatographic Separation Processes in the Pharmaceutical Industry: Preparative HPLC Versus Preparative SFC. *Green Chem.* **2009**, *11*, 1007-1012.

(43) Juvancz, Z.; Petersson, P. Enantioselective Gas Chromatography. *J. Microcolumn Sep.* **1996**, *8*, 99-114.

(44) Shaw, P. E.; Wilson, C. W. Organic-Acids in Orange, Grapefruit and Cherry Juices Quantified by High-Performance Liquid-Chromatography Using Neutral Resin or Propylamine Columns. *J. Sci. Food Agr.* **1983**, *34*, 1285-1288.

(45) Lensmeyer, G. L.; Kempf, T.; Gidal, B. E.; Wiebe, D. A. Optimized Method for Determination of Gabapentin in Serum by High-Performance Liquid-Chromatography. *Ther. Drug Monit.* **1995**, *17*, 251-258.

(46) Cavazza, A.; Bartle, K. D.; Dugo, P.; Mondello, L. Analysis of Oxygen Heterocyclic Compounds in Citrus Essential Oils by Capillary Electrochromatography and Comparison with HPLC. *Chromatographia* **2001**, *53*, 57-62.

(47) Cikalo, M. G.; Bartle, K. D.; Robson, M. M.; Myers, P.; Euerby, M. R. Capillary Electrochromatography. *Analyst* **1998**, *123*, 87R-102R.

(48) Pretorius, V.; Hopkins, B. J.; Schieke, J. D. Electro-osmosis - New Concept for High-Speed Liquid-Chromatography *J. Chromatogr.* **1974**, *99*, 23-30.

(49) Jorgenson, J. W.; Lukacs, K. D. High-Resolution Separations Based on Electrophoresis and Electroosmosis. *J. Chromatogr.* **1981**, *218*, 209-216.

(50) Gusev, I.; Huang, X.; Horvath, C. Capillary Columns with In Situ Formed Porous Monolithic Packing for Micro High-Performance Liquid Chromatography and Capillary Electrochromatography. *J. Chromatogr. A* **1999**, *855*, 273-290.

(51) Skerikova, V.; Jandera, P. Effects of the Operation Parameters on Hydrophilic Interaction Liquid Chromatography Separation of Phenolic Acids on Zwitterionic Monolithic Capillary Columns. *J. Chromatogr. A* **2010**, *1217*, 7981-7989.

(52) Huang, G.; Zeng, W.; Lin, X.; Xie, Z. Silica-Based Zwitterionic Monolithic Stationary Phase for Separation of Neutral and Ionized Solutes Using Pressurized CEC *J. Sep. Sci.* **2010**, *33*, 1625-1632.

(53) Zhang, L.; Ping, G.; Zhang, L.; Zhang, W.; Zhang, Y. Preparation and Characterization of Monolithic Columns for Capillary Electrochromatography with Weak Electroosmotic Flow *J. Sep. Sci.* **2003**, *26*, 331-336.

(54) Karenga, S.; Rassi, Z. E. Controlling Retention, Selectivity and Magnitude of EOF by Segmented Monolithic Columns Consisting of Octadecyl and Naphthyl Monolithic Segments – Applications to RP-CEC of Both Neutral and Charged Solutes. *Electrophoresis* **2011**, *32*, 1033-1043.

(55) Peters, E. C.; Petro, M.; Svec, F.; Frechet, J. M. J. Molded Rigid Polymer Monoliths as Separation Media for Capillary Electrochromatography. *Anal. Chem.* **1997**, *69*, 3646-3649.

(56) Knox, J. H.; Grant, I. H. Electrochromatography in Packed Tubes Using 1.5 to 50 Mu-M Silica-Gels and ODS Bonded Silica-Gels *Chromatographia* **1991**, *32*, 317-328.

(57) Smith, N.; Evans, M. The Analysis of Pharmaceutical Compounds Using Capillary Electrochromatography. *Chromatographia* **1994**, *38*, 649-657.

(58) Wiedmer, S. K.; D'Orazio, G.; Smatt, J.-H.; Bourdin, D.; Banos-Perez, C.; Sakeye, M.; Kivilompolo, M.; Kopperi, M.; Ruiz-Jimenez, J.; Fanali, S.; Riekkola, M.-L. Polyethylenimine-Modified Metal Oxides for Fabrication of Packed Capillary Columns for Capillary Electrochromatography and Capillary Liquid Chromatography. *J. Chromatogr. A* **2011**, *1218*, 5020-5029.

(59) Ratnayake, C. K.; Oh, C. S.; Henry, M. P. Characteristics of Particle-Loaded Monolithic Sol–Gel Columns for Capillary Electrochromatography: I. Structural, Electrical and Band-Broadening Properties. *J. Chromatogr. A* **2000**, *887*, 277-285.

(60) Wu, J.-T.; Huang, P.; Li, M. X.; Lubman, D. M. Protein Digest Analysis by Pressurized Capillary Electrochromatography Using an Ion Trap Storage/Reflectron Time-of-Flight Mass Detector. *Anal. Chem.* **1997**, *69*, 2908-2913.

(61) Matyska, M. T.; Pesek, J. J.; Boysen, I.; Hearn, T. W. Characterization and Applications of Etched Chemically Modified Capillaries for Open-Tubular Capillary Electrochromatography. *Electrophoresis* **2001**, *22*, 2620-2628.

(62) Pesek, J. J.; Matyska, M. T.; Sentellas, S.; Galceran, M. T.; Chiari, M.; Pirri, G. Multimodal Open-Tubular Capillary Electrochromatographic Analysis of Amines and Peptides. *Electrophoresis* **2002**, *23*, 2982-2989.

(63) Kitagawa, F.; Kamiya, M.; Otsuka, K. The Use of Multilayered Capillaries for Chiral Separation by Electrochromatography. *J. Chromatogr. B* **2008**, *875*, 323-328.

(64) Li, M.; Liu, X.; Jiang, F. Y.; Guo, L. P.; Yang, L. Enantioselective Open-Tubular Capillary Electrochromatography Using Cyclodextrin-Modified Gold Nanoparticles as Stationary Phase. *J. Chromatogr. A* **2011**, *1218*, 3725-3729.

(65) Chen, J. L.; Hsieh, K. H. Nanochitosan Crosslinked with Polyacrylamide as the Chiral Stationary Phase for Open-Tubular Capillary Electrochromatography. *Electrophoresis* **2011**, *32*, 398-407.

(66) Pesek, J. J.; Matyska, M. T.; Tran, H. Applications of Chemically Modified and Coated Etched Capillaries for the Separation of Basic Molecules. *J. Sep. Sci.* **2001**, *24*, 729-735.

(67) Yang, L.; Guihen, E.; Holmes, J. D.; Loughran, M.; O'Sullivan, G. P.; Glennon, J. D. Gold Nanoparticle-Modified Etched Capillaries for Open-Tubular Capillary Electrochromatography. *Anal. Chem.* **2005**, *77*, 1840-1846.

(68) Luces, C.; Fakayode, S. O.; Lowry, M.; Warner, I. M. Protein Separations Using Polyelectrolyte Multilayer Coatings with Molecular Micelles in Open Tubular Capillary Electrochromatography *Electrophoresis* **2008**, *29*, 889-900.

(69) Wang, J.; Warner, I. M. Chiral Separations Using Micellar Electrokinetic Capillary Chromatography and a Polymerized Chiral Micelle. *Anal. Chem.* **1994**, *66*, 3773-3776.

(70) Akbay, C.; Rizvi, S. A. A.; Shamsi, S. A. Simultaneous Enantioseparation and Tandem UV−MS Detection of Eight β-Blockers in Micellar Electrokinetic Chromatography Using a Chiral Molecular Micelle. *Anal. Chem.* **2005**, *77*, 1672-1683.

(71) Billiot, F. H.; Billiot, E. J.; Warner, I. M. Comparison of Monomeric and Polymeric Amino Acid Based Surfactants for Chiral Separations. *J. Chromatogr. A* **2001**, *922*, 329-338.

(72) Moore Jr, L.; LeJeune, Z. M.; Luces, C. A.; Gates, A. T.; Li, M.; El-Zahab, B.; Garno, J. C.; Warner, I. M. Lysine-Based Zwitterionic Molecular Micelle for Simultaneous Separation of Acidic and Basic Proteins Using Open Tubular Capillary Electrochromatography. *Anal. Chem.* **2010**, *82*, 3997-4005.

(73) Wang, J. A.; Warner, I. M. Chiral Separations Using Micellar Electrokinetic Capillary Chromatography and a Polymerized Chiral Micelle. *Anal. Chem.* **1994**, *66*, 3773-3776.

(74) Zhou, S. Y.; Tan, J. J.; Chen, Q. H.; Lin, X. C.; Lu, H. X.; Xie, Z. H. Carboxymethylchitosan Covalently Modified Capillary Column for Open Tubular Capillary Electrochromatography of Basic Proteins and Opium Alkaloids. *J. Chromatogr. A* **2010**, *1217*, 8346-8351.

(75) Hsieh, Y. L.; Chen, T. H.; Liu, C. Y. Capillary Electrochromatographic Separation of Proteins on a Column Coated with Titanium Dioxide Nanoparticles. *Electrophoresis* **2006**, *27*, 4288-4294.

(76) Qu, Q. S.; Liu, D. P.; Mangelings, D.; Yang, C.; Hu, X. Y. Permanent Gold Nanoparticle Coatings on Polyelectrolyte Multilayer Modified Capillaries for Open-Tubular Capillary Electrochromatography. *J. Chromatogr. A* **2010**, *1217*, 6588-6594.

(77) Fanali, S.; Catarcini, P.; Blaschke, G.; Chankvetadze, B. Enantioseparations by Capillary Electrochromatography. *Electrophoresis* **2001**, *22*, 3131-3151.

(78) Rizvi, S. A. A.; Shamsi, S. A. Synthesis, Characterization, and Application of Chiral Ionic Liquids and Their Polymers in Micellar Electrokinetic Chromatography. *Anal. Chem.* **2006**, *78*, 7061-7069.

(79) Wren, S. A. C.; Rowe, R. C. Theoretical Aspects of Chiral Separation in Capillary Electrophoresis .1. Initial Evaluation of a Model. *J. Chromatogr.* **1992**, *603*, 235-241.

(80) Hadley, M. R.; Harrison, M. W.; Hutt, A. J. Use of Chiral Zwitterionic Surfactants for Enantiomeric Resolutions by Capillary Electrophoresis. *Electrophoresis* **2003**, *24*, 2508-2513.

(81) Cavazzini, A.; Kaczmarski, K.; Szabelski, P.; Zhou, D.; Liu, X.; Guiochon, G. Modeling of the Separation of the Enantiomers of 1-Phenyl-1-propanol on Cellulose Tribenzoate. *Anal. Chem.* **2001**, *73*, 5704-5715.

(82) He, C.; Hendrickx, A.; Mangelings, D.; Smeyers-Verbeke, J.; Heyden, Y. V. Monolithic Silica Capillary Columns with Immobilized Cellulose Tris(3,5-dimethylphenylcarbamate) for Enantiomer Separations in CEC *Electrophoresis* **2009**, *30*, 3796-3803.

(83) Chankvetadze, B.; Kartozia, I.; Okamoto, Y.; Blaschke, G. The Effect of Pore Size of Silica Gel and Concentration of Buffer on Capillary Chromatographic and Capillary Electrochromatographic Enantioseparations Using Cellulose Tris(3,5-dichlorophenylcarbamate). *J. Sep. Sci.* **2001**, *24*, 635-642.

(84) Simmons, T. J.; Lee, S. H.; Miao, J. J.; Miyauchi, M.; Park, T. J.; Bale, S. S.; Pangule, R.; Bult, J.; Martin, J. G.; Dordick, J. S.; Linhardt, R. J. Preparation of Synthetic Wood Composites Using Ionic Liquids. *Wood Sci. Technol.* **2011**, *45*, 719-733.

(85) Vitz, J.; Erdmenger, T.; Haensch, C.; Schubert, U. S. Extended Dissolution Studies of Cellulose in Imidazolium Based Ionic Liquids. *Green Chem.* **2009**, *11*, 417-424.

(86) Lovell, C. S.; Walker, A.; Damion, R. A.; Radhi, A.; Tanner, S. F.; Budtova, T.; Ries, M. E. Influence of Cellulose on Ion Diffusivity in 1-Ethyl-3-Methyl-Imidazolium Acetate Cellulose Solutions. *Biomacromolecules* **2010**, *11*, 2927-2935.

(87) Xu, S.; Cruchon-Dupeyrat, S. J. N.; Garno, J. C.; Liu, G. Y.; Jennings, G. K.; Yong, T. H.; Laibinis, P. E. In Situ Studies of Thiol Self-Assembly on Gold from Solution Using Atomic Force Microscopy. *J. Chem. Phys.* **1998**, *108*, 5002-5012.

CHAPTER 2
LYSINE-BASED ZWITTERIONIC MOLECULAR MICELLE FOR SIMULTANEOUS SEPARATION OF ACIDIC AND BASIC PROTEINS USING OPEN TUBULAR CAPILLARY ELECTROCHROMATOGRAPHY*

2.1 Introduction

Chromatographic separation and quantification of proteins in complex biological mixtures remains a challenge for many bioanalytical applications including bioprocessing,[1] sensory devices,[2] tags in drug delivery,[3] and medical diagnostics.[4] Many proteins have been identified as biomarkers for detection of diseases such as lysozyme for Sjögren' s syndrome,[5] ribonuclease A for ovarian cancer,[6] and myoglobin for cardiac injury due to anthrax lethal toxin in mice.[7] However, separation of an individual protein from a pool of proteins in a biological fluid or tissue is a daunting task.

High performance liquid chromatography (HPLC) can be used for protein separation; however, HPLC analyses often require large sample volumes and relatively high protein concentrations. In addition, HPLC techniques are typically limited to separation of proteins based on either their size and/or charge, i.e. size-exclusion and ion-exchange. Recently, polymer beads grafted with a zwitterionic stationary phase have been applied to HPLC analyses for separating two acidic and three basic proteins simultaneously.[8] In contrast, electrophoretic techniques, such as capillary electrophoresis (CE), can achieve separation of proteins based on both the size and charge of the protein.

Apart from its use in protein separations, CE is routinely used for separation of a variety of small organic molecules including drugs,[9] amino acids,[10] and deoxyribonucleic acids.[11] Some advantages of CE over HPLC are higher resolution, higher efficiencies, smaller sample consumption, and shorter analysis time. However, the use of CE for the separation of

biomolecules such as proteins can be difficult due to analyte-wall adsorption resulting in peak tailing, unstable baseline, and poor reproducibility.[12, 13] One strategy for minimizing protein-wall adsorption is to use packed-column capillary electrochromatography (PC-CEC). PC-CEC is a technique in which the capillary is filled with micron-sized particles modified with chemical moieties such as octadecyl silica beads.[14] Separations are based on the electrophoretic mobilities of the analytes as well as interactions with the stationary phase. This technique typically yields highly efficient separations. However, it also requires frits at the ends of the capillary, which inevitably produce bubbles and affect the performance of the separation.[15]

Open tubular capillary electrochromatography (OT-CEC), a hybrid of CE and HPLC, is another mode of chromatography in which a capillary is coated with a stationary phase (e.g. charged polymers) to facilitate interactions with analytes.[16, 17] Chen *et al.* have applied positively charged amine based polymers as coatings to capillaries in separating peptides and basic proteins.[18] Advantages of this approach include low polymer consumption, ease of preparation, and possible coupling to mass spectrometry.[19] Coupling to mass spectrometers can be accomplished with OT-CEC because the stationary phase is electrostatically bound to the capillary wall, rather than present in the background electrolyte (BGE) as in micellar electrokinetic chromatography (MEKC).[20] Capillaries modified with either adsorbed or covalently attached polyelectrolyte coatings can be used for OT-CEC, which can minimize electrostatic interactions between the protein and capillary wall.[17, 21-25]

Questions yet to be addressed when using molecular micelles as coatings for OT-CEC are the nature and resilience of individual micellar structures and the thickness of the coatings that are formed on columns. It is not known if micelles undergo significant structural rearrangements when interacting with charged surfaces of the silica capillaries, or if the spherical geometries of

the micelles persist. In this chapter, atomic force microscopy (AFM) is used to image zwitterionic micelles in order to evaluate the structural persistence, shape, and film thickness under conditions that simulate buffered conditions within the capillary column. Polished, flat silicon substrates were used for AFM studies of micelle attachment under conditions that mimic the buffered media and immersion intervals that were used for OT-CEC with zwitterionic micelles. Studies using AFM are emerging as a practical strategy for probing mechanisms of surface adsorption and the integrity of micellar structures. For example, pioneering studies have been achieved by Manne and Gaub, who first resolved the structure of micelles of quaternary ammonium surfactants in 1995 in liquid media.[26] For their cationic surfactant system, an adsorbate layer of roughly spherical micelles was observed to form on silicon substrates; however, surfaces of mica, MoS_2, and graphite produced different surface morphologies, ranging from a flat bilayer to stripes of cylindrical or even hemispherical aggregated structures.

Scanning probe characterizations have also been accomplished for micelles adsorbed at the solid-liquid interface comprised of cationic surfactants,[27-29] polymers,[30, 31] and block copolymers.[32-38] Primary types of surfaces which have been used for such studies include mica, glass, gold, or graphite. It has been demonstrated that the morphology observed for micelles on various surfaces is often different from that observed in bulk solution. Thus, the substrate plays a critical role in determining the micelle surface structure. Surface immobilization of the micellar structures results from strong electrostatic interactions between the surface and surfactants. Depending on the surfactant concentration, interactions with highly charged surfaces have been shown to change micellar structures to form self-assembled layer-like structures in which surfactant molecules organize and interact with other surfactant molecules and with the underlying surface. Thus, to achieve molecularly-resolved images of micelle layers,

in situ AFM protocols were accomplished in liquid solutions to prevent micelles from floating in solution or from being destroyed by the AFM tip during contact-mode scanning.

Previously, we have demonstrated the effectiveness of several amino acid-based molecular micelles in MEKC and as coatings in OT-CEC for separation of achiral[39, 40] and chiral molecules,[41-44] as well as proteins.[16] It has been demonstrated that molecular micelle coated capillaries provide enhanced separation performance over capillaries coated with conventional micelles.[41-44] The principal advantage of molecular micelles is that they can be used at concentrations well below the monomer's critical micelle concentration (CMC) due to covalent bonds formed between individual monomers. Therefore, this eliminates the dynamic equilibrium of conventional micelles. Recently, Luces *et al*. have demonstrated amino acid based molecular micelles in polyelectrolyte coatings (PEMs) for the separation of four basic proteins.[16] These amino acid based anionic molecular micelles (e.g. sodium poly(N-undecanoyl-L-leucyl-alaninate), sodium poly(N-undecanoyl-L-leucyl-valinate)) were used in combination with cationic polymers to form the multilayers. A PEM coating consists of alternating layers of positively and negatively charged polymers. We hypothesized that a zwitterionic coating would eliminate the need for multiple layers due to its dual charge property. At acidic pH values the polymer has a more overall positive charge, while at basic pH values a net negative charge is achieved. This property allows separation of acidic and basic proteins simultaneously.

The use of conventional zwitterionic micellar coatings for OT-CEC has been reported.[23, 24] Of the many different types of OT-CEC capillary coatings, zwitterionic systems are of particular interest because they can provide a means of controlling the ionic characteristics of the capillary surface and the electroosmotic flow (EOF) as a function of pH.[45-48] Herein, we report the synthesis of a novel lysine-based zwitterionic molecular micelle and its first application to

protein separations in OT-CEC.

2.2 Experimental

2.2.1 Materials and Reagents

N-hydroxysuccidimide, N, N'-dicyclohexylcarbodiimde (DCC), 10-undecylenic acid, sodium bicarbonate, and acetone were purchased from Sigma (Milwaukee, WI). N_{ε}–Boc-L-lysine, dichloromethane (DCM), trifluoroacetic acid (TFA), ethyl acetate (EtOAc), tetrahydrofuran (THF), hydrochloric acid, lysozyme (chicken egg white), cytochrome *c* (bovine heart), α-chymotrypsinogen A (bovine pancreas), ribonuclease A (bovine pancreas), deoxyribonuclease I (bovine pancreas), α-lactalbumin (bovine milk), β-lactoglobulin A (bovine milk), β-lactoglobulin B (bovine milk), myoglobin (equine heart), albumin (bovine serum), human sera, and individual human sera proteins (IgG, transferrin, and albumin) were purchased from Sigma-Aldrich (St. Louis, MO). All proteins were ≥ 85% pure. Sodium phosphate dibasic, sodium hydroxide, and methanol were purchased from Fisher Scientific (Fair Lawn, NJ). All materials were used as received without further treatment or purification. The pH of the BGE, 20 mM phosphate buffer, was adjusted using 1 M HCl or 1 M NaOH. The buffer was filtered using a 0.45 μm polypropylene filter (Nalgene, Rochester, NY) and sonicated for 15 minutes prior to use. Protein stock solutions were prepared at 4 mg/mL in the BGE and diluted to a working concentration of 0.4 mg/mL.

2.2.2 Synthesis of a Lysine-based Zwitterionic Molecular Micelle

The poly-ε-sodium-undecanoyl lysinate (poly-ε-SUK) shown in Figure 2.1 was prepared by use of a modified method reported earlier.[20] The undecylenic acid N-hydroxysuccinimide ester and ε-Boc-lysine were stirred for 48 hrs to form the lysine based surfactant monomer. Sodium bicarbonate was used to neutralize the carboxylic acid to obtain the sodium salt of the surfactant

monomer. The mass spectrum as well as ^{1}H and ^{13}C NMR characterization (in D_2O) are shown in the APPENDIX I (I-A, I-B, and I-C, respectively). Elemental analysis values obtained ε-Boc-SUK, shown in APPENDIX I-D, were in close agreement with theoretical values (C 61.81%, H 9.05%, O 18.41%, N 6.45%). The polymerization of epsilon-butoxycarbonyl-sodium-undecenoyl lysinate (ε-Boc-SUK) was achieved by γ-irradiation of a 0.1 M surfactant solution using a ^{60}Co source. The ^{1}H and ^{13}C NMR characterization (in D_2O) are shown in the APPENDIX II (II-A and II-B, respectively). Deprotection of the Boc group from the resulting

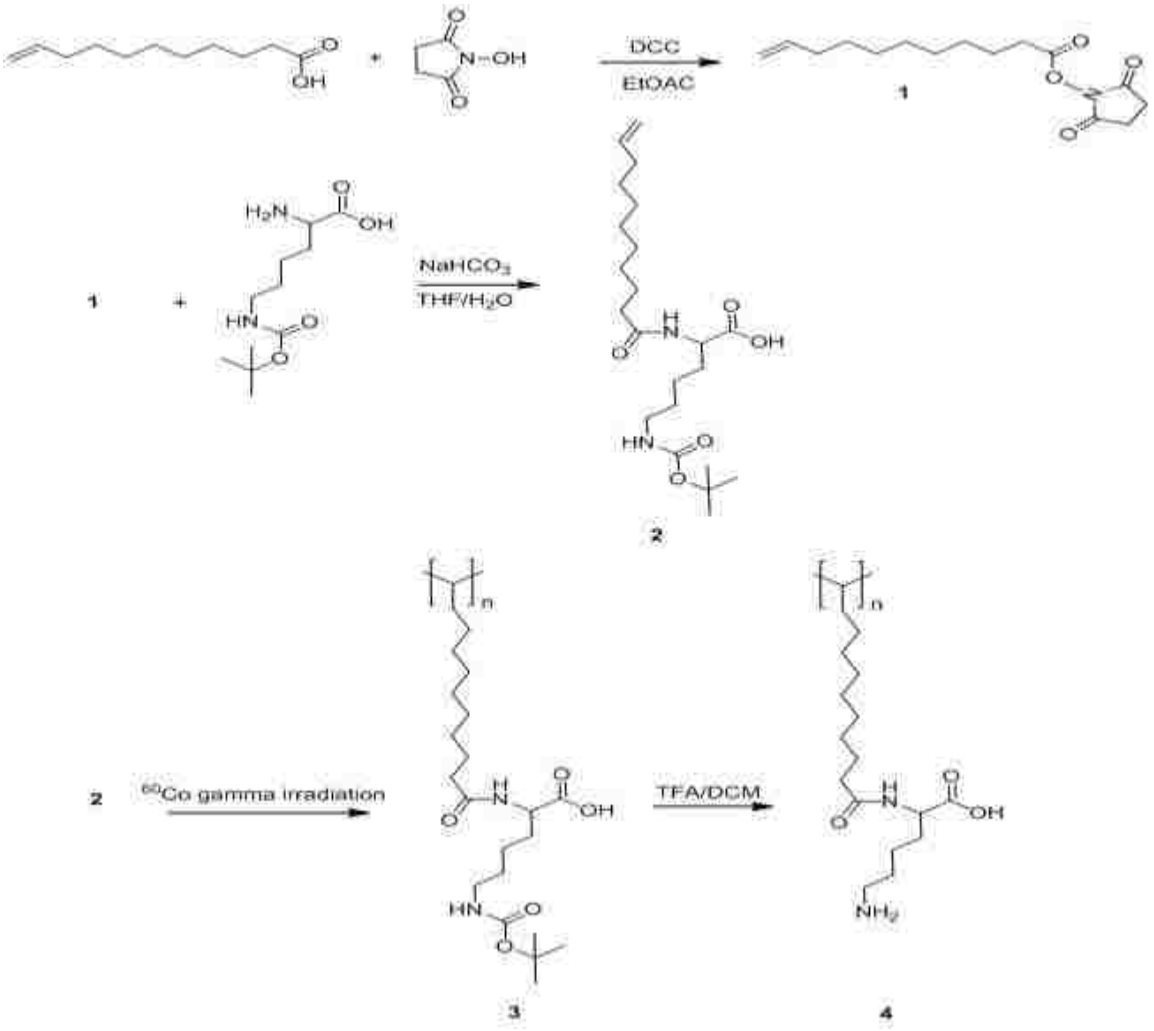


Figure 2.1 Synthesis of poly-ε-SUK.

polymer was accomplished with a 50%TFA/DCM solution.[49]

2.2.3 Tensiometric Characterization of Micellar Solutions

Surface tension measurements for all micellar solutions were acquired in triplicate at room temperature using a KSV Sigma 703 Digital Tensiometer. Special care was taken to thoroughly clean the platinum-iridium ring of the tensiometer with deionized water and acetone prior to each measurement. The cmc of ε-Boc-SUK surfactant in water (7.78mM) was determined by measuring the surface tension of the surfactant solution over a range of concentrations. The resultant plot of surface tension versus ε-Boc-SUK concentration is shown in the APPENDIX I-E. Note that the interception of the two lines indicates the CMC of ε-Boc-SUK. The CMC value obtained for ε-Boc-SUK was 9.05 mM**.**

2.2.4 Atomic Force Microscopy

Images of the poly-ε-SUK films were acquired at ambient conditions with a model 5500 scanning probe microscope (SPM) equipped with Picoscan v5.3.3 software (Agilent Technologies, Inc., Chandler, AZ). Topographic images were acquired using contact mode in buffer with a scan rate of 3.0 nm/s for 512 lines/frame. The AFM probes used for surface characterizations were oxide-sharpened silicon nitride tips, MSCT-AUHW (Veeco, Santa Barbara, CA) with an average force constant of 0.5 N/m. To minimize tip-sample adhesion, the AFM tips were coated with octadecyltrichlorosilane (OTS) purchased from Gelest, Inc, (Morrisville, PA).[50] To coat the AFM tip, the cantilever was first exposed to UV light (254 nm) for 1 h, then immersed in a solution of 1 mM OTS in a 7:3 v/v mixture of hexadecane and chloroform for 1 h. The tips were then rinsed in chloroform, dried in air and stored until needed. Images were processed using Gwyddion version 2.5, open source software for data visualization and analysis, supported by the Czech Metrology Institute.[51]

Substrates used for AFM investigations were pieces of polished silicon wafers (Virginia Semiconductors, Fredericksburg, VA) that were cleaned by immersion in piranha solution for 1 h. Pirahna solution was freshly prepared by mixing sulfuric acid and 30% hydrogen peroxide (3:1 v/v). Caution should be taken since piranha is highly reactive and corrosive. The surfaces were then rinsed with copious amounts of deionized water. To simulate the conditioning of a fused-silica capillary column, the cleaned substrates were sonicated in 1 M NaOH for 30 min and deionized water for 15 min. To prepare films of poly-ε-SUK micelles, clean substrates were immersed in 0.4% (w/v) solution of poly-ε-SUK in 20 mM phosphate buffer pH 3.0 for 20, 90, and 420 min, respectively. The samples were then analyzed immediately using contact-mode AFM in 20 mM phosphate buffer, pH 3.

2.2.5 OT-CEC Column Fabrication and Separation Methods

Separations were performed using a Beckman P/ACE MDQ capillary electrophoresis system equipped with a UV photodiode array detector (Fullerton, CA). Fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ). Each capillary was 50 cm total length (40 cm effective length) and had an internal diameter of 50 μm for all separations. Capillaries were conditioned by rinsing with 1 M NaOH for 30 minutes followed by deionized water for 15 min. The conditioned capillaries were then coated by rinsing with poly-ε-SUK solutions. Coated capillaries were flushed with the corresponding 20 mM sodium phosphate dibasic buffer. All rinses were performed using a pressure of 20 psi. Separations were performed under reverse or normal polarity at temperature of 15 to 25°C with an applied voltage of 10 to 25 kV. Electrokinetic injections were performed by applying +/-5 kV for 5 s. Electropherograms were collected at 200 nm.

2.3 Results and Discussion

OT-CEC protein separations are influenced by at least three factors: electrophoretic mobility, stationary phase interactions, and the EOF. Proteins are separated in CE due to differences in electrophoretic mobility based on their charge to size ratio. Protein interactions with the stationary phase are also an important factor to consider in order to provide greater peak resolution. The EOF is the bulk flow of the solution and a major component of an OT-CEC method. The EOF is established by the charge of and/or on the capillary wall, an applied electric field, and attraction of ions of the BGE to the capillary surface charge.[52, 53] Due to the isoelectric point of the zwitterionic coating, the overall charge can be more positive if the pH of the BGE is below the pI of the coating or more negative if above. Thus, μ_{EOF} can be manipulated through controlled changes in pH.

2.3.1 Evaluation of Zwitterionic Character of the Micellar Stationary Phase as a Function of pH

Tunability of the EOF is an important factor for a zwitterionic coating capable of separation at both acidic and basic pH values. Control of the EOF is due to protonation of both the amine and carboxyl groups of the zwitterionic coating, resulting in an overall positive charge in acidic media. Conversely, deprotonation of the amine and carboxyl groups results in an overall negative charge. Increased dominance of one of the charged species relates to increased electroosmotic mobility of the EOF marker, i.e. μ_{EOF}. In Figure 2.2, the influence of the μ_{EOF} is demonstrated for pH values ranging from 3.0 to 9.0. To acquire these data, each new coating was applied to a bare capillary at the corresponding separation pH. The capillary was then flushed for 2 minutes with the same buffer. The absence of the EOF marker in reverse or normal mode at pH values near the isoelectric point of the zwitterionic molecular micelle was due to severely diminished EOF. At pH values approaching the isoelectric point, i.e. approximately pH

5.5, the zwitterionic molecular micelle had a net charge of zero, and thus no attraction to the cationic and anionic components in the solution, which caused stagnation of the bulk flow. Deviations from the isoelectric point yielded an increased μ_{EOF} due to either higher net positive or net negative charge. Coated capillaries reflected an anodic EOF at low pH and conversely a

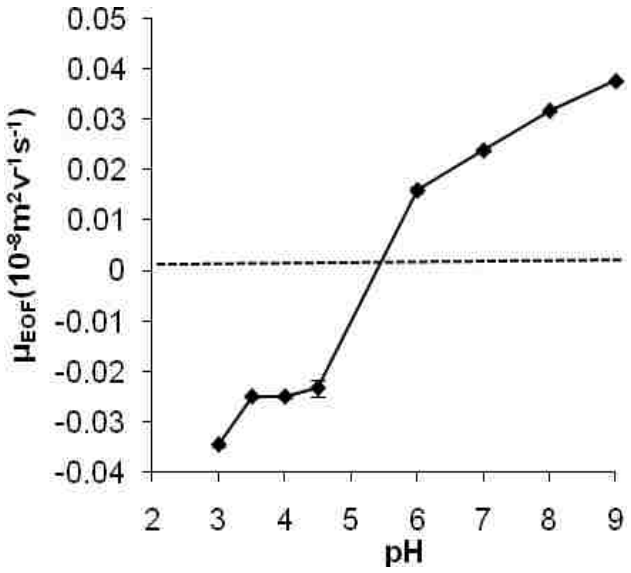


Figure 2.2 Effect of BGE pH on μ_{EOF} using poly-ε-SUK coating. **Conditions: Coating time:** 20 min at pH 3; **NaCl concentration:** 20mM; **Background electrolyte:** 20mM sodium phosphate dibasic; **Applied voltage:** 25 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50μm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** +/-5 kV for 5 s; **Detection:** 254 nm.

cathodic EOF at high pH. Such changes cannot be observed in a bare capillary which can only exhibit a cathodic EOF as has been previously reported by Wang et al.[52]

2.3.2 Characterizations of Zwitterionic Micellar Coating on Silicon using AFM

It is expected that increasing the thickness of a stationary phase in OT-CEC will yield more interactions with analytes.[52, 54] Nanofabrication with AFM was used to evaluate the changes in thickness for coatings formed on the silicon surface. Changes in the thickness of micellar coatings were evaluated at acidic pH, using the conditions optimized for protein

separation. To prevent the surface from supersaturation at higher concentrations of polymer, a parallel study using AFM with varying polymer coating times was investigated while holding concentration constant. As a baseline, AFM topography views of the clean, uncoated silicon surface are presented side-by-side with the coated substrate in Figure 2.3. Considerable changes are observed for the AFM topography images of the clean surface of silicon (Figure 2.3A) versus the film of poly-ε-SUK formed after 420 min immersion (Figure 2.3B). The silicon surface has a local surface roughness measuring 0.75 nm for the 4×4 μm^2 area displayed in Figure 2.3A. The brighter colors represent taller features, whereas shallow features are darker. The images displayed are representative of views for many different areas of the sample. The cleaned surface of silicon exhibits a somewhat irregular and uneven morphology at the nanoscale, with depressions or pits ranging from 0.3 to 2.9 nm in depth. The silicon surface does not contain visible contaminants or residues, indicating success of the cleaning procedure. Complete coverage of the surface with a film of micelles was apparent after 420 min of immersion in poly-ε-SUK solution (Figure 2.3B). The surface is coated with densely packed micelles, which range in height from 0.8 to 6 nm, corresponding to single and multiple layers. The shapes of the molecular micelles are nearly spherical, and are consistent with observations gleaned from previous reports for different types of micelles formed on mica[55] and silicon.[32] The slightly non-spherical shape of the micelles may be attributed to the shape of the probe, caused by the well-known tip-surface convolution effects of AFM tip geometry. The holes and roughness of the underlying substrate are still apparent after forming a coating of poly-ε-SUK. The surface roughness measured for the topography frame in Figure 2.3B measures 1.3 nm. The size of the micelles is quite uniform and monodisperse, as evident in the topograph and corresponding histogram of Figure 2.3C. Most of the micelles (52%) measure from 1.6 to 2.4 nm in height,

with an overall average size of 2.3 ± 1.6 nm. However, the roughness of the bare silicon substrate (0.75 nm) complicates the precise measurement of the micelle diameter, and the peaks and valleys of the substrate contribute to the overall dimensions plotted in the histogram. Nanoshaving was used to evaluate the local thickness of poly-ε-SUK coatings deposited onto silicon substrates. Tip induced displacement of surface films of multilayers of polymeric micelles was previously demonstrated by Emoto, et al.[56] Nanoshaving is accomplished by applying a sufficiently high force to the AFM tip to remove or "shave" away a small region of the film in order to expose a bare area of the silicon substrate. The uncovered area then provides a baseline for measuring the film thickness.[57] For the film of adsorbed zwitterionic micelles, the nanoshaved rectangular area of the silicon surface measures 600 × 900 nm^2 and is clearly visible in both the topography and lateral force images of Figures 2.4A and 2.4B, respectively. Note that the area was swept seven times with an applied set point of 10 nN to ensure complete removal of poly-ε-SUK. Micelle nanostructures are visible throughout the scan area except within the nanoshaved pattern. The lateral force image (Figure 2.4B) shows that micelles have been cleanly removed from the substrate, since the exposed polished silicon wafer has a smooth, homogeneous contrast as compared to the unshaved region. A representative cursor line profile across the pattern is presented in Figure 2.4C. The local thickness of the film measured 2.4 ± 0.7 nm, in agreement with the height values measured for individual micelles from Figure 2.3. To better evaluate the film thickness of poly-ε-SUK formed under different immersion intervals, additional nanoshaving experiments were conducted (AFM images not shown). These results are summarized in Table 2.1 for thickness values obtained from cursor measurements of nanoshaved patches from several nanoshaving experiments. After only 20 min of immersion, incomplete surface coverage was observed. As one would expect, as the length of immersion

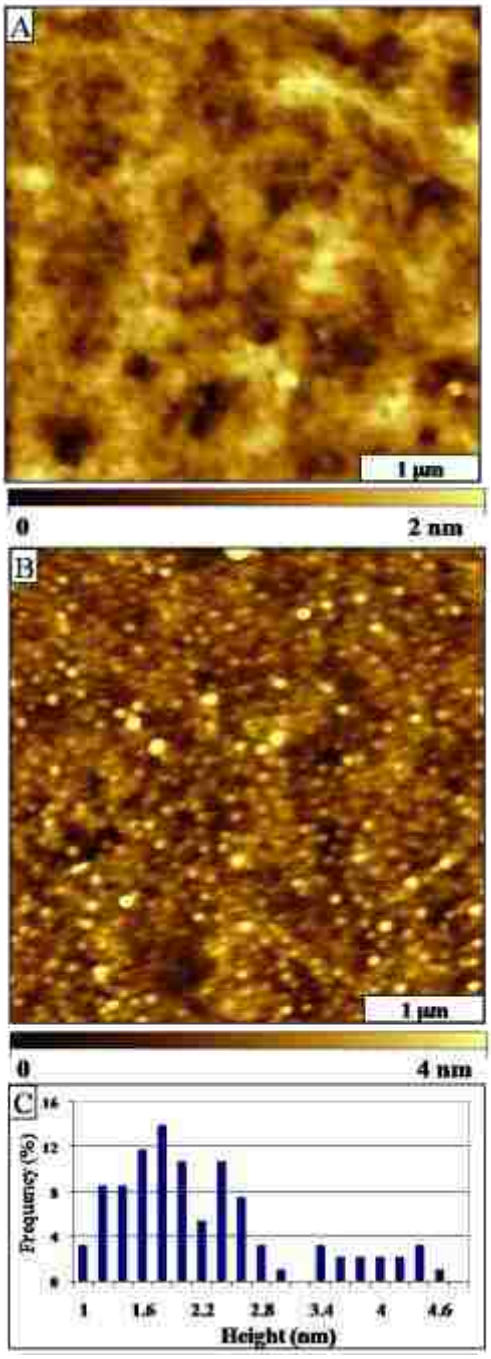


Figure 2.3 Changes in surface morphology viewed with contact-mode AFM topographs. [A] Clean surface of polished silicon; [B] after immersion in 0.4% (w/v) poly-ε-SUK; [C] size distribution for the micelles measured from individual cursor height profiles.

Table 2.1 Thickness of micelle layers formed on silicon after different immersion intervals.

Immersion time (min)	Film thickness (nm)
20	0.98 ± 0.12
120	1.9 ± 0.38
420	2.4 ± 0.74

was increased, a thicker layer micelles was observed to form.

In situ AFM characterization of films of lysine-based zwitterionic molecular micelles demonstrate that uniform and structurally precise micelle structures have been produced, with regular spherical shapes that persist after adsorption on silicon. The supramolecular assembly of zwitterionic micelles into multilayered coatings can be controlled by immersion intervals, within time frames of a few hours. Although micelles have been previously shown to disassemble and form bilayer coatings on certain substrates, our results with *in situ* AFM clearly demonstrate that controlling pH and solvent parameters ensure conditions for robust, persistent molecular micellar structures.

2.3.3 Simultaneous Acidic and Basic Protein Separation by OT-CEC under Acidic Conditions

The use of poly-ε-SUK as a coating in OT-CEC allowed minimization of protein-wall adsorption and provided a stationary phase for analyte interaction. Figure 2.5 is a display of the effect of poly-ε-SUK concentration on the simultaneous separation of 10 acidic and basic proteins (Table 2.2) under cathodic EOF. The polymer concentration ranged from 0.3 to 0.5% (w/v). At lower concentrations (0.3% w/v), long migration times were observed, as well as low separation reproducibility which is likely the result of partial coverage of the polymer on the capillary wall. Having non-uniform coating allowed protein-wall adsorption which may have resulted in low peak efficiencies. Correspondingly, not all individual proteins could be

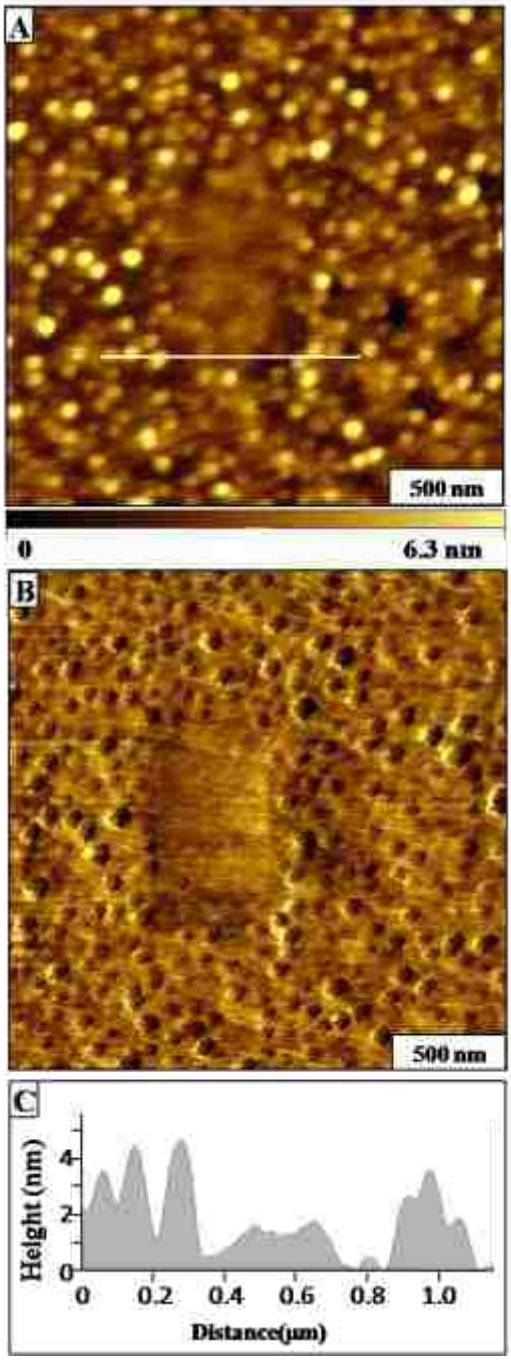


Figure 2.4 Nanoshaved area produced by increasing the force applied to an AFM tip. [A] Contact-mode topograph; [B] corresponding friction image; [C] height profile for the line in **A**.

identified. R_{s1} denotes the resolution between the first and second peaks, R_{s2} for the second and third peaks, R_{s3} for the third and fourth peaks, and so on.

Table 2.2 Protein system selected for OT-CEC separation.

Proteins	Source	pI	MW
α-lactalbumin	bovine milk	4.8	14.2
β-lactoglobulin A	bovine milk	5.1	36.7
β-lactoglobulin B	bovine milk	5.3	36.6
Albumin	bovine serum	4.7	69.0
deoxyribonuclease I	bovine pancreas	6.7	31.0
Myoglobin	equine heart	6.8	17.0
α-chymotrypsinogen A	bovine pancreas	9.2	25.0
ribonuclease A	bovine pancreas	9.3	13.7
cytochrome *c*	bovine heart	10.2	12.4
Lysozyme	chicken egg white	11.1	14.3

Using 0.4% (w/v), 9 protein peaks were resolved (R_{s1} = 3.42 R_{s2} = 1.02 R_{s3} = 2.07 R_{s4} = 0 R_{s5} = 1.83 R_{s6} = 5.13 R_{s7} = 0.59 R_{s8} = 1.32 R_{s9} = 4.58). Partial separation occurred between β-lactoglobulin A, and β-lactoglobulin B while α-lactalbumin and ribonuclease A coeluted. All other peaks were baseline resolved owing to thicker coating which in turn promoted interactions with the analytes.

As the polymer concentration was increased to 0.5% (w/v) protein migration times increased and efficiencies decreased, while protein peak migration order was the same as 0.4%(w/v), with 9 protein peaks being resolved (R_{s1} = 3.29 R_{s2} = 0.51 R_{s3} = 0.95 R_{s4} = 0 R_{s5} = 1.90 R_{s6} = 3.16 R_{s7} = 0.41 R_{s8} = 1.54 R_{s9} = 4.70). Due to increased thickness of the coating, further increases in elution times produced longer analysis times. Therefore, 0.4% (w/v) was chosen as the optimal polymer concentration value as a result of this study.

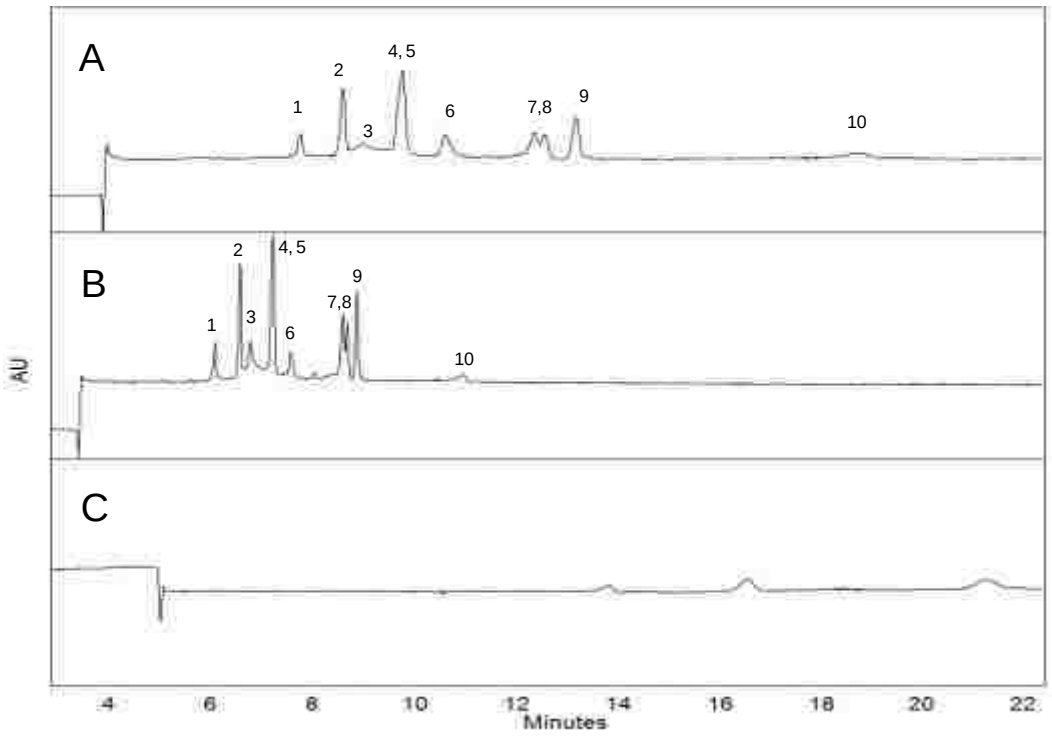


Figure 2.5 Influence of polymer concentration on separation of 10 acidic and basic proteins. **Conditions: Polymer concentration:** (A) 0.5% (B) 0.4% (C) 0.3% (w/v) poly-ε-SUK; **Coating time:** 20 min; **Background electrolyte:** 20mM sodium phosphate dibasic (pH 3); **Applied voltage:** 25 kV; **Temperature:** 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5s; **Detection:** 200 nm; **Analytes: 1.** deoxyribonulease I, 2. α-chymotrypsinogen A, **3.** albumin, **4**. α-lactalbumin, **5**. ribonuclease A, **6**.myoglobin **7.** β-lactoglobulin A, **8**. β-lactoglobulin B, **9**. lysozyme, **10**. cytochrome *C*.

2.3.4 Influence of NaCl Concentration on Protein Separation

Previous studies have demonstrated that the presence of NaCl in capillary coatings increases the thickness of the coating[56, 58] and in turn increases protein separation resolution.[16, 39, 59] Figure 2.6 shows the effect of NaCl concentration in poly-ε-SUK coatings on the above protein system in a selected concentration range of 15-25 mM (Table 2.3). When 25 mM NaCl was used, 8 peaks were observed. Protein peaks 4, 5 (α-lactalbumin and ribonuclease A) and 7, 8 (β-lactoglobulin A and β-lactoglobulin B) were not resolved. Using 20 mM NaCl, all 10

protein peaks were distinctively resolved. All peaks with the exception of peaks 4, 5 (α-lactalbumin and ribonuclease A) and 7, 8 (β-lactoglobulin A and β-lactoglobulin B) were baseline resolved. At 15 mM, 9 protein peaks eluted. Protein peaks 2, 3 (α-chymotrypsinogen A and albumin) were not resolved while 4, 5 (α-lactalbumin and ribonuclease A) and 7, 8 (β-lactoglobulin A and β-lactoglobulin B) were partially resolved. Therefore, 20 mM NaCl was selected as the optimal concentration and adopted for all subsequent separations.

2.3.5 Simultaneous Acidic and Basic Protein Separation by OT-CEC under Basic Conditions

Due to the zwitterionic properties of the coating, separations performed under cathodic EOF can be achieved as quickly as under anodic EOF. To examine the performance of this coating under anodic EOF conditions, the separation was performed at pH 11.5. Figure 2.7 shows the optimized separation of acidic and basic proteins under basic experimental conditions. The voltage was lowered to 10 kV and the poly-ε-SUK coating time was lowered to 15 min in contrast to the optimized separation at acidic pH. All other variables were held constant (0.4% (w/v) poly-ε-SUK, 20 mM sodium phosphate, 15°C). The EOF was observed at 9.8 minutes and 9 protein peaks were resolved. All resolved protein peaks had at least baseline resolutions, i.e. greater than 1.5. However, peaks 7 and 8 (β-lactoglobulin A and β-lactoglobulin B) co-eluted and were not resolved under any variations of voltage, salt concentration, temperature, or other experimental conditions.

2.3.6 Reproducibility of a Lysine-Based Zwitterionic Molecular Micelle-Coated Capillary

To determine the stability of the poly-ε-SUK coating, run-to-run, day-to-day, and week-to-week studies were investigated. The reproducibility of the EOF was evaluated by monitoring the average retention time of the EOF marker and % relative standard deviation (%RSD). The

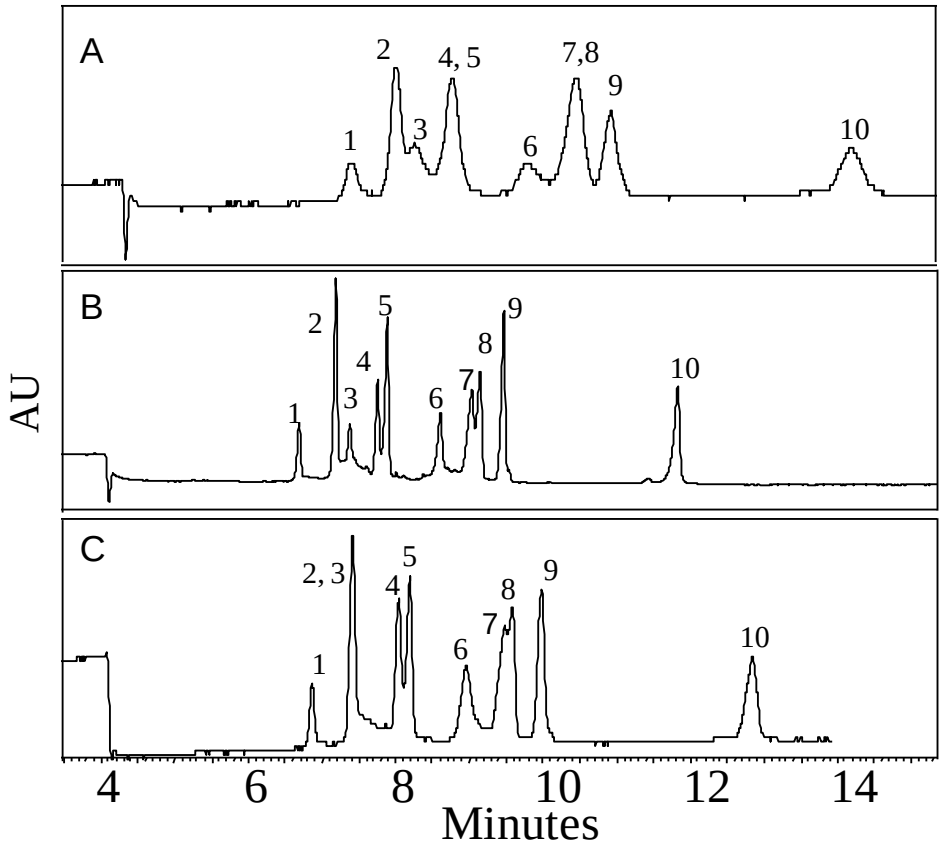


Figure 2.6 Effects of NaCl concentration on separation of 10 acidic and basic proteins. **Conditions: Polymer concentration:** 0.4%(w/v) poly-ε-SUK; **Coating time:** 20 min; **NaCl concentration:** (A) 25mM (B) 20mM (C) 15mM; **Background electrolyte:** 20mM sodium phosphate dibasic (pH 3); **Applied voltage:** 25 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50μm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. deoxyribonulease I, 2. α-chymotrypsinogen A, 3. albumin, **4**. α-lactalbumin, **5**. ribonuclease A, **6**.myoglobin **7.** β-lactoglobulin A, **8**. β-lactoglobulin B, **9**. lysozyme, **10**. cytochrome *c*.

results of this study are summarized in Table 2.4. Each capillary was prepared according to the coating procedure previously described for the optimized separation at acidic pH. The run-to-run reproducibility for the EOF was calculated based on 50 consecutive runs using the same capillary. The day-to-day reproducibility for the EOF was evaluated based on the use of one capillary for 5 days with replicate analyses; the week-to-week reproducibility was evaluated

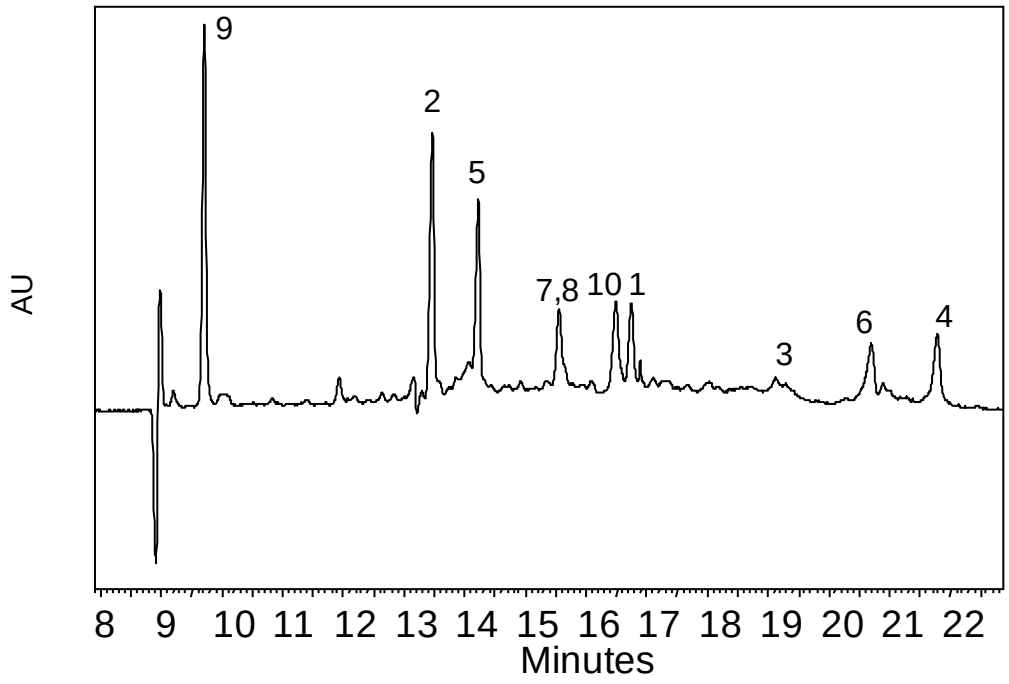


Figure 2.7 Optimized separation of acidic and basic proteins under basic experimental conditions. **Conditions: Polymer concentration:** 0.4%(w/v) poly-ε-SUK; **Coating time:** 15 min; **NaCl concentration:** 20mM; **Background electrolyte:** 20mM sodium phosphate dibasic (pH11.5); **Applied voltage:** 10 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. deoxyribonulease I, 2. α-chymotrypsinogen A, 3. albumin, **4**. α-lactalbumin, **5**. ribonuclease A, **6**.myoglobin **7.** β-lactoglobulin A, **8**. β-lactoglobulin B, **9**. lysozyme, **10**. cytochrome *c*.

Table 2.3 Resolutions calculated from protein separations.

		R_{s1}	R_{s2}	R_{s3}	R_{s4}	R_{s5}	R_{s6}	R_{s7}	R_{s8}	R_{s9}
pH 3	25mM NaCl	3.01	2.94	0.76	2.30	0.88	0.21	1.08	7.80	0
	20mM NaCl	2.26	1.50	2.55	1.01	4.52	1.96	0.52	1.96	13.62
	15mM NaCl	1.17	0.34	0.60	0	1.17	0.92	0	0.45	4.05
pH 11.5	20mM NaCl	19.65	4.15	5.53	0	3.10	0.95	3.81	2.25	3.37

*0.4% (w/v) poly-ε-SUK was used for each salt concentration

based on 5 different capillaries with 3 consecutive runs for each. All measured %RSD were below 4%, indicative of highly acceptable reproducibilities.

Table 2.4 Reproducibility studies of the poly-ε-SUK coating.
Conditions: Polymer concentration: 0.4 %(w/v) poly-ε-SUK; **Coating time:** 20 min; **NaCl concentration:** 20mM; **Background electrolyte:** 20mM sodium phosphate dibasic (pH 3); **Applied voltage:** 15 kV; **Temperature:** 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50μm i.d.; **EOF marker:** acetone; **Injection:** 5 kV for 5 s; **Detection:** 254 nm.

	Ave. EOF migration time(min)	**%RSD of EOF**
Run to Run (n=50 runs)	**3.95**	**1.24**
Day to Day (n=5 days)	**4.08**	**1.20**
Week to Week (n=3 weeks)	**4.19**	**1.85**
Capillary to Capillary (n=5 capillaries)	**4.09**	**3.15**

2.3.7 Investigation of Column Robustness

The column robustness was investigated based on changing one variable (+/-) 10% while keeping other optimized variables the constant. Variables of interest for column robustness included: polymer concentration, coating time, and salt concentration. Polymer concentration values were 0.44 and 0.36% (w/v). Coating time values were varied at 22 and 18 mins. Sodium chloride concentration values were 22 and 18 mM. Resolution values are calculated in Table 2.5 below.

At a concentration of 0.44% (w/v) poly-ε-SUK, only eight peaks were observed in Figure 2.8. Analysis time was increased for higher concentrations of polymer used. Increased concentration results in increased interactions for protein analytes. Resolution increased for all protein peaks with the exception of peaks 2 and 3 (α-chymotrypsinogen A and albumin) as well

as peaks 6 and 7 (myoglobulin and β-lactoglobulin A) which in each case complete coelution of peaks was observed. A decrease in resolution was observed for other peaks which could be due

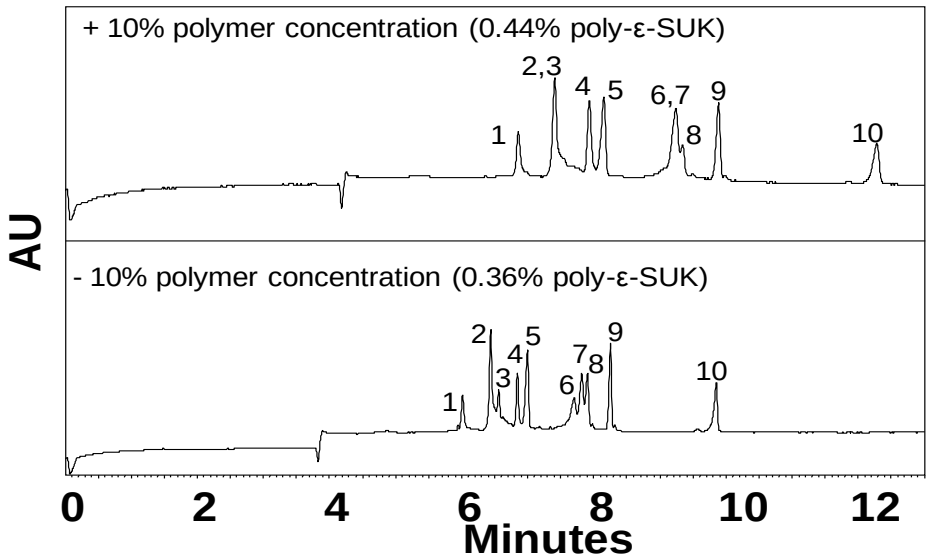


Figure 2.8 Column robustness investigating +/- 10% variations in optimized polymer concentration. **Conditions: Polymer concentration:** 0.44%, 0.36% (w/v) p-ε-SUK; **Coating time:** 20 min; **NaCl concentration:** 20mM; **Background electrolyte:** 20mM sodium phosphate dibasic (pH 3); **Applied voltage:** 25 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. deoxyribonuclease I, 2. α-chymotrypsinogen A, 3. albumin, **4**. α-lactalbumin, **5**. ribonuclease A, **6**. myoglobin **7.** β-lactoglobulin A, **8**. β-lactoglobulin B, **9**. lysozyme, **10**. cytochrome *c*.

to band broadening. Decreases in resolution were observed by peaks 5 and 6 (myoglobin/β-lactoglobulin A and β-lactoglobulin B) as well as lysozyme and cytochrome *c*. The loss of resolution can be attributed to band broadening of protein analytes.

All protein peaks were observed using a concentration of 35% (w/v) poly-ε-SUK in Figure 2.8. Separation analysis time was decreased due to fewer interactions with all analytes. Resolution was decreased for peaks 2 and 3 (α-chymotrypsinogen A and albumin), peaks 6 and 7 (myoglobulin and β-lactoglobulin A), and peaks 9 and 10 (lysozyme and cytochrome *c*). However, resolution increased for all other peaks. This increase can be due to having a decrease

in concentration, which would result in decreased interaction and lesser band broadening to enhance resolution.

Table 2.5. Resolutions calculated from column robustness experiments using poly-ε-SUK.

	R_{s1}	R_{s2}	R_{s3}	R_{s4}	R_{s5}	R_{s6}	R_{s7}	R_{s8}	R_{s9}
0.44% (w/v)	3.95	0	3.55	1.46	5.22	0	0.47	3.02	13.54
0.36% (w/v)	4.24	1.19	2.89	1.58	7.04	0.69	0.55	3.06	13.34
22 min coat	4.27	1.31	2.72	1.58	4.13	1.20	0.59	2.39	12.51
18 min coat	3.51	3.59	0	1.49	4.16	0.74	0.48	2.60	10.36
22mM NaCl	4.16	1.09	2.86	1.40	4.00	1.08	0.71	2.91	11.85
18mM NaCl	3.87	1.33	2.40	0.81	3.50	1.76	0.54	2.10	11.44

*all other variables were held constant as from Figure 2.7 B unless stated differently above

For coating at 22 mins, separation analysis times were longer due to higher concentrations of poly-ε-SUK on the capillary wall to result in higher interactions in Figure 2.9. All protein peaks are observed using the 22 min coating time. Higher resolution was observed for all peaks except peaks 2 and 3 (α-chymotrypsinogen A and albumin), peaks 5 and 6 (ribonuclease A and myogloblin), peaks 6 and 7 (myoglobulin and β-lactoglobulin A), and peaks 9 and 10 (lysozyme and cytochrome *c*). Lower resolution was observed due to band broadening.

Coating times at 18 mins, separation analysis times were longer in Figure 2.9. Using decreased coating times may result in less surface coverage of poly-ε-SUK. Protein analytes may have interacted with the capillary wall to result in longer analysis times. When using a shorter coating time, interactions between protein analytes and the polymer coating should be decreased. Only 8 protein peaks were observed. Protein peaks 3 and 4 (albumin and α-lactalbumin) coeluted. A decrease in resolution was observed for all other protein peaks with the

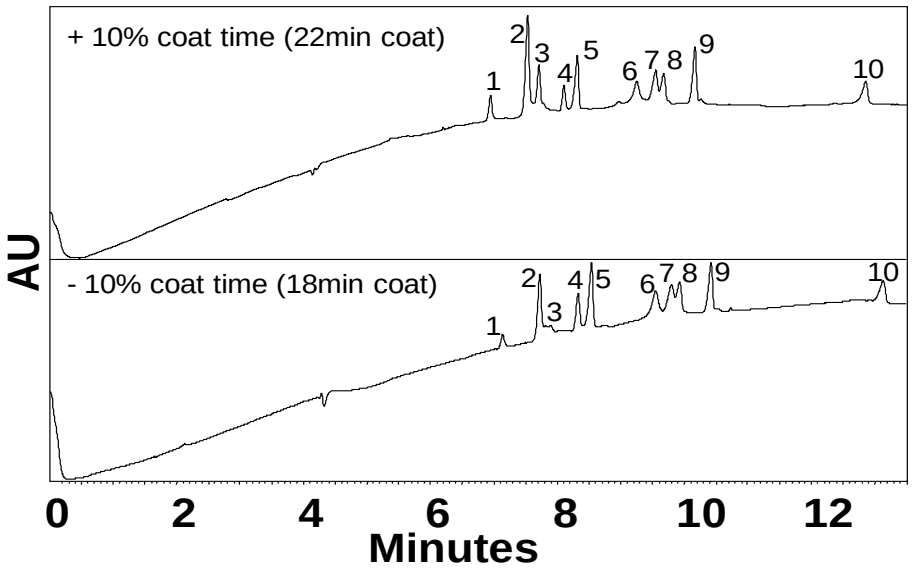


Figure 2.9 Column robustness investigating +/- 10% variations in optimized polymer coat time. **Conditions: Polymer concentration:** 0.4%(w/v) p-ε-SUK; **Coating time:** 18, 22 min; **NaCl concentration:** 20mM; **Background electrolyte:** 20mM sodium phosphate dibasic (pH 3); **Applied voltage:** 25 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. deoxyribonuclease I, 2. α-chymotrypsinogen A, 3. albumin, **4**. α-lactalbumin, **5**. ribonuclease A, **6**.myoglobin **7.** β-lactoglobulin A, **8**. β-lactoglobulin B, **9**. lysozyme, **10**. cytochrome *c*.

exception of peaks 1 and 2 (deoxyribonuclease I and α-chymotrypsinogen A), peaks 2 and 3 (α-chymotrypsinogen A and albumin), peaks 4 and 5 (α-lactalbumin and ribonuclease A), and peaks 8 and 9 (β-lactoglobulin B and lysozyme). An increase in resolution may be due to having less band broadening of peaks with lower polymer concentration on the capillary wall.

The salt concentration (NaCl) was varied from 18 to 22 mM in Figure 2.10. All protein peaks were observed for both changes in salt concentration. The same trend was observed for all protein peaks except peaks 3 and 4 (albumin and α-lactalbumin) and peaks 4 and 5 (α-lactalbumin and ribonuclease A). An increase in resolution was observed for higher salt concentrations while a decrease was observed for lower concentrations. These protein analytes are more sensitive to polymer swelling for the concentrations observed.

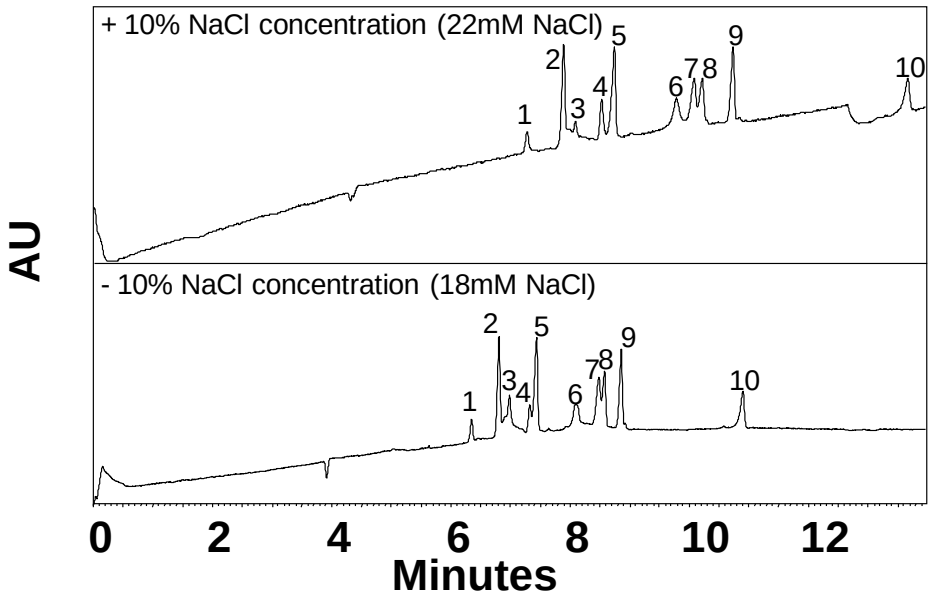


Figure 2.10 Column robustness investigating +/- 10% variations in optimized NaCl concentration.

Conditions: Polymer concentration: 0.4%(w/v) p-ε-SUK; **Coating time:** 20 min; **NaCl concentration:** 18, 22mM; **Background electrolyte:** 20mM sodium phosphate dibasic (pH 3); **Applied voltage:** 25 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. deoxyribonuclease I, 2. α-chymotrypsinogen A, 3. albumin, **4**. α-lactalbumin, **5**. ribonuclease A, **6**.myoglobin **7.** β-lactoglobulin A, **8**. β-lactoglobulin B, **9**. lysozyme, **10**. cytochrome *c*.

2.3.8 Separation of Proteins from a Human Serum Sample using a Lysine-Based Zwitterionic Molecular Micelle Coating

A sample of human serum, used as received from Sigma Aldrich, was evaluated using the optimized conditions for both acidic and basic pH values. The results of these studies are provided in Figure 2.11. For the acidic pH separation, the human serum was diluted with buffer by a factor of 8. However, under basic conditions a more concentrated mixture with a dilution factor of 2 was used. Peak areas were observed to correlate with the amount of protein found from normal human serum samples reported in the literature (IgG 8-18 mg/mL, transferrin 2-3 mg/mL, and albumin 35-50 mg/mL).[41, 60] The separations achieved at acidic and basic pH values were obtained under the same optimized conditions used in Figures 2.6B and 2.7, respectively.

Contributions from sample impurities along with degradation products of the three major proteins may lead to peaks of low efficiencies since protein standards did not exhibit such

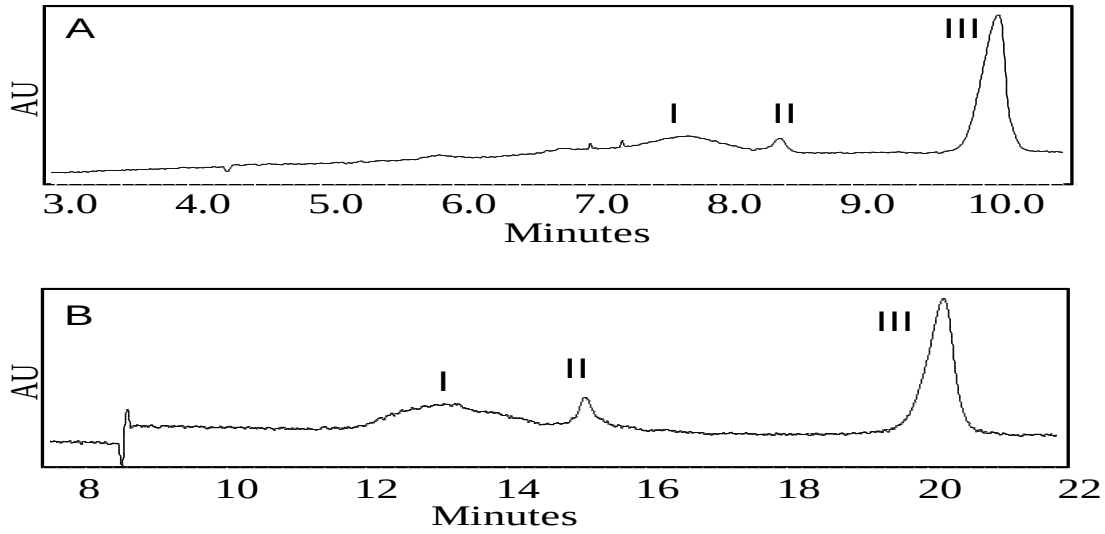


Figure 2.11 Separation of Human Serum Proteins using Zwitterionic Coating. **Conditions:** (A) same as Figure 6B, pH 3 (B) same as Figure 7, pH 11.5; **Human Serum:** (A) dilution factor of 8, (B) dilution factor of 2; **Peaks:** I. IgG II. Transferrin, III. Albumin.

behavior. Both acidic and basic optimized conditions resulted in the same elution order of proteins. However, separation under basic conditions provided better resolution than under acidic conditions. Thus, this separation shows the versatility of using lysine-based molecular micelles for the separation of proteins from human serum with no purification of the sample.

2.3.9 Reproducibility of a Lysine-Based Zwitterionic Molecular Micelle-Coated Capillary for Proteins in Human Serum

The reproducibility of the poly-ε-SUK coating was evaluated using the human serum sample in Table 2.6. Protein analytes (IgG, transferring, and albumin) were observed on the same capillary for three consecutive runs. One column was used for acidic pH while the other was for basic pH. All runs show less than 1% RSD in the migration time of the analyte peak of interest. The poly-ε-SUK coated column exhibited excellent reproducibilities for the human serum sample.

Table 2.6 Reproducibility of protein in human serum peak migration times using optimized acidic and basic conditions.

pH	Protein	Average migration time	%RSD (n=3)
Acidic	IgG	6.29	0.67
	Transferrin	6.80	0.86
	Albumin	7.66	0.85
Basic	IgG	13.27	0.56
	Transferrin	15.19	0.49
	Albumin	20.37	0.76

2.4 Concluding Remarks

The synthesis, characterization, and application of a lysine-based zwitterionic molecular micelle (poly-ε-SUK) for capillary coatings in OT-CEC are presented in this study. The versatility of poly-ε-SUK is demonstrated through simultaneous separation of acidic and basic proteins at both low and high pH values. Most of the proteins studied were baseline resolved. This novel zwitterionic coating also contributed to variations in the μ_{EOF} as a function of systematic changes in pH. It was found that μ_{EOF} approached zero as the pH approached the estimated pI of the zwitterionic molecular micelle (~pH 5.5). High stability of the coating was demonstrated with up to 50 runs on the same capillary, resulting in an RSD of 1.24%. Views acquired with AFM demonstrate that regular, robust spherical geometries of micelles are formed as multilayers on silicon, which retain their structural integrity after surface adsorption. Proteins from an untreated human serum sample were separated and identified under both acidic and basic pH conditions to demonstrate the general utility for separation of complex samples. As a result of these findings, poly-ε-SUK is considered a unique coating with great potential for

simultaneous separation of acidic and basic protein systems.

2.5 References

(1) Kim, S.; Jimenez-Gonzalez, C.; Dale, B. E. Enzymes for Pharmaceutical Applications-a Cradle-to-Gate Life Cycle Assessment. *Int. J. Life C. Ass.* **2009**, *14*, 392-400.

(2) Colombo, M.; Ronchi, S.; Monti, D.; Corsi, F.; Trabucchi, E.; Prosperi, D. Femtomolar Detection of Autoantibodies by Magnetic Relaxation Nanosensors. *Anal. Biochem.* **2009**, *392*, 96-102.

(3) Enback, J.; Laakkonen, P. Tumour-Homing Peptides: Tools for Targeting, Imaging and Destruction. *Biochem. Soc. T.* **2007**, *35*, 780-783.

(4) Kojima, T.; Yoshikawa, K.; Saga, S.; Yamada, T.; Kure, S.; Matsui, T.; Uemura, T.; Fujimitsu, Y.; Sakakibara, M.; Kodera, Y.; Kojima, H. Detection of Elevated Proteins in Peritoneal Dissemination of Gastric Cancer by Analyzing Mass Spectra Data of Serum Proteins. *J. Surg. Res.* **2009**, *155*, 13-17.

(5) Ryu, O. H.; Atkinson, J. C.; Hoehn, G. T.; Illei, G. G.; Hart, T. C. Identification of Parotid Salivary Biomarkers in Sjogren's Syndrome by Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and Two-Dimensional Difference Gel Electrophoresis. *Rheumatology* **2006**, *45*, 1077-1086.

(6) Ye, B.; Skates, S.; Mok, S. C.; Horick, N. K.; Rosenberg, H. F.; Vitonis, A.; Edwards, D.; Sluss, P.; Han, W. K.; Berkowitz, R. S.; Cramer, D. W. Proteomic-Based Discovery and Characterization of Glycosylated Eosinophil-Derived Neurotoxin and COOH-Terminal Osteopontin Fragments for Ovarian Cancer in Urine. *Clin. Cancer Res.* **2006**, *12*, 432-441.

(7) Moayeri, M.; Crown, D.; Dorward, D. W.; Gardner, D.; Ward, J. M.; Li, Y.; Cui, X.; Eichacker, P.; Leppla, S. H. The Heart is an Early Target of Anthrax Lethal Toxin in Mice: A Protective role for Neuronal Nitric Oxide Synthase (nNOS). *Plos Pathogens* **2009**, *5*, 1-13.

(8) Gong, B. L.; Bo, C. M.; Zhu, J. X.; Yan, C. Synthesis of Zwitterionic Stationary Phase Based on Hydrophilic Non-Porous Poly(glycidymethacrylate-co-ethylenedimethacrylate) Beads and Their Application for Fast Separation of Proteins. *J. Appl. Polym. Sci.* **2009**, *113*, 984-991.

(9) Mie, A.; Ray, A.; Axelsson, B. O.; Jornten-Karlsson, M.; Reimann, C. T. Terbutaline Enantiomer Separation and Quantification by Complexation and Field Asymmetric Ion Mobility Spectrometry-Tandem Mass Spectrometry. *Anal. Chem.* **2008**, *80*, 4133-4140.

(10) Charvatova, J.; Deyl, Z.; Kasicka, K.; Kral, V. Open Tubular Capillary Electrochromatography of Underivatized Amino Acids Using Rh(III) tetrakis(phenoxyphenyl)porphyrinate as Wall Modifier. *J. Chromatogr. A* **2003**, *990*, 159-167.

(11) Strege, M.; Lagu, A. Separation of DNA Restriction Fragments by Capillary Electrophoresis Using Coated Fused Silica Capillaries. *Anal. Chem.* **1991** *63*, 1233-1236.

(12) Jorgenson, J. W.; Lukacs, K. D. Capillary Zone Electrophoresis. *Science* **1983**, *222*, 266-272.

(13) Wang, C.; Lucy, C. A. Mixed Cationic/Anionic Surfactants for Semipermanent Wall Coating in Capillary Electrophoresis. *Electrophoresis* **2004**, *25*, 825-832.

(14) Piraino, S. M.; Dorsey, J. G. Comparison of Frits Used in the Preparation of Packed Capillaries for Capillary Electrochromatography. *Anal. Chem.* **2003**, *75*, 4292-4296.

(15) Carney, R. A.; Robson, M. M.; Bartle, K. D.; Myers, P. Investigation into the Formation of Bubbles in Capillary Electrochromatography. *J. High Res. Chromatog.* **1999**, *22*, 29-32.

(16) Luces, C. A.; Fakayode, S. O.; Lowry, M.; Warner, I. M. Protein Separations Using Polyelectrolyte Multilayers Coatings with Molecular Micelles in Open Tubular Capillary Electrochromatography. *Electrophoresis* **2008**, *29*, 889-900.

(17) Baryla, N. E.; Lucy, C. A. Simultaneous Separation of Cationic and Anionic Proteins Using Zwitterionic Surfactants in Capillary Electrophoresis. *Anal. Chem.* **2000**, *72*, 2280-2284.

(18) Wu, Y.; Xie, J.; Wang, F.; Chen, Z. Electrokinetic Separation of Peptides and Proteins using a Polyvinylamine-Coated Capillary with UV and ESI-MS Detection *J. Sep. Sci.* **2008**, *31*, 814-832.

(19) Yang, Y. Z.; Boysen, R. I.; Matyska, M. T.; Pesek, J. J.; Hearn, M. T. W. Open-Tubular Capillary Electrochromatography Coupled with Electrospray Ionization Mass

Spectrometry for Peptide Analysis. *Anal. Chem.* **2007**, *79*, 4942-4949.

(20) Terabe, S.; Otsuka, K.; Ando, T. Electrokinetic Chromatography with Micellar Solution and Open-Tubular Capillary. *Anal. Chem.* **1985**, *57*, 834-841.

(21) Gong, B. Y.; Ho, J. W. Effect of Zwitterionic Surfactants on the Separation of Proteins by Capillary Electrophoresis. *Electrophoresis* **1997**, *18*, 732-735.

(22) Jiang, W.; Awasum, J. N.; Irgum, K. Control of Electroosmotic Flow and Wall Interactions in Capillary Electrophoresis Capillaries by Photografted Zwitterionic Polymer Surface Layers. *Anal. Chem.* **2003**, *75*, 2768-2774.

(23) Yeung, K. K.; Lucy, C. A. Suppression of Electroosmotic Flow and Prevention of Wall Adsorption in Capillary Zone Electrophoresis Using Zwitterionic Surfactants. *Anal. Chem.* **1997**, *69*, 3435-3441.

(24) Baryla, N. E.; Melanson, J. E.; McDermott, M. T.; Lucy, C. A. Characterization of Surfactant Coatings in Capillary Electrophoresis by Atomic Force Microscopy. *Anal. Chem.* **2001**, *73*, 4558-4565.

(25) MacDonald, A. M.; Lucy, C. A. Highly Efficient Protein Separations in Capillary Electrophoresis Using a Supported Bilary/Diblock Copolymer Coating. *J. Chromatogr. A* **2006**, *1130*, 265-271.

(26) Manne, S.; Gaub, H. E. Molecular-Organization of Surfactants at Solid-Liquid Interfaces. *Science* **1995**, *270*, 1480-1482.

(27) Chatjaroenporn, K.; Baker, R. W.; FitzGerald, P. A.; Warr, G. G. Structure Changes in Micelles and Adsorbed Layers during Surfactant Polymerization. *J. Colloid Interf. Sci.* **2009**, *336*, 449-454.

(28) Sakai, H.; Nakamura, H.; Kozawa, K.; Abe, M. Atomic Force Microscopy Observation of the Nanostructure of Tetradecyltrimethylammonium Bromide Films Adsorbed at the Mica/Solution Interface. *Langmuir* **2001**, *17*, 1817-1820.

(29) Subramanian, V.; Ducker, W. A. Counterion Effects on Adsorbed Micellar Shape: Experimental Study of the Role of Polarizability and Charge. *Langmuir* **2000**, *16*, 4447-4454.

(30) Li, S. L.; Palmer, A. F. Structure and Mechanical Response of Self-Assembled Poly(butadiene)-b-poly(ethylene oxide) Colloids Probed by Atomic Force Microscopy. *Macromolecules* **2005**, *38*, 5686-5698.

(31) Ma, N.; Zhang, H. Y.; Song, B.; Wang, Z. Q.; Zhang, X. Polymer Micelles as Building Blocks for Layer-by-Layer Assembly: An Approach for Incorporation and Controlled Release of Water-Insoluble Dyes. *Chem. Mater.* **2005**, *17*, 5065-5069.

(32) Hamley, I. W.; Connell, S. D.; Collins, S. In Situ Atomic Force Microscopy Imaging of Adsorbed Block Copolymer Micelles. *Macromolecules* **2004**, *37*, 5337-5351.

(33) Bougard, F.; Jeusette, M.; Mespouille, L.; Dubois, P.; Lazzaroni, R. Synthesis and Supramolecular Organization of Amphiphilic Diblock Copolymers Combining Poly(N,N-dimethylamino-2-ethyl methacrylate) and Poly(epsilon-caprolactone). *Langmuir* **2007**, *23*, 2339-2345.

(34) Karymov, M. A.; Prochazka, K.; Mendenhall, J. M.; Martin, T. J.; Munk, P.; Webber, S. E. Chemical Attachment of Polystyrene-Block-Poly(methacrylic acid) Micelles on a Silicon Nitride Surface. *Langmuir* **1996**, *12*, 4748-4753.

(35) Lee, M. Y.; Kim, S. H.; Ganapathy, H. S.; Kim, S. W.; Lim, K. T. Characterization of Micellar Film Morphologies of Semifluorinated Block Copolymers by AFM. *Ultramicroscopy* **2008**, *108*, 1210-1214.

(36) Seo, Y.; Im, J. H.; Lee, J. S.; Kim, J. H. Aggregation Behaviors of a Polystyrene-b-poly(methyl methacrylate) Diblock Copolymer at the Air/Water Interface. *Macromolecules* **2001**, *34*, 4842-4851.

(37) Sakai, K.; Vamvakaki, M.; Smith, E. G.; Wanless, E. J.; Armes, S. P.; Biggs, S. Adsorption Characteristics of Zwitterionic Diblock Copolymers at the Silica/Aqueous Solution Interface. *J. Colloid Interf. Sci.* **2008**, *317*, 383-394.

(38) Kohori, F.; Sakai, K.; Aoyagi, T.; Yokoyama, M.; Sakurai, Y.; Okano, T. Preparation and Characterization of Thermally Responsive Block Copolymer Micelles Comprising Poly(N-isopropylacrylamide-b-DL-lactide). *J. Control. Release* **1998**, *55*, 87-98.

(39) Kapnissi, C. P.; Akbay, C.; Schlenoff, J. B.; Warner, I. M. Analytical Separations Using Molecular Micelles in Open-Tubular Capillary Electorchromatography. *Anal. Chem.* **2002**, *74*, 2328-2335.

(40) Mwongela, S. M.; Numan, A.; Gill, N. L.; Agbaria, R. A.; Warner, I. M. Separation of Achiral and Chiral Analytes Using Polymeric Surfactants With Ionic Liquids as Modifiers in Micellar Electrokinetic Chromatography. *Anal. Chem.* **2003**, *75*, 6089-6096.

(41) Kamande, M. W.; Zhu, X.; Kapnissi-Christodoulou, C. K.; Warner, I. M. Chiral Separations Using a Polypeptide and Polymeric Dipeptide Surfactant Polyelectrolyte Multilayer Coating in Open-Tubular Capillary Electrochromatography. *Anal. Chem.* **2004**, *76*, 6681-6692.

(42) Shamsi, S. A.; Valle, B. C.; Billiot, F.; Warner, I. M. Polysodium N-Undecanoyl-L-leucylvalinate: A Versitile Chiral Selector for Micellar Electrokinetic Chromatography. *Anal. Chem.* **2003**, *75*, 379-387.

(43) Billiot, F. H.; Billiot, E. J.; Warner, I. M. Comparison of Monomeric and Polymeric Amino Acid Based Surfactants for Chiral Separations. *J. Chromatogr. A* **2001**, *922*, 329-338.

(44) Wang, J.; Warner, I. M. Chiral Separations Using Micellar Electrokinetic Capillary Chromatography and a Polymerized Chiral Micelle. *Anal. Chem.* **1994**, *66*, 3773-3776.

(45) Cunliffe, J. M.; Baryla, N. E.; Lucy, C. A. Phospholipid Bilayer Coatings for the Separation of Proteins in Capillary Electrophoresis. *Anal. Chem.* **2002**, *74*, 776-783.

(46) Pesek, J. J.; Matyska, M. T.; Sentellas, S.; Galceran, M. T.; Chiari, M.; Pirri, G. Multimodal Open-Tubular Capillary Electrochromatographic Analysis of Amines and Peptides. *Electrophoresis* **2002**, *23*, 2982-2989.

(47) Dong, X.; Dong, J.; Ou, J.; Zhu, Y.; Zou, H. Capillary Electrochromatography with Zwitterionic Stationary Phase on the Lysine-Bonded Poly(glycidyl methacrylate-co-ethylene dimethacrylate) Monolithic Capillary Column. *Electrophoresis* **2006**, *27*, 2518-2525.

(48) Fu, H.; Xie, C.; Dong, J.; Huang, X.; Zou, H. Monolithic Column with Zwitterionic Stationary Phase for Capillary Electrochromatography. *Anal. Chem.* **2004**, *76*, 4866-4874.

(49) Shendage, D. M.; Frohlich, R.; Haufe, G. Highly Efficient Stereoconservative Amidation and Deamidation of Alpha-amino Acids. *Org. Lett.* **2004**, *6*, 3675-3678.

(50) Knapp, H. F.; Stemmer, A. Preparation, Comparison and Performance of Hydrophobic AFM Tips. *Surf. Interface Anal.* **1999**, *27*, 324-331.

(51) http://gwyddion.net/.

(52) Wang, A. J.; Witos, J.; D'Ulivo, L.; Vainikka, K.; Riekkola, M. L. Noncovalent Poly(1-vinylpyrrolidone)-Based Copolymer Coating for the Separation of Basic Proteins and Lipoproteins by CE. *Electrophoresis* **2009**, *30*, 3939-3946.

(53) Huang, X. H.; Gordon, M. J.; Zare, R. N. Current-Monitoring Method for Measuring the Electroosmotic Flow-Rate in Capillary Zone Electrophoresis. *Anal. Chem.* **1988**, *60*, 1837-1838.

(54) Salloum, D. S.; Schlenoff, J. B. Protein Adsorption Modalities on Polyelectrolyte Multilayers. *Biomacromolecules* **2004**, *5*, 1089-1096.

(55) Zhang, Q.; Remsen, E. E.; Wooley, K. L. Shell Cross-Linked Nanoparticles Containing Hydrolytically Degradable, Crystalline Core Domains. *J. Am. Chem. Soc.* **2000**, *122*, 3642-3651.

(56) Emoto, K.; Nagasaki, Y.; Kataoka, K. A Core-Shell Structured Hydrogel Thin Layer on Surfaces by Lamination of a Poly(ethylene glycol)-b-poly(D,L-lactide) Micelle and Polyallylamine. *Langmuir* **2000**, *16*, 5738-5742.

(57) Liu, G. Y.; Xu, S.; Qian, Y. L. Nanofabrication of Self-Assembled Monolayers Using Scanning Probe Lithography. *Accounts Chem. Res.* **2000**, *33*, 457-466.

(58) Graul, T. W.; Schlenoff, J. B. Capillaries Modified by Polyelectrolyte Multilayers for Electrophoretic Separations. *Anal. Chem.* **1999**, *71*, 4007-4013.

(59) Kamande, M. W.; Zhu, X. F.; Kapnissi-Christodoulou, C.; Warner, I. M. Chiral Separations Using a Polypeptide and Polymeric Dipeptide Surfactant Polyelectrolyte Multilayer Coating in Open-Tubular Capillary Electrochromatography. *Anal. Chem.* **2004**, *76*, 6681-6692.

(60) Putnam, F. W., Ed. *The Plasma Proteins: Structure, Function, and Genetic Control, Vol. 5. 2nd Ed*, 1987.

CHAPTER 3
FACILE PREPARATION OF CELLULOSE COATED CAPILLARIES USING A ROOM TEMPERATURE IONIC LIQUID FOR CHIRAL SEPARATIONS

3.1 Introduction

The separation of chiral compounds is an essential task in the pharmaceutical industry, mainly due to drug purity. In particular, the Food and Drug Administration and the European Medicines Agency require single enantiomer drug purity for chiral compounds, whose enantiomers differ qualitatively or quantitatively in their pharmacological action or toxicological profile.[1] Therefore, pharmaceutical industries, in order to enhance drug purity, require more cost efficient and highly selective stationary phases as well as less column preparation time. Chiral separations can be achieved by the use of a chiral selector as a pseudostationary phase or a stationary phase such as native and derivatized cyclodextrins,[2, 3] polymeric surfactants,[4, 5] polymers,[6, 7] and modified nanoparticles[8, 9] have demonstrated great enantioseparation ability for various chiral compounds. However, many of these stationary phases are costly and/or time consuming. Hence, the use of a more abundant material should reduce the cost of starting materials. Moreover, it has been observed that in a chiral selector, an increase in density of stereogenic centers can result in more interactions, which provides a highly selective stationary.[10] Natural biopolymers such as polysaccharides have a high density of stereogenic centers due to chiral sugars, which constitute the repeat unit of the macromolecule. For instance, cellulose contains glucose molecules, each having five chiral centers. Therefore, cellulose is considered an attractive molecule for chiral separations. In addition, cellulose is the most abundant organic substance on earth,[11] which in turn makes it very cost efficient.

Even though cellulose and most of its derivatives are water insoluble, there are reports of water soluble cellulose derivatives, such as cationized hydroxyethylcellulose, which can be adsorbed to the capillary wall through electrostatic interactions.[12] However, these molecules lose their effectiveness after several runs because the coating is washed away by the background electrolyte (BGE), mainly due to the hydrophilicity of the molecule. Water insoluble cellulose derivatives have also garnered considerable attention as stationary phases because of their effectiveness in capillary electrochromatography (CEC) separations. These stationary phases are generated via coating polysaccharides to an aminopropyl derivatized silica gel in non-aqueous CEC[13] or packing the column with cellulose acetate fibers,[14] or chemically modifying the polysaccharides to the capillary wall.[15-18] Although these methods offer effective separations, the effective separations the column preparation times are long and laborious. An alternative approach to these methods is the use of polysaccharides as coatings constructed to the capillary walls in the open-tubular CEC mode. This approach can reduce column preparation time and decrease the long analysis times that are commonly associated with packed columns.

Some traditional solvents used for the dissolution of cellulose are volatile[19]or highly basic.[20] Room temperature ionic liquids (RTILs), on the other hand, are attractive solvents that can be used for the dissolution of cellulose. RTILs are molten salts with melting points at or below 100 °C.[21, 22] In comparison to other salts, such as NaCl, RTILs have lower melting points due to the asymmetric packing of their cations and anions. Ionic compounds can also have very unique properties depending on the type of the cation and anion pairing. For instance, an anionic molecule such as europium can be used for visual detection due to its luminescence.[23] In addition, a RTIL composed of iron (IV) chloride can could be utilized for site specific targeting via magnetic fields.[24] Furthermore, an ionic liquid, which contains 1-butyl-3-methylimidazolium

[BMIM] cation was used by Tian *et al*. as a coating, in CEC, and it demonstrated exhibited very good reproducibilities.[25] RTILs can have up to 10^9 different combinations,[26] some of which have been shown to dissolve cellulose as well as its deriviatives.[27-29]

In this study, water insoluble cellulose-based polysaccharides were dissolved in order to provide a RTIL, in order to provide a facile preparation method by way of flushing a coating onto the capillary wall. Three different cellulose derivatives (cellulose acetate, cellulose acetate phthalate, and cellulose acetate butyrate) (Figure 3.1) were each investigated as chiral stationary phases in OT-CEC. Each polysaccharide was dissolved into 1-butyl-3-methylimidazolium acetate, [BMIM][OAc], (Figure 3.1), and each solution was used to fill the fused-silica capillaries. The capillaries were then rinsed with water to remove excess cellulose/ionic liquid solution, thus, leaving a coating on the capillary walls. Finally, the performance of the constructed stationary phases as chiral media were evaluated by use of thiopental, sotalol, labetalol, and ephedrine, as chiral analytes (Figure 3.2). We found that the constructed stationary phases were stable and some separations resulted in fast analysis times, e.g., as low as 2.3 minutes. To the best of our knowledge, this is the first report on the use of cellulose based polysaccharides dissolved in a RTIL for the separation of chiral molecules.

3.2 Experimental

3.2.1 Materials and Reagents

Sodium phosphate monobasic monohydrate, sodium phosphate dibasic dihydrate, sodium hydroxide, hydrochloric acid, and methanol were purchased from Fisher Scientific (Fair Lawn, NJ). Cellulose acetate (CA), cellulose acetate phthalate (CAP), cellulose acetate butyrate (CAB), rhodamine 6G (R6G), and 1-butyl-3-methylimidazolium acetate [BMIM][OAc] were obtained from Sigma-Aldrich (Milwaukee, WI, USA). The chiral analytes thiopental, sotalol, labetalol,

and ephedrine were purchased from Sigma Chemical Company (St. Louis, MO). All materials were used as received without further treatment or purification.

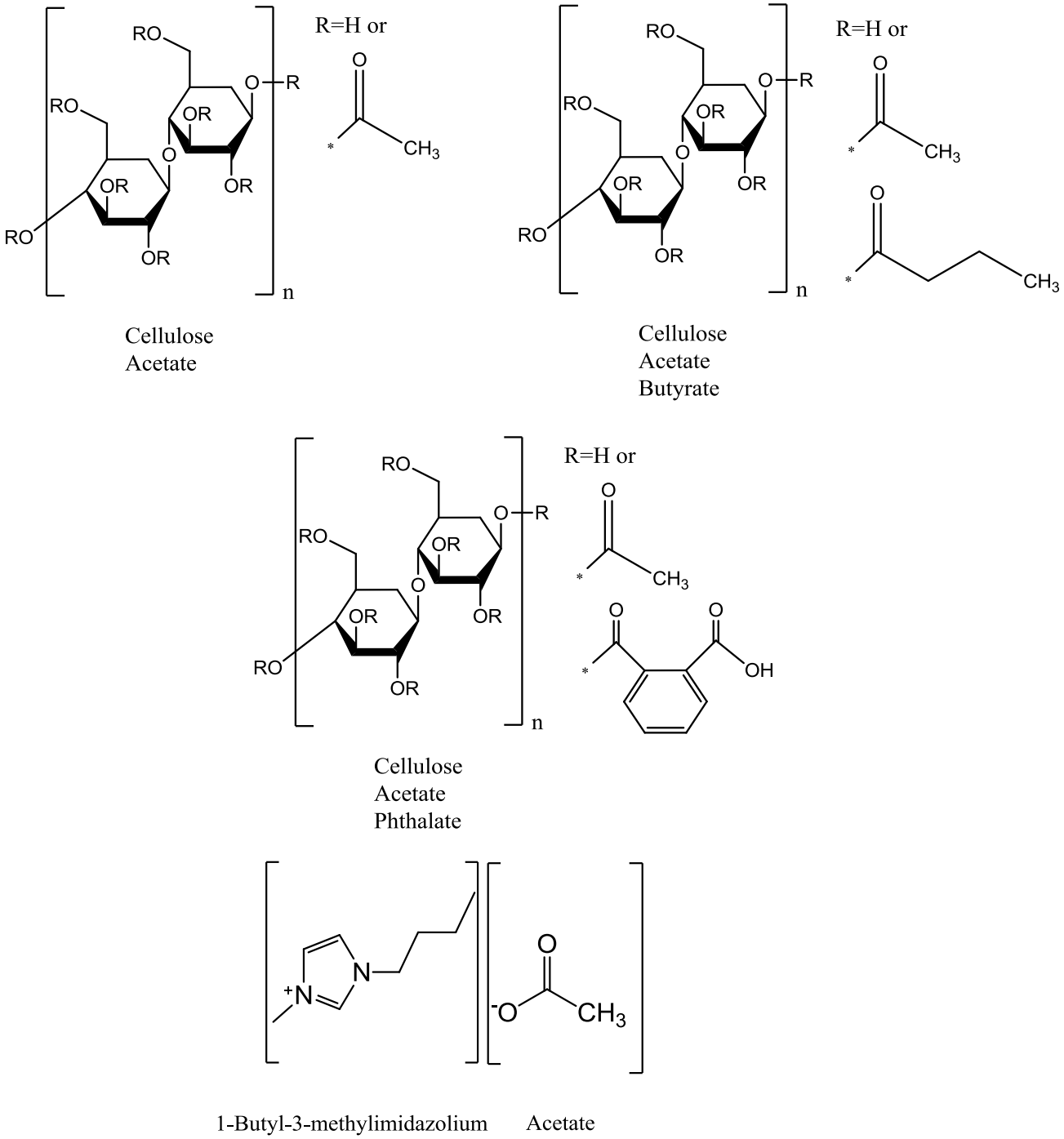


Figure 3.1 Structures of cellulose derivatives and ionic liquid.

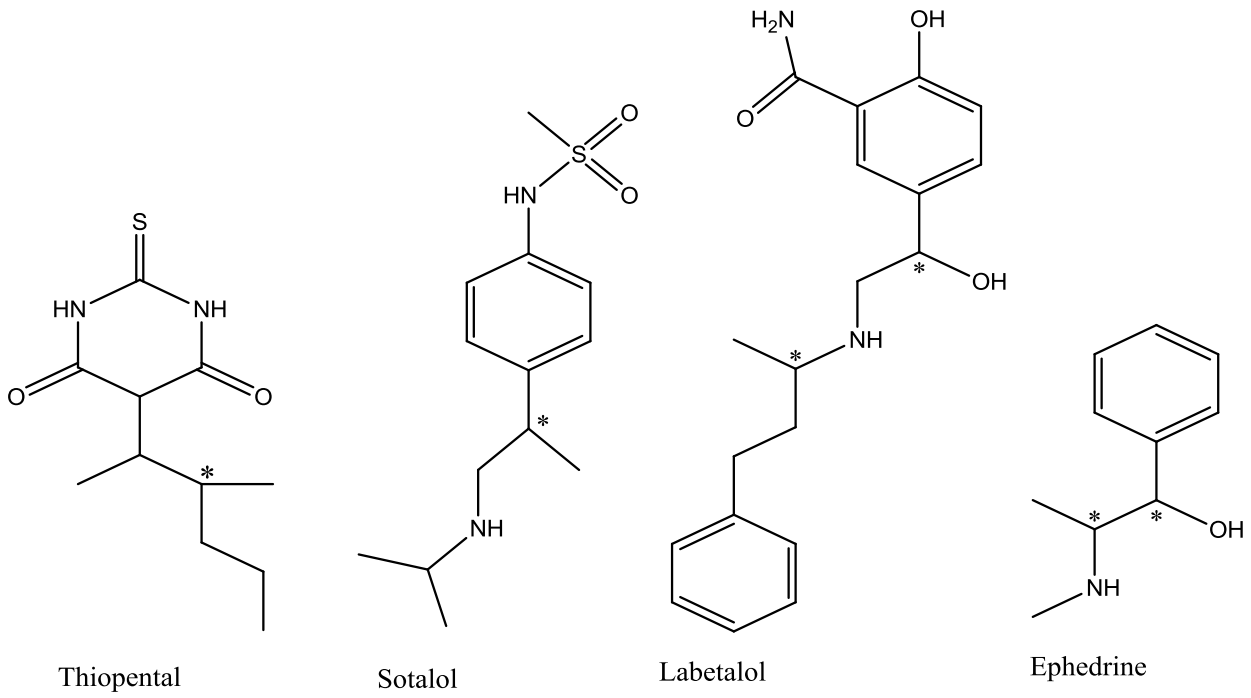


Figure 3.2 Structure of chiral drugs.

3.2.2 Sample and Background Electrolyte Preparation

The chiral analyte stock solutions were prepared at 2mg/mL in methanol and diluted to a working concentration of 0.2 mg/mL in 50:50 methanol/water. An appropriate amount of a buffer was dissolved in water to give the appropriate concentration, and the pH of the BGE was adjusted using 1 M hydrochloric acid (HCl). All BGEs were filtered using a 0.45 μm polypropylene filter (Nalgene, Rochester, NY) and sonicated to ensure proper degassing for 15 minutes prior to use.

3.2.3 Instrumentation and Preparation Procedure for Polysaccharide Coating

A Beckman P/ACE MDQ capillary electrophoresis system was used for CEC separations. A UV photodiode array (Fullerton, CA) was used for the detection of the analyte molecules. Separations were performed using fused-silica capillaries purchased from Polymicro

Technologies (Phoenix, AZ). Each capillary had 50 cm total length (40 cm effective length) and 50μm internal diameter. Conditioning of the capillaries was accomplished by flushing with 1 M NaOH for 30 minutes followed by deionized water for 15 min. Capillaries were dried using argon gas for a minimum of one hour. The dried capillaries were then filled, using a syringe pump, which consisted of the polysaccharide of interest dissolved, at various concentrations, in [BMIM][OAc]. Filled capillaries were flushed with water until aqueous droplets could be observed on the opposite end. All rinses, using the CE instrument, were performed at a pressure of 20 psi. The temperature of the capillary cassette ranged from 15 to 25°C and the applied voltage ranged from 15 to 30 kV. Pressure injections were performed by applying 5 psi for 5 s, and the separations were conducted under normal polarity. Data obtained was collected at 200 nm.

Capillary images were acquired using a Leica DM-RXA fluorescence microscope with a 20x 0.7 NA objective and a Leica N2.1 (TRITC) filter cube. Data was processed using slidebook (ver 4.1) imaging software. The capillary was treated with polysaccharide of interest using the procedure as mentioned earlier. After rinsing the capillary with water, a solution of 10mM R6G was flushed throughout the capillary. The capillary was then dried using a stream of argon gas. Double sided tape was placed on microscope slide to immobilize the capillary. The capillary was inverted through the tape on glass slide for imaging.

3.3 Results and Discussion

3.3.1 Characterization of Polysaccharide Coated Capillary Using a Leica DMRXA Fluorescence Microscope

A visual representation of the coating was taken using fluorescence microscopy for a cross section of the end of a capillary. A representative image using 2.75% (w/w) CA coated capillary

with R6G is compared to the bare capillary can be viewed below in Figure 3.3. The ~4μm thick coating is indicated by the image on the left.

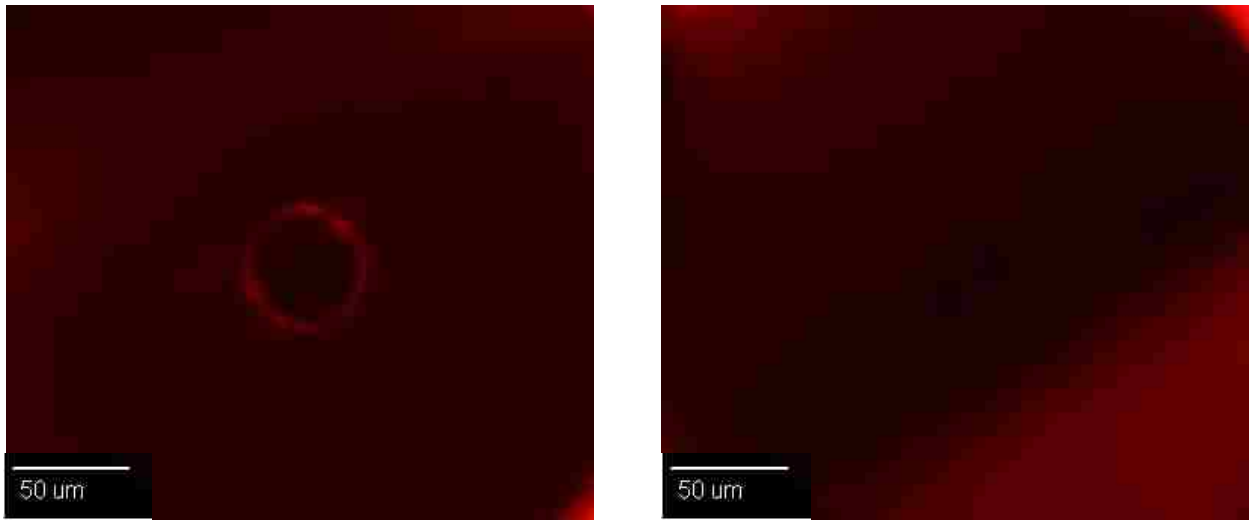


Figure 3.3 Cellulose acetate/R6G coated capillary in comparison to the bare capillary.

3.3.2 Optimized Separation Conditions for Thiopental Using Cellulose Acetate, Cellulose Acetate Phthalate, and Cellulose Acetate Butyrate

The effect of polymer concentration on the separation of four chiral analytes, mentioned earlier, was investigated by keeping the voltage and the temperature constant at 30 kV and 15°C, respectively. Polymer concentrations were varied from 0.25% to 3.50% (w/w). In each case, when the concentration was optimized, other experimental parameters were examined, such as applied voltage and column temperature.

The concentration of CA for the enantiomeric separation of thiopental was varied between 1.25 and 1.75% (w/w) in figure 3.4. A concentration of 1.50% (w/w) resulted in baseline resolution ($R_s \geq 1.5$) of the enantiomers of thiopental. An increasing or decreasing of

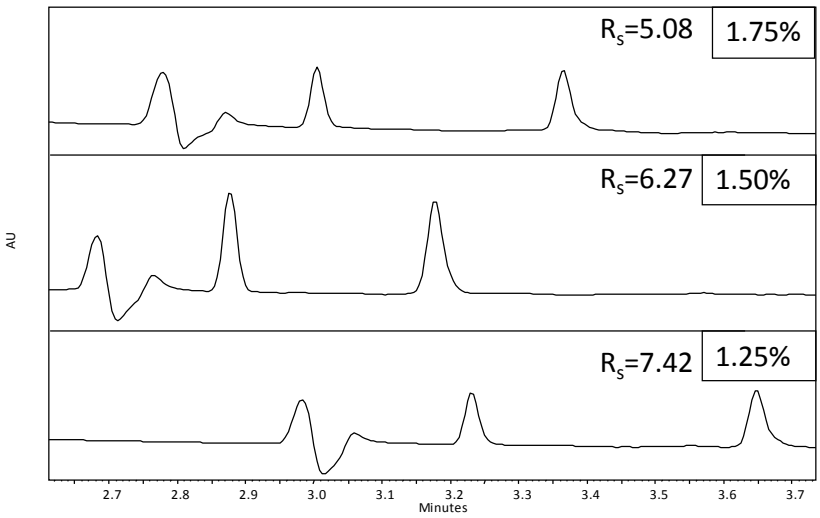


Figure 3.4 Effect of CA concentration on the separation of thiopental. **Conditions: Polymer concentration:** 1.25, 1.50, 1.75% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 7.2; **Applied voltage:** 30 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

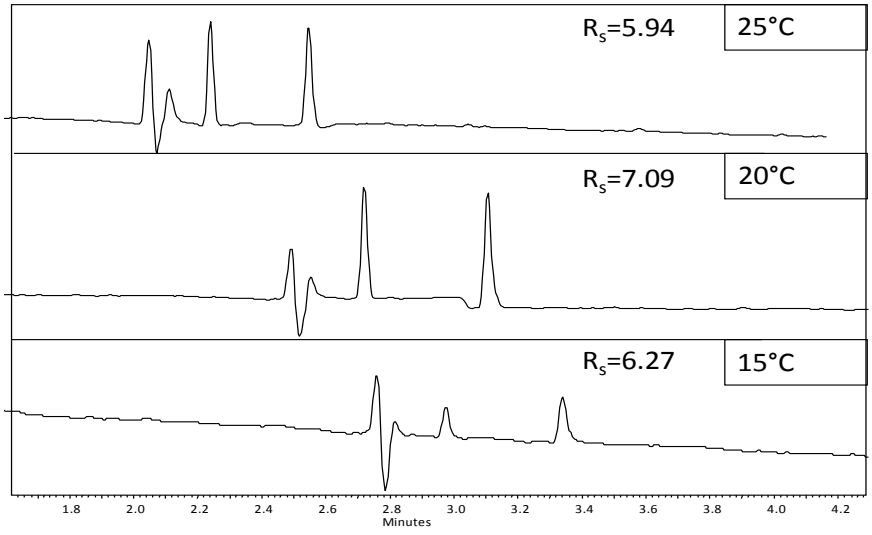


Figure 3.5 Effect of temperature on the separation of thiopental using the CA coating. **Conditions: Polymer concentration:** 1.50% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 7.2; **Applied voltage:** 30 kV; **Temperature:** 15, 20, 25˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

the polymer concentration resulted in higher resolution; however, longer migration times of the analyte. Therefore, 1.50% (w/w) was chosen as the optimal CA concentration.

Further optimization studies were performed by varying the temperature in Figure 3.5. When the temperature was increased to 25°C in Figure 3.5, the enantiomeric separation was performed in a very short analysis times (2.6 mins). However, due to low peak efficiency, a temperature of 20°C was chosen for further studies. The effect of voltage on the enantiomeric separation of thiopental was also investigated. A decrease in the voltage from 30 kV to 15 kV did not have a major impact on resolution. Therefore, an applied voltage of 30 kV was chosen as the optimum. An applied voltage of 30kV with a temperature of 20°C were chosen as optimized as optimized conditions for the separation of thiopental enantiomers (R_s = 6.27) when CA was used as a stationary phase.

The second cellulose derivative that was investigated as a stationary phase was CAP. The examined CAP concentration ranged from 0.50 to 1.00% (w/w) in Figure 3.6. When the concentration was 0.75% (w/w), thiopental enantiomers were baseline resolved in 3.5 mins. Polymer concentrations that deviated from 0.75% (w/w) resulted in longer analysis times, probably due to the non-charged polysaccharide coating covering the charged silanol groups on the capillary wall. When the column temperature increased in Figure 3.7, resolution increased from 5.60 to 7.31. Optimized conditions were achieved using a voltage of 30 kV and a temperature of 25°C to result in R_s = 7.31 by use of a CAP-modified capillary.

As far as the CAB-modified capillary column is concerned, a concentration range of 1.25-1.75% (w/w) CAB was selected for the separation of thiopental. A concentration of 1.50% (w/w) CAB resulted in a baseline separation (R_s = 5.40) within just 3.1 mins. In addition, a

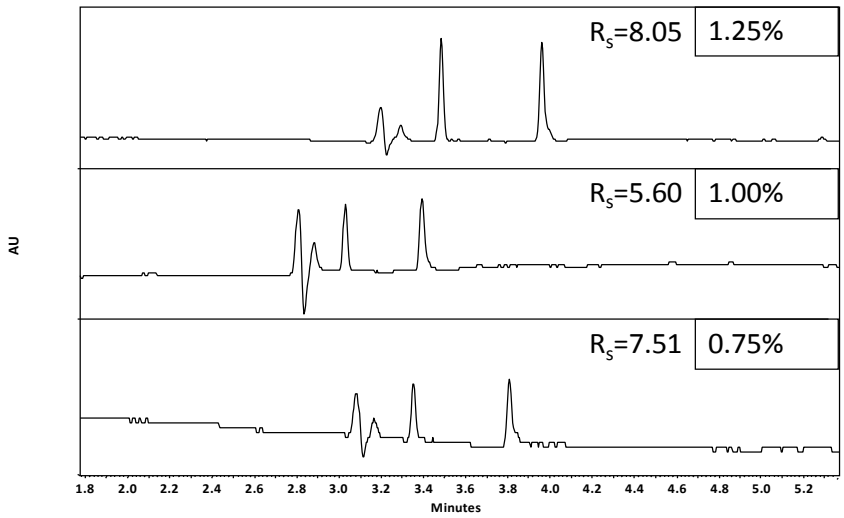


Figure 3.6 Effect of CAP concentration on the separation of thiopental. **Conditions: Polymer concentration:** 0.75, 1.00, 1.25% (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 7.2; **Applied voltage:** 30 kV; **Temperature:** 15˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

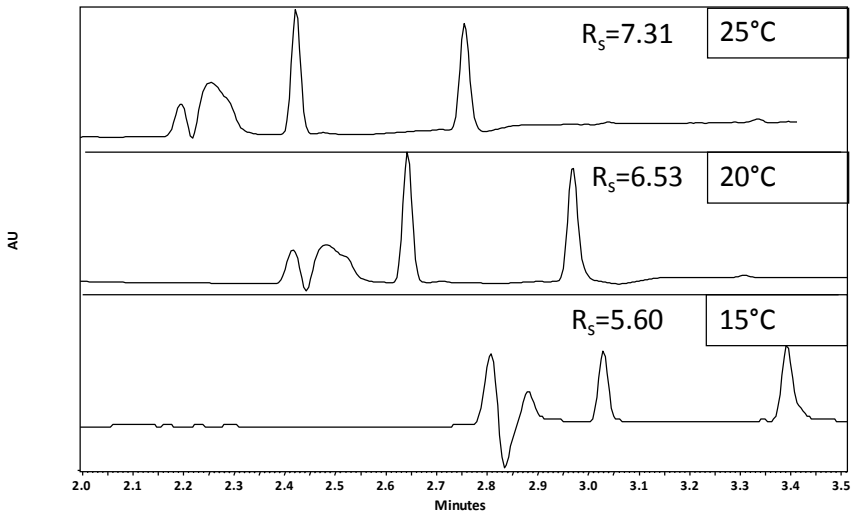


Figure 3.7 Effect of temperature on the separation of thiopental using the CAP coating. **Conditions:** Polymer concentration: 1.00% (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 7.2; **Applied voltage:** 30 kV; **Temperature:** 15, 20, 25˚C; Capillary: 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

temperature of 25 °C and an applied voltage of 30 kV provided the best results, in regard to resolution, efficiency, and analysis time Figure 3.9.

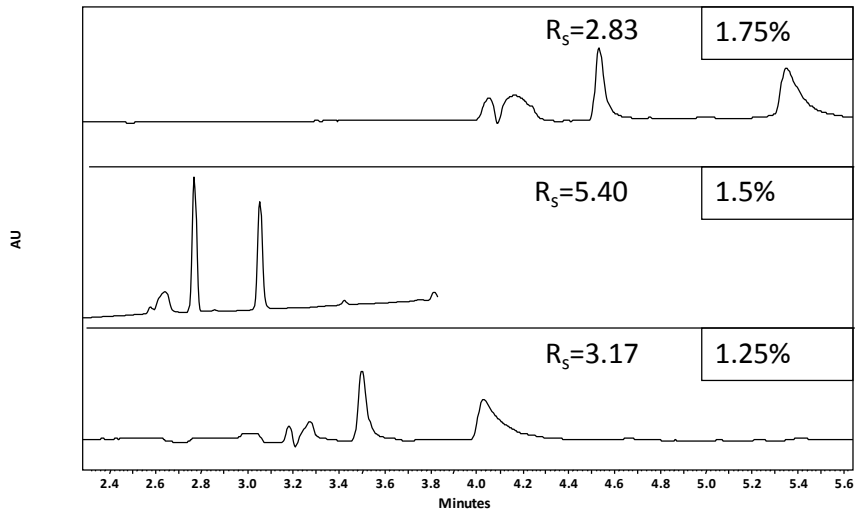


Figure 3.8 Effect of CAB concentration on the separation of thiopental. **Conditions:** Polymer concentration: 1.25, 1.50, 1.75% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 7.2; **Applied voltage:** 30 kV; **Temperature:** 15˚C; Capillary: 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

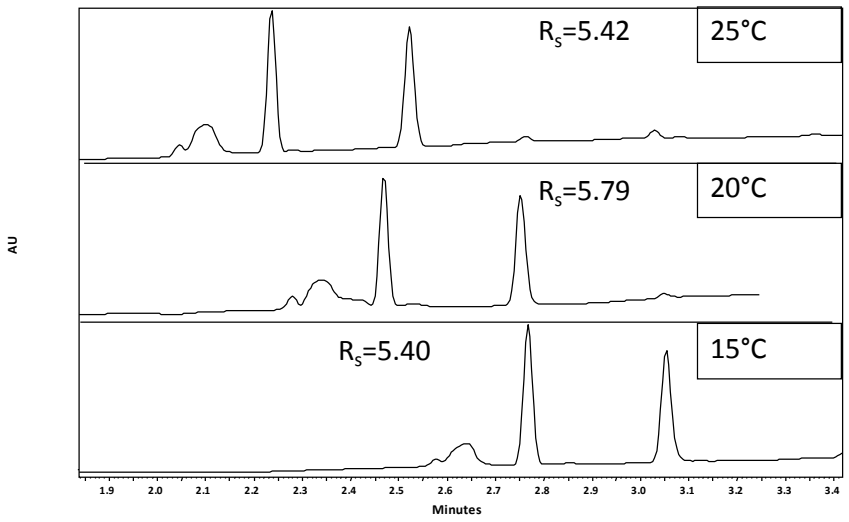


Figure 3.9 Effect of temperature on the separation of thiopental using the CAB Coating. **Conditions:** Polymer concentration: 1.25, 1.50% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 7.2; **Applied voltage:** 30 kV; **Temperature:** 15, 20, 25˚C; Capillary: 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

In conclusion, resolutions greater than baseline resolution were achieved for the separation of thiopental enantiomers using each one of the cellulose derivatives. Voltage was held constant at 30 kV and not varied due to already achieving baseline resolution as decreasing voltage would result in longer analysis times. As a result of high voltages being used, increasing the temperature of the systems decreased analysis times. Thus, fast and efficient separations were observed.

3.3.3 Optimized Separation Conditions for Sotalol Using Cellulose Acetate, Cellulose Acetate Phthalate, and Cellulose Acetate Butyrate.

The influence of each of the three cellulose derivatives on the enantiomeric separation of sotalol was also investigated. The electropherograms obtained by use of each cellulose derivative are demonstrated in Figures 3.10 – 3.15. The concentration of CA was varied from 2.25 – 2.75% (w/w) in Figure 3.10. When the coating was constructed by using 2.50% (w/w) CA, only partial separation was attained for sotalol (R_s = 0.56) in a migration time of 2.7 min. When the CA concentration was increased from 2.25 to 2.50% (w/w) the resolution slightly increased, while when the concentration increased to 2.75% (w/w) no separation was achieved. In addition, a decrease in resolution was observed by increasing the column temperature in Figure 3.11, while the electroosmotic mobility increased, likely due to a decrease in electrolyte viscosity. Voltages lower than 30 kV resulted in no separation of the enantiomers of sotalol in Figure 3.12. There was a decrease in resolution when temperature was increased.

The separation of sotalol enantiomers was then examined by using the CAP as coating. No separation was observed for CAP concentration study at lower temperatures. However, using a concentration of 1.25% at higher voltages resulted in partial separation of enantiomers as demonstrated in Figure 3.13. Loss of resolution was observed when temperatures were

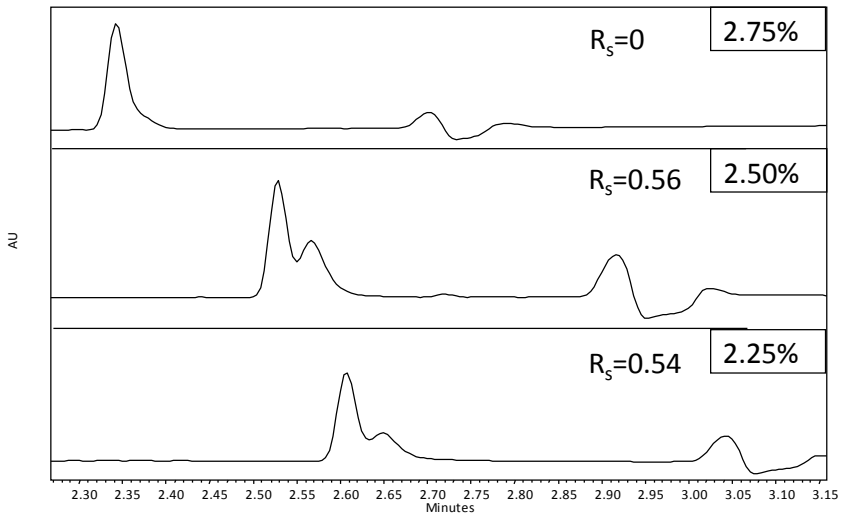


Figure 3.10 Effect of CA concentration on the separation of sotalol. **Conditions: Polymer concentration:** 2.25, 2.50, 2.75% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 30 kV; **Temperature:** 15˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

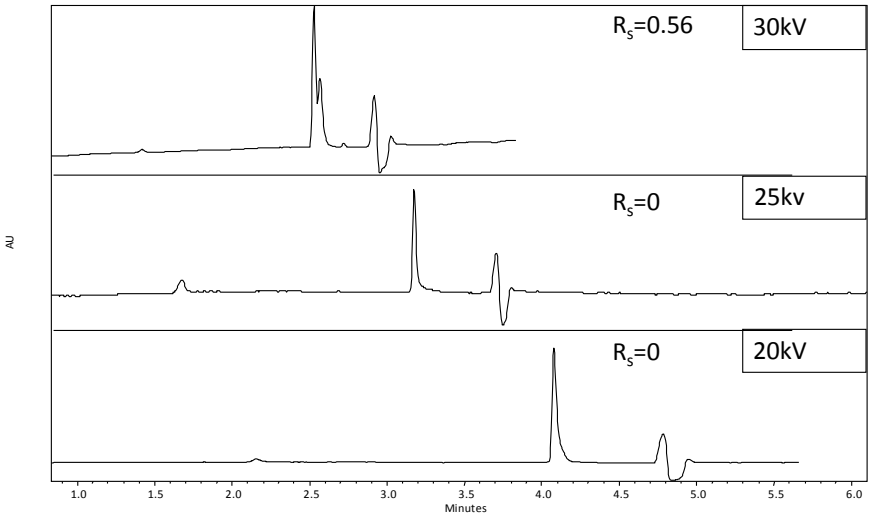


Figure 3.11 Effect of voltage on the separation of sotalol using the CA coating. **Conditions: Polymer concentration:** 2.50% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 30, 25, 20 kV; **Temperature:** 15˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

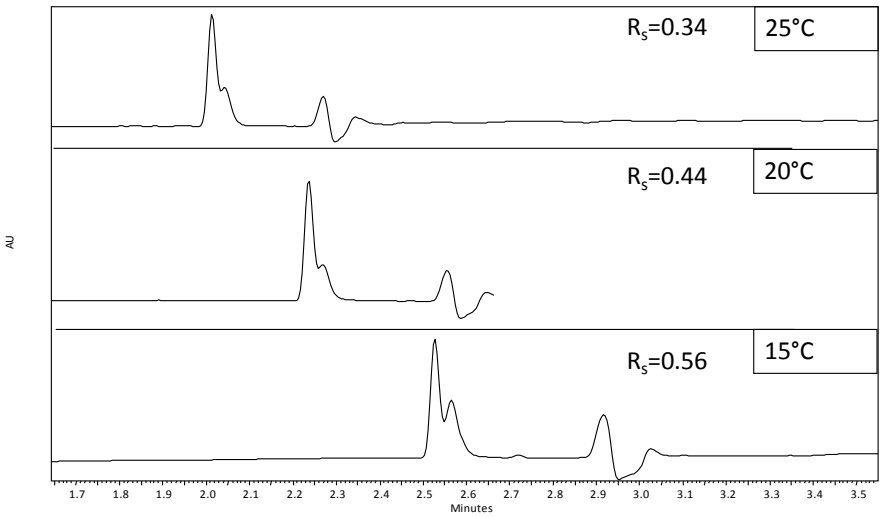


Figure 3.12 Effect of temperature on the separation of sotalol using the CA coating. **Conditions: Polymer concentration:** 2.50% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 30 kV; **Temperature:** 15, 20, 25˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

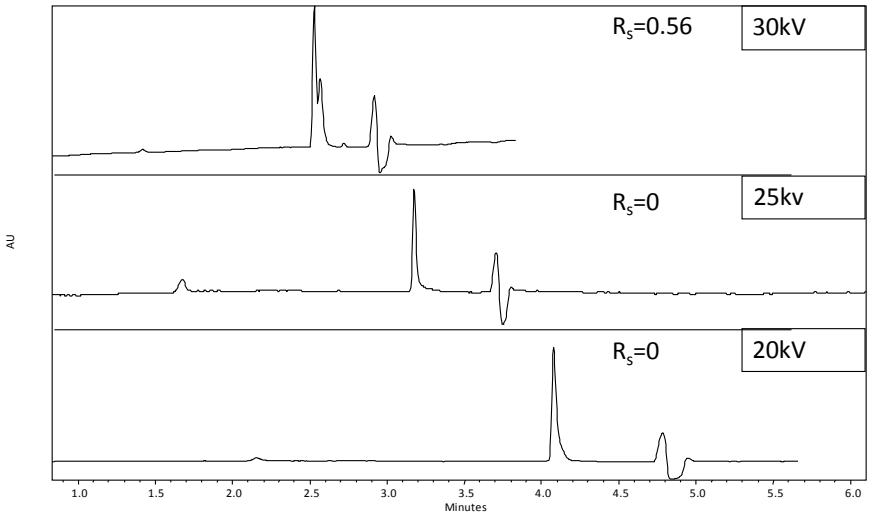


Figure 3.13 Effect of voltage on the separation of sotalol using the CAP coating. **Conditions: Polymer concentration:** 1.25% (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50mM Phosphate pH 8.2; **Applied voltage:** 20, 25, 30 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

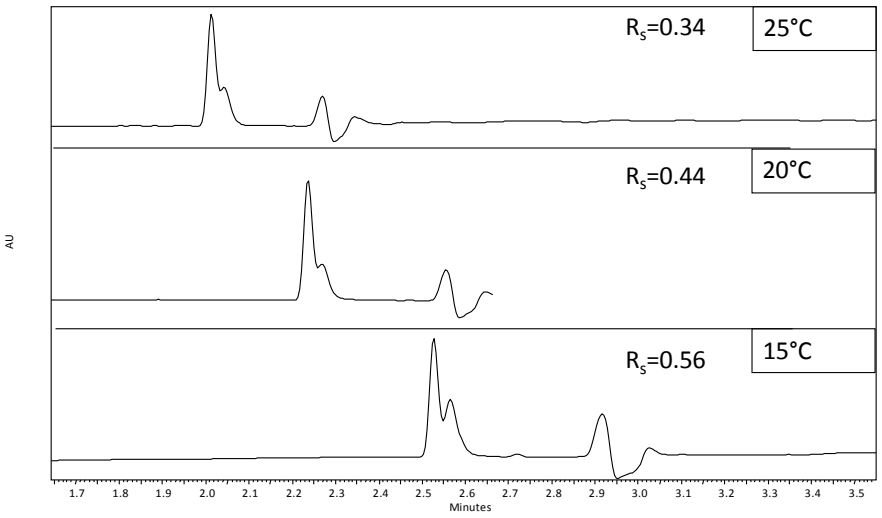


Figure 3.14 Effect of temperature on the separation of sotalol using the CAP coating. **Conditions: Polymer concentration:** 1.25% (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 30 kV; **Temperature:** 15, 20, 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

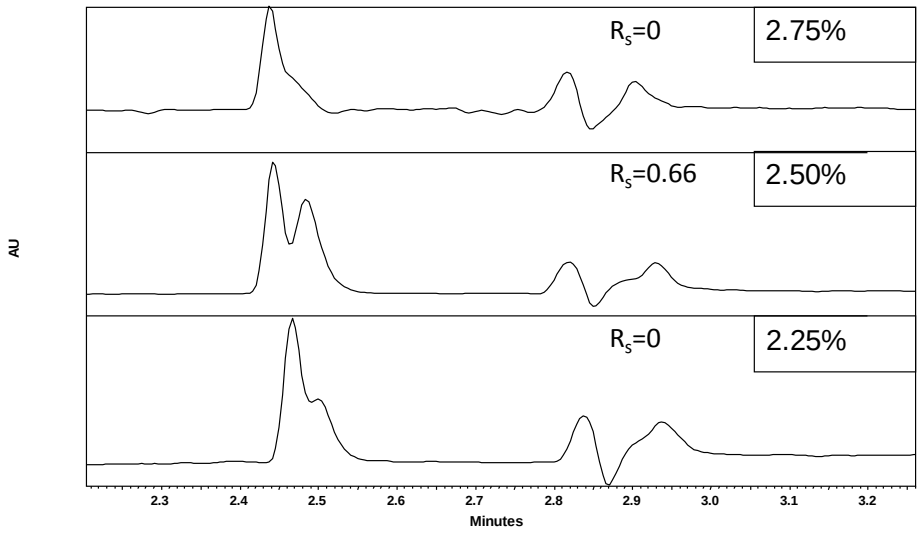


Figure 3.15 Effect of CAB concentration on the separation of sotalol. **Conditions: Polymer concentration:** 2.25, 2.50, 2.75% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 30 kV; **Temperature:** 15˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

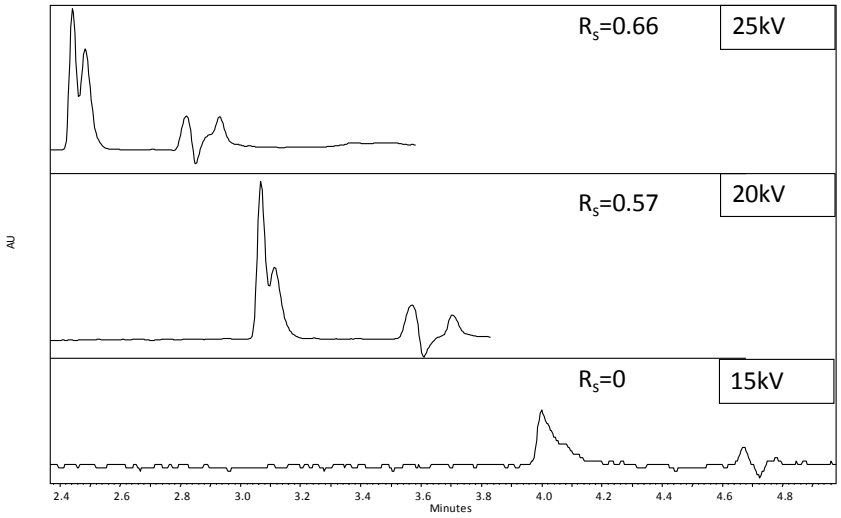


Figure 3.16 Effect of voltage on the separation of sotalol using the CAB coating. **Conditions: Polymer concentration:** 2.50% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 15, 20, 25 kV; **Temperature:** 15˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

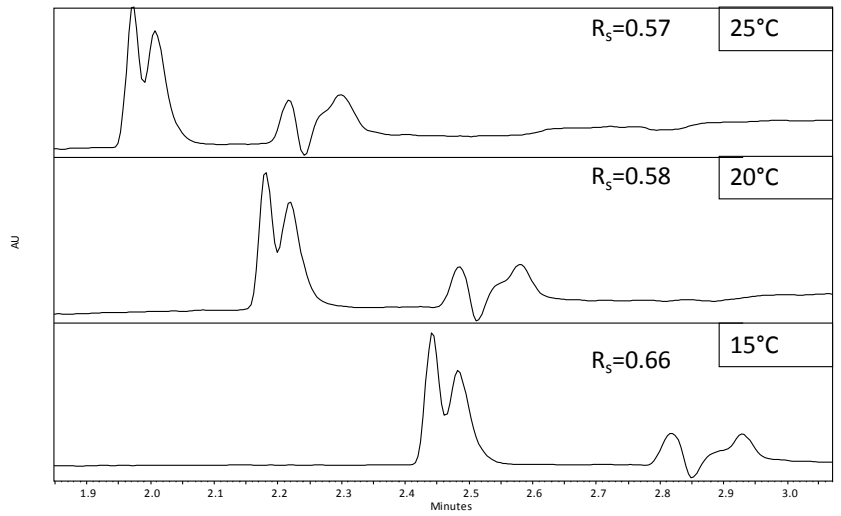


Figure 3.17 Effect of temperature on the separation of sotalol using the CAB coating. **Conditions: Polymer concentration:** 2.50% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** kV; **Temperature:** 15, 20, 25˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

increased as in Figure 3.14. When CAB, and particularly a concentration of 2.50% (w/w) was used as a separation medium, sotalol enantiomers were partially separated, with a resolution

value of 0.66 (Figure 3.15). Another observation with the CAP-and the CAB-modified capillary columns was the decrease in resolution by decreasing the applied voltage or by increasing the column temperature in Figure 3.16 and Figure 3.17, respectively. In addition, the electropherograms involving sotalol indicate that efficiency of the second enantiomeric peak is low. As reported elsewhere for the beta blockers[4] this is probably due to increased interactions of the second enantiomer with the stationary phase.

3.3.4 Optimized Separation Conditions for Ephedrine Using Cellulose Acetate, Cellulose Acetate Phthalate, and Cellulose Acetate Butyrate

Ephedrine is a molecule with two asymmetric centers resulting in four diastereomeric compounds [(+)-ephedrine, (-)-ephedrine, (+)-pseudoephedrine, and (-)-pseudoephedrine]. Ephedrine is another analyte that was used for the evaluation of the performance of each cellulose-modified capillary column.

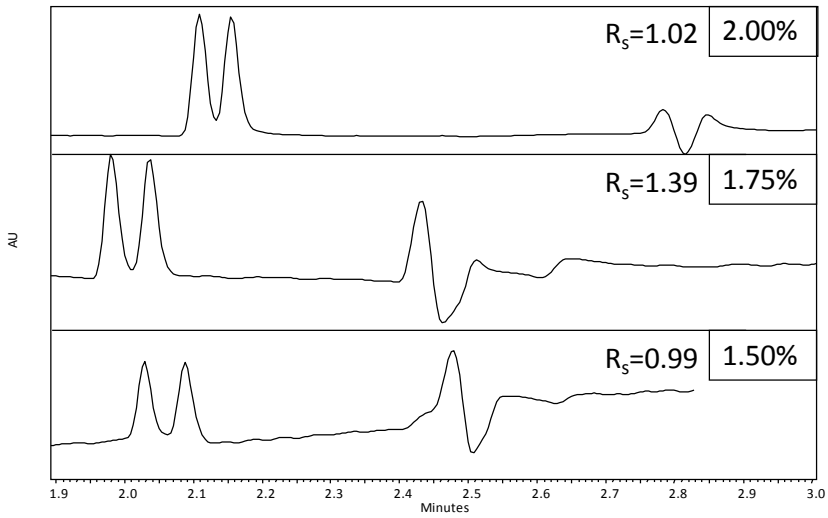


Figure 3.18 Effect CA concentration on the separation of ephedrine. **Conditions: Polymer concentration:** 1.50, 1.75, 2.00% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 30 kV; **Temperature:** 15˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

In this study, the concentration of CA was varied from 1.50 - 2.00% (w/w) in Figure 3.18. A resolution of 1.07 and elution times of 1.97 and 2.05 were obtained when the concentration was 1.75% (w/w) CA. Each elution time corresponded to the peaks with a resolution of 1.39 with one peak elution time at 1.97 min while the other was at 2.05 min. The two peaks were composed of (±)-pseudoephedrine and (±)-ephedrine, respectively. A further increase in polymer concentration resulted in a slight increase in resolution and analysis time.

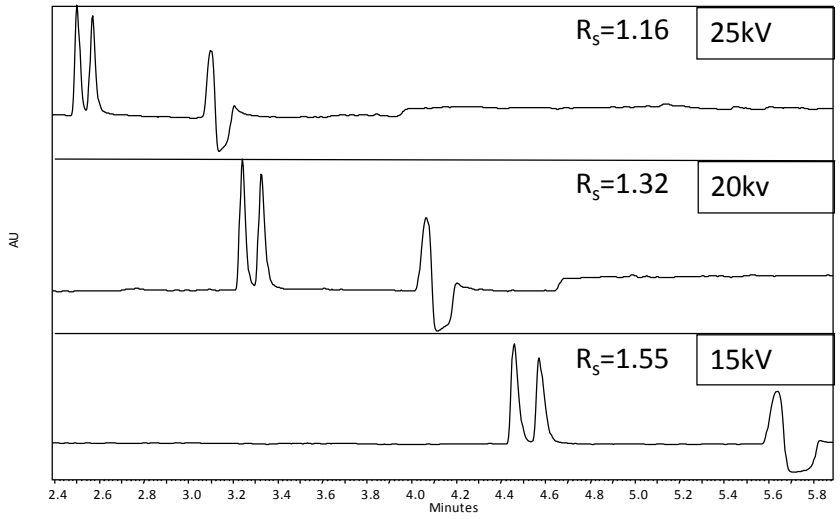


Figure 3.19 Effect of voltage on the separation of ephedrine using the CA coating. **Conditions: Polymer concentration:** 1.75% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 15, 20, 25 kV; **Temperature:** 15˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

When the applied voltage and column temperature was decreased from 30 kV to 15 kV, resolution increased as demonstrated in Figure 3.19. However, in Figure 3.20 as temperature increased from 15°C to 25°C, resolution decreased significantly due to the analyte staying on the column a shorter analysis time and acquiring a lower amount of interactions with the stationary phase.

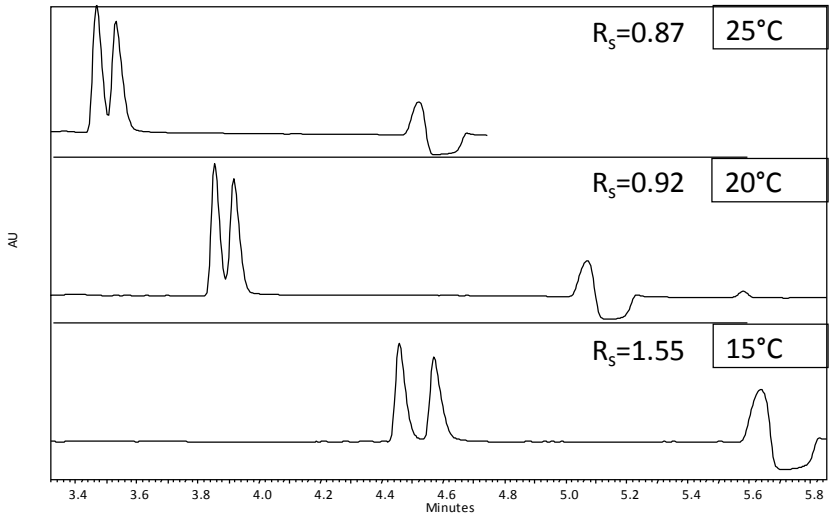


Figure 3.20 Effect of temperature on the separation of ephedrine using the CA coating. **Conditions: Polymer concentration:** 1.75% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 15 kV; **Temperature:** 15, 20, 25˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

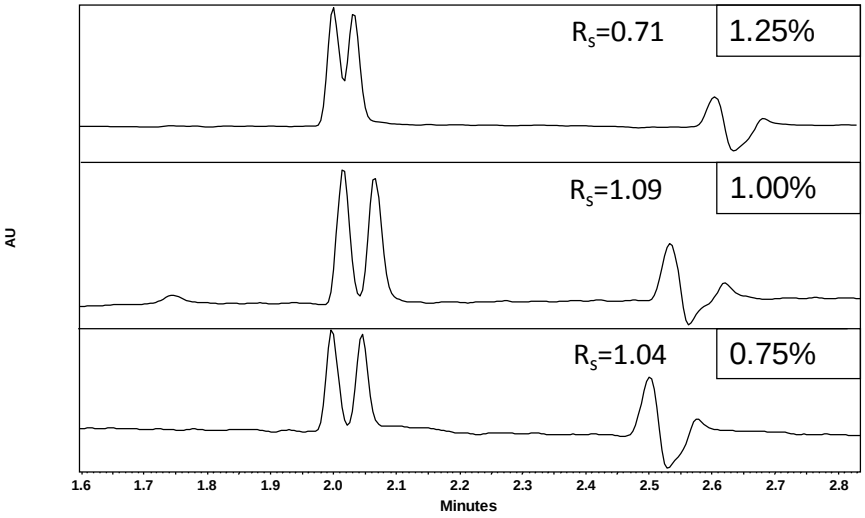


Figure 3.21 Effect CAP concentration on the separation of ephedrine. **Conditions: Polymer concentration:** 0.75, 1.00, 1.25% (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 8.2; **Applied voltage:** 30 kV; **Temperature:** 15˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

The polymer concentration range using CAP was 0.50 – 1.00% (w/w) in Figure 3.21, while the range using CAB was 1.50 – 2.00% (w/w) in Figure 3.24. Concentration of 0.75%

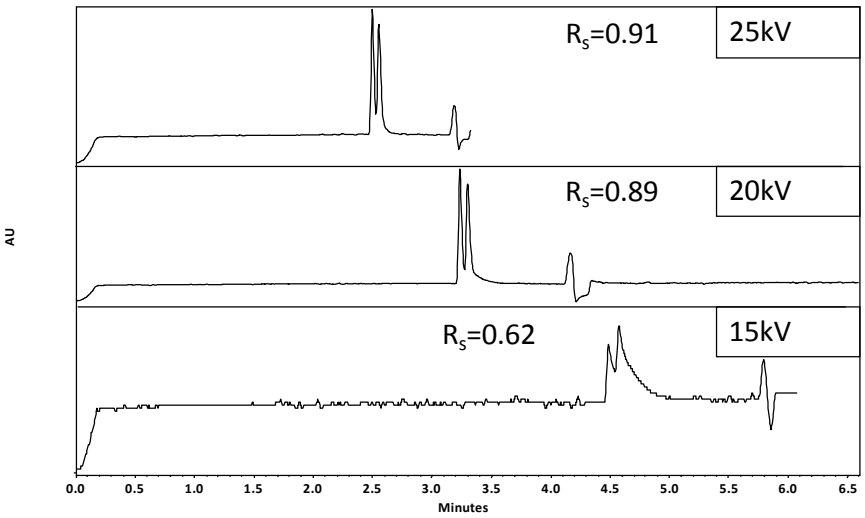


Figure 3.22 Effect of voltage on the separation of ephedrine using the CAP coating. **Conditions: Polymer concentration:** 1.00% (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 15, 20, 25 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

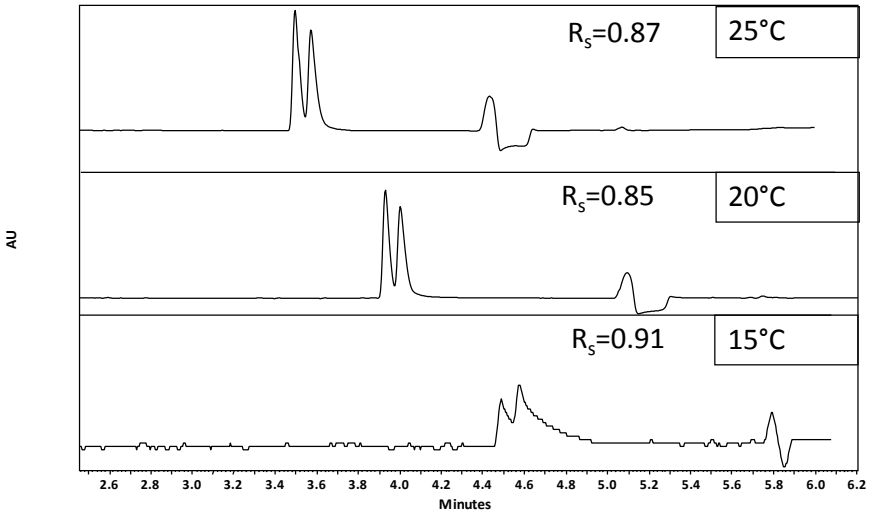


Figure 3.23 Effect of temperature on the separation of ephedrine using the CAP coating. **Conditions: Polymer concentration:** 1.00 % (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 30 kV; **Temperature:** 15, 20, 25˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

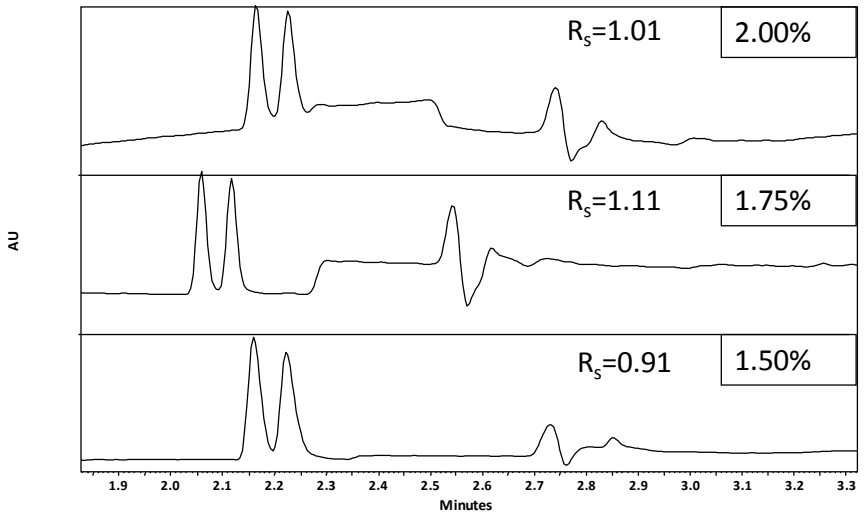


Figure 3.24 Effect of CAB concentration on the separation of ephedrine. **Conditions: Polymer concentration:** 1.50, 1.75, 2.00% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50mM Phosphate pH 9.7; **Applied voltage:** 30 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

(w/w) CAP and 1.75% (w/w) CAP and CAB, respectively, were able to separate (±)-pseudoephedrine from (±)-ephedrine was observed. Decreases in voltage resulted in lower resolution values of both CAP and CAB in Figures 2.22 and 2.25, respectively. Similarly, decreases in temperatures resulted in lower resolution values of CAP and CAB in Figures 2.23 and 3.26, respectively. In the case of CAB, after optimizing temperature, the best resolution achieved was 2.03. In addition, according to the last figure, a third peak was observed with optimized conditions indicating the separation of diastereomers of (±)-pseudoephedrine. The elution order for the two peaks observed was (±)-pseudoephedrine followed by (±)-ephedrine with each biopolymer. Two peaks were observed using CA and CAP. However, there was the appearance of a third peak indicating the partial separation of chiral analytes (+)-pseudoephedrine and (-)-pseudoephedrine using CAB as a stationary phase.

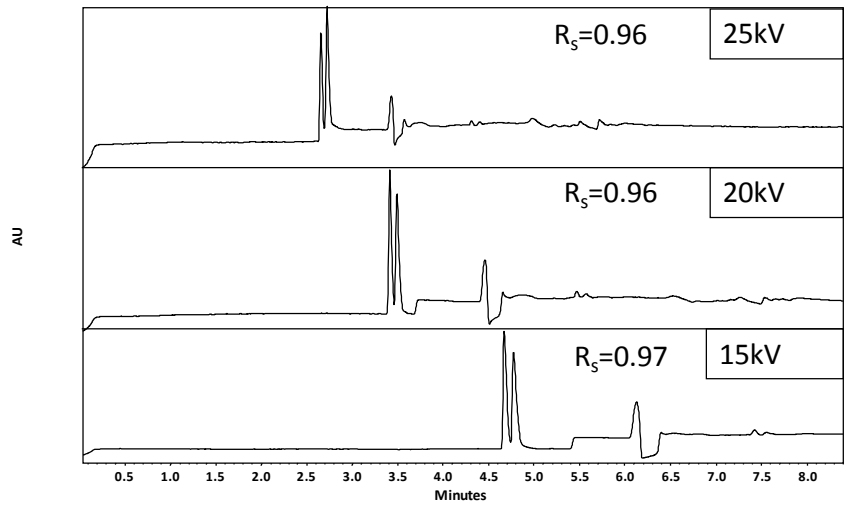


Figure 3.25 Effect of voltage on the separation of ephedrine using the CAB coating. **Conditions: Polymer concentration:** 1.75% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 15, 20, 25 kV; **Temperature:** 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

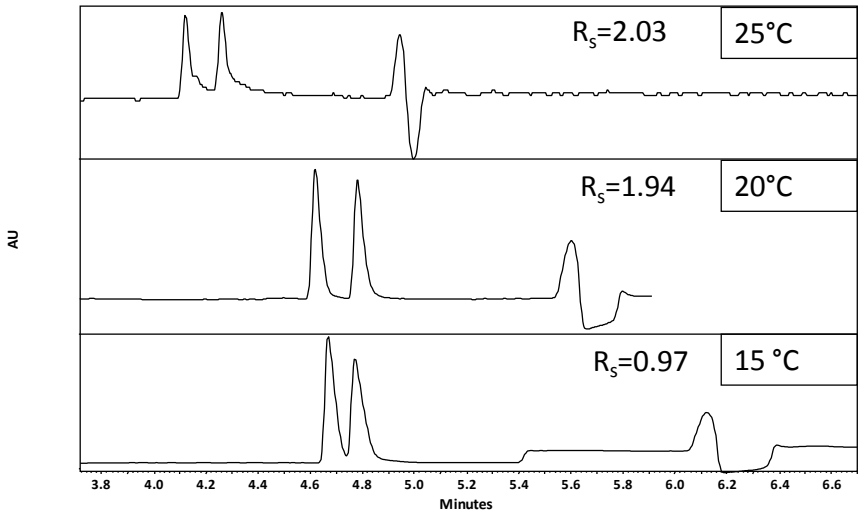


Figure 3.26 Effect of temperature on the separation of ephedrine using the CAB coating. **Conditions: Polymer concentration:** 1.75% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 15 kV; **Temperature:** 15, 20, 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

3.3.5 Optimized Separation Conditions for Labetalol Using Cellulose Acetate, Cellulose Acetate Phthalate, and Cellulose Acetate Butyrate

Labetalol is a betablocker that has two asymmetric centers as mentioned earlier for ephedrine. Due to the lack of pure optically standards of labetalol, diastereomer peaks could not be identified. The examined polymer concentration range using CA was 1.50 – 2.00% (w/w) in Figure 3.27. Only two of the four analyte peaks of interest were observed. There was a loss of resolution in varying the polymer concentration. Thus, 1.75% (w/w) was chosen as the optimal polymer concentration. An increase in the voltage led to an increased resolution in Figure 3.28. Moreover, as temperature was increased from 15°C to 25°C resolution was increased in Figure 3.29.

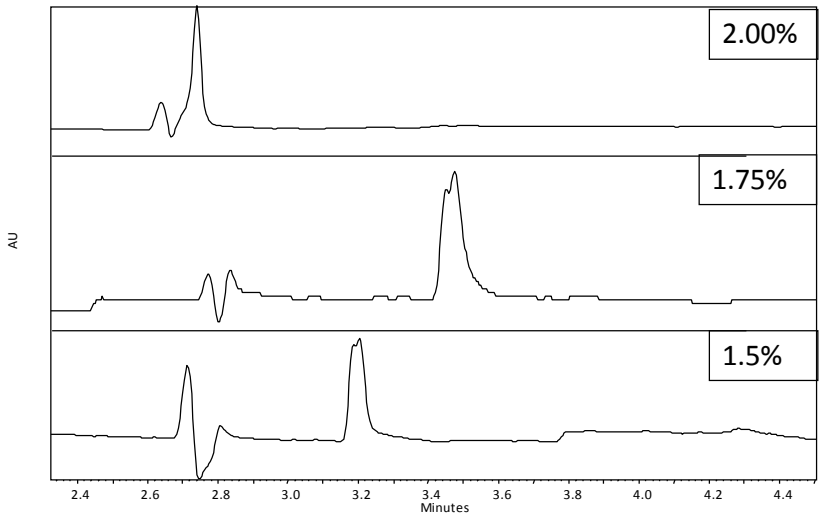


Figure 3.27 Effect of CA concentration on the separation of labetalol. **Conditions: Polymer concentration:** 1.50, 1.75, 2.00% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 30 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

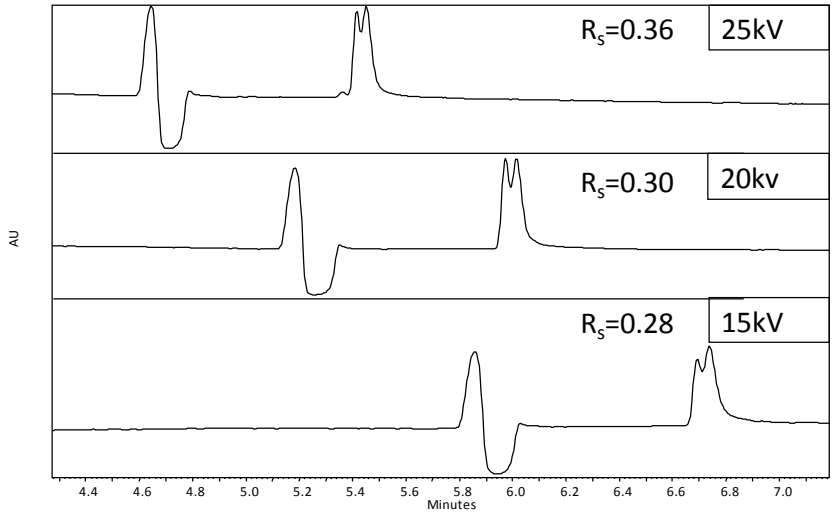


Figure 3.28 Effect of voltage on the separation of labetalol using the CA coating. **Conditions: Polymer concentration:** 1.75% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 15, 20, 25 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

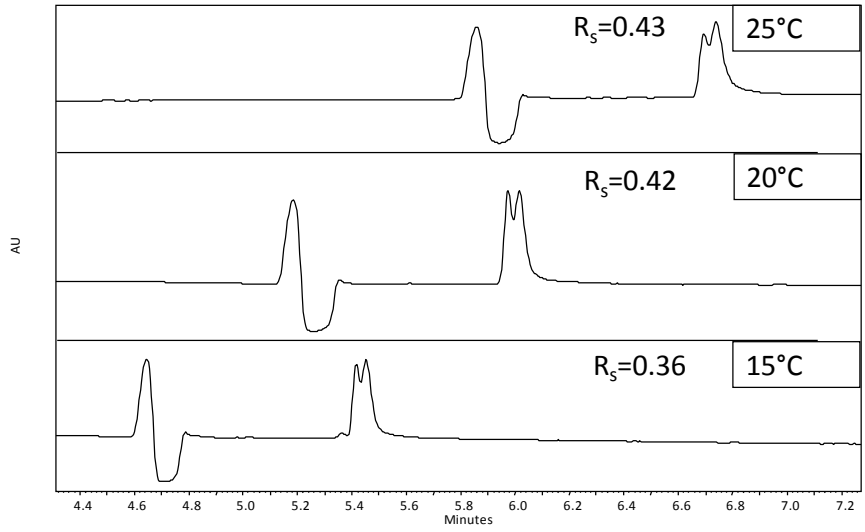


Figure 3.29 Effect of temperature on the separation of labetalol using the CA coating. **Conditions: Polymer concentration:** 1.75% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 15, 20, 25 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

The polymer concentration range of CAP was 0.75 – 1.25% (w/w) in Figure 3.30. The separation of labetalol using 1.00% (w/w) CAP also results in a partial separation. At a

concentration of 1.00% (w/w) CAP two peaks are observed while variations in polymer concentration result in no separation or a loss in resolution. When the voltage was decreased from 30 kV to 15 kV, resolution increased in Figure 3.31. Similarly, it was observed when temperatures were lowered from 25°C to 15°C resolution increased in Figure 3.32.

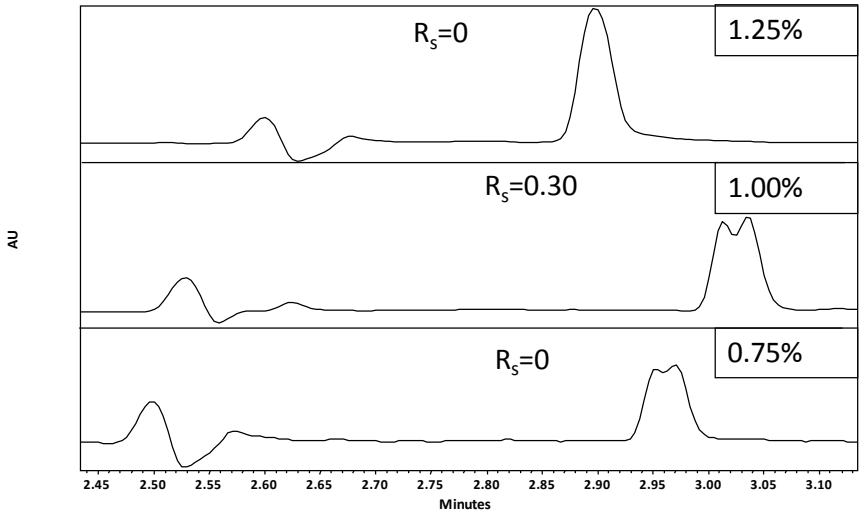


Figure 3.30 Effect of CAP concentration on the separation of labetalol. **Conditions: Polymer concentration:** 0.75, 1.00, 1.25% (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 30 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

A polymer concentration range of 1.50 – 2.00% (w/w) CAB was used to evaluate labetalol in Figure 3.33. Using a concentration of 1.75% (w/w) CAB resulted in two peaks for the separation of labetalol. Variations in polymer concentration using CAB led to either no separation or a loss in resolution. When the voltage was decreased from 30 kV to 15kV, resolution increased in Figure 3.34. Similarly as temperature was decreased resolution also increased in Figure 3.35.

Only two peaks were observed using each one of the biopolymer as a coating. Partial separation with the highest resolution and most efficient peaks were accomplished using CAB.

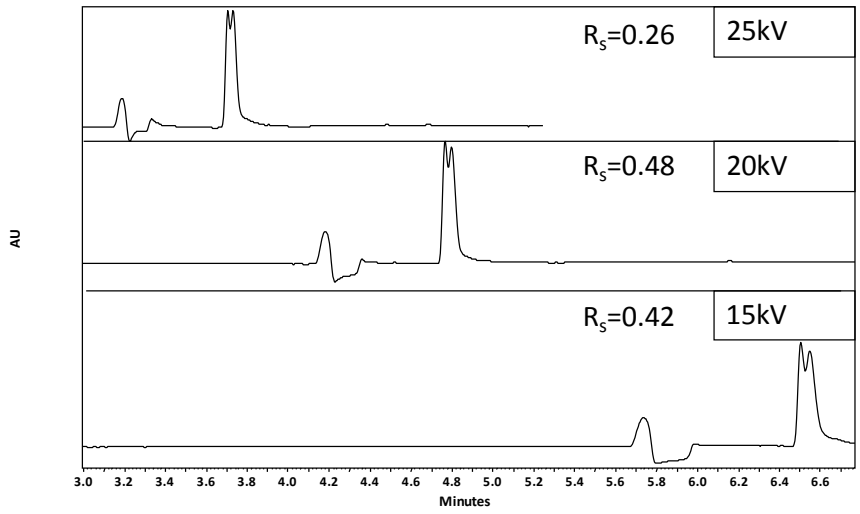


Figure 3.31 Effect of voltage on the separation of labetalol using the CAP coating. **Conditions: Polymer concentration:** 1.75% (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 15, 20, 25 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

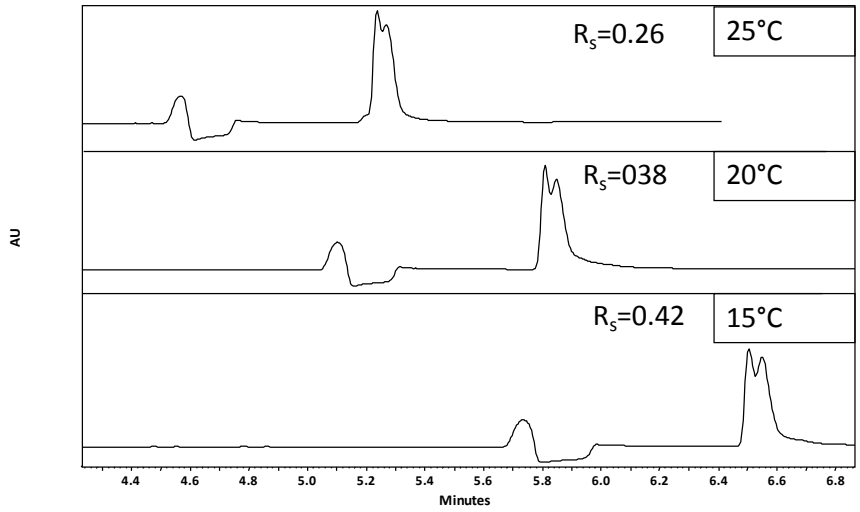


Figure 3.32 Effect of temperature on the separation of labetalol using the CAP coating. **Conditions: Polymer concentration:** 1.75% (w/w) CAP in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 25 kV; **Temperature:** 15, 20, 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

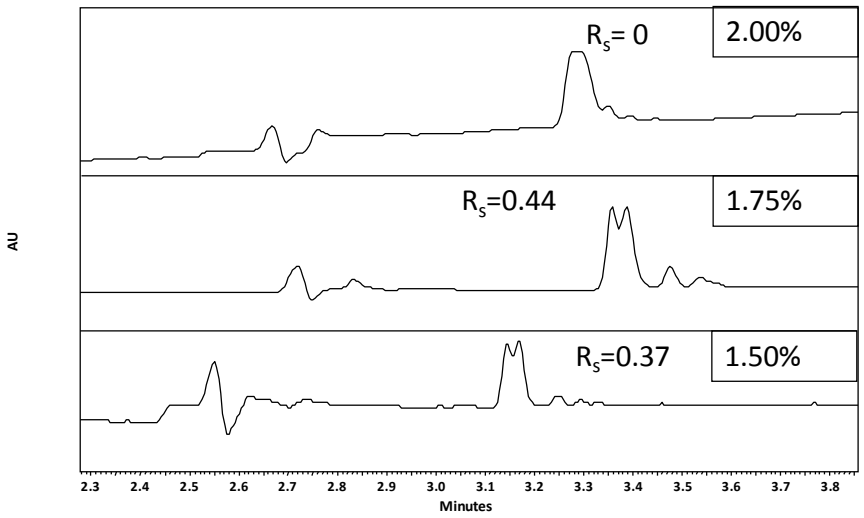


Figure 3.33 Effect of CAB concentration on the separation of labetalol. **Conditions: Polymer concentration:** 1.50, 1.75, 2.00% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 30 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

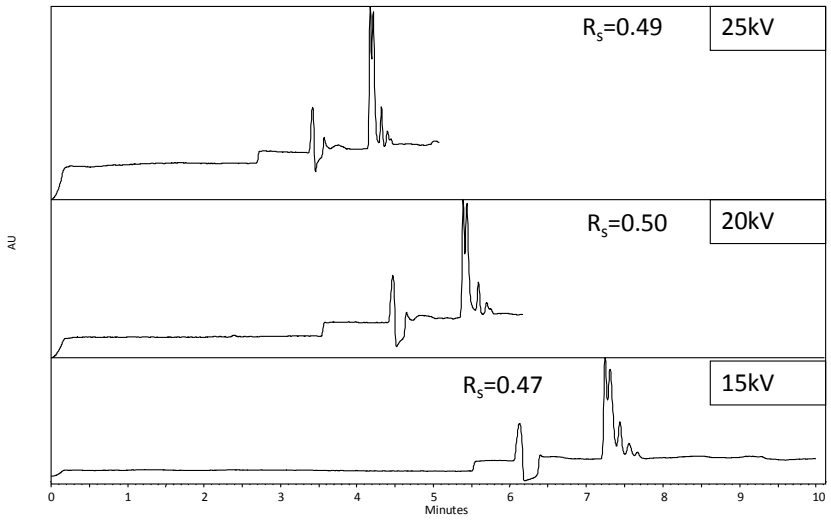


Figure 3.34 Effect of voltage on the separation of labetalol using the CAB coating. **Conditions: Polymer concentration:** 1.75% (w/w) CAB in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 15, 20, 25kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

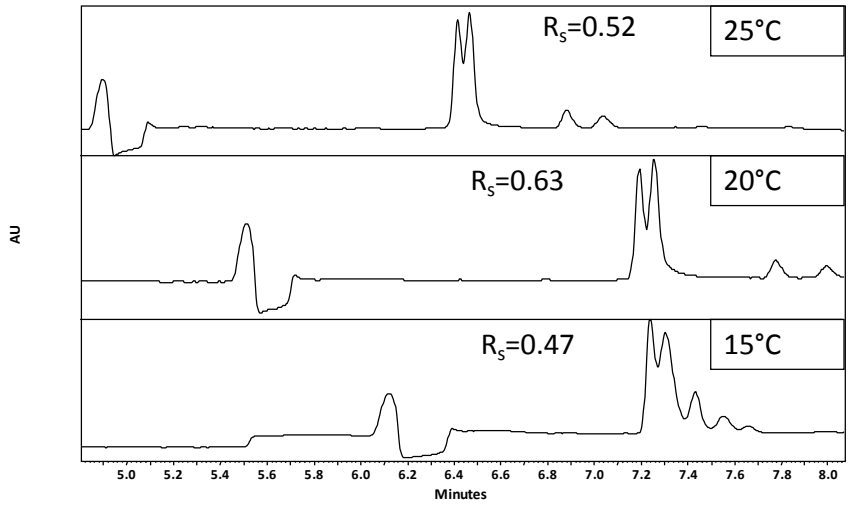


Figure 3.35 Effect of temperature on the separation of labetalol using the CAB coating. **Conditions: Polymer concentration:** 1.75% (w/w) cellulose acetate butyrate in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 9.7; **Applied voltage:** 15 kV; **Temperature:** 15, 20, 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.2 mg/mL; **Injection:** 0.5 psi for 5 s; **Detection:** 200 nm.

Moreover, these separations occurred in less than 8 mins. Analysis times using CAB were longer due to more interactions with the stationary phase.

3.3.6 Reproducibility of Polysaccharide Coated Capillaries

An important factor to consider when cellulose-coated capillaries are used is the reproducibility The stability of the cellulose-based coatings were evaluated using run-to-run and capillary-to-capillary studies in Table 3.1. Each polysaccharide was evaluated using conditions similar to that with the separation of thiopental. EOF was monitored via the methanol marker. Run-to-run studies were based on 50 consecutive runs on the same capillary. Capillary-to-capillary studies were based on four capillaries with replicate analyses. Studies show less than 7% RSD of the EOF for each coating.

Table 3.1 Reproducibility studies of the polysaccharide coatings. **Conditions: Polymer concentration:** 1.5% (w/w) cellulose acetate, 0.75% (w/w) cellulose acetate phthalate, or 1.5% (w/w) cellulose acetate butyrate in [BMIM][OAc]; **Background electrolyte:** 50 mM Phosphate pH 7.2; **Applied voltage:** 30 kV; **Temperature:** 15 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 μm i.d.; **EOF marker:** methanol; **Injection:** 0.5 psi for 5 s; **Detection:** 254 nm.

	Ave. EOF migration time (min)	%RSD of EOF
Run to Run (n=50 runs)		
Cellulose Acetate	3.35	2.52
Cellulose Acetate Phthalate	2.88	3.68
Cellulose Acetate Butyrate	3.17	6.04
Capillary to Capillary (n=5 capillaries)		
Cellulose Acetate	3.18	6.94
Cellulose Acetate Phthalate	3.02	3.34
Cellulose Acetate Butyrate	3.00	3.57

3.4 Concluding Remarks

A simple and facile method of preparing cellulose based stationary phases used for OT-CEC chiral separations has been applied. RTIL, [BMIM][OAc], facilitated the cellulose stationary phase fabrication due to its solubility with the cellulose. Removal of the [BMIM][OAc] was accomplished with a simple rinse due to the miscibility of the RTIL with water. The separation of chiral drugs was evaluated using commercially available cellulose derivatives. Baseline resolution was received using the cellulose derivatives for the separation of thiopental, while only partial separation could be achieved for the separation of sotalol.

Similarly, partial separation was observed for the diastereomers of labetalol. However, baseline resolution was achieved for ephedrine diastereomers. It is worth noting the appearance of a third peak indicates the separation of (+)-pseudoephedrine and (-)-pseudoephedrine. Column stability was good with RSD less than 7% of the EOF with 50 consecutive runs with each of the cellulose derivatives. With the high density of stereogenic chiral centers in cellulose based compounds, the addition of more hydrophobic groups may increase chiral selection. With a copious library of RTILs to choose from, the dissolution of cellulose derivatives should relatively straightforward. This method may be applied towards many hydrophobic chiral selectors for separations in OT-CEC.

3.5 References

(1) Scriba, G. K. E. Fundamental Aspects of Chiral Electromigration Techniques and Application in Pharmaceutical and Biomedical Analysis. *J. Pharmaceut. and Biomed.* **2011**, *55*, 688-701.

(2) Zhang, Z.; Wu, M.; Wu, R. a.; Dong, J.; Ou, J.; Zou, H. Preparation of Perphenylcarbamoylated β-Cyclodextrin-silica Hybrid Monolithic Column with "One-Pot" Approach for Enantioseparation by Capillary Liquid Chromatography. *Anal. Chem.* **2011**, *83*, 3616-3622.

(3) Gong, Y.; Lee, H. K. Application of Cyclam-Capped β-Cyclodextrin-Bonded Silica Particles as a Chiral Stationary Phase in Capillary Electrochromatography for Enantiomeric Separations. *Anal. Chem.* **2003**, *75*, 1348-1354.

(4) Kamande, M. W.; Zhu, X.; Kapnissi-Christodoulou, C.; Warner, I. M. Chiral Separations Using a Polypeptide and Polymeric Dipeptide Surfactant Polyelectrolyte Multilayer Coating in Open-Tubular Capillary Electrochromatography. *Anal. Chem.* **2004**, *76*, 6681-6692.

(5) Luces, C. A.; Warner, I. M. Achiral and Chiral Separations Using MEKC, Polyelectrolyte Multilayer Coatings, and Mixed Mode Separation Techniques with Molecular Micelles. *Electrophoresis* **2010**, *31*, 1036-1043.

(6) Qu, P.; Lei, J.; Ouyang, R.; Ju, H. Enantioseparation and Amperometric Detection of Chiral Compounds by in Situ Molecular Imprinting on the Microchannel Wall. *Anal. Chem.* **2009**, *81*, 9651-9656.

(7) Hosoya, K.; Hira, N.; Yamamoto, K.; Nishimura, M.; Tanaka, N. High-Performance Polymer-Based Monolithic Capillary Column. *Anal. Chem.* **2006**, *78*, 5729-5735.

(8) Krenkova, J.; Lacher, N. A.; Svec, F. Control of Selectivity via Nanochemistry: Monolithic Capillary Column Containing Hydroxyapatite Nanoparticles for Separation of Proteins and Enrichment of Phosphopeptides. *Anal. Chem.* **2010**, *82*, 8335-8341.

(9) Li, M.; Liu, X.; Jiang, F.; Guo, L.; Yang, L. Enantioselective Open-Tubular Capillary Electrochromatography Using Cyclodextrin-Modified Gold Nanoparticles as Stationary Phase. *J. Chromatogr. A* **2011**, *1218*, 3725-3729.

(10) Cavazzini, A.; Kaczmarski, K.; Szabelski, P.; Zhou, D.; Liu, X.; Guiochon, G. Modeling of the Separation of the Enantiomers of 1-Phenyl-1-propanol on Cellulose Tribenzoate. *Anal. Chem.* **2001**, *73*, 5704-5715.

(11) Takegawa, A.; Murakami, M.-a.; Kaneko, Y.; Kadokawa, J.-i. Preparation of Chitin/Cellulose Composite Gels and Films with Ionic Liquids *Carbohyd. Polym.* **2010**, *79*, 85-90.

(12) Yang, R.; Shi, R.; Peng, S.; Zhou, D.; Liu, H.; Wang, Y. Cationized Hydroxyethylcellulose as a Novel, Adsorbed Coating for Basic Protein Separation by Capillary Electrophoresis *Electrophoresis* **2008**, *29*, 1460-1466.

(13) Girod, M.; Chankvetadze, B.; Blaschke, G. Enantioseparations in Non-Aqueous Capillary Electrochromatography Using Polysaccharide Type Chiral Stationary Phases *J. Chromatogr. A* **2000**, *887*, 439-455.

(14) Jinno, K.; Wu, J.; Sawada, H.; Kiso, Y. Cellulose Acetate Fiber as Stationary Phase in Capillary Electrochromatography. *J. High Res. Chromatog.* **1998**, *21*, 617-619.

(15) Hendrickx, A.; Mangelings, D.; Heyden, Y. V. Comparative Enantioseparations of Pharmaceuticals in Capillary Electrochromatography on Polysaccharide-Based Chiral Stationary Phases Containing Selectors With or Without Chlorinated Derivatives. *Electrophoresis* **2010**, *31*.

(16) Qin, F.; Xie, C.; Feng, S.; Ou, J.; Kong, L.; Ye, M.; Zou, H. Monolithic Silica Capillary Column with Coated Cellulose Tris(3,5-dimethylphenylcarbamate) for Capillary Electrochromatographic Separation of Enantiomers. *Electrophoresis* **2006**, *27*, 1050-1059.

(17) Dong, X.; Wu, R. a.; Dong, J.; Wu, M.; Zhu, Y.; Zou, H. The Covalently Bonded Cellulose Tris(3,5-dimethylphenylcarbamate) on a Silica Monolithic Capillary Column for Enantioseparation in Capillary Electrochromatography. *J. Chromatogr. B* **2008**, *875*, 317-322.

(18) Kumar, A. P.; Park, J. H. Enantioseparation on Cellulose Dimethylphenylcarbamate-Modified Zirconia Monolithic Columns by Reversed-Phase Capillary Electrochromatography *J. Chromatogr. A* **2010**, *1217*, 4494-4500.

(19) Zhang, Y.-J.; Zhang, Y.-P.; Duan, Q.-r.; Bai, L.-Y.; Chen, J.; Zhou, X.-M. Enantioseparation Using a Cellulose-Based Stationary Phase by Capillary Liquid Chromatography. *J. Liq. Chromatogr. R. T.* **2010**, *33*, 1733-1744.

(20) Isogai, A.; Atalla, R. H. Dissolution of Cellulose in Aqueous NaOH Solutions *Cellulose* **1998**, *5*, 309-319.

(21) Rogers, R. J.; Seddon, K. R. Ionic Liquids - Solvents of the Future? . *Science* **2003**, *302*, 792-793.

(22) Baker, G.; Baker, S. N.; Pandey, S.; Bright, F. V. An Analytical View of Ionic Liquids. *Analytst* **2005**, *130*, 800-808.

(23) Dumke, J. C.; El-Zahab, B.; Challa, S.; Das, S.; Chandler, L.; Tolocka, M.; Hayes, D. J.; Warner, I. M. Lanthanide-Based Luminescent NanoGUMBOS *Langmuir* **2010**, *26*, 15599-15603.

(24) Tesfai, A.; El-Zahab, B.; Kelley, A. T.; Li, M.; Garno, J. C.; Baker, G. A.; Warner, I. M. Magnetic and Nonmagnetic Nanoparticles from a Group of Uniform Materials Based on Organic Salts. *ACS Nano* **2009**, *3*, 3244-3250.

(25) Tian, Y.; Feng, R.; Liao, L.; Liu, H.; Chen, H.; Zeng, Z. Dynamically Coated Silica Monolith with Ionic Liquids for Capillary Electrochromatography. *Electrophoresis* **2008**, *29*, 3153-3159.

(26) Mora-Pale, M.; Meli, L.; Doherty, T. V.; Linhardt, R. J.; Dordick, J. S. Room Temperature Ionic Liquids as Emerging Solvents for the Pretreatment of Lignocellulosic Biomass *Biotechnol. and Bioeng.* **2011**, *108*, 1229-1245.

(27) Lovell, C. S.; Walker, A.; Damion, R. A.; Radhi, A.; Tanner, S. F.; Budtova, T.; Ries, M. E. Influence of Cellulose on Ion Diffusivity in 1-Ethyl-3-Methyl-Imidazolium Acetate Cellulose Solutions. *Biomacromolecules* **2010**, *11*, 2927-2935.

(28) Vitz, J.; Erdmenger, T.; Haensch, C.; Schubert, U. S. Extended Dissolution Studies of Cellulose in Imidazolium Based Ionic Liquids. *Green Chem.* **2009**, *11*, 417-424.

(29) Kosan, B.; Michels, C.; Meister, F. Dissolution and Forming of Cellulose with Ionic Liquids *Cellulose* **2008**, *15*, 59-66.

CHAPTER 4
PROTEIN SEPARATIONS USING POLYSACCHARIDE-BASED COATED CAPILLARIES

4.1 Introduction

Cancer is the second leading cause of death in the world.[1] Early treatment could increase survival rates. Therefore, early detection of various types of cancer could lead to rapid and more effective treatment of the disease. Protein biomarkers can be analyzed as an indicator for the various types of cancer such as ribonuclease A for ovarian cancer.[2] However, in early development stages these biomarkers can be in low abundance. Therefore, the use of sensitive instruments for the detection of disease-related biomarkers is highly required. Detection techniques such as high performance liquid chromatography (HPLC) cannot be used for the detection of small volume samples (i.e. nano- or picoliter) and capillary electrophoresis (CE) suffers from analytes adsorbing to the capillary wall.[3, 4] A hybrid technique of interest is open tubular capillary electrochromatography (OT-CEC), which applies the selectivity of HPLC and efficiency of CE.[5-7] OT-CEC can use sample volumes being as a low as the nanoliter scale and reduce adsorption of protein analytes to the capillary wall due to the applied stationary phase.[4, 8] There are many stationary phases used for OT-CEC; however, most have high cost associated due to synthesis procedures to form the stationary phase. Thus, the use of more abundant materials would prove to be beneficial.

Cellulose is a highly hydrophobic polysaccharide and the most abundant organic mass on the planet which makes it cost efficient. The use of cellulose has garnered much interest in separation science due to its effectiveness as a stationary phase.[9-11] For instance, water soluble hydroxyethylcellulose-graft-poly(2(dimethylamino)ethyl

methacrylate was applied as a physically adsorbed coating for the separation of basic proteins by Cao et al.[12] Results show that manipulating the grafting ratio of poly(2-(-(dimethyamino)ethyl methacrylate) grafts can affect the charge of the capillary wall and allow the possible analysis of acidic and basic proteins on the same column. However, hitherto, the use of cellulose in OT-CEC have required chemical modification to the capillary wall or the use of harsh solvents.[13, 14] Furthermore, the dissolution of cellulose could become a troublesome even with the addition of hydrophobic groups (e.g., benzene) which may improve the selectivity.[15] Therefore, the use of a solvent to dissolve many derivatives of cellulose would be highly resourceful. One such solvent with the ability to have a wide range of hydrophobicities for the dissolution of compounds is room temperature ionic liquids (RTILs).

RTILs are solvents composed of anions and cations with melting points near room temperature.[16] RTILs have many unique properties such as low volatility and the ability to dissolve highly hydrophobic material while remaining water soluble.[17, 18] The latter property has been exploited in the preparation of cellulose coated capillaries for OT-CEC. The dissolution of cellulose into RTILs has been previously reported.[17-20]

In this study, cellulose-based derivatives were dissolved into 1-butyl-3-methylimidazolium acetate [BMIM][OAc] and filled into the capillary. A water rinse was then applied to remove [BMIM][OAc] leaving the capillary wall coated with the polysaccharide. The best resolution values, in combination with rapid analysis times, were obtained using CA. Nanoparticles of chitosan have been investigated recently and results show higher resolution achieved versus a non-nanoparticle based stationary phase.[21, 22] Modified gold nanoparticles have also been employed as stationary phases.[23-

[25] Nilsson *et al*. found nanoparticles containing certain surface-to-volume proportions can provide interactions for an effective stationary phase.[26] Therefore, nanoparticles of the polysaccharide CA were synthesized and compared to the wall coating of CA.

4.2 Experimental

4.2.1 Materials and Reagents

Cellulose acetate (CA), cellulose acetate phthalate (CAP), cellulose acetate butyrate (CAB) and 1-butyl-3-methylimidazolium acetate [BMIM][OAc] in Figure 4.1 as well as lysozyme, ribonuclease A, cytochrome *c*, and α-chymotrypsinogen A were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Acetone, sodium phosphate dibasic dihydrate, hydrochloric acid, and methanol were obtained from Fisher Scientific (Fair Lawn, NJ). All materials were used as received without further treatment or purification. Background electrolyte (BGE), 20 mM phosphate dibasic buffer, was adjusted from pH 3.5 to 2.5 using 1 M HCl. The buffer was filtered using a 0.45 μm polypropylene filter (Nalgene, Rochester, NY) and sonicated for 15 minutes prior to use. Protein analytes were prepared at 2 mg/mL concentration stock solutions in water and diluted to a concentration of 0.4 mg/mL.

4.2.2 Cellulose Acetate Nanoparticle Preparation

Nanofibers were prepared by dissolving 1 mM cellulose acetate in 100 μL of acetone. The solution was then added to 5 mL of water and sonicated using a probe sonicator for 10 mins. A sample was placed on a grid for imaging, using a JEOL 100-CX transmission electron microscope (TEM), shown in Figure 4.7. The solution was then centrifuged at 14,000 rpm for 15 minutes. After centrifugation, the supernatant was

removed and nanofibers were allowed to dry overnight at ambient conditions. Fibers were then resuspended into phosphate buffer at pH 3.

4.2.3 Instrumentation and Preparation Procedure for Polysaccharide Coating

The protein separation was conducted on a Beckman P/ACE MDQ capillary

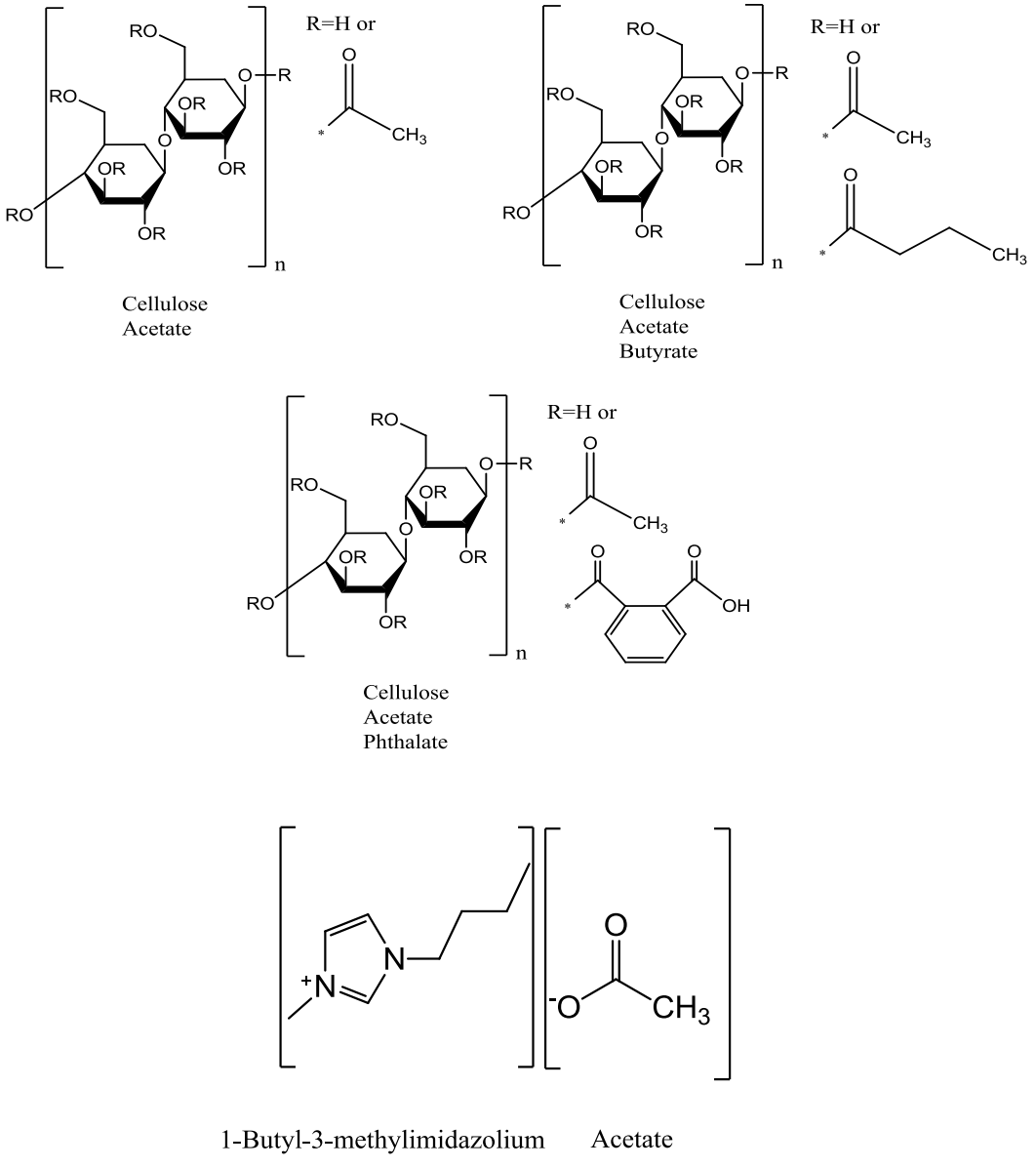


Figure 4.1 Structures of cellulose derivatives and ionic liquid.

electrophoresis system for CEC separations. Protein analytes were detected at 200 nm using a UV photodiode array (Fullerton, CA). Separations were performed using fused-silica capillaries purchased from Polymicro Technologies (Phoenix, AZ). Each capillary had a total length of 50 cm (40 cm effective length) and an internal diameter of 50 μm. Conditioning of capillaries was accomplished by flushing with 1 M NaOH for 30 minutes followed by deionized water for 15 min. Capillaries were dried using argon gas for at least one hour. The dried capillaries were then filled with a solution of the polysaccharide of interest dissolved at various concentrations in [BMIM][OAc] using a Harvard apparatus 22 syringe pump. Filled capillaries were flushed with water until aqueous droplets could be observed on the opposite end. Capillaries were then placed into the CE instrument and rinsed with the corresponding separation buffer for 20 mins. For the preparation of cellulose acetate nanofiber coatings, capillaries were rinsed with buffer for four minutes then nanofiber solutions were flushed through the capillary at appropriate coating times. All rinses using the CE instrument were performed at a pressure of 20 psi. Separations were conducted under normal polarity at temperature of 15 to 25°C with an applied voltage of 20 to 30 kV. Electrokinetic injections were performed by applying 5 kV for 5 s. Resolutions are shown for example as R_{s1} to denote the resolution between peaks 1 and 2, followed by R_{s2} for peaks 2, and 3 and so on.

Capillary images were acquired using a Leica DM-RXA fluorescence microscope with a 20x 0.7 NA objective and a Leica N2.1 (TRITC) filter cube. Data was processed using slidebook (ver 4.1) imaging software. The capillary was treated with polysaccharide of interest using the procedure as mentioned earlier. After rinsing the capillary with water, a solution of 10mM rhodamine 6G (R6G) was flushed throughout

the capillary. The capillary was then dried using a stream of argon gas. An image was taken along the length of the capillary to observe the uniformity of the coating throughout the capillary.

4.3 Results and Discussion

4.3.1 Separation of Basic Proteins with Varying Buffer pH

Proteins analytes were evaluated under acidic conditions using pH values of 2.5 through 3.5 in Figure 4.2. Electroosmotic flow was decreased in separations due to the reduction in surface charge by having the neutral polysaccharide coating.[27] The highest

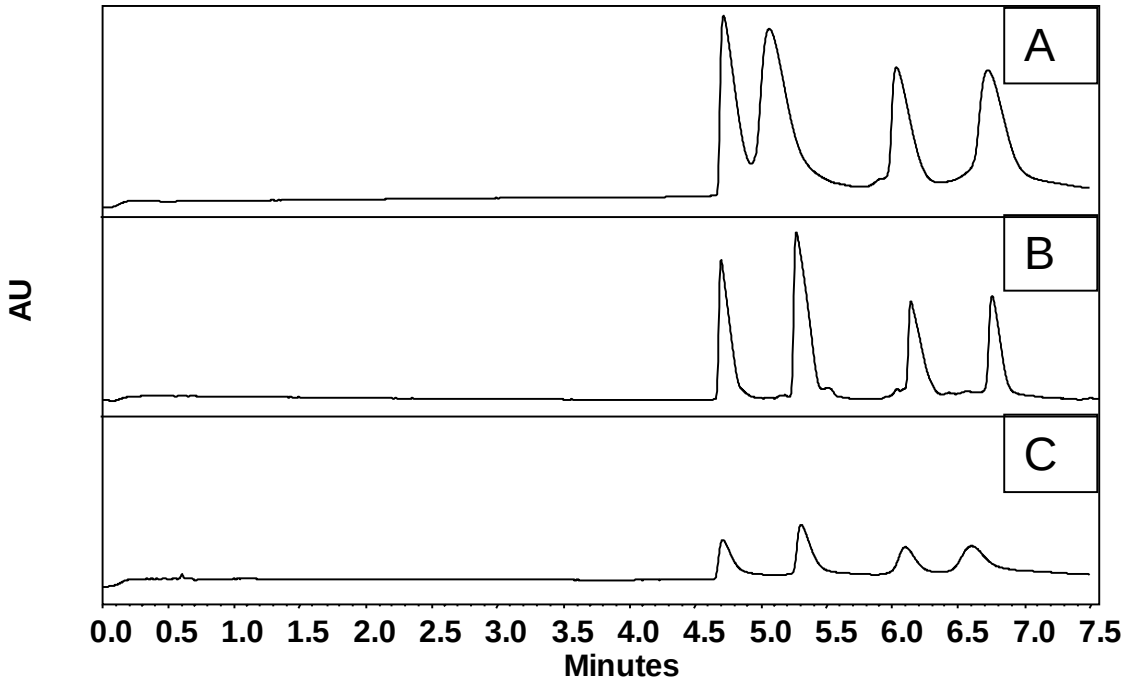


Figure 4.2 Effect of pH on the separation of four basic proteins using cellulose acetate coating. **Conditions: Polymer concentration:** 2.75% (w/v) cellulose acetate in [BMIM][OAc]; **Background electrolyte:** 20 mM Sodium Phosphate dibasic pH (A) 3.5, (B) 3.0, (C) 2.5; **Applied voltage:** 25 kV; **Temperature:** 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. lysozyme, 2. ribonuclease A, 3. cytochrome *c*, 4. α-chymotrypsinogen A.

resolution obtained was for pH 3 where resolution values were at least baseline resolved, R_s = 1.5, (R_{s1} = 2.56, R_{s2} = 3.48, R_{s3} = 2.75). While at pH 2.5 peak intensities were decreased as well as resolution (R_{s1} = 2.36, R_{s2} = 2.52, R_{s3} = 1.12). At pH 3.5 peak intensities were greater; however, there was a loss of resolution between protein peaks lysozyme and ribonuclease A (R_{s1} = 0.85, R_{s2} = 2.21, R_{s3} = 1.59). There were very small noticeable differences in the individual migration times of protein analytes with varying pH. Differences in resolution can be attributed to multiple factors such as BGE viscosity and interactions of analytes with the stationary phase.

4.3.2 Effect of CA Concentration on the Separation of Four Basic Proteins

Polymer concentration was varied from 2.50 – 3.00% (w/w) as shown in Figure 4.3. The optimal polymer concentration was observed at 2.75% (w/w) CA with resolution values of (R_{s1} = 2.56, R_{s2} = 3.48, R_{s3} = 2.75). Analysis time was faster than other concentrations possibly due to having a sufficient amount of coating on the capillary wall for interactions to occur. At 3.00% (w/w) all proteins were baseline resolved or greater (R_{s1} = 1.59, R_{s2} = 5.12, R_{s3} = 3.95); however, peaks were wider and the analysis time were longer, which indicated more interactions with a thicker CA coating. As polymer concentration was lowered to 2.50% (w/w) peaks became less efficient and reproducibility was reduced. Resolutions between ribonuclease A, cytochrome *c*, and α-chymotrypsinogen A being reduced (R_{s1} = 2.80, R_{s2} = 2.68, and R_{s3} = 1.18), respectively. Reproducibility may be due to not having enough coverage of the polysaccharide stationary phase to reduce protein adsorption to the capillary wall.

4.3.3 Investigation of Polymer Variation on the Separation of Four Basic Proteins

Polysaccharides CAP and CAB were optimized by polymer concentration for the best separation of the basic protein sample. Most separations occurred using 2.00% (w/w) of CAP and CAB, respectively, with the results being compared to using the optimized concentration of CA coating in Figure 4.3. Using the CAP polymer coating resulted with resolutions being the lowest (R_{s1} = 1.81, R_{s2} = 1.97, R_{s3} = 0.92) of the three polymers. CAB and CA had resolutions that were similar (R_{s1} = 2.56, R_{s2} = 3.26, R_{s3} = 2.68) and (R_{s1} = 2.56, R_{s2} = 3.48, R_{s3} = 2.75), respectively. However, CA had a shorter

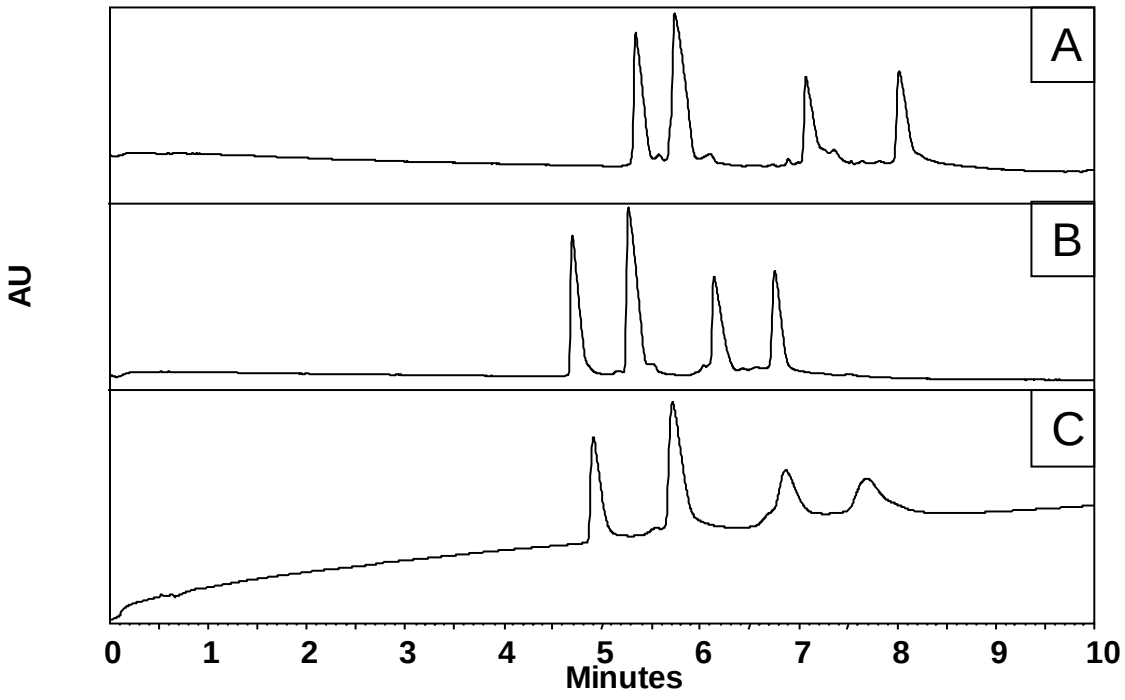


Figure 4.3 Effect of polymer concentration on the separation of four basic proteins using CA. **Conditions: Polymer concentration:** (A) 3.00% (w/w) (B) 2.75% (w/w) (C) 2.5% (w/w) cellulose acetate in [BMIM][OAc]; **Background electrolyte:** 20mM Sodium Phosphate dibasic pH 3.0; **Applied voltage:** 25 kV; **Temperature:** 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. lysozyme, 2. ribonuclease A, 3. cytochrome *c*, 4. α-chymotrypsinogen A.

analysis time and was chosen as the polymer of interest for the rest of the experiments.

4.3.4 Effect of Voltage on the Separation of Four Basic Proteins Using CA Coating

The voltage was varied from 30 to 20 kV which can be observed in Figure 4.5. Resolution increased as voltages were decreased due to allowing analytes more time to interact with the stationary phase. However, at lower voltages 25 kV and 20 kV analysis time increased with increasing resolution (R_{s1} = 2.03, R_{s2} = 2.69, R_{s3} = 1.78) and (R_{s1} =2.43, R_{s2} =2.96, R_{s3} = 1.96), respectively. Since all protein peaks were at least baseline resolved (R_s = 1.98, R_s = 2.57, R_s = 1.60) for the highest voltage, 30 kV was selected for the swiftest separation.

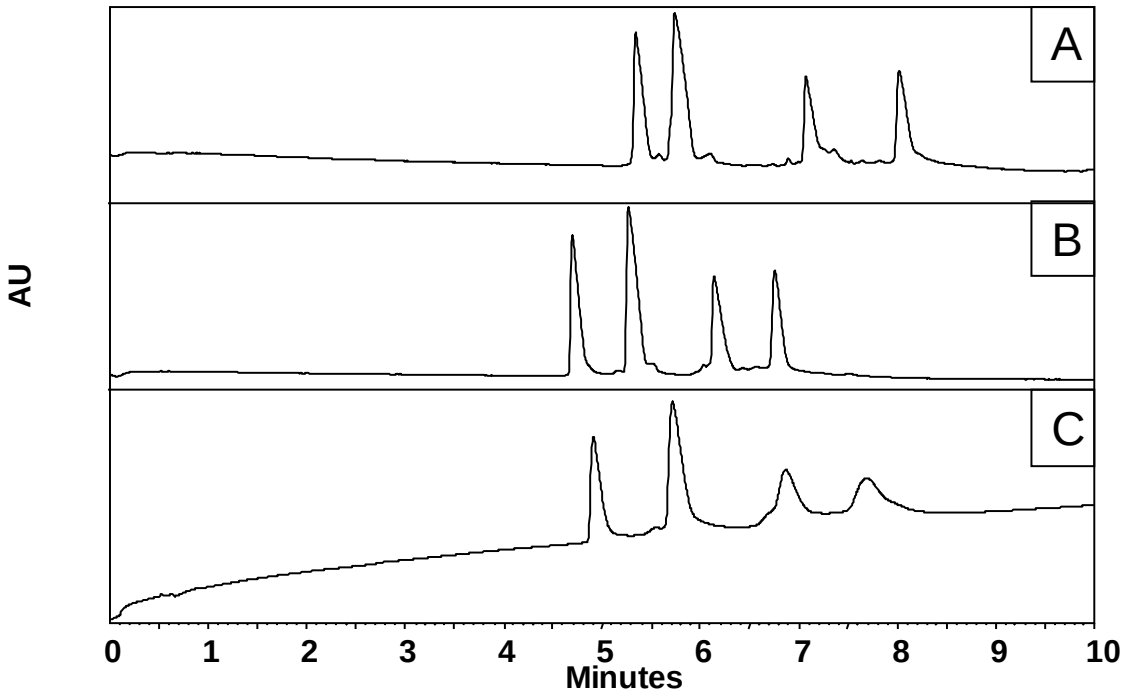


Figure 4.4 Effect of polymer variation on the separation of 4 basic proteins. **Conditions: Polymer concentration:** (A) 2.00% CAP (B) 2.00% (w/v) CAB (C) 2.75% (w/w) CA in [BMIM][OAc]; **Background electrolyte:** 20 mM Sodium Phosphate dibasic pH 3.0; **Applied voltage:** 25 kV; **Temperature:** 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. lysozyme, 2. ribonuclease A, 3. cytochrome *c*, 4. α-chymotrypsinogen A.

4.3.5 Effect of Temperature on the Separation of Four Basic Proteins Using CA Coating

The effect of temperature (15-25°C) on protein separations was investigated in this study shown in Figure 4.6. Having a similar trend as the voltage, using lower temperatures allowed analytes to interact longer with the stationary phase to result in higher resolutions. The lowest temperature, 15°C, resulted with the highest resolution values (R_{s1} = 2.68, R_{s2} = 2.90, R_{s3} = 1.60); however, analysis times increased. At 20°C resolution decreased (R_{s1} = 2.23, R_{s2} = 2.86, R_{s3} = 1.58) with faster analysis times. The

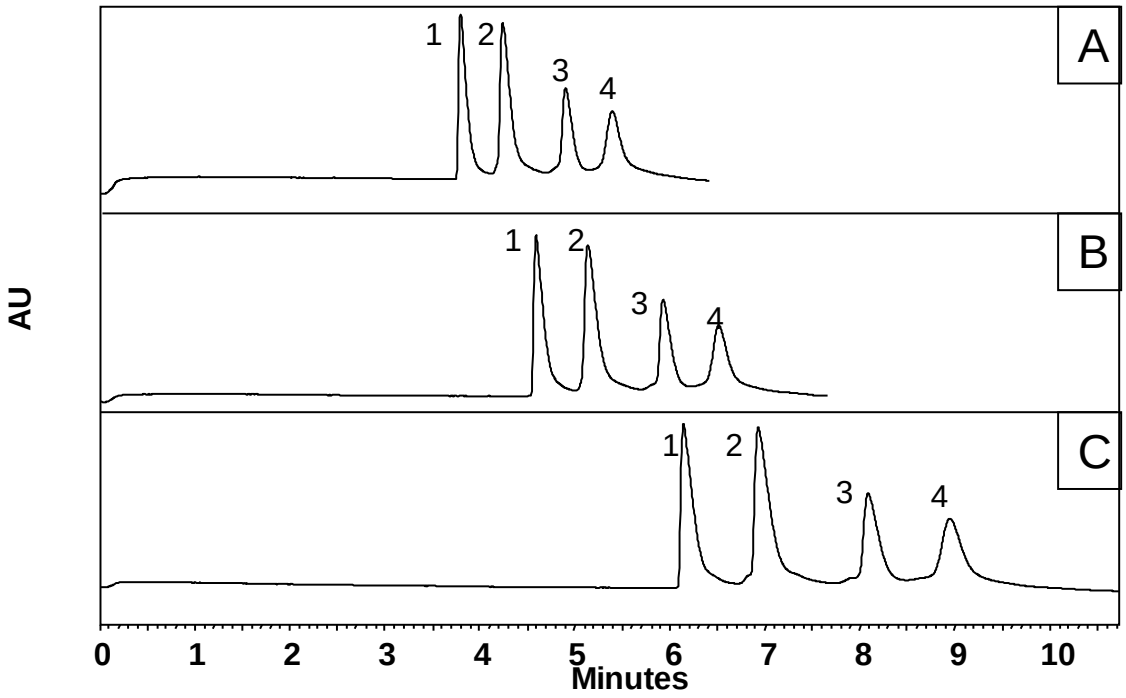


Figure 4.5 Effect of voltage on the separation of four basic proteins using CA coating. **Conditions: Polymer concentration:** 2.75% (w/v) cellulose acetate in [BMIM][OAc]; **Background electrolyte:** 20mM Sodium Phosphate dibasic pH 3.0; **Applied voltage:** (A) 30 kV (B) 25 kV (C) 20kV; **Temperature:** 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. lysozyme, 2. ribonuclease A, 3. cytochrome *c*, 4. α-chymotrypsinogen A.

optimized temperature was chosen to be 25°C from the previous voltage study due to having the fastest analysis time, while maintaining baseline resolution.

4.3.6 Effect of Using CA Nanofibers with Varying Coating Time on the Separation of Four Basic Proteins

Nanofibers of CA (Figure 4.7A) were suspended in 20 mM sodium phosphate dibasic buffer pH 3 for the analysis of the basic protein biomarkers under previous conditions in Figure 4.8. The suspended solution was flushed through the capillary at 5, 10, and 15 min coat times. Results show that using a 5 min coat time resulted in proteins were not fully resolved (R_{s1} = 1.18, R_{s2} = 1.14, R_{s3} = 0.66) with low reproducibility. This

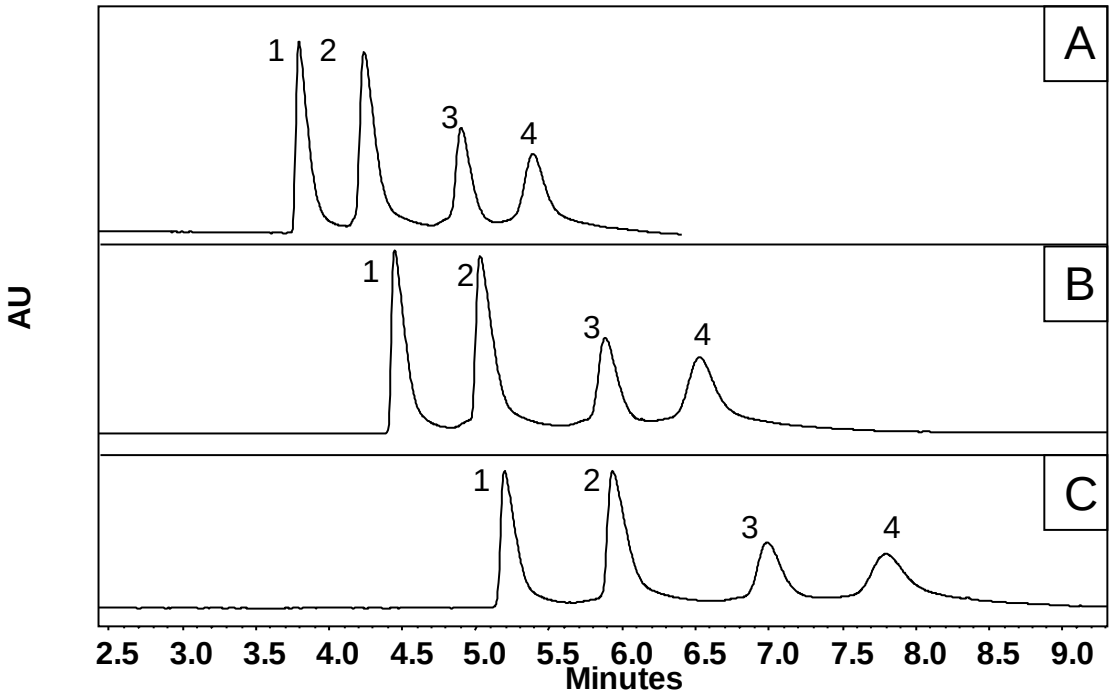


Figure 4.6 Effect of temperature variation on the separation of four basic proteins using CA coating. **Conditions: Polymer concentration:** 2.75% (w/v) cellulose acetate in [BMIM][OAc]; **Background electrolyte:** 20mM Sodium Phosphate dibasic pH 3.0; **Applied voltage:** 30 kV; **Temperature:** (A) 25 ˚C (B) 20°C (C) 15°C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. lysozyme, 2. ribonuclease A, 3. cytochrome *c*, 4. α-chymotrypsinogen A.

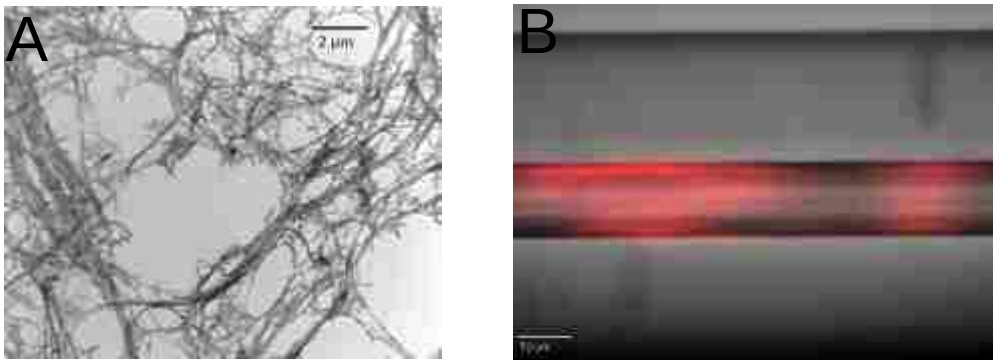


Figure 4.7 (A) TEM image of CA nanofibers. (B) Fluorescence microscope image of nanofibers coated on the capillary wall.

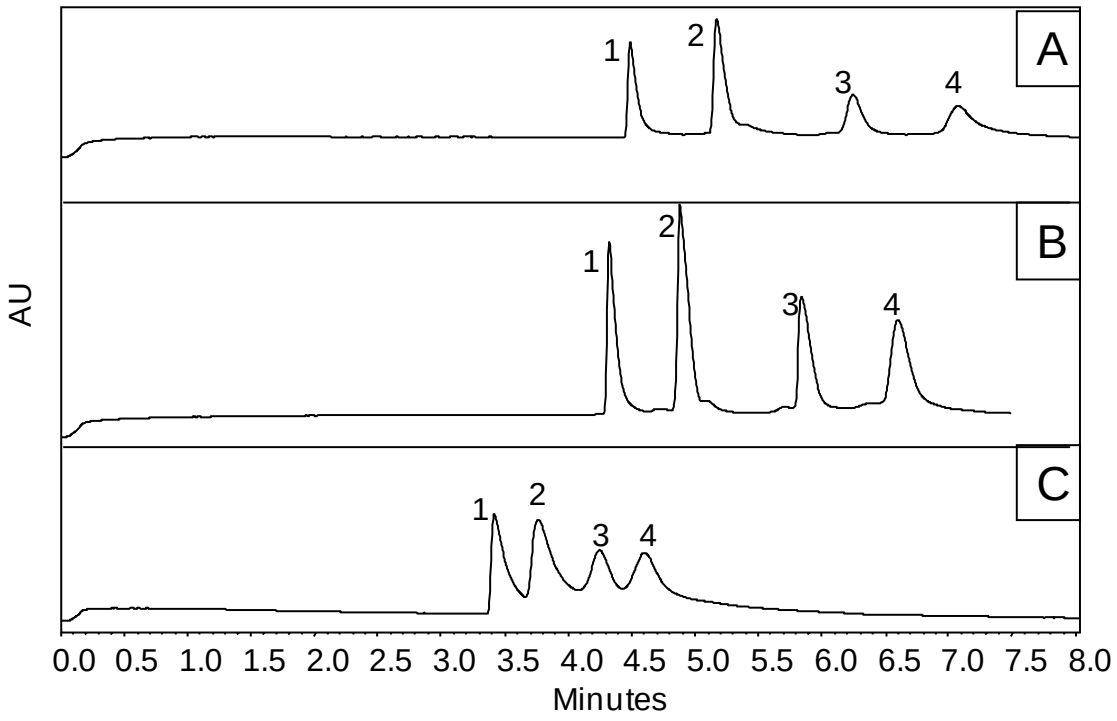


Figure 4.8 Effect of coating time on the separation of four basic proteins using cellulose acetate nanofibers. **Conditions: Polymer:** cellulose acetate; **Coating Time:** (A) 15 min (B) 10 min (C) 5 min; **Background electrolyte:** 20mM Sodium Phosphate dibasic pH 3.0; **Applied voltage:** 30 kV; **Temperature:** 25 ˚C; **Capillary:** 50 cm (total length), 40 cm (effective length), 50 µm i.d.; **Analyte concentration:** 0.4 mg/mL; **Injection:** 5 kV for 5 s; **Detection:** 200 nm; **Analytes:** 1. lysozyme, 2. ribonuclease A, 3. cytochrome *c*, 4. α-chymotrypsinogen A.

may be due to not having full coverage of the capillary wall resulting in the protein analytes adsorbing to the capillary wall. As the coating time was increased to 10 min, protein separations were more reproducible and resolution was increased (R_{s1} = 2.83, R_{s2} = 4.14, R_{s3} = 2.43) with better peak efficiency. Although resolution increased (R_{s1} = 3.56, R_{s2} = 2.73, R_{s3} = 2.59) for 15 min coating time, peak efficiency had decreased due to increased interactions with the stationary phase. Moreover, analysis time was increased for longer coating times. The CA coating and nanofibers baseline resolved all protein peaks. However, analyte peaks eluted slower for the nanofibers than for the CA coating possibly due to increased adsorption of the proteins to the silanol groups from the capillary wall. Non-uniform coverage, of the CA nanofibers coated at 10 minutes on the capillary wall, is indicated by the darker shades of the fluorescence image of the internal diameter of the capillary (Figure 4.8B). Having more uniform coverage would result in better reproducibilities obtained for the nanofiber coating.

4.4 Concluding Remarks

Cellulose-based polysaccharides stationary phases have been used for the separation of basic proteins in OT-CEC. Coatings were applied to capillary walls using simple preparation methods with commercially available materials rather than lengthy organic synthesis for polysaccharides covalently bonded to the capillary wall. Proteins separations were fast (< 10mins) and the majority of the separations resulted in baseline resolved peaks. The use of CA nanofibers was explored as a stationary phase for OT-CEC in which baseline resolution was also acheived for all protein peaks. These findings show promising results for protein analysis using polysaccharide stationary phases in OT-CEC.

4.5 REFERENCES

(1) Murphy, S. L.; Xu, J.; Konchanek, K. D. Deaths: Preliminary Data for 2010. *National vital statistics reports: from the Center for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics* **2012**, *60*, 1-69.

(2) Ryu, O.; Atkinson, J.; Hoehn, G.; Illei, G.; Hart, T. Identification of Parotid Salivary Biomarkers in Sjogren's Syndrome by Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and Two-Dimensional Difference Gel Electrophoresis. *Rheumatology* **2006**, *45*, 1077-1086.

(3) Pacakova, V.; Stulik, K.; Hau, P. T.; Jelinek, I.; Vins, I.; Sykora, D. Comparison of High-Performance Liquid-Chromatography and Capillary Electrophoresis for the Determination of Some Bee Venom Components. *J. Chromatogr. A* **1995**, *700*, 187-193.

(4) Jorgenson, J. W.; Lukacs, K. D. Zone Electrophoresis in Open-Tubular Glass-Capillaries. *Anal. Chem.* **1981**, *53*, 1298-1302.

(5) Huang, X.; Zhang, J.; Horvath, C. Capillary Electrochromatography of Proteins and Peptides with Porous Layer Open-Tubular Columns. *J. Chromatogr. A* **1999**, *858*, 91-101.

(6) Kapnissi, C. P.; Akbay, C.; Schlenoff, J. B.; Warner, I. M. Analytical Separations Using Molecular Micelles in Open-Tubular Capillary Electrochromatography. *Anal. Chem.* **2002**, *74*, 2328-2335.

(7) Liu, Z.; Zou, H. F.; Ye, M. L.; Ni, J. Y.; Zhang, Y. K. Study of Physically Adsorbed Stationary Phases for Open Tubular Capillary Electrochromatography. *Electrophoresis* **1999**, *20*, 2891-2897.

(8) Moore, L., Jr; LeJeune, Z. M.; Luces, C. A.; Gates, A. T.; Li, M.; El-Zahab, B.; Garno, J. C.; Warner, I. M. Lysine-Based Zwitterionic Molecular Micelle for Simultaneous Separation of Acidic and Basic Proteins Using Open Tubular Capillary Electrochromatography. *Anal. Chem.* **2010**, *82*, 3997-4005.

(9) Yang, R.; Wang, Y. L. Y. Hydroxyethylcellulose-Graft-Poly (4-vinylpyridine) as a Novel, Adsorbed Coating for Protein Separation by CE *Electrophoresis* **2009**, *30*, 2321-2327.

(10) Lv, C. G.; Liu, Y. Q.; Mangelings, D.; Vander Heyden, Y. Enantioselectivity of Monolithic Silica Stationary Phases Immobilized with Different Concentrations Cellulose Tris (3,5-dimethylphenylcarbamate), Analyzed with Different Mobile Phases in Capillary Electrochromatography. *Electrophoresis* **2011**, *32*, 2708-2717.

(11) Peterson, E. A.; Sober, H. A. Chromatography of Proteins .1. Cellulose Ion-Exchange Adsorbents. *J. Am. Chem. Soc.* **1956**, *78*, 751-755.

(12) Cao, F. H.; Luo, Z. F.; Zhou, D.; Zeng, R. J.; Wang, Y. M. Hydroxyethylcellulose-Graft-Poly (2-(dimethylamino)ethyl methacrylate) as Physically Adsorbed Coating for Protein Separation by CE. *Electrophoresis* **2011**, *32*, 1148-1155.

(13) Isogai, A.; Atalla, R. H. Dissolution of Cellulose in Aqueous NaOH Solutions *Cellulose* **1998**, *5*, 309-319.

(14) Zhang, Y.-J.; Zhang, Y.-P.; Duan, Q.-r.; Bai, L.-Y.; Chen, J.; Zhou, X.-M. Enantioseparation Using a Cellulose-Based Stationary Phase by Capillary Liquid Chromatography. *J. Liq. Chromatogr. R. T.* **2010**, *33*, 1733-1744.

(15) Okamoto, Y.; Aburatani, R.; Fukumoto, T.; Hatada, K. Chromatographic Resolution .17. Useful Chiral Stationary Phases for Hplc - Amylose Tris(3,5-Dimethylphenylcarbamate) and Tris(3,5-Dichlorophenylcarbamate) Supported on Silica-Gel. *Chem. Lett.* **1987**, 1857-1860.

(16) Walden, P. *Bulletin de l'Academie Imperiale des Sciences de St. Petersburg* **1914**, 405-422.

(17) Lovell, C. S.; Walker, A.; Damion, R. A.; Radhi, A.; Tanner, S. F.; Budtova, T.; Ries, M. E. Influence of Cellulose on Ion Diffusivity in 1-Ethyl-3-Methyl-Imidazolium Acetate Cellulose Solutions. *Biomacromolecules* **2010**, *11*, 2927-2935.

(18) Vitz, J.; Erdmenger, T.; Haensch, C.; Schubert, U. S. Extended Dissolution Studies of Cellulose in Imidazolium Based Ionic Liquids. *Green Chem.* **2009**, *11*, 417-424.

(19) Simmons, T. J.; Lee, S. H.; Miao, J. J.; Miyauchi, M.; Park, T. J.; Bale, S. S.; Pangule, R.; Bult, J.; Martin, J. G.; Dordick, J. S.; Linhardt, R. J. Preparation of Synthetic Wood Composites Using Ionic Liquids. *Wood Sci. Technol.* **2011**, *45*, 719-733.

(20) Kosan, B.; Michels, C.; Meister, F. Dissolution and Forming of Cellulose with Ionic Liquids. *Cellulose* **2008**, *15*, 59-66.

(21) Chen, J. L.; Hsieh, K. H. Nanochitosan Crosslinked with Polyacrylamide as the Chiral Stationary Phase for Open-Tubular Capillary Electrochromatography. *Electrophoresis* **2011**, *32*, 398-407.

(22) Chen, J. L. Molecularly Bonded Chitosan Prepared as Chiral Stationary Phases in Open-Tubular Capillary Electrochromatography: Comparison with Chitosan Nanoparticles Bonded to the Polyacrylamide Phase. *Talanta* **2011**, *85*, 2330-2338.

(23) Li, M.; Liu, X.; Jiang, F. Y.; Guo, L. P.; Yang, L. Enantioselective Open-Tubular Capillary Electrochromatography Using Cyclodextrin-Modified Gold Nanoparticles as Stationary Phase. *J. Chromatogr. A* **2011**, *1218*, 3725-3729.

(24) Qu, Q. S.; Zhang, X. X.; Shen, M.; Liu, Y.; Hu, X. Y.; Yang, G. J.; Wang, C. Y.; Zhang, Y. K.; Yan, C. Open-Tubular Capillary Electrochromatography Using a Capillary Coated with Octadecylamine-Capped Gold Nanoparticles. *Electrophoresis* **2008**, *29*, 901-909.

(25) Yang, L.; Guihen, E.; Holmes, J. D.; Loughran, M.; O'Sullivan, G. P.; Glennon, J. D. Gold Nanoparticle-Modified Etched Capillaries for Open-Tubular Capillary Electrochromatography. *Anal. Chem.* **2005**, *77*, 1840-1846.

(26) Nilsson, C.; Harwigsson, I.; Becker, K.; Kutter, J. P.; Birnbaum, S.; Nilsson, S. Nanoparticle-Based Capillary Electroseparation of Proteins in Polymer Capillaries under Physiological Conditions. *Electrophoresis* **2010**, *31*, 459-464.

(27) Zheng, J.; Bragg, W.; Hou, J. G.; Lin, N.; Chandrasekaran, S.; Shamsi, S. A. Sulfated and Sulfonated Polysaccharide as Chiral Stationary Phases for Capillary

Electrochromatography and Capillary Electrochromatography-Mass Spectrometry. *J. Chromatogr. A* **2009**, *1216*, 857-872.

CHAPTER 5
SUMMARY OF RESULTS AND FUTURE STUDIES

In this dissertation, various stationary phases were evaluated for the separation of protein analytes as well as chiral drug molecules using OT-CEC. Novel micelles were used as coatings for the simultaneous separation of acidic and basic proteins under acidic and basic conditions. An alternate approach to applying polysaccharides to capillary walls by using a room temperature ionic liquid was used for the separation of chiral drugs and protein analytes in OT-CEC, respectively. This chapter summarizes the studies conducted in this dissertation and provides recommendations for potential future studies.

Chapter 1 of this dissertation gives a review of background information relevant to the research presented. In Chapter 2, novel lysine-based molecular micelles, poly-ε-SUK, were synthesized and applied as a stationary phase in OT-CEC. Ten protein analytes, 6 acidic and 4 basic, were chosen as a model system for separations. Due to the zwitterionic nature of the lysine-based molecular micelles, the analytes were evaluated under cathodic and anodic EOF. Under optimized acidic conditions, anodic EOF, all ten proteins were successfully separated. When using cathodic EOF, basic conditions, two proteins could not be separated and co-eluted as one peak. However, other protein peaks had resolution values of 0.95 or greater. A human serum sample was also evaluated to assess the applicability of the molecular micelle coating towards a real sample. Three major protein peaks, with no purification of the sample, were observed under acidic and basic conditions. Reproducibility studies also show high stability of the column with 50 consecutive runs yielding 1.24% RSD of the EOF. Future studies involve the synthesis of the novel molecular micelle poly-α-SUK and its use as a coating for the separation of protein analytes. The pH sensitive amine would be the α-amino group rather than

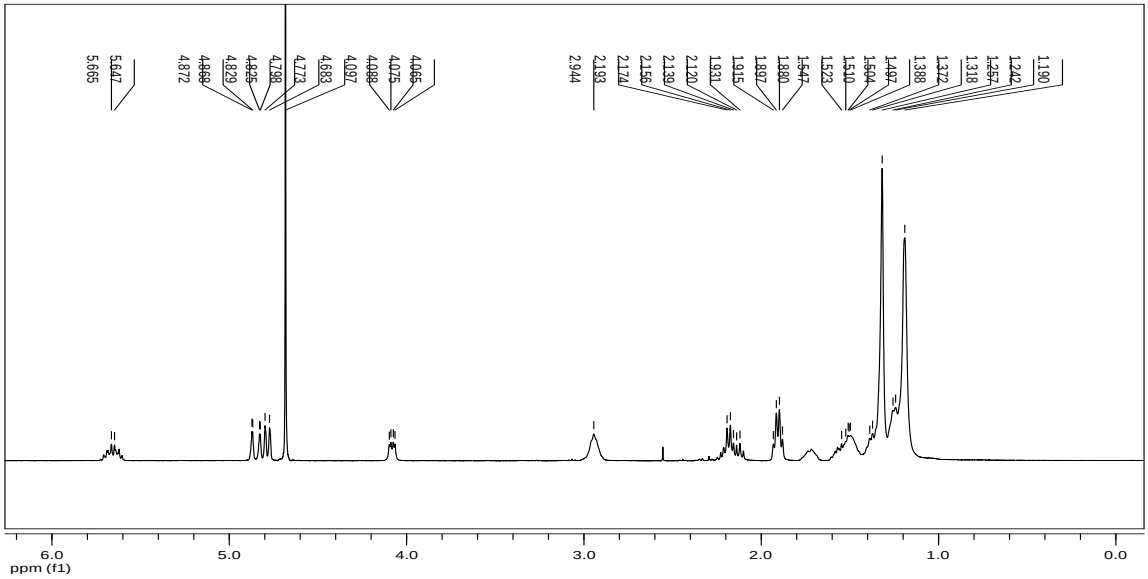
the ε-amine. This molecular micelle may provide insight on types of interactions occurring throughout the capillary. Moreover, various chiral separations will also be performed using both poly-ε-SUK and poly-α-SUK to determine the effect of the chiral center placement, within the chiral selector, on the separation of chiral compounds.

In Chapter 3, the use of polysaccharide adsorbed stationary phases for the analysis of chiral drug analytes was evaluated. A polysaccharide of choice (i.e., cellulose acetate, cellulose acetate phthalate, or cellulose acetate butyrate) was dissolved into 1-butyl-3-methylimidazolium acetate and then filled into a capillary using a syringe pump. The capillary was then rinsed with water to remove the water soluble ionic liquid leaving the water insoluble polysaccharide stationary phase for use in OT-CEC. During this study, the chiral drug analytes thiopental, sotalol, ephedrine, and labetalol were investigated. While thiopental was baseline resolved for all three cellulose derivatives, sotalol was only partially separated. Labetalol and ephedrine are diastereomeric compounds. Only two major peaks were observed with labetalol and partial separation could be achieved using each one of the chiral selectors. For the separation of ephedrine only two peaks were observed using chiral selectors cellulose acetate and cellulose acetate phthalate; however, the appearance of a third peak, using cellulose acetate butyrate, indicated the enantiomeric separation of (±)-psuedoephedrine. Each of the polysaccharide coatings were used for stability studies and exhibited between 2.52 through 7% RSD of the EOF. Future investigations include the use of amylose, another highly hydrophobic polysaccharide, and its modified forms for the separation of chiral analytes. These experiments would prove the usefulness of the method developed for coating cellulose derivatives on the capillary wall as discussed in Chapter 3. Higher chiral selectivity should be expected due to helical structure of amylase based on previous literature.

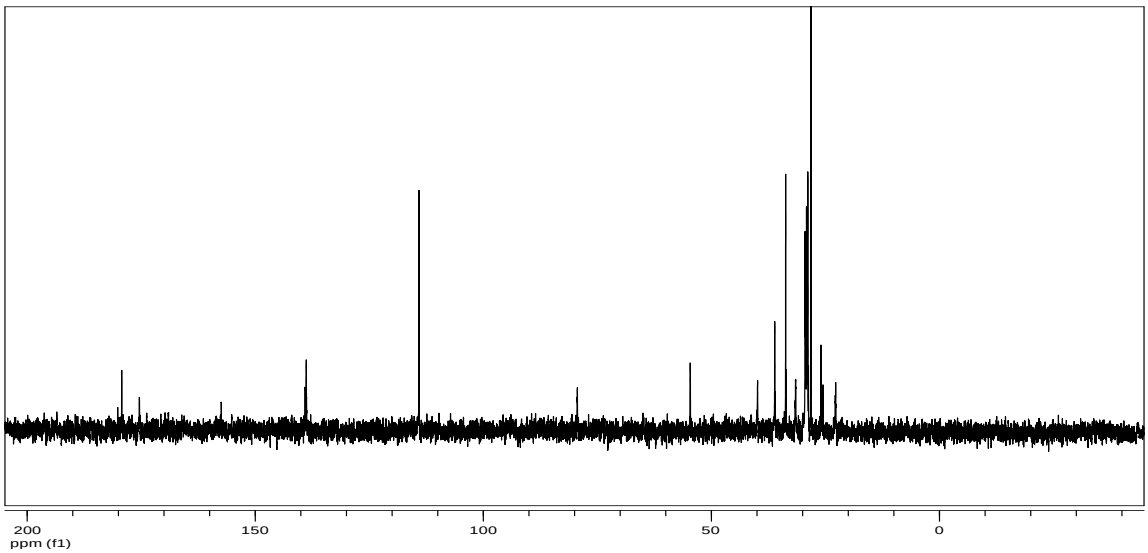
Chapter 4 involves the use of cellulose derivatives being applied to capillary walls as in Chapter 3. This stationary phase was evaluated in the separation of basic proteins in OT-CEC. Protein separations were optimized using polymer concentration, temperature, voltage, and buffer pH. Nanofibers were also formed using CA and applied as a stationary phase. These alternate approaches of applying chiral polysaccharide stationary phases are more rapid than covalently modifying the polymers to the capillary wall. Thus, column preparation time is decreased. Future work involves the use of human serum samples to determine if the polysaccharide coating could be used to distinguish protein biomarkers found in clinical samples.

APPENDIX I
CHARACTERIZATION OF THE LYSINE-BASED SURFACTANT

Appendix I-A. Electrospray Ionization mass spectrum of ε-Boc-SUK. The peak at 435 amu is representative of ε-Boc-SUK.



Appendix I-B. ^{1}H NMR spectrum of ε-Boc-SUK. Peaks at 4.8 ppm and 7.2 ppm are representative of the terminal double bond.



APPENDIX 1-C. ^{13}C NMR spectrum of ε-Boc-SUK. The carboxyl group of the Boc group is at 158 ppm. The double bond is observed at 114 and 138 ppm. The other two carboxyls are at 178 and 180 ppm, respectively.

CUSTOMER #:

HUFFMAN
LABORATORIES, INC.
Quality Analytical Services Since 1956
4630 Indiana Street • Golden, CO 80403
Phone: (303) 278-4455 • FAX: (303) 278-7013

DATE 11/5/07
LAB# 195807
P.O. SEE BELOW
RECD 10/30/07

ANALYSIS REPORT

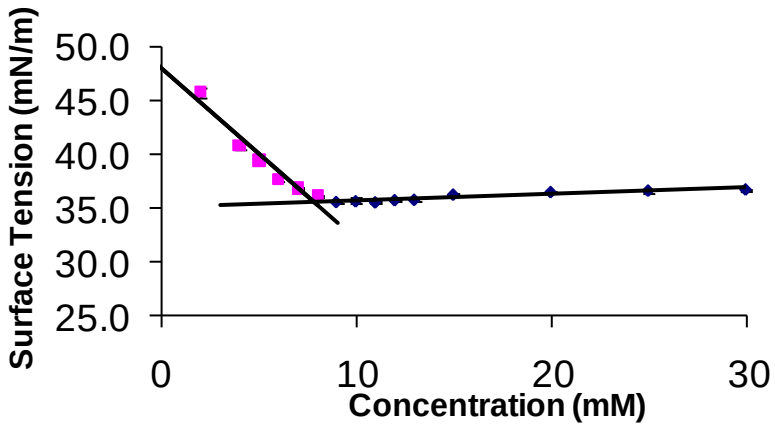
LEONARD MOORE
LOUISIANA STATE UNIV
133 CHOPPIN HALL
BATON ROUGE LA 70803

P.O. #

SEQUENCE/ SAMPLE ID	01 E (EPSILON)
Carbon-------%	59.92
Hydrogen -----%	9.03
Nitrogen------%	6.03
Oxygen--------%	18.69

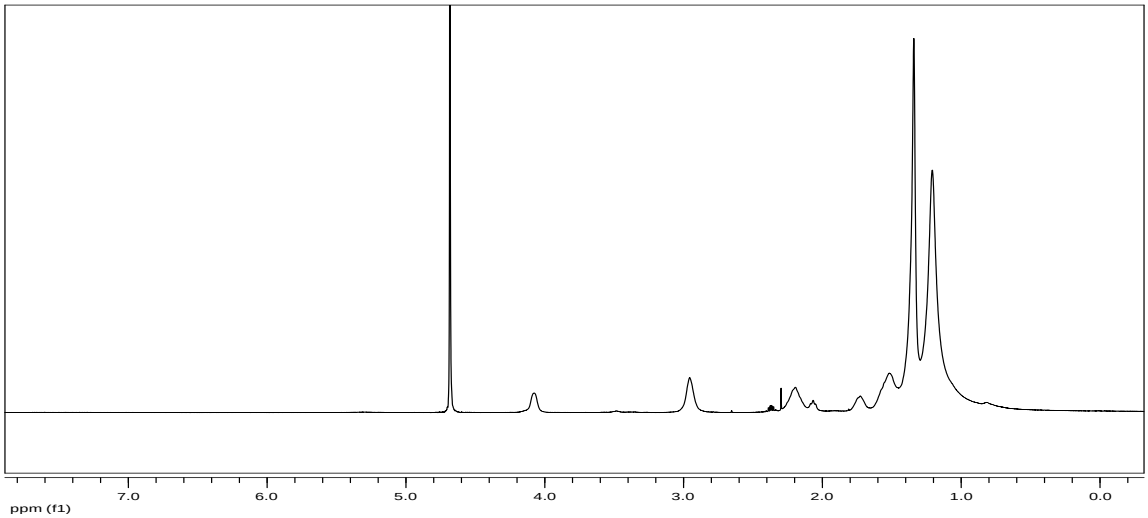
Sample E(EPSILON) was dried under vacuum at 105 degrees C for 2 hours prior to analysis and is reported on a dried sample basis.

APPENDIX 1-D. Elemental analysis of ε-Boc-SUK

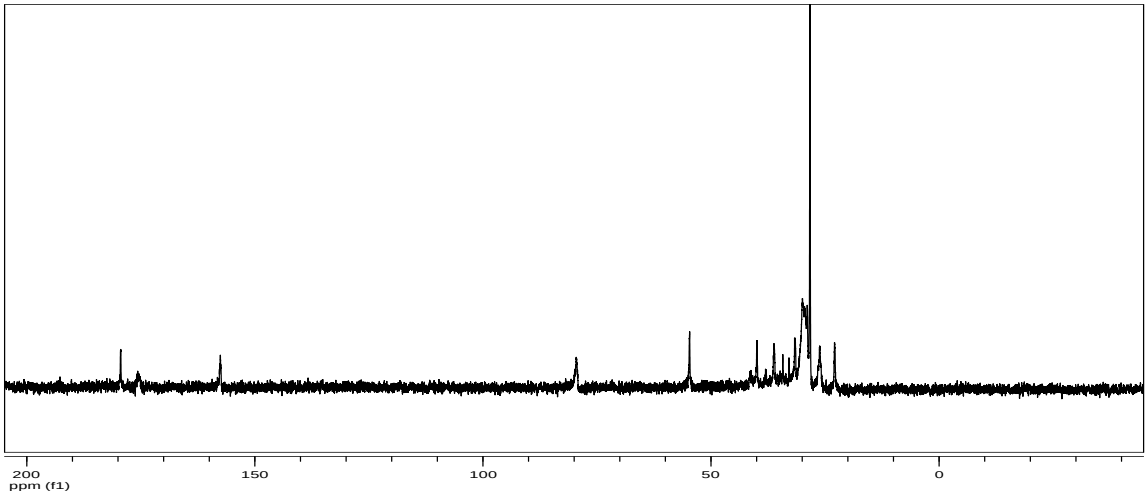


APPENDIX 1-E. Determination of ε-Boc-SUK CMC using Tensiometry.

APPENDIX II
CHARACTERIZATION OF THE LYSINE-BASED MOLECULAR MICELLE



Appendix II-A. ^{1}H NMR spectrum of poly-Boc-ε-SUK. The double bond peaks were not observed after polymerization.



APPENDIX II-B. ^{13}C NMR spectrum of poly-Boc-ε-SUK. Peaks at 114 and 138 ppm shift not observed indicating the polymerization of the double bond.

APPENDIX III
LETTER OF PERMISSION

Copyright Clearance Center RightsLink

Home | Account Info | Help

ACS Publications

Title: Lysine-Based Zwitterionic Molecular Micelle for Simultaneous Separation of Acidic and Basic Proteins Using Open Tubular Capillary Electrochromatography

Logged in as: Leonard Moore Jr.

LOGOUT

Author: Leonard Moore et al.
Publication: Analytical Chemistry
Publisher: American Chemical Society
Date: May 1, 2010

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

BACK CLOSE WINDOW

VITA

Leonard Moore, Jr., was born to Leonard and Betty Moore of Bastrop, Louisiana. He attended Henry V. Adams Elementary, Bastrop Middle School, and Bastrop High School. After his junior year in high school, Leonard was selected to participate in Grambling State University's High Ability Program. Upon graduating from high school, Leonard received the *Georgia Pacific Waymond Powell Scholarship*. He attended Grambling State University where he received dual baccalaureates in both chemistry and physics (2004). As an undergraduate, he participated in summer research internships at Grambling State University, Pennsylvania State University, as well as NASA Ames in Mountain View, California. Leonard received numerous awards, including the *Louis Stoke Louisiana Alliance for Minority Participation Scholarship (LS-LAMP)* and *Minority Research to Access Careers (MARC) Scholarship*, during his undergraduate years. After graduating from Grambling State University, Leonard enrolled at Louisiana State University, Baton Rouge, Louisiana to obtain a doctorate in chemistry under the advisement of Dr. Isiah M. Warner. In 2010, Leonard was selected to attend the Proctor & Gamble Research and Technical Careers in Industry (RTCI) Conference. Leonard has received awards during his graduate career which include: *National Science Foundation (NSF) Bridge to Doctorate Fellowship (BDP), Agilent-National Organization for the professional advancement of Black Chemists and Chemical Engineers (NOBCChE) Professional Development Award*, and *Graduate Alliance for Education in Louisiana (GAELA) Supplement Award*. His research publications and presentations include:

Moore, Jr., Leonard; Stavrou, Giannis; Fernand, Vivian E.; Kapnissi-Christodoulou, Constantina P.; and Warner, Isiah M. "Facile Preparation of Cellulose Coated Capillaries Using a Room Temperature Ionic Liquid for Chiral Separations", *to be submitted to Journal of Chromatography A.*

Moore, Jr., Leonard; Stavrou, Giannis; Hamdan, Suzana; Fernand, Vivian E.; Kapnissi-Christodoulou, Constantina P.; and Warner, Isiah M. "Facile Preparation of Cellulose Coated Capillaries Using a Room Temperature Ionic Liquid for Protein Separations", *Manuscript in Preparation*.

LeJeune, Zorabel M.; **Moore Jr., Leonard**; Warner, Isiah M.; and Garno, Jayne. C. "*In situ* AFM Investigation on the pH dependence of Zwitterionic Molecular Micelle Assembly on Silica", *to be submitted to Langmuir*.

Hamdan, Suzana; **Moore, Jr., Leonard**; Das, Susmita; Spivak, David; Warner, Isiah M. "Synthesis of Molecularly Imprinted Polymeric NanoGUMBOS", Manuscript in Preparation.

Tsioupi, Despina A.; Nicolaou, Irene; **Moore, Leonard**; and Kapnissi-Christodoulou, Constantina P. "Chiral Separation of Huperzine A Using CE – Method Validation and Application in Pharmaceutical Formulations", *Electrophoresis*, **2011**, 33 (3), 516-522.

Moore, Jr., Leonard; LeJeune, Zorabel M.; Luces, Candace A.; Gates, Arther T.; Li, Min.; El-Zahab, Bilal.; Garno, Jayne C.; and Warner, Isiah M. "Lysine-Based Zwitterionic Molecular Micelle for the Simultaneous Separation of Acidic and Basic Proteins using Open Tubular Capillary Electrochromatography", *Analytical Chemistry*, **2010**, 82 (10), 3997–4005.

Gates, Arther T.; **Moore, Jr., Leonard**; Sylvain, Monica R.; Jones, Christina M.; Lowry, Mark; El-Zahab, Bilal, Robinson, James W.; Strongin, Robert M.; and Warner, Isiah M. "Mechanistic Investigation of N-Homocysteinylation-Mediated Protein-Gold Nanoconjugate Assembly" *Langmuir*, **2009**, 25, 9346-9351.

Invited Lectures

"Protein Separations using a Zwitterionic Micelle Coating in Open Tubular Capillary Electrochromatography"
(LSU Mass Spectrometry Cyber Symposium – **2009**)

Guest Speaker at NASA Explorer School

(H.V. Adams Elementary - **2005)**

Oral Presentations

"An Investigation of Lysine-Based Molecular Micelle as a Coating for Protein Separations in Open Tubular Capillary Electrochromatography"
(NOBCChE National Conference – **2008**)

"Synthesizing Carbon Nanotubes Using an Inductively Coupled Plasma Reactor"
(Annual Biomedical Research Conference for Minority Students – **2004**)

Poster Presentations

"Synthesizing Carbon Nanotubes Using an Inductively Coupled Plasma Reactor"

(National Society of Black Physicists – **2004**)

(Louis Stokes – Louisiana Alliance for Minority Participation Conference – **2004**)

"Using Biological Adhesions to form Colloidal Crystals"

(Annual Biomedical Research Conference for Minority Students – **2003**)

(Louisiana Academy of Sciences – **2002**)

"Synthesis and Characterization of Novel Polyimides"
(Louisiana Academy of Sciences – **2001**)